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Stromal cell regulation of homeostatic and inflammatory lymphoid organogenesis

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Summary

Secondary lymphoid organs function to increase the efficiency of interactions between rare, antigen-specific lymphocytes and antigen presenting cells, concentrating antigen and lymphocytes in a supportive environment that facilitates the initiation of an adaptive immune response. Homeostatic lymphoid tissue organogenesis proceeds via exquisitely controlled spatiotemporal interactions between haematopoietic lymphoid tissue inducer populations and multiple subsets of non-haematopoietic stromal cells. However, it is becoming clear that in a range of inflammatory contexts, ectopic or tertiary lymphoid tissues can develop inappropriately under pathological stress. Here we summarize the role of stromal cells in the development of homeostatic lymphoid tissue, and assess emerging evidence that suggests a critical role for stromal involvement in the tertiary lymphoid tissue development associated with chronic infections and inflammation.

Keywords: cytokines; inflammation; mucosal associated lymphoid tissue ; spleen/lymph nodes.

Introduction

Secondary lymphoid organs (SLOs) function to increase the efficiency of interactions between rare, antigen-specific lymphocytes and antigen-presenting cells, concentrating antigen and lymphocytes in a supportive environment that facilitates the initiation of an adaptive immune response. Homeostatic lymphoid tissue organogenesis proceeds via exquisitely controlled spatiotemporal interactions between haematopoietic lymphoid tissue inducer populations and multiple subsets of non-haematopoietic stromal cells. However, it is becoming clear that in a range of inflammatory contexts, ectopic or tertiary lymphoid organs (TLOs) can develop inappropriately under pathological stress. Here we summarize the role of stromal cells in the development of homeostatic lymphoid tissue, and assess emerging evidence that suggests a critical role for stromal involvement in the TLO development associated with chronic infections and inflammation.

Stromal cell–haematopoietic cell interactions govern homeostatic lymphoid tissue development

Peripheral lymphoid tissue generation occurs sequentially in the developing mouse embryo from embryonic days E11 to E16.[1,2] Lymph node (LN) development is thought to be initiated by the production of retinoic acid, which acts on mesenchymal stromal cells at predetermined anatomical sites to induce expression of the chemokine CXCL13[3] (Fig. 1). It has been proposed that outgrowing nerves are responsible for the production of retinoic acid in development, as they express RALDH2, an enzyme required for the conversion of retinal to retinoic acid.[3] A CXCL13 gradient attracts $CXCR5^+$ haematopoietic cells to the LN anlagen; the first cells to arrive are lymphoid tissue-inducer cells (LTis),[4] derived from fetal liver progenitor cells that can also give rise to B cells, T cells, natural killer cells and dendritic cells.[5] The LTis express lymphotoxin (LT) $\alpha_1\beta_2$ ($LT\alpha_1\beta_2$), a cytokine that is the major determinant of SLO development.[6–8] $LT\alpha_1\beta_2$ is a heterotrimeric complex, comprising membrane-bound $LT\beta$ and soluble $LT\alpha$. Together these bind to the lymphotoxin-β receptor ($LT\beta R$) that is predominantly expressed by mesenchymal stromal cells.

Interestingly, the first $CXCR5^+$ LTis recruited to the site of LN formation express receptor activator of nuclear factor-κB ligand (RANKL), rather than $LT\alpha_1\beta_2$.[9,10] Indeed the initial clustering of LTis can occur without $LT\alpha_1\beta_2$ expression by LTis[9] or $LT\beta R$ expression on mesenchymal stromal cells.[11] Therefore, initiation of LT expression by

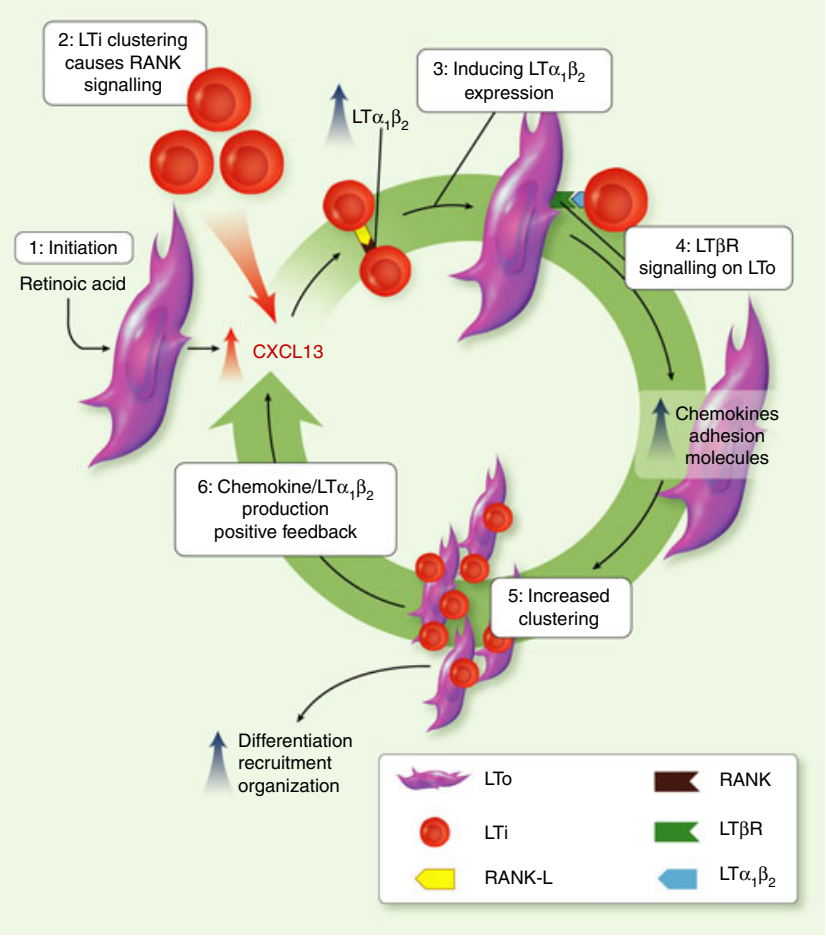

Figure 1. Sequential stromal cell–haematopoietic cell interactions govern secondary lymphoid organ (SLO) development (adapted from ref. 2). Lymph node (LN) development is initiated by retinoic acid acting on a population of stromal cells(s) that induces CXCL13 expression at predetermined anatomical sites (1). $CXCR5^+$ lymphoid tissue-inducer (LTi) cells migrate on this CXCL13 gradient and cluster, with subsequent receptor activator of nuclear factor-κB (RANK)/ RANK ligand (RANKL) signalling inducing expression of lymphotoxin (LT) by LTIs (2 + 3). The LT signals through the LTβ receptor on stromal cells causing them to differentiate into lymphoid tissue organizers (LTos) (4). This activation drives the expression of chemokines and adhesion molecules that lead to the further attraction, clustering and local retention of LTis (and other lymphocytes: 5), production of more LT and the generation of a positive feedback loop that increases clustering and facilitates LN expansion (6).

LTis is probably through clustering and subsequent RANK/RANKL signalling. The $LT\alpha_1\beta_2$ then signals via LTβR to drive mesenchymal stromal cells to differentiate into lymphoid tissue organizer cells (LTos),[9] accompanied by the up-regulation of chemokine (e.g. CXCL13, CCL19 and CCL21) and adhesion molecule (e.g. vascular cell adhesion molecule-1, intercellular adhesion molecule-1, mucosal addressin cell adhesion molecule-1)[12] expression in the LN anlagen. Chemokines, as well as the up-regulated expression of RANKL and interleukin-7 (IL-7) by LTos,[9,10] induce the recruitment and survival of further cells to the expanding LN anlagen.[13] The arrival of more $LT\alpha_1\beta_2$-expressing cells, which includes few LTis[14] but after birth is dominated by lymphocytes (both T and B cells),[15,16] creates a positive feedback loop (Fig. 1), further increasing signalling through the LTβR and the subsequent expression of LTo-derived factors. Using conditional ablation of the *Ltbr* gene exclusively in VE-$Cadherin^+$ endothelial stromal cells, Onder *et al.*[17] recently revealed that the development of multiple peripheral LNs required LT signalling specifically into this $LT\beta R^+$ stromal compartment. Interestingly, not all LNs required endothelial sensitivity to $LT\alpha_1\beta_2$, as the mesenteric LNs of the intestine were fully intact in these mice, hinting at a requirement for distinct $LT\beta R^+$ stromal cell populations in the development of anatomically disparate peripheral LNs *in vivo*. Other homeostatic SLOs develop in a fundamentally similar way to the LN with only minor differences between tissues. For instance in the Peyer's patches of the small intestine, although ligands of the receptor tyrosine kinase RET acting on a distinct population of $CD45^+$ IL-$7R\alpha^-$ $CD11c^+$ cells contributes to stromal activation in the developing anlagen,[18] LTis and $LT\alpha_1\beta_2$ are still important in this developmental process,[4] although it

is not clear if LT$\alpha_1\beta_2$ expression is induced by RANK as in early LN development. However, the earliest steps in homeostatic intestinal SLO development are still under intense investigation.[19]

Lymphoid tissue organizers differentiate into the various non-haematopoietic stromal subtypes present in the adult SLO via LTβR signalling,[20] although the ontogeny and lineage relationships of the various stromal cell subsets within the LN is still under investigation.[21,22] Mesenchyme-derived stromal cells can be divided into several subsets including follicular dendritic cells (FDCs), marginal reticular cells and populations of fibroblastic reticular cells (FRCs). Lymph node stromal endothelial cells can be divided into blood endothelial cells and lymphatic endothelial cells,[23] and all SLOs contain high endothelial venules composed of endothelial cells with distinct morphology and phenotype. Four CD45$^-$ stromal subsets can therefore be identified by a dual CD31 (PECAM-1) and Podoplanin (gp38) stain.[23] Identification of further subsets can be achieved using a range of different surface markers (Table 1). Interestingly, the CD31$^-$ Podoplanin$^-$ double-negative population (previously of unknown function) has recently been shown to contain a novel subset of fibroblastic contractile pericytes, related to both smooth muscle cells and FRCs.[24] However, this population does not account for all the stromal cells lying in the double-negative gate, and suggests further stromal subset heterogeneity within lymphoid tissue.

Structural and functional features of SLO stromal cells

Once SLOs are formed, a major functional role of stromal cells is undoubtedly the maintenance of SLO structural integrity, and many subsets secrete large amounts of extracellular matrix (Table 1). The FRCs form collagen-rich reticular fibres, which they then surround to form conduits for afferent lymph.[25] These function by allowing for the transport of low-molecular-weight antigen and so facilitate antigen presentation by antigen-presenting cells in the T-cell zone.[26] Similar conduits have been found in the subcapsular sinus of the lymph node that are specialized for transport of antigen to the B-cell zone[27] and may be formed by marginal reticular cells that are present at this distinct location.[28]

Stromal cells also play a vital role in lymphocyte trafficking by maintaining a functional separation of B-cell and T-cell zones via specific chemokine expression. The FRCs in the T-cell zone express CCL19 and CCL21, which act to recruit CCR7$^+$ naive T cells.[29] The importance of

Table 1. Secondary lymphoid organ (SLO) stromal cell subsets

Stromal subset	Location in SLO	Selected surface markers	Function
Podoplanin (gp38) and/or CD31 positive			
Fibroblastic reticular cells (gp38$^+$ CD31$^-$)	T-cell zone	CCL19, CCL21, IL-7, MHC Class I, laminin, desmin, fibrillin, fibronectin, collagen I, II and IV, ICAM-1, VCAM-1	Production of reticular network and conduit system to increase antigen-presenting cell–T-cell interactions and T-cell homeostasis
Lymphatic endothelial cells (gp38$^+$ CD31$^+$)	Afferent/efferent lymphatics	ER-TR7 antigen, PROX1, LYVE1, CCL21, S1P, VE-cadherin, ICAM-1, VCAM-1	Transport of lymph and antigen/cells it contains
Blood endothelial cells (gp38$^-$ CD31$^+$)	High endothelial venules	Peripheral lymph node adressin (peripheral LN), MAdCAM-1 (mucosal LN) CCL21, CD34, JAM, ESAM1, VE-cadherin	Entry of blood cells to SLO
Follicular dendritic cells (gp38$^+$ CD31$^-$)	B-cell follicles	MAdCAM-1, CXCL12, CXCL13, BAFF, Complement receptors, including CD35–CR1) laminin, desmin, αSMA	Capture of antigen, B-cell presentation and chemokine production
Marginal reticular cells (gp38$^+$ CD31$^-$)	Subcapsular sinus	ER-TR7 antigen, ICAM1, VCAM1, MAdCAM1, RANKL, CXCL13 laminin, desmin	Conduits, chemokines and structure
Double negative (gp38$^-$ CD31$^-$)			
Extrathymic AIRE-expressing cells	T-cell zone	EpCAM, MHC Class II, MHC Class I	Peripheral tolerance
Fibroblastic contractile pericytes	Medulla and cortex	ITGA7, Unknown	Unknown
Bulk population	Unknown	Unknown	5–10% of stromal population, unknown function

EpCAM, epithelial cell adhesion molecule; ICAM-1, intercellular adhesion molecule 1; LN, lymph node; MAdCAM-1, mucosal addressin cell adhesion molecule 1; RANKL, receptor activator of nuclear factor-κB ligand; VCAM-1, vascular cell adhesion molecule 1.
References: 2,23,24,26,111,118,119

the stromal chemokine gradient induced is shown by aberrant SLO structure and T-cell distribution in the *plt/plt* mutant mouse,[30] which lacks CCL19 and CCL21 expression. In contrast, FDCs and marginal reticular cells express CXCL13,[31,32] which acts on CXCR5 to attract B cells to the B-cell zone of SLOs. As naive T cells and B cells do not express CXCR5 and CCR7, respectively (except for T-follicular helper cells, which express enough CXCR5 to enter the B-cell zone[33]), the stromal chemokine gradients restrict lymphocytes to their respective zones during steady-state conditions. Moreover, stromal chemokine production can even play a role in the further differentiation of lymphocytes. Recently, a key role for stromal cells in the functional activation of T helper cells in the LN has been revealed, whereby stromal cell production of CXCL9 optimizes the polarization of CXCR3$^+$ T cells toward an interferon-γ^+ T helper type 1 phenotype *in vivo*.[34] Multiple stromal subsets also provide vital survival signals to peripheral lymphocytes, e.g. FRC and lymphatic endothelial cell-derived IL-7 for T cells[23,35] and FDC-derived BAFF for B cells.[36]

Stromal cells control the influx and retention of naive lymphocytes to SLOs via chemokines, yet they may also control the egress of lymphocytes via sphingosine-1-phosphate (S1P) signalling.[37] Levels of S1P are much lower in SLOs than in the circulation because of increased SLO expression of S1P-lyase.[38] Cyclic expression of the S1P receptor on lymphocytes competes with CCR7 or CXCR5 signalling to determine lymphocyte retention versus egress.[39] It is highly plausible that SLO stromal cells constitutively express S1P-lyase to maintain this S1P gradient. Furthermore, differential stromal subset expression of oxysterol determines B-cell positioning within lymphoid tissue,[40] adding a further level of complexity to the regulation of lymphocyte localization by stromal cells within SLOs.

Stromal cells and SLO plasticity

During inflammation or infection, SLO stromal networks have a degree of plasticity. For example T-cell and B-cell networks grow and remodel[41,42] accompanied by changes to homeostatic chemokine expression[43] and lymphatics,[44–46] enabling lymphocyte motility. Data have revealed a key role for IL-7-expressing stromal cells in the infection-induced remodelling of murine LN, with lymphatic endothelial cells found to be the major producers of IL-7 using an *in vivo* IL-7 fate-mapping system and the staining of human LN sections.[35] Importantly, the *in vivo* ablation of IL-7-expressing stromal cells abolished infection-driven changes in LN architecture, highlighting the crucial role that these cells play in both the development and subsequent remodelling of the LN. Interestingly FRCs are capable of directly modifying LN endothelial cell growth and expansion,[45] suggesting that both stromal–stromal and stromal–leucocyte interactions regulate the processes underlying the formation and remodelling of lymphoid tissues.

In addition to the developmentally imprinted homeostatic tissues discussed above, 'intermediate' lymphoid tissues exist that can be considered as somewhere between predetermined and inflammatory lymphoid tissues. Isolated lymphoid follicles (ILFs) are primarily B-cell follicle-containing lymphoid structures that form at predetermined sites along the length of the mesenteric wall of the small intestine.[47] The ILFs develop from cryptopatches, clusters of LTi cells seen in both mouse[48] and human[49] intestine. As with the LN, LTi–stromal interactions are vital in ILF formation[50] mediated via LTβR signalling,[47,51] which is aided by the recruitment of naive LT$\alpha_1\beta_2$-expressing B cells.[52] Recent work has also revealed that the cytokine IL-22 may also be involved in the maintenance of ILFs during bacterial-induced inflammation.[53] Mice kept in a specific-pathogen-free environment develop few and small ILFs,[51] whereas infection with *Salmonella enterica* greatly enlarges individual ILFs, but importantly does not increase their overall number.[54] The ILFs therefore represent a partially programmed lymphoid tissue lying between ectopic and predetermined. Their anatomical location is predetermined and their developmental processes show many similarities to LN expansion, yet their formation is dependent upon environmental signals, namely microbial stimulation.[54,55]

Stromal cells: a crucial role in ectopic TLO development?

Truly distinct from developmentally encoded lymphoid tissue are ectopic or TLOs, also known as tertiary lymphoid tissue. The TLOs spontaneously develop as a result of chronic inflammation,[56] normally due to chronic infection, autoimmunity or tumours. Such tissues can rapidly form stable structures during inflammation, and yet equally as easily regress, as seen in the dynamic development of TLOs during chronic *Helicobacter pylori* infection.[57] The fundamentals underpinning SLO development also lie at the heart of TLO development: inflammatory cytokine expression (LT/tumour necrosis factor-α); stromal activation and chemokine production; and high endothelial venule development.[58,59] As seen in transplantation studies,[60,61] activated stromal cells alone are capable of initiating TLO formation in some instances, indicating their overriding capacity to contribute to TLO development. Nevertheless, the precise signals leading to stromal activation during TLO development *in vivo* are still unclear. The majority of mechanistic data on the development of TLOs are derived from transgenic mice expressing molecules in ectopic sites. Although these are narrow models that lack the complexity that undoubtedly underpins *in vivo* TLO generation, they do offer a

Table 2. Transgenic ectopic expression models used to study tertiary lymphoid organ (TLO) development

Model	TLO characteristics	Reference
LTα	Lymphotoxin α (LTα) expressed under control of the rat insulin promoter (RIP-LTα) developed functional secondary lymphoid organ (SLO)-like tissue organized by stromal cells via CXCL13 and CCL21, in both kidney and pancreas	120
LTα/LTβ	Double LTα and LTβ transgenic under the control of the rat insulin promoter mouse saw more developed TLO in the kidney and pancreas than a single RIP-LTα transgenic model	6
LIGHT	RIP-LIGHT transgenic mice on a NOD (diabetic) background rapidly develop ectopic pancreatic TLOs via LTβR and HVEM signalling	121
CXCL13	RIP-BLC transgenic mice developed very well defined TLO in the pancreas with clear T-cell and B-cell zones, stromal cells expressing CCL21 and high endothelial venules	122
CCL21	RIP-SLC and TG-SLC (CCL21 expression under the thyroglobulin promoter) transgenic mice did produce TLO in the pancreas and thyroid, respectively, but to a less developed degree, with mainly haematopoietic infiltrates. CCL21 less important in stromal network development? But CCL21 is still vital in T-cell recruitment and extravasation, showing that at least some of the molecular interactions in TLO and SLOs are preserved	123–125
IL-5	Constitutive interleukin-5 expression under the promoter of a lung-derived protein, CC10, causes the formation of inducible bronchial-associated lymphoid tissue (iBALT). However, interleukin-5 is an aggressive effector cytokine, therefore TLO formation is probably secondary to the observed lung pathology	126

glimpse into TLO development that would otherwise be hard to observe. Table 2 highlights animal models of TLO development that use either LTβR signalling, homeostatic chemokine or non-homeostatic cytokine transgenic expression.

If TLO and SLO development is conceptually similar, what is the source of $LT\alpha_1\beta_2$ in TLO development? One possibility is that TLOs are formed by LTis in much the same way as in SLOs, but there is conflicting evidence to support this hypothesis. Interleukin-7 (a key survival factor for LTis in developing SLOs) transgenic mice develop a large number of LNs and Peyer's patches, as well as the formation of organized TLOs after immunization with antigen, in a process that is dependent upon $LT\alpha_1\beta_2$ and the LTi-associated transcription factor retinoic acid-related orphan receptor γt (RORγt).[62] However, a CCL21 transgenic model of TLO development lacking LTis still develops TLOs,[63] with $CD3^+$ $CD4^+$ T cells the first to arrive at the site of TLO development, indicating an LTi-independent mechanism that may be unique to TLOs. Formation of TLOs during inflammation of the intestine is able to occur in the absence of RORγt (and hence LTis),[64,65] although with the recent identification of multiple innate lymphoid cell (ILC) populations, which express similar levels of $LT\alpha_1\beta_2$ to their LTi cousins,[66,67] the extent to which RORγt-independent ILCs can contribute to intestinal TLO generation requires further investigation.[68] As B and T cells both express $LT\alpha_1\beta_2$,[69] are relatively much more abundant in chronically inflamed tissues than LTis (or other ILCs), and activated conventional lymphocytes are known to play a role in TLO generation in the skin,[60] it is likely that B and T cells contribute significantly to TLO development during inflammation. In addition, there is emerging evidence for a potential role of Th17 cells in the development of TLOs during experimental autoimmune encephalomyelitis, a murine model of central nervous system inflammation.[70,71] Nevertheless a definitive comparison between the TLO-inducing capacities of ILCs versus T and/or B cells *in vivo* has not yet been attempted.

The precise mechanisms leading to stromal activation and TLO generation in multiple tissue sites are not yet fully defined. This includes doubt as to whether tissue stromal cells simply convert to a 'lymphoid-like' phenotype during inflammation,[72] or whether LTos in TLOs arise from distinct progenitors. The tools to begin assessing this second hypothesis have only recently been developed, with sophisticated genetic lineage tracing and ablation systems leading to the identification of a pro-fibrotic stromal cell population in murine skin that arises during inflammation from a fetal progenitor developmentally distinct from muscle and skin tissue cells.[73] In addition, recent work has revealed that FDCs arise from perivascular platelet-derived growth factor receptor β^+ stromal progenitors in lymphoid and non-lymphoid tissues, with this process occurring during chronic inflammation.[74] Interestingly, the development of LN stromal cell subsets from adipocyte precursors has been recently reported.[75] As chronic inflammation of the intestine is associated both with TLOs[76] and substantial mesenteric fat deposits around the inflamed organ[77] it is possible that inflamed adipose tissue may provide precursors that subsequently develop into TLO-associated stromal networks in the gut. The specific precursor(s) responsible for differentiating into the various stromal subsets remain elusive, but may well be tissue-specific and disease-specific. Fibroblast-like cells are a potential candidate; fibrocytes are capable of differentiating into FDCs and have been implicated in human inflammatory disease;[78–81] fibroblasts themselves are capable of expressing adhesion molecules and producing homeostatic chemokines (so mimicking SLO stroma);[82] and large

Table 3. Examples of ectopic tertiary lymphoid organ (TLO) formation in human autoimmune/chronic inflammatory diseases

Disease	Prevalence (%)	Characteristics
Rheumatoid arthritis	10–35	Well-developed TLO appears in synovial membrane, separated functional areas, high endothelial venules (HEVs), stromal chemokines (CXCL13, CCL21)
Hashimoto's thyroiditis	100	Well developed TLO in thyroid, functional germinal centres with stromal support [CCL21, CXCL12, CXCL13, peripheral lymph node addressin (PNAd)]
Sjögren's syndrome	17	TLO appears in salivary glands, well-developed germinal centres with follicular dendritic cell support and chemokines (CXCL13, CCL21, CXCL12, PNAd)
Multiple sclerosis	30–40	Seen in brains of patients with secondary progressive multiple sclerosis. Mainly less developed, with germinal centres, CCL19 and CCL21 as well as lymphangiogenesis
Crohn's disease	–	Development of both T-cell and B-cell compartments with HEVs in inflamed bowel. Could potentially be hyperplasia of gut mucosa-associated lymphoid tissue, undetermined
Atherosclerosis	32	Develops in arteries. Highly developed, conduits, separated into functional T-cell and B-cell areas. Evidence to show that smooth muscle cells differentiate into various stromal-like subsets and produce stromal chemokines (CXCL13, CCL19, CXCL16).
Helicobacter pylori infection	27–100	Gastric mucosa-associated lymphoid tissue forms in stomach, CXCL13 expression as well as the formation of HEVs expressing PNAd and mucosal addressin cell adhesion molecule 1
Graft rejection	–	TLO seen in human kidney and heart transplants, with the development of germinal centres and lymphangiogenesis

References: 20,58,127,128

numbers of intestinal fibroblast-like cells up-regulate Podoplanin expression during intestinal inflammation.[72] Nevertheless, there is still much to be revealed about the specific stromal subsets and/or stromal alterations that underlie TLO generation during inflammation, including in the gut.[83]

As Table 3 shows, the structural make up of TLOs varies. Most TLOs will develop supportive and effective B-cell zones, sometimes capable of antigen-driven B-cell maturation, somatic hypermutation and class-switching.[84] This can occur via FDC expression of activation-induced cytidine deaminase,[85] with these processes accompanied by significant lymphangiogenesis[86–88] and vascular remodelling.[56] The level of T-cell zone development varies greatly; although the CCL21 expression often observed in TLOs would suggest that T-cell-zone-associated LTos may be present. Indeed the TLOs associated with rheumatoid arthritis appear to have distinct spatial segregation of T-cell and B-cell zones akin to that observed in SLOs, with this micro-anatomical localization governed by CXCL13 and CCL21 expression.[85] Whether the corresponding LTo stromal subsets are present in these TLOs is not entirely clear.

Beneficial and detrimental properties of TLOs to the host

The importance of SLO stromal cells in microbial defence is well documented. During inflammation, FRCs up-regulate anti-microbial genes[24] and the disruption of stromal networks (via viral infection) leaves the host susceptible to secondary infection,[43] an immunodeficiency that is reversed by the restoration of stromal architecture via LT expression by LTis.[89] Whether specific stromal populations in TLOs versus SLOs have a differential capacity to induce an antimicrobial state is not known. However, viral infection models hint at a major role for TLOs in the defence against pathogens. Well-developed inducible bronchial-associated lymphoid tissue (iBALT) is a form of TLO formed during acute influenza infection,[90] via stromal chemokine expression[91] in a process that is stabilized by myeloid cells.[92] Other processes, including the expression of IL-17 by T cells, appear to contribute to iBALT generation in some experimental contexts,[93] however, the absolute requirement for this cytokine in iBALT generation is unclear.[94,95] Interestingly mice that lack SLOs, but retain iBALT, can withstand higher inoculations of virus[90] and have a fully intact memory $CD8^+$ T-cell compartment in the context of influenza infection.[96] Hence TLOs can assume a host-protective role in some infectious contexts by providing a microenvironment that supports the local generation of a protective immune response. Further support for a role of TLOs in a protective response to infectious insult, comes from evidence that antigen persistence in itself is important for the maintenance of TLO structure during chronic infection. So the eradication of *Helicobacter pylori* antigen via antibiotics leads to drastic mucosa-associated lymphoid tissue regression,[57] presumably because the TLO has performed its function.

Although it is clear that TLO formation can help to increase the efficiency of antigen presentation to lymphocytes for a protective immune response, TLOs can also initiate immune responses that may be responsible for inducing or exacerbating an autoimmune response. Although there is no definitive causal link between TLO

presence and disease, in certain autoimmune diseases such as multiple sclerosis (or the murine model experimental autoimmune encephalomyelitis), TLO presence correlates with increased disease severity.[97,98] TLOs in the pancreas skew B cells toward an autoreactive phenotype during diabetes[99] and a recently described model of murine salivary gland pathology is characterized by TLO formation, ectopic stromal chemokine expression and $GL7^+$ germinal centre development that initiates autoimmunity by breaking self-tolerance to antigen.[59] Similarly, the TLOs in salivary glands of patients suffering from Sjögren syndrome sustain high levels of the B-cell survival factor BAFF,[100] potentially leading to the expansion of self-reactive B-cells escaping peripheral negative selection, that can then promote pathology.

Tertiary lymphoid organs also form in diseases that may be inflammatory but are (at least partially) antigen independent. For example; TLO formation and aberrant stromal chemokine expression in the terminal ileum of colitic TNFΔRE mice, which lack a negative regulator of tumour necrosis factor-α signalling and are therefore predisposed to joint and gut inflammation, drives the accumulation of effector T-cell populations and exacerbates disease;[101] and multiple stromal-derived factors contribute to TLO generation and the perpetuation of inflammation during rheumatoid arthritis.[82] The TLOs can also develop during atherosclerosis, and intriguingly the development of these structures coincides with the attraction/retention of both effector and regulatory T-cell populations in the artery, highlighting the potential for TLOs to simultaneously localize potentially damaging and protective immune cell types to the same tissue site.[102]

TLO stromal cells as therapeutic targets in inflammatory disease

The stromal cell networks of TLOs could be a future therapeutic target for (auto)immune disease. First, blocking the stromal-led development or maintenance of TLOs is a possibility; this has been shown in pre-clinical models by inhibiting LTβR signalling via administration of a LTβR-immunoglobulin fusion protein.[103] This strategy has reduced clinical symptoms in experimental autoimmune encephalomyelitis,[104,105] decreased insulitis in NOD mice,[106] reduced corneal pathology in a model of Sjögren syndrome,[107] inhibited the development of intestinal pathology in models of inflammatory bowel disease[108] and ameliorated pathology in collagen-induced arthritis.[109] However, efficacy data for this approach in humans are currently lacking. Beyond the targeting of lymphotoxin, recent pre-clinical data have revealed that biological CXCL13 blockade can disrupt splenic germinal centre structures after immunization, and ameliorate pathology during collagen-induced arthritis.[110] However, administration of a therapeutic anti-CXCL13 monoclonal antibody in a distinct model of inflammation had no impact on the structure of established ectopic follicles (e.g. in salivary glands), presumably because of functional redundancy in pathways downstream of this stromal chemokine. In some inflammatory contexts adjunctive blockade of multiple stromal pathways may therefore be required to modulate TLO formation.

Stromal cells also appear to be naturally immunosuppressive. As well as maintaining peripheral tolerance via tissue-specific antigen expression,[111] in SLOs they have been shown to directly suppress T-cell proliferation via nitric oxide production[112] and regulate $CD8^+$ T-cell function via PD-L1 expression during viral infection.[113] In addition it appears that stromal cells of multiple organs are naturally predisposed to the generation of immunoregulatory myeloid cell populations.[114] Therefore techniques could be developed to selectively activate or target stromal cells for the initiation of tolerogenic or regulatory responses, although much needs to be revealed regarding the differential mechanisms underlying inflammatory and tolerogenic stromal cell activation before this becomes a realistic option. Nevertheless, a similar conceptual approach is under intense investigation in the field of tumour therapeutics, where antibody–drug conjugates targeting tumour stroma for therapeutic manipulation have been developed and show promise in pre-clinical models.[115]

Conclusion and future directions

A critical outstanding question is to define the relative contribution of inflammatory lymphoid tissue (i.e. TLOs) versus homeostatic lymphoid tissue (i.e. SLOs) to inflammatory pathology. As is clear from this review, many of the developmental pathways between TLOs and SLOs are shared, particularly at the stromal cell and chemokine level, and so differentiating between them functionally will prove challenging. Interestingly it would appear that many features of immune responses generated from SLOs versus TLOs differ significantly, at least in the context of chronic allograft rejection,[116] but the specific contributions of stromal cells to these differences are not known. Unravelling the ontogeny of stromal cell subsets in homeostatic and inflammatory lymphoid tissues is another important area for future research. Newly developed tools[73,117] offer the promise of developmentally tracking and functionally manipulating the stromal cell networks that underlie lymphoid organogenesis, yet multiple outstanding questions remain as to the precise functions of these critical cell populations during homeostasis and inflammatory disease. Extending our knowledge of stromal cell biology will enable the development of novel therapeutic strategies for severely debilitating inflammatory conditions, treatments for which are currently lacking or sub-optimal.

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