

CXCL13 and CCL21 as drivers of tertiary lymphoid structure assembly in the tumor microenvironment

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Abstract. Although Immune Checkpoint Blockade (ICB) has transformed modern oncology, its efficacy remains limited due to the absence of organized immune infiltration in "cold" Tumor Microenvironments (TMEs). Tertiary Lymphoid Structures (TLSs), the pivotal ectopic lymphoid organs, have been shown to orchestrate localized, adaptive, anti-tumor immunity within non-lymphoid tissues. This review focuses on two master chemokine drivers, CXCL13 and CCL21, based on their roles in orchestrating TLS neogenesis, structural maturation, and functional compartmentalization. This paper examines how CCL21 triggers early lymphoid aggregation by recruiting CCR7⁺ T cells and mature dendritic cells, as well as facilitating the integration of High Endothelial Venules (HEVs). Meanwhile, CXCL13 acts as a primary architectural morphogen that drives CXCR5⁺ B-cell recruitment, follicular segregation, and germinal center maintenance via a self-amplifying lymphotoxin-mediated feedback loop. This paper also emphasizes the clinical significance and prognostic value of chemokine-dense TLSs as predictive biomarkers of positive patient outcomes and ICB responsiveness. Finally, this paper evaluates emerging bioengineering modalities, including chemokine-delivering biomaterial scaffolds, targeted vascular conditioning, and synthetic cellular therapies. These modalities are designed to induce functional TLSs in refractory malignancies.

Keywords: Tertiary Lymphoid Structures (TLS), CXCL13, CCL21, tumor microenvironment, immune checkpoint blockade

1. Introduction

Immunotherapy, or the study of harnessing and modulating the immune system for the prevention and treatment of diseases, has become a cornerstone of modern cancer therapy [1]. However, a major challenge limiting the efficacy of these treatments is the presence of "cold tumors", which respond poorly to immune checkpoint inhibitors. These tumors are characterized by a highly immunosuppressive Tumor Microenvironment (TME), impaired antigen presentation, and a paucity of Tumor-Infiltrating Lymphocytes (TILs) [2]. Historically, the spatial organization of TILs was underappreciated, and these infiltrates were often regarded as disorganized, passive aggregates of immune cells. However, advances in high-throughput spatial omics have revealed that these infiltrates can form highly organized, functional immune niches known as Tertiary Lymphoid Structures (TLSs), representing a major shift in the understanding of anti-tumor immunity [3].

Unlike Secondary Lymphoid Organs (SLOs), such as lymph nodes, which arise through genetically programmed embryonic development, TLSs develop ectopically in non-lymphoid tissues in response to persistent inflammation. They are absent under normal physiological conditions, emerge after birth, and are induced by chronic inflammatory stimuli, including chronic infections, autoimmune diseases, transplant rejection, and cancer [4]. Although TLSs share key architectural and cellular features with SLOs, they are dynamic structures that progress through distinct stages of maturation, ranging from early lymphocytic aggregates to fully mature germinal center–positive TLSs [4].

Here, this study provides a thorough overview of the distinct yet collaborative contributions of C-X-C motif Ligand 13 (CXCL13) and C-C motif Ligand 21 (CCL21) in initiating and assembling TLS. This work traces their spatiotemporal coordination in the TME to bridge the gap between basic chemokine signaling and modern translational oncology. Ultimately, this study provides a unified conceptual framework to facilitate the development of novel immune-based therapeutics and implant-based treatment strategies that can transform treatment-resistant "cold" tumors into immune-responsive "hot" microenvironments.

2. Anatomical and cellular architecture of TLS

As pointed out in the introduction, a functional TLS isn't merely a collection of immune cells but a well-structured ectopic lymphoid organ mirroring the organized layout of SLOs. This unique arrangement allows for effective local priming and robust effector responses.

2.1. Spatial compartmentalization of B-cell and T-cell zones

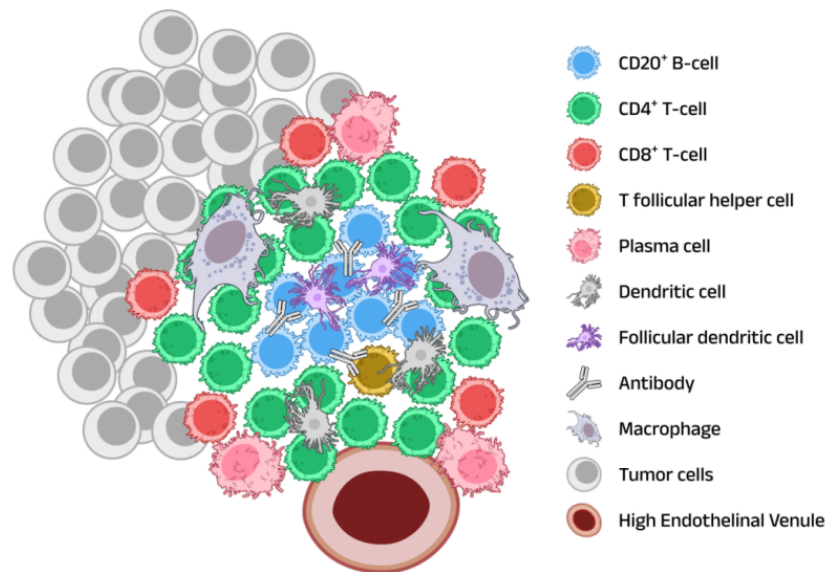


Figure 1. Spatial organization of a mature Tertiary Lymphoid Structure (TLS) within the Tumor Microenvironment (TME)

A central germinal center-like B-cell zone is supported by Follicular Dendritic Cells (FDCs), surrounded by a distinct T-cell zone containing CD4⁺ and CD8⁺ T cells, along with mature Dendritic Cells. High Endothelial Venules (HEVs) act as the primary entry portals for circulating lymphocytes, establishing a localized and highly organized hub for adaptive anti-tumor activity [5].

At the simplest level, TLSs are non-encapsulated clusters of immune cells, but more organized TLSs show a clearer separation, driven by relevant chemokines signaling the organization of B cells and T cells into distinct zones via stromal remodeling and specialized vasculature [6, 7].

As shown in Figure 1, a mature TLS is characterized by two zones: an inner CD20⁺ B-cell zone and an outer CD3⁺ T-cell zone [8]. The inner B-cell zone is typically rich in follicular CD20⁺ B cells resembling those found in lymph nodes, but it also commonly contains plasma cells and Follicular Dendritic Cells (FDCs), which help to retain antigen and support B-cell selection. Functionally, this zone is where B cells proliferate, undergo somatic hypermutation and class switching, resulting in high-affinity antibody responses [4, 5]. The T-cell zone is enriched with CD4⁺ T follicular helper (Tfh) cells, CD8⁺ cytotoxic T cells, Th1 cells, and sometimes regulatory T cells (Treg). Often it contains mature Dendritic Cells like DC-LAMP⁺ and CD83⁺ Dendritic Cells as well, whose job is to present antigens to the T cells mentioned above. Similar to the B-cell zones, the T-cell zone is where T cells are activated, helper signals for B cells are generated, and cytotoxic or regulatory signals are coordinated [5, 6, 8].

The proximity of these two zones is significant, since the interaction between the zones provides localized antigen presentation, B-cell class switching, and somatic hypermutation, allowing the immune system to generate tumor-specific effector responses without relying on the migration of lymphocytes back to the distant lymph nodes. Such an organization drastically reduces temporal and spatial barriers for an effective humoral and cellular immune attack against the neoplastic cells that are rapidly mutating [8].

2.2. Integration of high-endothelial venules and Dendritic Cells

A critical structural feature of a mature TLS is the presence of High Endothelial Venules (HEVs). These specialized post-capillary venules feature cuboidal endothelial cells that express Peripheral Node Addressin (PNAd) and L-selectin ligands, acting as exclusive entry gates for circulating lymphocytes [9, 10]. During entry, lymphocytes roll along the HEV endothelium via L-selectin and PNAd interactions, where they encounter localized chemokines, such as CCL21, which trigger integrin activation. This cascade facilitates firm adhesion and transmigration into the tumor stroma [9]. Consequently, HEVs operate with much higher selectivity than typical tumor vasculature, ensuring a continuous, regulated influx of naïve and central memory lymphocytes required to sustain the localized immune response [11].

Once these lymphocytes navigate the HEV portals, they require immediate spatial and antigenic instruction, a process orchestrated by local Dendritic Cells (DCs). DCs establish the localized chemokine environment and stromal architecture that enable TLSs to function as ectopic lymphoid organs [12, 13]. Within mature TLSs, these cells consistently express Lysosome-Associated Membrane Glycoprotein 3 (LAMP3) and localize directly within the T-cell zones [8]. Recent studies emphasize that type 1 conventional Dendritic Cells (cDC1s) are central to anti-tumor TLS programs. cDC1 maturation strictly depends on Interferon- γ (IFN- γ). The genetic ablation of Major Histocompatibility Complex classes I and II (MHC I and II) on these cells completely abolishes TLS maintenance, Tfh cell survival, and germinal center formation [14]. However, this architecture exhibits dangerous phenotypic plasticity. In progressive disease states, such as Papillary Thyroid Carcinoma (PTC), LAMP3⁺ DCs can adopt a pro-tumorigenic role by secreting CCL17 and CCL22 to recruit Tregs, thereby mediating immune evasion [15].

2.3. Maturation continuum and phenotypic plasticity

TLSs do not appear instantaneously, but develop through a stepwise process that moves from loose immune cell clusters into a mature, lymph node-like structure with germinal centers (see Figure 2) [16].

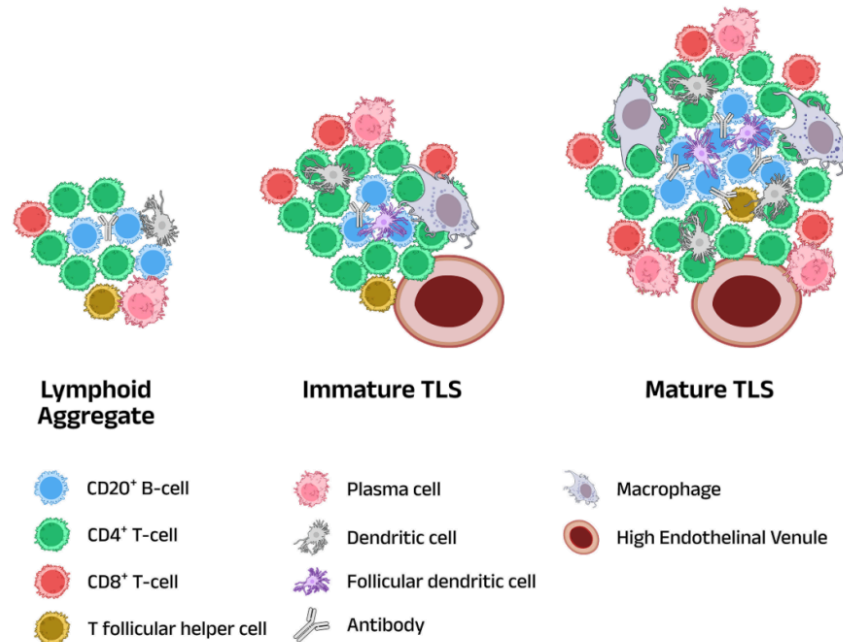


Figure 2. Morphological stages of TLS maturation

The progression from a disorganized lymphoid aggregate to an immune-competent, mature TLS is defined by three key structural milestones: the integration of High Endothelial Venules (HEVs), the spatial segregation of T-cell and B-cell zones, and the establishment of functional germinal centers supported by follicular dendritic cell networks [17].

The earliest recognizable phase begins when lymphocytes and antigen-presenting cells accumulate in inflamed tissue, typically around a nascent or stromal niche, such as an HEV-rich region. During this stage, the aggregate is largely disorganized, and FDCs are either entirely absent or just beginning to appear [16]. As development continues, a clear separation begins to occur between the B-cell and T-cell zones. The FDC network becomes more apparent in the B-cell zone, and the DC network is already present and active, supporting local immune activation. This stage is often considered a primary follicle-like TLS because it exhibits more structure, though it lacks a fully mature germinal center [17]. During the final maturation stage, the TLS develops a clear germinal center and complete B-cell follicular architecture. At this point, it resembles a true lymphoid organ with organized B-cell maturation, T-cell support, and active, local, antigen-driven responses [18].

It is important to note that the presence of immature TLSs may not simply be benign, but may be actively detrimental because they can represent an ongoing inflammatory niche that recruits and organizes immune cells without producing effective, well-coordinated immunity [19]. In environments heavily influenced by metabolic disruptions, such as those involving tryptophan catabolism and the production of kynurenine, or those with high levels of Transforming Growth Factor- α (TGF- α) and vascular endothelial growth factor, TLS may fail to mature and instead adopt an immunosuppressive state [20].

3. Lymphoid Tissue inducer and organizer (LTi-LTo) paradigm

To better describe how TLSs form, it is important to understand the Lymphoid Tissue inducer (LTi) and Lymphoid Tissue organizer (LTo) paradigm. This interaction is usually triggered in chronically inflamed

tissues [17]. In this paradigm, there are two types of cells: LTi cells and LTo cells. LTi cells act as initiators, and LTo cells act as organizers. A key signal pair is the expression of membrane Lymphotoxin (LT α 1 β 2) by LTi cells and the expression of Lymphotoxin- β Receptor (LT β R) by LTo cells. This activates NF κ B-dependent programs in stromal cells [17, 21]. After the initial LTi-LTo interaction, additional immune cells amplify the structure. Stromal and immune cells communicate with each other to organize the vasculature, maintain lymphocyte trafficking, and stabilize tissue architecture. This creates a self-reinforcing loop [17]. However, it is important to note that in adult TMEs, this role exhibits significant plasticity. Circulating CD11⁺ Gr1⁺ myeloid cells, T helper 17 cells, CD8⁺ T cells, and even subsets of B cells can adopt an inducer-like phenotype [20]. This helps explain why TLSs are heterogeneous across cancers, autoimmune diseases, infections, and transplants [21]. The direct molecular consequence of this NF κ B-dependent stromal activation is the robust induction and secretion of unique homeostatic chemokines. When activated, these LTo-like stromal cells begin pumping out localized gradients of CXCL13 and CCL21. These two chemokines serve as the main architectural beacons, physically recruiting and organizing the circulating immune cells needed to build the TLS and in effect translating the initial LTi-LTo contact signal into massive spatial reorganization.

4. CXCL13: orchestrating humoral immunity and germinal center dynamics

In the many different types of chemokine, CXCL13 is one of the most important B-cell chemoattractants involved in the formation of TLS [22]. This section will analyse how CXCL13 acts as the master architectural regulator of the B-cell zone and germinal center maturation [23].

4.1. Cellular sources and the CXCR5 receptor axis

Within the TME, CXCL13 can originate from both immune and non-immune cells, and the dominant source can depend on the tumor type and local signaling [24]. While FDCs are the classic source in SLOs, the neoplastic environment features CXCL13 expression from multiple unconventional cell types. TFH13 cells, a prognostic subpopulation of CD4⁺ Tfh cells, act as profound producers of CXCL13 in solid tumors such as breast cancer and melanoma [3]. Other sources of CXCL13 include hypoxia- and TGF- α -stimulated cancer-associated fibroblasts, PD-1⁺ exhausted CD8⁺ T cells and, occasionally, the neoplastic cells themselves [20].

CXCL13 works by activating the CXCL13–CXCR5 axis. CXCR5 is a G-protein–coupled receptor expressed on B cells and certain T-cell subsets, especially Tfh and some CXCR5⁺ CD8⁺ T cells in tumors [25]. When CXCL13 binds CXCR5, it triggers the dissociation of G proteins into G α and G β subunits, initiating downstream signaling cascades, ultimately shaping cell dynamics, guiding B cells toward the emerging follicular zones, and maintaining their positioning within the TLS [26, 27].

4.2. Positive feedback loops in germinal center maintenance

The CXCL13 network is often described as self-amplifying since it recruits and organizes the immune cells that sustain more CXCL13 signaling [28]. The sequence of events operates as follows: First, CXCL13 is secreted by initial organizer cells or Tfh, which recruit CXCR5⁺ B cells into the tissue. Once recruited, these B cells upregulate membrane-bound Lymphotoxin α 1 β 2 (LT α β), which binds to the lymphotoxin- β receptor on adjacent stromal and FDCs. This stimulates these cells to undergo further maturation and produce larger quantities of CXCL13, thus amplifying the cycle [27]. This means that CXCL13 not only recruits cells once, but also helps to create a niche that continues to produce the recruitment signal [29]. This reciprocal stimulation is fundamental to the transition from an immature primary follicle to a fully functional and mature germinal center.

In mature TLS, CXCL13 is also closely tied to the presence and maintenance of germinal-center-like B-cell zones [24]. Within the established germinal center, highly concentrated CXCL13 gradients help guide the cyclical movement of B cells between the dark zone (where somatic hypermutation of B-cell receptor genes occurs) and the light zone (where antigen selection via FDCs takes place) [30]. This precise spatial coordination enables the generation of high-affinity and tumor-specific memory B cells and plasma cells directly within the local tissue.

5. CCL21: spatial coordination of cellular immunity and HEV integration

Alongside the regulation of the humoral compartment by CXCL13, CCL21 is crucial for the organization of cellular immunity in conjunction with its functional partner, CCL19.

5.1. The CCL19/CCL21/CCR7 axis in T-cell and DC recruitment

The CCL19/CCL21/CCR7 axis is the T-cell zone–organizing system in the TLS. It helps with the recruitment of naïve T cells, central memory T cells, and DCs into the T-cell zone [31]. In this axis, CCR7 acts as the receptor, while CCL19 and CCL21 act as the ligands, with CCL21 predominantly synthesized by organizer-like stromal cells, lymphatic endothelial cells, HEVs, and specialized subsets of fibroblastic reticular cells [3], and CCL21 and CCR7 being primarily expressed on naïve T cells, central memory T cells, and mature DCs [31].

Within this framework, CCL21 acts as the ultimate spatial coordinator for antigen presentation, co-attracting both CCR7⁺ naïve T cells and CCR7⁺ antigen-loaded DCs to the same anatomical niche within the peritumoral stroma. This drastically increases the probability of cognate T-cell receptor engagement [32]. Furthermore, such expression of CCL21 by high endothelial cells ensures that circulating lymphocytes rolling along the endothelium receive the required integrin-activation signals to arrest, undergo diapedesis, and enter into the tumor tissues without vascular leakage [32]. Together, these components create positional cues that support TLS organization, T-cell priming, and local immune interaction maintenance [33].

5.2. Spatiotemporal synergy between CXCL13 and CCL21

CXCL13 and CCL21 are often described as complementary chemokines that together help to organize TLS by dividing the labor between B-cell and T-cell compartments [24, 34]. In this synergy, CCL21 acts early in the neogenesis cascade, providing the infrastructural base by creating the initial T-cell and DC aggregate and establishing the vascular gateways; subsequently, CXCL13 gradients rise, which facilitates the massive recruitment of B cells, T cells, and Tfh cells, compartmentalizing the aggregate and driving the maturation of the germinal center [8]. In fact, studies in inflammatory tissues and cancer demonstrated that CXCL13 and CCL21 often co-occur during progressive lymphoid neogenesis, including the early aggregates before full compartmentalization between T-cell and B-cell zones [24, 34]. In tumor models, combined CXCL13 and CCL21 expression is associated with stronger TLS induction and can improve immune cell density within tumors, the reason why certain TLS-induction strategies intentionally use both signals rather than either one alone [35]. Table 1 includes a more detailed comparison to illustrate the complementary relationship between CXCL13 and CCL21. This matrix highlights the distinct molecular targets, primary cellular sources within the tumor microenvironment, and the specific architectural roles these chemokines execute during Tertiary Lymphoid Structure (TLS) neogenesis.

Table 1. Comparative summary of the CXCL13 and CCL21/CCL19 chemokine axes

Features	CXCL13	CCL21 / CCL19
Primary Cellular Source in TME	FDCs, inflammatory stromal/immune cells	Stromal L To cells, stromal cells, fibroblasts
Target Receptor	CXCR5	CCR7
Main Recruited Cells	B cells, CXCR5+ Tfh cells	Naïve T cells, mature DCs
Architectural Role	Organizing B-cell zone, driving germinal center formation, positive feedback loop maintenance	Organizing T-cell zones, facilitating intra-tumoral antigen presentation and HEV diapedesis
References	[27, 36, 37]	[36, 38]

Note: TME = Tumor Microenvironment; FDC = Follicular Dendritic Cell; DC = Dendritic Cell.

6. Clinical implications: prognostic value and ICB responsiveness

Such insights into the molecular and cellular biology of CXCL13 and CCL21 in mature TLS provide profound prognostic value in terms of patient survival and decision-making. Across multiple studies, patients whose tumors contain well-organized TLS tend to have a more positive prognosis, and these TLSs often contain both CD20⁺ B cells and CD8⁺ T cells in close proximity [39]. In fact, the co-existence of CD8⁺ T cells and CD20⁺ B cells within organized TLS is one of the strongest positive prognostic indicators across numerous metastatic melanoma, soft-tissue sarcoma, renal cell carcinoma, and non-small cell lung cancer [39, 40]. Tumors rich in mature TLS exhibit a unique transcriptomic signature, characterized by B-cell markers and high CXCL13 expression, which actively predicts successful clinical responses to PD-1 and PD-L1 blockade therapies [41].

The underlying mechanism of this is clear. Mature TLS operate as local staging grounds for formidable anti-tumor immunity by locally generating tumor-specific memory B cells and plasma cells, which in turn produce anti-tumor antibodies, such as those targeting endogenous retroviruses in lung adenocarcinoma models [24]. They also prevent or even reverse CD8⁺ T cell exhaustion via direct co-stimulation and cytokine support from neighboring B cells and T cells [42]. This implies that the presence and maturity of TLS provides a much more refined prognostic tool compared to merely quantifying generalized tumor-infiltrating lymphocytes, since it captures the structural capability of the TME to sustain an adaptive immune response [3].

7. Bioengineering and therapeutic induction of TLS

Finally, the prognostic value of CXCL13 and CCL21 shows the exciting possibility within tumor immunology: engineering the TME to artificially induce TLS formation, converting immunologically "cold" tumors into highly reactive "hot" ones.

7.1. Exogenous chemokine delivery and biomaterial scaffolds

A classic approach for inducing TLS is to overexpress or locally deliver TLS-inducing chemokines, such as CXCL13, CCL 19, and CCL 21, to recruit lymphocytes and seed lymphoid-like aggregates [24]. In murine melanoma models, this direct co-administration of CXCL13 and CCL21, alongside anti-PD-L1 antibodies, resulted in significant increases of localized TLS formation, B-cell activation, and tumor growth suppression

[24]. However, the main drawback of this approach is evident. Although this strategy shows significant promise in animal models, systemic chemokine expression remains risky due to concerns related to off-target inflammation and tissue damage, and TLS induced in this way can be heterogeneous and not fully equivalent to spontaneous tumor TLS [43]. Free, soluble chemokines also degrade rapidly in vivo, which can cause systemic toxicity or inappropriate systemic immune activation [44].

To combat this "wrong dose, wrong place, wrong duration" problem, the field is now shifting toward spatiotemporally controlled biomaterial scaffolds. Some promising instances include collagenous scaffolds and collagen sponges loaded with lymphoid-inducing cytokines as the platform for generating artificial TLS-like structures with distinct B-cell and T-cell zones, FDC/FRC-like features, and HEV-like vasculature [45]. Another approach would be the deployment of thermosensitive hydrogels embedded with lipid-coated mesoporous microparticles, providing a slow and sustained release that mimics the natural developmental gradients of a nascent lymph node, which generates synthetic TLS niches that mount robust and antigen-specific T-cell responses against melanoma [44].

7.2. Vascular targeting and synthetic cellular therapies

An alternative to delivering exogenous chemokines is to stimulate the existing tumor stroma to produce them endogenously. The two most relevant endogenous production strategies fall under the categories of vascular targeting of host tissues and synthetic cellular therapies engineered to secrete chemokines on site.

A major idea that has emerged recently is that endothelial cells are not merely passive conduits, but rather, they can be reprogrammed to produce chemokines that influence immune cell entry and the vascular state. A 2023 study revealed that conditioning endothelial cells to mount a vascular-selective cytokine response improved CD8⁺ T cells entry into tumors, supporting the idea that vascular targeting can create a local, chemokine-rich gateway for immunity [46]. A more advanced application of this concept is LIGHT-VTP, a vascular-targeted peptide linked to the LIGHT cytokine. This approach induces robust CCL21 expression in the vascular bed and tumor-resident macrophages. This drives HEV neogenesis that expresses the MECA-79 antigen, which leads to the formation of TLSs. Furthermore, intratumoral injection of lymphotoxin- β receptor agonist antibodies activates NF κ B signaling in the stroma, effectively replacing the natural initiation process of TLS assembly [20].

Another way to generate chemokines endogenously is through synthetic cellular therapies, in which an engineered immune cell acts as the "factory" rather than the endothelium. For TLS-related goals, engineered T cells, DCs, macrophages, and other cellular platforms are attractive options because they can produce chemokines in a sustained and spatially restricted manner after homing into tissue [45, 47]. An example of this strategy is an engineered *Salmonella typhimurium* strain that expresses LIGHT (TNFSF14) and colonizes tumors, inducing mature TLS formation in colorectal cancer models. This study showed that LIGHT-HVEN signaling on group 3 innate lymphoid cells is crucial for TLS maturation and T-cell-mediated antitumor immunity [48]. This demonstrates that synthetic cells can influence TLS architecture beyond merely recruiting lymphocytes.

8. Conclusion

In conclusion, CXCL13 and CCL21 are not just transient chemostatic gradients; they are the fundamental structural architects of TLSs. These chemokines act as the molecular glue that spatializes T cells, B cells, and DCs into functional germinal centers and HEV networks. Thus, they represent a vital biological bridge between chronic tumor inflammation and durable adaptive immunity.

The gap between biochemical discovery and practical clinical application remains substantial. There is an urgent need for a standardized immunohistochemical scoring system to quantify TLS maturity clinically, as differentiating between suppressive, immature aggregates and functional, mature structures remains a significant diagnostic challenge. Additionally, the dual nature of chemokines presents a challenge: elevated systemic levels of CXCL13, without the structural confinement of an organized TLS, can inadvertently recruit pro-tumorigenic regulatory cell populations or fuel specific lymphoma subtypes.

Future studies should focus on therapeutic strategies that limit chemokine delivery to the peritumoral stroma. This ensures the induction of TLS and enhances anti-tumor immunity without inducing systemic autoimmune toxicity, and therapeutically targeting CXCL13 and CCL21 could change the immunological landscape of the TME. These interventions offer a mechanistic blueprint for successful conversion of treatment-refractory "cold" tumors into immune-infiltrated "hot" environments via artificial orchestration of TLS neogenesis. Ultimately, such structural reprogramming might help to overcome resistance to immune checkpoint blockade and represent a new paradigm in solid tumor oncology.

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