B cells in the formation of Tertiary Lymphoid Organs in autoimmunity, transplantation and tumorigenesis.

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Highlights

- TLS develop in target organs of autoimmune diseases, transplantation and cancer.
- TLS can function as germinal centres supporting B-cell selection/differentiation.
- TLS can be destructive or have beneficial effects at the site of inflammation/disease.
- Therapeutic targeting of TLS results in beneficial effects in patients, though inhibition may lead to immune suppression while stimulation may lead to autoimmunity.

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Abstract

Tertiary lymphoid organs named also tertiary lymphoid structures (TLS) often occur at sites of autoimmune inflammation, organ transplantation and cancer. Although the mechanisms for their formation/function are not entirely understood, it is known that TLS can display features of active germinal centres supporting the proliferation and differentiation of (auto)-reactive B cells. In this Review, we discuss current knowledge on TLS-associated B cells with particular reference on how within diseased tissues these structures are linked to either deleterious or protective outcomes in patients and the potential for therapeutic targeting of TLS through novel drugs.

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Introduction

Tertiary lymphoid organs can develop at non-lymphoid sites in the target organs of chronically inflammatory and autoimmune diseases, allograft rejection, and solid tumours. They are commonly known as tertiary lymphoid structure (TLS) or ectopic lymphoid structure (ELS). Here, we will define them as TLS throughout the text. Unlike secondary lymphoid organs , TLS lack a capsule and afferent lymphatic vessels and they are transient structures which resolve after antigen clearance [1, 2]. TLS are characterised by B- and T-cell segregation, ectopic expression of lymphoid chemokines CXCL13, CCL19 and CCL21 that regulate T and B lymphocyte compartmentalisation, high endothelial venules, and often by the presence of CD21+ follicular dendritic cell (FDC) network which sustains the local humoral B-cell response. The general cellular and molecular mechanisms underlying their formation and pathogenic function have been extensively reviewed elsewhere [1-4*]; thus, for the purpose of this Review, we focus solely on the importance of B cells associated with TLS in the context of autoimmune diseases, transplantation and cancer highlighting their potential for deleterious and/or beneficial effects in diseases.

B cells contribution in TLS in autoimmune diseases.

TLS can form in some patients in the target organ of several autoimmune diseases such as the inflamed synovium in rheumatoid arthritis (RA), salivary and lacrimal glands in Sjögren's syndrome (SS), the central nervous system in multiple sclerosis, in diabetic pancreas, and in the intestine in inflammatory bowel diseases [3*-5*]. In the target organs of rheumatic autoimmune diseases, TLS can display features of active germinal centres (GCs), including the expression of the enzyme activation-induced cytidine deaminase (AID) which regulates immunoglogulin gene affinity maturation via the process of somatic hypermutation, and they support a local antigen-driven B-cell proliferation and differentiation into antibody-producing plasma cells [6-9]. Growing evidences have demonstrated that TLS in RA are actually required for B-cell affinity maturation at ectopic sites since, in the absence of functional GCs the B cells that enter into the chronically inflamed synovial tissue do not acquire further diversification [7]. Accordingly, the expression of AID, which also initiates immunoglobulin isotype class-switch recombination, is limited to TLS+ tissues and correlates with the presence of CD21+ FDC networks [6, 10, 11]. There is now conclusive evidence that TLS are actively implicated in sustaining autoimmunity to disease-specific antigens in the target organs of autoimmune diseases although this concept was initially challenged in RA since TLS are also found in seronegative RA patients (i.e., without circulating autoantibodies such as anti-citrullinated protein antibodies (ACPA) and rheumatoid factor) [12] and previous studies fail to show a direct correlation between the presence of TLS and circulating or synovial fluid ACPA which might be explained by the production of autoantibodies at extra-articular sites, such as secondary lymphoid organs [12-14]. Conversely, we and others have produced evidence that confirmed the active role of TLS in the perpetuation of local autoimmunity by showing that i) the engraftment of TLS+ RA synovial tissue or SS salivary glands into severe combined immunodeficiency (SCID) mice results in the release of human class-switched ACPA [6] or anti-Ro/SSA and anti-La/SSB human IgG [15] into the mice circulation, respectively; ii) >30% of the synovial B-cell response in TLS+ RA patients is directed toward citrullinated antigens, supporting the concept that the presence of TLS in RA synovial tissue supports a selection toward ACPA-producing B cells [8*, 16]. As mentioned above, TLS support the local proliferation and differentiation of autoreactive B cells in a disease-specific manner suggesting that the maintenance of TLS is also sustained, at least in part, by an autoantigen-dependent process with the differentiation of autoreactive plasma cells toward disease-specific autoantigens (**Figure 1**) [15]. Recently, functional TLS have been described in an experimental autoimmune encephalomyelitis model of central nervous system (CNS) autoimmunity. Horn and colleagues [17*] demonstrated the expression of AID, hence SHM and CSR in meningeal TLS providing evidence of TLS functionality and *in situ* B-cell antigen-driven affinity maturation. Using deep sequencing technology, the authors showed the presence of mutated Ig-VH within meningeal TLS which were absent in secondary lymphoid organs, thus supporting the concept that meningeal TLS are partially independent structures in sustaining B-cell differentiation at the local site. These data corroborate the concept that TLS can be actively involved in B-cell affinity maturation at the local site of inflammation.

Although direct evidence of the importance of B cells in antigen presentation and cross-talk to Th cells in the context of TLS is currently missing, it is highly likely that TLS maintenance and function are regulated by the interaction with specialised T helper cell subsets, particularly T follicular helper (T_{FH}) cells, which are critical in the regulation of active GC responses in secondary lymphoid organs. The possible pathogenic role of T_{FH} cells in the activation and affinity maturation of B cells in TLS via the interaction between inducible T-cell co-stimulator (ICOS) and its ligand (ICOSL), CD40 and its ligand, CD40L, and IL-21 release which is critical for B-cell survival, proliferation and differentiation into plasma cells has been reviewed elsewhere [3*].

B cells within TLS in organ transplantation: rejection or tolerance?

Rejection during organ transplantation is caused by an alloimmune response to donor-specific human leukocytes antigens (HLA) which ultimately results into engrafted organ damage and failure. Rejected grafts are characterised by infiltration of several immune cells including B cells and plasma cells. In contrast to acute rejection where the infiltrating cells do not acquire a proper organisation, in chronic graft rejection the immune cells can organise in TLS [18]. B cells seem to play a central role in both initiation and organization of TLS within the graft. In particular, B cells might substitute lymphotoxin-$\alpha1\beta2$-expressing lymphoid tissue inducer cells in the initiation of TLS [5, 18]. Gene expression studies on renal allograft biopsies have revealed that B cells are recruited into TLS by the interaction of specific chemokines with their receptors (CXCL10 and its receptor CXCR3 [19]; CXCL13 and CXCR5 [20]; CCL3, CCL5, CCL7 and CCR1 [21]) [5, 22]. Presence of B cell-producing autoantibodies after organ transplantation have been also correlated with chronic graft rejection. Interestingly, non-canonical anti-HLA antibodies have been shown to have an adverse effect on graft survival suggesting a breach of self-tolerance in TLS within the rejected graft [23*]. In a recent study Lu *et al* [24*] showed that the presence of CD20+ B cells in allograft rejection can be used as predictive marker of a poorer kidney allograft outcome since it was associated with an increased risk of graft loss. However, recent evidences have demonstrated the role of IL-10-producing B cells in regulating donor specific T-cell response and in contributing to long-term graft tolerance supporting a role of B cells in graft tolerance [25].

TLS-associated B cells in cancer.

While the overall evidence for TLS in solid tumours points towards a protective anti-tumour immunity exerted by TLS in cancer, as discussed below and as previously reviewed [2], whether B cells play a deleterious or beneficial role in anti-tumour immune response is still debated [26]. Depletion of B cells in tumour mice models [27*] and treatment with Rituximab, a humanised monoclonal antibody directed against human CD20 [28], led to the reduction of tumour size in colorectal cancer. Several mechanisms can potentially explain the pro-tumoral role of B cells in cancer: production of TGF-β and IL-10 responsible of an immune-suppressive environment or antibody production [29] and complement system activation providing a pro-angiogenic and pro-tumoral environment. However, B cells have been clearly shown to correlate with an improved overall survival when present in aggregates forming tumour-associated TLS [30-33]. TLS have been described in most of common as well as rare solid tumours. Their presence mostly correlates with better patient prognosis, therefore highlighting a critical role of TLS in development of anti-tumour immunity (**Figure 1**). A model for immune processes within tumour-associated TLS has been described in a previous review [34]. In ectopic lymphoid-like structures B cells can act as antigen presenting cells or undergo maturation in GCs, expressing AID and Bcl-6, and produce tumour associated (TA)-specific immunoglobulines. *In situ* antigen-driven B-cell activation and antibody production has been shown in several cancers. Sequencing data of BCR of TLS-associated B cells showed clonal expansion [35, 36]. For instance, in patients with non-small-cell lung carcinoma (NSCLC) [30] and patients with lung squamous cell carcinomas (LSCC) [37**] the organisation of intra-tumoral B cells into B-cell follicles, but not the diffuse infiltration of lymphocytes, is associated with longer survival and TA-specific humoral responses [38**]. In pancreatic ductal adenocarcinoma (PDAC), generally considered an immunologically inert cancer, clusters of B cells, but not disorganised B-cell infiltration, correlate with better patient prognosis [33]. Recently, not only TLS density, but also TLS maturation, have been shown to jointly concur to a more accurate prognostic information on the risk of disease recurrence in untreated non-metastatic colorectal cancer (TLS immunoscore) [39*]. As support of the role of an active GCs and importance of presence of mature tumour-associated TLS, a study in chemotherapy-treated LSCC patients showed loss of prognostic value of TLS density after neo-adjuvant treatment that was associated with significantly less and smaller GCs when compared with untreated patients [37**].

TLS-associated B cells are oligoclonal and may act as crucial players not only in terms of TA-specific humoral response, but also cellular-mediated response as they can not only mature into TA-specific antibody producing cells , but also act as efficient APC within the tumour, capturing the antigen through their BCR and expressing co-stimulatory molecules upon activation, therefore inducing the generation of memory CD4+ T cells [40].

Concluding remarks: therapeutic agents to disrupt or enhance TLS

Considering their impact on patient prognosis, TLS could be envisioned as either targets for immunotherapy in autoimmunity and graft rejection or as vehicles for a boost in anti-tumour immunity if enhanced in patients with solid cancer (**Figure 2**). In chronic inflammation, autoimmune diseases or organ transplantation, and possibly in some types of cancer where TLS has been suggested to exert a deleterious effect (i.e. hepatocellular carcinoma), a potential therapeutic approach might involve the disruption of their architecture and prevention of their formation for therapeutic purposes. Several clinical trials of drugs targeting TLS formation and function are already in place in autoimmune diseases [2, 3*]. Drugs capable of blocking TLS initiation include compounds targeting the lymphotoxin-β pathway but also pro-inflammatory cytokines such as IL-17 and IL-22 which have emerged as key players in TLS development in animal models [41, 42]. Moreover, blocking B-T_{FH} cells interaction targeting ICOSL/ICOS, CD40L/CD40 or IL-21/IL-21R (for which there are ongoing clinical trials [3*]) could affect the downstream B-cell activation in TLS. Finally, targeting (auto)reactive long-lived plasma cells has also emerged as a promising therapeutic approach. New drugs include proteasome inhibitors and monoclonal antibodies targeting cell-surface molecules such as CD38 which is highly expressed on plasma cells [43*].

By contrast, in cancer, where organised lymphocytic infiltration in the tumour microenvironment concurs to a better outcome and is associated with host protection, one could attempt to locally induce them, thus circumventing the need to therapeutically vaccinate to undefined antigens. Lymphoid chemokines are overexpressed in TLS of melanoma [44], colorectal [45], and lung [46] cancer patients. Therefore, TLS modulation can be addressed by targeting lymphoid chemokines in order to induce B- and T-cell recruitment and therefore TLS neogenesis in cancers. *In vivo* studies have shown promising results. The transduction of tumour cells with a recombinant adeno-associated virus (rAAV) expressing CCL21, or intra-tumoral injections of rAAV-CCL21, resulted in the recruitment at the tumour site of CD11c+ dendritic cells (DCs) and in the activation of CD3+ CD69+ T cells in a mouse model of hepatocellular carcinoma [47]. In PDAC, injection of CCL21 in a subcutaneous model showed a beneficial effect, by inhibiting tumour growth, decreasing distant metastasis, and recruiting DCs and T cells [48]. We can speculate that this vaccine therapy would induce a recruitment of T and B lymphocytes and boost TLS formation within the tumours, sites of an effective anti-tumour immune response.

TLS development has been achieved in other studies after anti-tumour vaccination protocols. In patients with high-grade cervical intraepithelial neoplasia (CIN2/3) who received intramuscular therapeutic vaccination targeting HPV16 E6/E7 antigens formation of TLS was observed [49]. Similarly, TLS formation was observed in PDAC patients after vaccination with GM-CSF-secreting pancreatic tumour vaccine (GVAX), a granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting, allogeneic PDAC vaccine [50]. The number of TLS increased after combination of GVAX with cyclophosphamide [50]. Still, in cancer a substantial amount of work remains to be done in order to take advantages from the activation of both the *in situ* present- and the newly formed- TLS associated with anti-tumour immune response, and combine them with target therapies and immunotherapies. Finally, a critical area for research regarding the modulation of TLS in cancer immunotherapy will relate to the understanding of whether immunotherapies with immune checkpoint inhibitors such as monoclonal antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), exert their beneficial clinical efficacy by promoting TLS formation/function within the cancer tissue. By the same token, although highly effective in combination with chemotherapy, many studies have reported the adverse effects of immune checkpoints inhibitors, the so called immune-related adverse events (IRAEs), promoting inflammatory reactions commonly observed in autoimmune conditions including inflammatory arthritis, myositis, vasculitis, colitis, sialoadenitis and scleroderma [51**]. Once again, whether these adverse reactions are related to the *de novo* formation of TLS remains to be elucidated.

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* Special interest

** Outstanding interest

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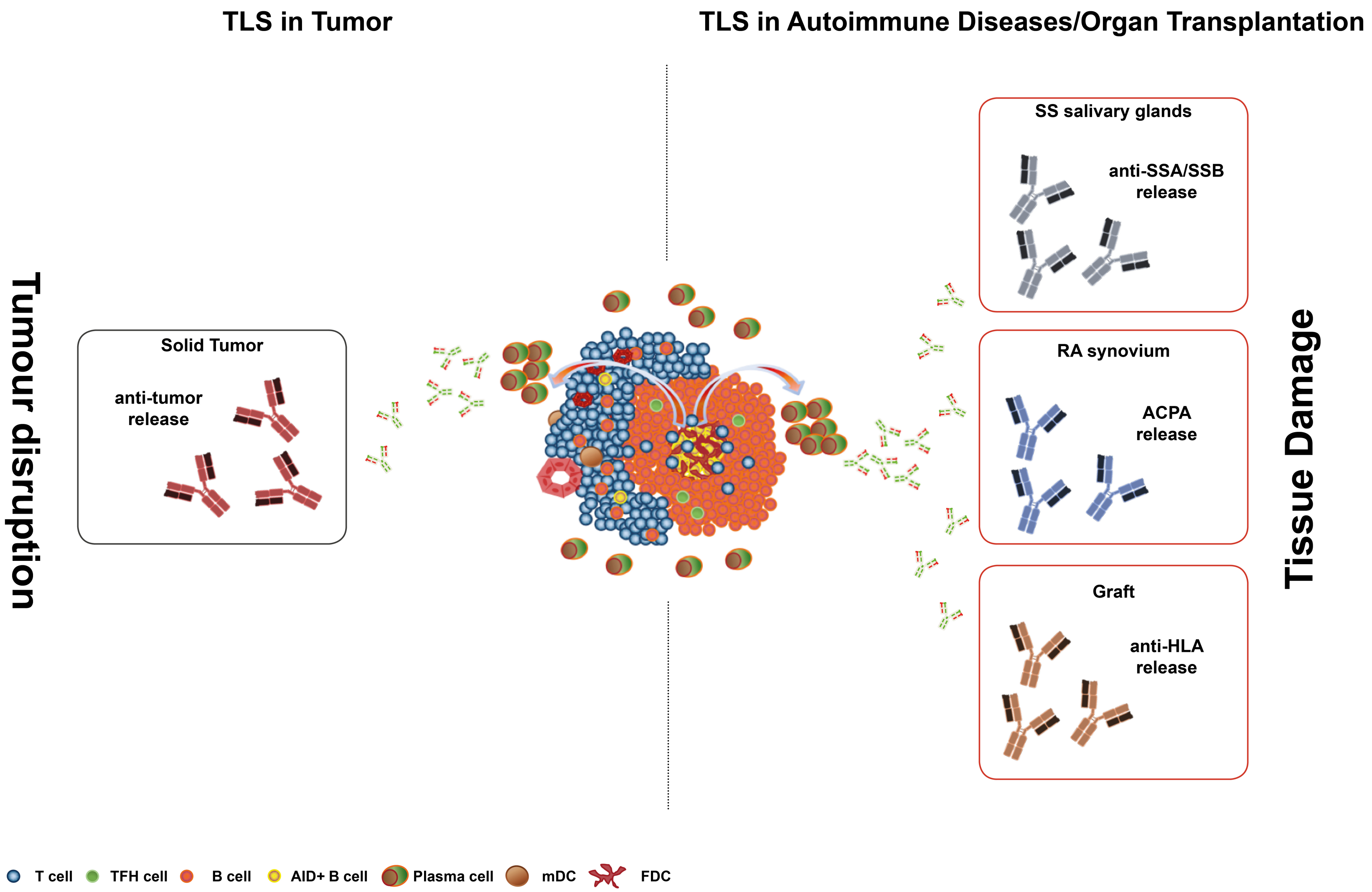
Figure legends

Figure 1. Effect of TLS in solid tumours, autoimmune diseases and organ transplantation.

Although the processes of TLS formation are similar, their effect seems to be disease- and antigen-disease specific. In solid tumours, TLS are responsible for the generation of an anti-tumor immune response. In the target organs of rheumatic autoimmune diseases, such as the SS salivary glands and RA synovium, TLS sustain an antigen-disease driven immune response leading to tissue damage. Similarly, in rejected grafts TLS can sustain a donor-specific anti-HLA response.

Figure 2. Clinical trials of therapeutic drugs enhancing or disrupting TLS formation.

Status of some studies identified in ClinicalTrials.gov reporting drugs targeting TLS in order to enhance TLS formation in cancer or disrupt TLS in autoimmune diseases and organ transplantation. PD-1 = programmed cell death protein-1; CTLA-4 = cytotoxic T lymphocyte-associated antigen-4; CCL21 = Chemokine (C-C motif) ligand 21; LT = lymphotoxin; ICOS = inducible T-cell co-stimulator; L = ligand; RA = rheumatoid arthritis; SS = Sjögren’s syndrome; SLE = systemic lupus erythematosus


TLS in Tumor
TLS in Autoimmune Diseases/Organ Transplantation
Tumour disruption
Solid Tumor
anti-tumor release
SS salivary glands
anti-SSA/SSB release
RA synovium
ACPA release
Graft
anti-HLA release
Tissue Damage
T cell
TFH cell
B cell
AID+ B cell
Plasma cell
mDC
FDC

Therapeutic Drugs

Targeted Pathway	Drug	Target	Disease	Clinical trial ID	Phase	Status
CCL21	autologous dendritic cell adenovirus CCL21 vaccine	CCL21	Non-Small Cell Lung Cancer (NSCLC)	NCT01574222	I	Terminated
	autologous dendritic cell-adenovirus CCL21 vaccine	CCL21	Lung Cancer	NCT00601094	I	Completed
	autologous dendritic cell-adenovirus CCL21 vaccine	CCL21	Melanoma (skin)	NCT00798629	I	Completed
PD-1	Nivolumab	PD-1 monoclonal antibody (IgG4)	Head/Neck Cancer	NCT03355560	II	Recruiting
	Pembrolizumab	PD-1 monoclonal antibody (IgG4)	Melanoma	NCT03200847	I/II	Recruiting
PD-L1	Atezolizumab	PD-1 monoclonal antibody (IgG1)	Non-Small Cell Lung Cancer (NSCLC)	NCT03526900	II	Recruiting
	Durvalumab	PD-1 monoclonal antibody (IgG1)	Non-Small Cell Lung Cancer (NSCLC)	NCT03620669	II	Recruiting
CTLA-4	Ipilimumab	CTLA-4 monoclonal antibody	Head/Neck Cancer	NCT02812524	I	Recruiting

Targeted Pathway	Drug	Target	Disease	Clinical trial ID	Phase	Status
LT-α/LT-β	Pateclizumab	LT-α monoclonal antibody	RA	NCT01225393	II	Completed
	Baminercept-α	LT-β receptor fusion protein	SS	NCT01552681	II	Terminated
			RA	NCT00664573	II	Terminated
IL-17	Secukinumab	IL-17A monoclonal antibody	RA	NCT01377012	III	Completed
				NCT01350804	III	Completed
	Ixekizumab	IL-17A monoclonal antibody	RA	NCT00966875	II	Completed
IL-21	NNC0114-0006	IL-21 monoclonal antibody	RA	NCT01647451	II	Completed
ICOS-ICOSL	AMG557	ICOSL monoclonal antibody	SLE	NCT01683695	I	Completed
			SS	NCT02334306	II	Completed
CD40-CD40L	CFZ533	CD40 monoclonal antibody	RA	NCT02089087	I	Completed
			SS	NCT02291029	II	Recruiting
			Kidney Transplantation	NCT02217410	I/II	Completed
Plasma Cells Proteasome	Bortezomib	Proteasome inhibitor	RA/SLE	NCT02102594	II	Recruiting
	Carfilzomib	Proteasome inhibitor	Transplant	NCT02442648	I	Recruiting
CD38	Daratumumab	CD38 monoclonal antibody	Multiple Myeloma	NCT03475628	II	Recruiting