Lymphoid neogenesis in chronic inflammatory diseases

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Abstract | The frequent observation of organized lymphoid structures that resemble secondary lymphoid organs in tissues that are targeted by chronic inflammatory processes, such as autoimmunity and infection, has indicated that lymphoid neogenesis might have a role in maintaining immune responses against persistent antigens. In this Review, we discuss recent progress in several aspects of lymphoid neogenesis, focusing on the similarities with lymphoid tissue development, the mechanisms of induction, functional competence and pathophysiological significance. As more information on these issues becomes available, a better understanding of the role of lymphoid neogenesis in promoting chronic inflammation might eventually lead to new strategies to target immunopathological processes.

Somatic hypermutation
An accumulation of point mutations in the variable-region genes of immunoglobulin heavy and light chains that gives rise to high-affinity antibodies specific for a given antigen in a process known as affinity maturation. B cells that express high-affinity immunoglobulins on their surface are selected by limited amounts of the antigen and competition for survival factors.

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Immune responses are primarily aimed at the eradication of pathogens. However, certain antigens are difficult to eradicate because of efficient countermeasures of the pathogen to the host immune response or because the antigen is a self or tumour antigen that is constantly replenished by the organism. In both cases, the result is a sustained immune response that produces local or systemic chronic inflammation. In the long term, there is considerable tissue destruction and loss of function with progressing clinical symptoms. It is a common finding that tissues that harbour the target antigen(s) of chronic immune responses are infiltrated by cellular effectors of the immune system, mainly T cells and macrophages, but also dendritic cells (DCs), B cells and plasma cells. Intriguingly, it has long been observed that these cellular elements often organize themselves anatomically and functionally as in secondary lymphoid organs (SLOs), leading to the *de novo* formation of B-cell follicles and T-cell areas[1–6]. This phenomenon has been termed lymphoid neogenesis or tertiary lymphoid organ (TLO) formation[7,8].

B- and T-cell priming, clonal expansion, antigen retention, somatic hypermutation, affinity maturation, immunoglobulin class switching, B-cell-receptor revision and maintenance of peripheral tolerance are crucial processes that take place in SLOs and can also occur in TLOs, probably contributing to the exacerbation of chronic inflammatory diseases. Lymphoid neogenesis has therefore received increasing attention and the list of diseases in which it has been observed has grown longer (TABLE 1). Also, the cellular and molecular basis of lymphoid organogenesis have been unveiled in recent years and this knowledge has been applied to the study of TLOs, showing remarkable similarity between the formation and structure of SLOs and TLOs[9–13]. Here, we review recent studies on the microarchitecture, molecular determinants and functional competence of ectopic lymphoid structures in chronic inflammatory diseases of different aetiologies, and we discuss the issues concerning their pathophysiological role and suitability as potential targets for immunotherapy.

Microarchitecture of tertiary lymphoid organs

The complex microarchitecture of SLOs — characterized by distinct B-cell (follicle) and T-cell (paracortex) areas, and specialized vascular and canalicular systems — is essential for regulating leukocyte traffic and compartmentalization. It therefore optimizes the efficiency of antigen sampling by naive lymphocytes and allows the appropriate activation and differentiation of antigen-responsive B and T cells (FIG. 1a). TLOs can develop in various inflamed tissues with a frequency that varies greatly depending on the anatomical site and disease (TABLE 1). Microanatomical and immunohistochemical analyses — carried out mainly in tissues from patients with autoimmune and infectious diseases — indicate that lymphoid neogenesis is a dynamic process during which sparse lymphocytic infiltrates evolve into aggregates that eventually organize in secondary B-cell follicles with germinal centres (GCs) and distinct T-cell areas containing DCs and high endothelial venules (HEVs)[8,14–17] (FIG. 1b). The most organized structures are generally found in highly infiltrated tissues, which indicates that TLO induction requires extensive local activation of immune

Immunoglobulin class switching
DNA rearrangement of the variable, diversity and junction (VDJ) regions from IgM to any of the IgG, IgA and IgE constant genes at the heavy-chain locus. Recombination occurs in repetitive sequences of DNA that are located upstream of each constant gene.

cells. Although different patterns of lymphoid arrangements usually coexist in the same patient, individual patients with rheumatoid arthritis tend to have the same type of synovial lymphoid infiltrate, indicating a role for host factors in TLO induction[18].

Ectopic GCs comprise proliferating B cells and networks of follicular dendritic cells (FDCs), which are essential for B-cell maturation owing to their ability to retain antigens on their membrane in the form of immunocomplexes, and to stimulate proliferation and prevent apoptosis of GC B cells[19,20]. Remarkably, naive B cells, centroblasts, centrocytes, memory B cells and plasma cells have all been detected in ectopic GCs or in the nearby biological fluids, which indicates that a complete B-cell maturation process takes place in ectopic GCs[15,21]. T cells are also a regular component of ectopic GCs, probably providing cognate T-cell help, which is required for the progression of the GC B-cell response[22].

Although the basic cellular constituents of B- and T-cell areas in SLOs and TLOs are similar, the overall structure of TLOs differs markedly from that of conventional SLOs (FIG. 1). Unlike lymph nodes, but

Table 1 | **Human chronic inflammatory diseases with lymphoid neogenesis**

Disease	Target tissue	Percentage of patients with ectopic follicles that contain CD21$^+$ CD35$^+$ FDCs or GCs	T-cell aggregates with CCL19$^+$CCL21$^+$stromal cells, DCs and HEV-like vessels	Antigen recognized by antibodies generated in ectopic GCs	References
Autoimmune diseases					
Rheumatoid arthritis	Diarthrodial joints	10–35%	Present; PNAD$^-$CCL21$^+$ blood vessels and PNAD$^+$CCL21$^+$ HEVs	Rheumatoid factor (?)	6,14,17,18, 41,45,71,80
Hashimoto's thyroiditis (hypothyroidism)	Thyroid gland	100%	Present; HECA-452$^+$CCL21$^+$ HEVs	Thyroglobulin, thyroperoxidase	1,4,15,43,44
Graves' disease (hyperthyroidism)	Thyroid gland	54–63%	Present; HECA-452$^+$CCL21$^+$ HEVs	Thyroglobulin, thyroperoxidase	1,44,15,43,44
Myasthenia gravis	Thymus	Mainly patients with early-onset myasthenia gravis	Present	Nicotinic acetylcholine receptor	2,5,78,79
Sjogren's syndrome	Salivary glands	17%	Present; PNAD$^+$CCL21$^+$ HEVs	SSA/Ro SSB/La	16,55,58,81
Multiple sclerosis	Central nervous system	30–40% of patients with secondary progressive multiple sclerosis	Absent	Not determined	3,46, R. Magliozzi, personal communication
Cryptogenic fibrosing alveolitis	Lungs	83–90%	Not determined	Not determined	83,84
Primary sclerosing cholangitis and primary biliary cirrhosis	Liver	None	Present; CCL21$^+$MADCAM1$^+$HEVs	Not determined	42,56
Other chronic inflammatory diseases					
Ulcerative colitis*	Gut	27%	Present; CCL21$^+$PNAD$^-$ blood vessels	Not determined	53,71,123,124
Crohn's disease*	Gut	Not determined	Present; CCL21$^+$PNAD$^-$ blood vessels	Not determined	53,71,123,124
Atherosclerosis	Arteries	32%	Present; HECA-452$^+$ HEVs	Not determined	106,107
Infectious diseases					
Chronic hepatitis C	Liver	33–85%	Not determined	Not determined	98,99
Helicobacter pylori-(or *Campylobacter pylori*)-induced gastritis	Stomach	27–100%	Present; PNAD$^+$ HEVs	Bacterial antigens	40,57,60,63,97
Chronic Lyme disease	Joints	17%	Present	Not determined	102,103
Tumours					
Ductal breast carcinoma	Breasts	33–100%	Not determined	Tumour-associated and normal breast tissue antigens	108,109

*In inflammatory bowel diseases, it is more difficult to distinguish between lymphoid neogenesis and hyperplasia of mucosa-associated lymphoid tissue. CCL21, CC-chemokine ligand 21; DC, dendritic cell; FDC, follicular dendritic cell; GC, germinal centre; HECA-452, high endothelial cell antigen-452; HEV, high endothelial venule; MADCAM1, mucosal addressin cell-adhesion molecule 1; PNAD, peripheral node addressin; SSA/Ro, Sjogren's syndrome antigen A (ribonucleoprotein autoantigen); SSB/La, Sjogren's syndrome antigen B (autoantigen La).

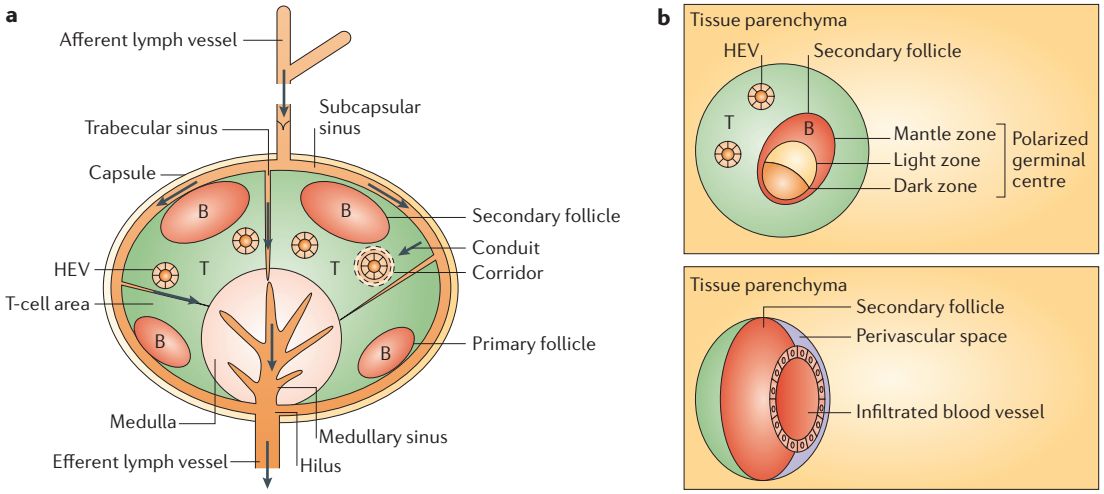

Figure 1 | **Basic structure of secondary and tertiary lymphoid organs. a** | The main structural components of a lymph node (secondary lymphoid organ, SLO) are shown. The cortex contains T-cell areas and B-cell areas that consist of primary follicles and, after antigen challenge, secondary follicles that contain germinal centres (GCs). Afferent lymph vessels carry interstitial fluid and antigen-loaded dendritic cells (DCs) into the lymph node, and high endothelial venules (HEVs) regulate the extravasation of naive B and T cells. **b** | A schematic depiction of tertiary lymphoid organs (TLOs). Secondary B-cell follicles, which are surrounded by T-cell aggregates with HEVs, are found in the tissue parenchyma, and in some tissues, such as the thymic medulla in myasthenia gravis[79] and the brain meninges in multiple sclerosis[46], they arise as perivascular expansions. Although atypical GC structures have been described[16,102], the architecture of ectopic GCs in TLOs is markedly similar to that of secondary B-cell follicles in SLOs. Detailed immunohistochemical analysis carried out in thyroid autoimmune disease has revealed the presence of polarized intrathyroidal GCs with a dark zone containing proliferating lymphocytes (centroblasts), surrounded by a light zone containing small lymphocytes (centrocytes) and enriched in follicular dendritic cells (FDCs), a mantle zone with T cells and mature DCs, and scattered plasma cells[15]. In most pathological tissues that have been analysed, except for multiple sclerosis[46], the inflamed endothelia acquire a HEV phenotype.

Receptor revision
A molecular process, also known as editing, that involves secondary variable-region gene rearrangements (either in the heavy- or light-chain loci) that generate a new B-cell receptor with altered specificity.

Canalicular system
A network of channels lined by fibroblastic reticular cells in the lymph-node cortex and paracortex. Conduits drain lymph (which consists mainly of water and low-molecular-weight molecules) from the subcapsular sinus to high endothelial venules (HEVs). Corridors are broad spaces around HEVs where emigrating lymphocytes are retained.

Germinal centre
A highly specialized and dynamic microenvironment that gives rise to secondary B-cell follicles during an immune response. It is the principal site of B-cell maturation, which leads to the generation of memory B cells and plasma cells that produce high-affinity antibodies.

High endothelial venule
(HEV). A specialized venule that occurs in secondary lymphoid organs, except the spleen. HEVs allow continuous transmigration of lymphocytes as a consequence of the constitutive expression of adhesion molecules and chemokines at their luminal surface.

Follicular dendritic cells
(FDCs). Specialized reticular fibroblasts located in the germinal centre that present antigen to B cells through antigen–antibody complexes and promote B-cell survival and proliferation.

Mucosa-associated lymphoid tissue
(MALT). Lymphoid tissue that enables antigen sampling from the mucosal surfaces and stimulation of cognate naive B and T cells. Its function is to ensure a rapid protective response to invading pathogens and the induction of tolerance to innocuous soluble antigens and commensal bacteria.

similar to mucosa-associated lymphoid tissue (MALT)[23], TLOs are not supplied by afferent lymph vessels and are not encapsulated, which implies that they are directly exposed to signals, such as stimulating antigens and cytokines, from the inflamed environment. It remains to be determined whether TLOs also lack the intricate canalicular system of conduits and corridors that, in SLOs, regulate lymph flow and the traffic of antigen-presenting cells, lymphocytes and low-molecular-weight molecules, including chemokines, to distinct areas of the lymphoid tissue[24]. This incomplete development of TLOs could result in unrestricted access of DCs, lymphocytes and macromolecules to the TLOs, favouring abnormal B- and/or T-cell activation.

Signals that induce lymphoid neogenesis

Clues to understanding the signals that lead to TLO formation in pathological conditions come from the study of signalling pathways involved in secondary lymphoid tissue organogenesis, signals generated during the immune response and signals generated in the tissue where the inflammatory reaction takes place.

Molecular determinants that are common to lymphoid organogenesis and lymphoid neogenesis. The organization of lymphoid tissue during development is the result of a highly coordinated interplay between haematopoietic cells, non-lymphoid stromal cells, adhesion molecules, chemokines, cytokines, and growth and survival factors. Studies in mutant mice and blocking experiments have identified a key requirement for tumour-necrosis factor (TNF)-family members — mainly lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$), and to some extent TNF — in the development and organization of spleen, lymph nodes and MALT[11,12,25,26]. Binding of $LT\alpha_1\beta_2$ and TNF to their respective receptors, LTβR and TNFR1, induces the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1), vascular cell-adhesion molecule 1 (VCAM1), mucosal addressin cell-adhesion molecule 1 (MADCAM1) and peripheral node addressin (PNAD), and of a set of chemokines known as lymphoid or homeostatic chemokines (CC-chemokine ligand 19 (CCL19), CCL21, CXC-chemokine ligand 12 (CXCL12) and CXCL13) that regulate lymphocyte homing and compartmentalization in lymphoid tissues[11–13] (FIG. 2). In the early stages of SLO development, membrane-bound $LT\alpha_1\beta_2$ expressed by $CD3^-CD4^+CD45^+$ haematopoietic inducer cells binds to LTβR on the surface of stromal organizer cells, and this interaction results in the attraction of additional haematopoietic cells, followed by HEV differentiation and the accumulation of naive lymphocytes[11]. In differentiated lymphoid tissue, B cells, and to a lesser extent T cells, are the main source of $LT\alpha_1\beta_2$, which together with TNF is required for adhesion-molecule and lymphoid chemokine expression by HEVs and stromal cells, the induction of FDC differentiation and function, and lymphocyte and DC homeostasis[26,27].

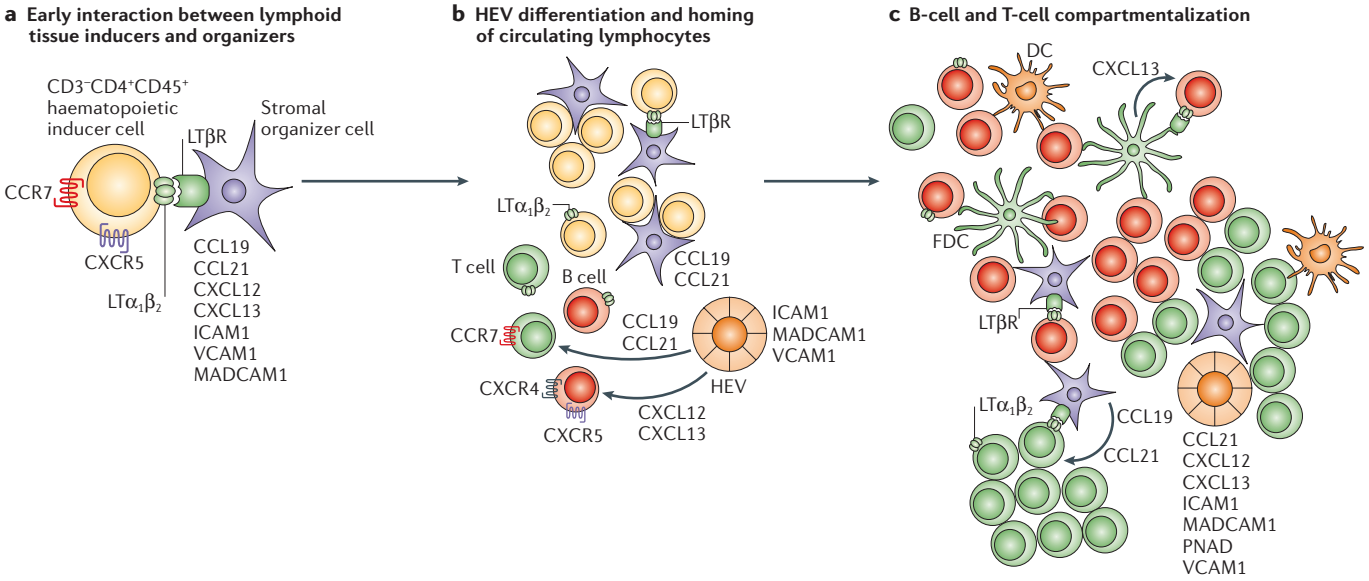

Figure 2 | **Molecular and cellular interactions in lymphoid tissue development and homeostasis.** A model of lymphoid organogenesis has been proposed based mainly on studies in lymph nodes[11]. **a** | One of the earliest events in lymph-node development is triggering of lymphotoxin-β receptor (LTβR) on stromal organizer cells by lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) that is expressed by $CD3^-CD4^+CD45^+$ haematopoietic inducer cells. This induces the expression of lymphoid chemokines and adhesion molecules, and favours the attraction of haematopoietic cells. **b** | Developing high endothelial venules (HEVs) express adhesion molecules (intercellular adhesion molecule 1 (ICAM1), vascular cell-adhesion molecule 1 (VCAM1), mucosal addressin cell-adhesion molecule 1 (MADCAM1) and, at later stages, peripheral node addressin (PNAD), and express lymphoid chemokines on their surface that promote the accumulation of naive lymphocytes from the blood. Both lymphoid tissue inducer cells and lymphocytes express the chemokine receptors CC-chemokine receptor 7 (CCR7) and CXC-chemokine receptor 5 (CXCR5), which bind CC-chemokine ligand 19 (CCL19) and CCL21, and CXCL13, respectively, and favour interactions with the stromal cells that produce these chemokines. **c** | Traffic of naive T cells through HEVs is regulated by CCL21 and PNAD, whereas B-cell trafficking also involves CXCL12 and, in some cases, CXCL13 (REFS 9,10,24). CCL19 and CCL21 produced by stromal cells and interdigitating dendritic cells (DCs) regulate the homing of $CCR7^+$ T cells and mature DCs to T-cell areas, whereas CXCL13 produced by follicular dendritic cells (FDCs) and germinal-centre (GC) DCs attract $CXCR5^+$ B cells into the follicles[9,10,122]. B and T cells become the main source of $LT\alpha_1\beta_2$, which, together with tumour-necrosis factor, is essential for lymphoid chemokine expression and lymphoid tissue homeostasis[27]. In turn, lymphoid chemokines induce $LT\alpha_1\beta_2$ expression by lymphocytes, creating a positive-feedback loop that maintains increased levels of all of these mediators in the lymphoid environment[9,38].

$CD3^-CD4^+CD45^+$ haematopoietic inducer cells
A population of haematopoietic precursors that colonize lymphoid tissues early in development and can differentiate into dendritic cells and natural killer cells, but not B or T cells. They are essential components of lymphoid organogenesis, owing to their ability to express $LT\alpha_1\beta_2$, interact with stromal cells and induce expression of adhesion molecules and chemokines that regulate lymphocyte migration and segregation in the lymphoid tissue.

Stromal organizer cells
Cells of mesenchymal origin that are activated by lymphoid cells through the lymphotoxin-β receptor to express adhesion molecules and chemokines that regulate lymphoid tissue development.

The first evidence that inflammation-associated lymphoid neogenesis could involve the same signalling pathways that regulate lymphoid organogenesis came from studies of transgenic mice[7]. Expression of LTα under control of the rat insulin promoter was shown to induce pancreatic expression of lymphoid chemokines and adhesion molecules, as well as induce leukocyte infiltration with some features of lymphoid tissue[28–30]. Remarkably, expression of both LTα and LTβ induced the formation of highly organized B- and T-cell compartments and complete HEV development[31]. Subsequent studies showed that engineering the expression of LTα or LIGHT (an alternative ligand for LTβR) by tumour cells leads to the formation of intratumoural lymphoid tissue that sustains an efficient immune response, indicating that signalling through LTβR is implicated in the induction of functionally competent TLOs[32–34]. In parallel, studies addressing the role of individual chemokines in lymphoid neogenesis showed that overexpression of CCL21 or CXCL13 in a non-lymphoid tissue is sufficient to activate a pathway of events that leads to B- and T-cell recruitment and segregation at these ectopic sites[35–39], whereas CCL19 and CXCL12 promote leukocyte accumulation but not lymphoid tissue organization[38]. These data indicate that lymphoid chemokine expression is an essential step in lymphoid neogenesis. However, the lack of FDC networks in the B-cell aggregates that form in *Cxcl13*-transgenic mice[37] indicates that completion of the GC microarchitecture requires additional inducing signals, such as those associated with immune activation (discussed next).

More direct evidence for the involvement of similar molecular mechanisms in SLO ontogeny and TLO formation was derived from immunohistochemical and gene-expression analyses of tissue specimens from patients[15–18,40–46] and experimental disease models[47–52]. Increased expression of lymphoid chemokines (CCL19, CCL21, CXCL12 and/or CXCL13)[16,17,40–46,53–55], HEV-associated molecules (MADCAM1, PNAD and/or the sulphotransferase high endothelial cell *N*-acetylglucosamine 6-*O*-sulphotransferase (HEC-GlcNAc6ST), which is the enzyme involved in PNAD synthesis)[17,52,56–58] and, in some cases, LTα or LTβ[18,43] has been shown in most of the chronically inflamed

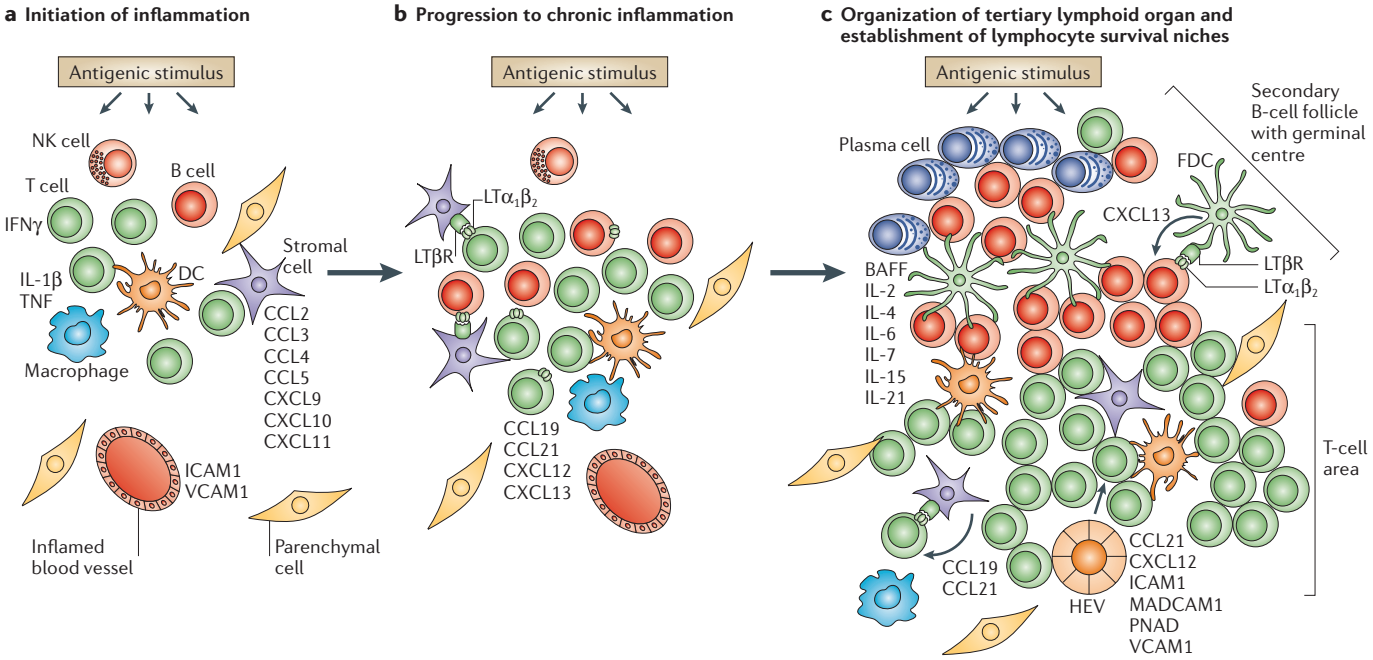

Figure 3 | **Schematic model of cellular and cytokine–chemokine networks in the initiation of inflammation and in the development of chronic inflammation and lymphoid neogenesis. a** | Inflammatory responses are initiated by innate immune cells (such as macrophages and dendritic cells, DCs), leading to the recruitment of peripherally primed lymphocytes into the target tissue. The process is regulated by cytokines, such as interferon-γ (IFNγ), interleukin-1β (IL-1β) and tumour-necrosis factor (TNF), that promote the synthesis of adhesion molecules and other inflammatory mediators, including chemokines, the expression of which is suppressed during the resolution phase of inflammation. **b** | Chronic antigen stimulation leads to persistent activation of innate and adaptive immune cells in the inflamed tissue and to increased expression of lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) by activated B and T cells, and of lymphoid chemokines by resident stromal cells, infiltrating macrophages, DCs and other parenchymal cells. Synthesis of lymphoid chemokines might be induced by $LT\alpha_1\beta_2$ and by pathogen components binding to Toll-like receptors (TLRs) (not shown) on innate immune cells[54,69]. **c** | Recruitment of B cells, T cells and DCs to tertiary lymphoid organs is thought to be facilitated by acquisition of a high endothelial venule (HEV)-like phenotype by activated endothelial cells. CC-chemokine ligand 19 (CCL19) and CCL21 produced by stromal cells (probably fibroblasts or fibroblast precursors) would favour the formation of T-cell areas. Under the influence of $LT\alpha_1\beta_2$, stromal cells would acquire the phenotypic and functional properties of follicular dendritic cells (FDCs), including CXC-chemokine ligand 13 (CXCL13) production, which promote germinal-centre organization. Cytokines that mediate lymphocyte survival and/or proliferation, such as B-cell-activating factor (BAFF), IL-2-family members (IL-2, IL-4, IL-7, IL-15 and IL-21) and IL-6, might contribute, together with antigen, to establish optimal niches for lymphocyte growth. ICAM1, intercellular adhesion molecule 1; LTβR, lymphotoxin-β receptor; MADCAM1, mucosal addressin cell-adhesion molecule 1; NK, natural killer; PNAD, peripheral node addressin; VCAM1, vascular cell-adhesion molecule 1.

tissues that harbour lymphoid-like structures. The cellular localization of these molecules in TLOs is generally consistent with that observed in B- and T-cell areas of SLOs (FIG. 2), although lymphoid chemokines, such as CXCL12 and CXCL13, can have a broader distribution in some inflamed tissues and can also be expressed by infiltrating macrophages and parenchymal cells[15,18,54]. The most probable sources of $LT\alpha_1\beta_2$ at inflammatory sites are infiltrating natural killer (NK) cells, B cells and T cells (mainly of the T helper 1 type)[18,43,54]. In these cell types, expression of membrane-bound $LT\alpha_1\beta_2$ is increased by both antigen-dependent and antigen-independent activation[27]. Although it is possible that the adult counterparts of fetal $LT\alpha_1\beta_2$-expressing $CD3^-CD4^+CD45^+$ lymphoid-tissue-inducer cells are recruited into inflamed tissues, evidence for their presence in TLOs is still inconclusive[33]. Remarkably, a correlation between the expression of LTα or LTβ and/or lymphoid chemokines and lymphoid tissue organization has been reported in human autoimmune lesions, which supports a causative involvement of these mediators in TLO formation[17,18,43,44]. However, it was also noted that lymphoid chemokines can be expressed in inflamed tissues without leading to the formation of lymphoid structures[17,18]. These data indicate that lymphoid chemokines, which are probably induced upstream of lymphoid tissue organization, are not sufficient to drive the complete process. This is consistent with findings in transgenic mice[37,38]. Therefore, although the precise sequence of events leading to TLO formation remains to be defined, the available evidence supports the concept that TLO induction and/or stabilization depends, at least in part, on the local availability of the same molecules that mediate physiological SLO organization. A hypothetical model of TLO formation involving LT- and chemokine-induced signalling in an inflammatory environment is shown in FIG. 3.

TLO-inducing signals that are generated during the immune response. SLO development is mainly induced by an LT- and/or chemokine-driven positive-feedback loop that leads to the accumulation and compartmentalization of naive lymphocytes. These lymphocytes will only subsequently encounter their activating antigens and, in the case of B cells, will enter GC reactions. By contrast, TLO formation under pathological conditions probably requires previous as well as persistent antigen exposure. Whether TLOs maintain an immune response that originates from circulating memory B and/or T cells or from naive cells remains to be defined. If it involves memory cells, TLOs would only amplify and perpetuate the ongoing response that was originally generated in canonical SLOs. If naive cells are the triggers, there could be a real qualitative contribution of the TLOs to the immune response, as the unique features of this type of lymphoid tissue could favour the recruitment of cells bearing a broader range of T-cell receptors or antibodies that can react with the activating antigens. The idea that antigen is crucial for maintaining TLO organization is supported by the presence of secondary B-cell follicles with ongoing GC reactions and only rare primary B-cell follicles (P. Armengol and R.P.-B., unpublished observations) in chronically inflamed tissues, and by the finding that ectopic GCs generate plasma cells that produce antibodies specific for antigens that are expressed in the target tissue[15,16,59–61] (TABLE 1). GCs are dynamic structures that evolve quickly during the primary immune response but are more stable under chronic antigenic stimulation, such as in the tonsil and probably in the TLOs[62]. This view is also supported by the finding that the number and size of secondary B-cell follicles that form in the gastric submucosa and lamina propria of patients with *Helicobacter pylori* infection are markedly reduced after the eradication of this Gram-negative pathogen[59,63].

B cells that give rise to ectopic GCs could be naive B cells or, most probably, memory B cells that are recruited from the periphery and after exposure to activating stimuli in the inflammatory environment, such as CD40 ligand (CD40L; also known as CD154) expressed by activated T cells[64], upregulate expression of $LT\alpha_1\beta_2$ and/or TNF, which promote B-cell aggregation and the differentiation of FDC networks. However, it is also possible that FDCs and FDC-bound antigens are not required in the early stages of ectopic GC formation and that tissue-infiltrating B cells aggregate, proliferate and undergo somatic hypermutation and affinity maturation before FDC differentiation occurs[65]. One possibility is that the high availability of antigen at inflammatory sites results in antigen presentation by B cells to other B cells, which circumvents the requirement of FDCs for ectopic GC induction. However, FDCs might be important to maintain the GC response by providing B-cell survival and proliferation factors, such as the TNF-family member B-cell-activating factor (BAFF), interleukin-6 (IL-6) and IL-15 (REF. 20).

Further evidence for the contribution of immune activation to lymphoid neogenesis has been provided by studies in animal models. Cupedo *et al.* have shown that CD40 stimulation with an agonistic antibody (that mimicks the interaction with CD40L-expressing T cells) is required for the complete organization of ectopic lymphoid structures that are induced in the skin of adult mice by intradermal injection of lymph-node-derived cells from newborn mice, probably through the activation of CD40-expressing B cells, DCs and/or macrophages[66]. A crucial role for DCs in directing inflammation towards lymphoid neogenesis is indicated by the study showing *de novo* formation of lymphoid structures in the pancreatic parenchyma of transgenic mice that express lymphocytic choriomeningitis virus (LCMV) glycoprotein in the pancreas and develop diabetes after repeated immunization with DCs that are pulsed with the same viral protein[67]. Although increased antigen presentation and T-cell activation might explain these findings, DCs themselves are an important source of chemokines and survival factors that might facilitate lymphocyte homing and compartmentalization in inflamed tissues. Notably, recent work indicates that an infectious challenge with influenza virus can trigger lymphoid chemokine expression and lymphoid neogenesis in the lungs even in LTα-deficient mice that lack SLOs[68]. Signals that are induced by microbial pathogens through pattern-recognition receptors, such as Toll-like receptors (TLRs), could substitute for $LT\alpha_1\beta_2$ and directly activate innate immune cells involved in the *de novo* formation of lymphoid tissue. Consistent with this hypothesis, lipopolysaccharide (a component of Gram-negative bacteria that binds TLR4) induces CXCL13 production by DCs and macrophages[54,69]. It can therefore be concluded that the state of activation of both innate and adaptive immune cells that are recruited to inflammatory sites might be crucial for increasing the local concentrations of key mediators (in particular, $LT\alpha_1\beta_2$ and lymphoid chemokines) above a threshold that is required for the initiation of lymphoid tissue organization.

Feedback from inflamed tissues. A restricted number of tissues, such as the thymus, thyroid gland, liver, gut, lungs and joint synovium, provide permissive environments for lymphoid neogenesis (TABLE 1). Conversely, organized lymphoid structures are not observed in diseased skin, although at this site inflamed blood vessels can acquire some properties of HEVs, such as CCL21 expression[70,71]. A unique situation is observed in the central nervous system (CNS), which is traditionally viewed as an immune-privileged site. In patients with multiple sclerosis and in animal models of this disease, ectopic GCs develop in the meninges, but do not develop in the white-matter lesions (plaques), despite extensive lymphocyte infiltration[46,51]. These observations indicate that each tissue reacts to inflammation with a unique response programme that shapes the subsequent development of the inflammatory process. This concept is supported by experimental studies showing that ectopic expression of CCL21 is sufficient to induce lymphoid neogenesis in the pancreas and thyroid, but not in the skin or CNS parenchyma[35,36,38,39,72].

The functional diversity of stromal cells, including endothelial cells, fibroblasts, smooth muscle cells and pericytes, at distinct anatomical sites could account

Immune-privileged site
Areas in the body with a decreased immune response to foreign antigens, including tissue grafts. These sites include the brain, eye, testis and uterus.

Meninges
Fibroblastic layers that ensheath the brain and spinal cord and line the subarachnoid space where the cerebrospinal fluid circulates. The meninges contain a resident population of macrophages and dendritic cells and are a less immune-privileged central-nervous-system compartment compared with the neural parenchyma.

for the different permissiveness and the heterogeneous configuration of ectopic lymphoid tissue in different locations. For example, the remarkable plasticity of the vascular endothelium and its ability to acquire the adhesive and chemoattractant properties of HEVs in response to inflammatory stimuli are thought to have an important role in facilitating lymphocyte traffic to inflamed tissues[24]. CCL21 expression has been detected in PNAD$^-$ blood vessels as well as in PNAD$^+$ HEVs and perivascular cells in T-cell areas of human TLOs[17,43,45,58,71,73], whereas contrasting reports exist on the vascular expression of CXCL13 (REFS 17,54,55,73). It should be pointed out that, unlike mouse HEVs, human HEVs do not synthesize lymphoid chemokines. Instead, lymphoid chemokines are transported to the HEV luminal surfaces through vascular transcytosis[74]. However, the lack of HEV-like vessels and the perivascular location of ectopic GCs in certain chronically inflamed tissues, such as the brain meninges in multiple sclerosis[46], indicate that other adhesion molecules and chemokines expressed on activated blood vessels are involved in TLO formation. Although expression of PNAD and CCL21 is expected to facilitate the recruitment of naive T cells, most studies in humans indicate that the T cells that accumulate in chronically inflamed tissues have an effector-memory phenotype and express a distinct pattern of chemokine receptors that bind inflammatory chemokines (such as CCR5 and CXCR3), as well as those that bind lymphoid chemokines (such as CCR7, CXCR4 and CXCR5)[43,73]. However, this T-cell phenotype might have been acquired locally after migrating into the tissue. It has been proposed that expression of lymphoid chemokine receptors on tissue-infiltrating lymphocytes is regulated by mediators that are released in the inflammatory environment itself and might be more important for the organization of B- and T-cell areas than for lymphocyte recruitment through the vascular endothelium[43,73].

Tissue fibroblasts are another stromal component that are thought to have an important role in TLO formation by providing the functional scaffold for the retention and compartmentalization of lymphocytes after extravasation into the inflamed tissue. Recent studies have shown that activated fibroblasts share several features with the stromal cells that form the reticular network of SLOs, including expression of cytoskeletal proteins, secretion of extracellular-matrix components, production of cytokines and chemokines (including CXCL12), and ability to support lymphocyte adhesion and survival[75,76]. As FDCs are thought to originate from mesenchymal stromal cells[20], *de novo* development of FDCs in ectopic GCs is most probably a result of the differentiation of local fibroblasts or differentiation from fibroblast precursors that are located in the inflamed tissue or recruited from the blood[76]. This could explain why the fibroblast-rich meninges, but not the neural parenchyma, are the preferential site for GC formation in CNS autoimmune disease[46,51]. In addition to stromal cells, activated parenchymal cells, such as thyroid-gland epithelial cells and brain astrocytes, produce CXCL12, which might have an important role in retaining B and T cells, plasma cells and DCs in the inflamed tissue[43,77]. It therefore seems that within the target tissue, multiple cellular components actively participate in lymphoid neogenesis, either becoming integrated in the lymphoid tissue architecture or contributing to the establishment of a lymphoid-like chemoattractive environment.

Disease-specific features of TLOs

Autoimmune diseases. Most of the conditions in which lymphoid neogenesis has been documented are organ-specific autoimmune disorders, in which both B- and T-cell responses against tissue antigens have been implicated (TABLE 1). This is not surprising because the chronic immune stimulation that is inherent to autoimmune diseases probably favours TLO formation. Among them, lymphoid neogenesis is most prominent in thyroid autoimmune diseases, such as Hashimoto's thyroiditis and autoimmune thyrotoxicosis (Graves' disease)[1,4,15,44], and myasthenia gravis[2,5,78,79], for which the antigenic targets of the humoral immune response have been best characterized. Rheumatoid arthritis[6,14,18,80] and Sjogren's syndrome[16,55,81] — two clinically associated conditions that share features of B-cell polyclonal stimulation and increased levels of rheumatoid factor — show a relatively lower frequency of GC formation. The discovery of GCs in the CNS of patients with multiple sclerosis has provided the long-sought anatomical basis for the intrathecal synthesis of immunoglobulins and the presence of oligoclonal bands in the cerebrospinal fluid (CSF), which are a hallmark of this disease[46,82]. Although healthy adult humans do not have bronchus-associated lymphoid tissue, few reports have documented the presence of ectopic GCs in lung autoimmune disease, mainly in cryptogenic fibrosing alveolitis[83,84]. In liver autoimmune diseases such as primary sclerosing cholangitis, HEVs have been observed but ectopic GCs have not, which supports the involvement of a primarily T-cell-mediated immune response[42,56].

The most important questions relating to the process of lymphoid neogenesis in autoimmune diseases are: do TLOs have the functional properties of SLOs? What are the antigenic stimuli that trigger their formation? And, to what extent do TLOs contribute to the ongoing inflammatory process? In some autoimmune diseases, TLOs fulfil many of the criteria for having a pathological role in the disease process, including the presence of ongoing GC reactions and the production of relevant pathogenic autoantibodies (TABLE 1). Molecular analyses of the rearranged immunoglobulin V genes of B cells that were isolated from biopsied GCs of patients with Sjogren's syndrome[85], rheumatoid arthritis[60,86] and myasthenia gravis[61], and from the CSF (which is adjacent to the meninges where ectopic GCs develop) of patients with multiple sclerosis[82,87] have shown that B cells undergo antigen-driven clonal expansion and somatic hypermutation at these ectopic sites. These processes might lead to the generation and selection of memory B cells and plasma cells that produce high-affinity autoantibodies. Expression of the recombination-activating genes (RAG1 and RAG2), which are involved in the rearrangement of V(D)J (variable, diversity and junction) gene segments in primary lymphoid organs,

has been shown in the lymphocytic infiltrates that were isolated from autoimmune lesions[15,88,89]. This finding indicates that active peripheral rearrangements of immunoglobulin V genes (receptor revision) occur at these sites and raises the possibility that new specificities of autoreactive B cells are generated in the inflamed tissue. Increased generation of autoreactive B-cell clones could also result from the avoidance of peripheral-tolerance checkpoints, which leads to disturbance of the selection process. For example, autoreactive B cells could escape apoptosis and negative selection in ectopic GCs, owing to the sustained production of the B-cell survival

Table 2 | **Mouse models of inflammation that are accompanied by lymphoid neogenesis**

Disease	Target tissue	Microarchitecture of lymphoid infiltrates	Immunological correlates	References
Autoimmune disease				
Autoimmune gastritis	Stomach	B-cell follicles with FDCs but rare PNA$^+$ GCs; no CCL21$^+$PNAD$^+$ HEVs	Increased titre of serum gastric-mucosa-specific autoantibodies; T_H1-cell-biased microenvironment	49
Autoimmune thyroiditis*	Thyroid gland	B-cell follicles and HEVs	Serum colloid-specific antibodies	125
Prediabetic NOD mice	Pancreatic islets; salivary and lacrimal glands	Lymphoid aggregates with CCL21$^+$ stromal cells and CCL21$^+$HEC-GlcNAc6ST$^+$MADCAM1$^+$ HEVs	Not determined	30,47,52
Collagen-induced arthritis	Joints	B-cell follicles with GL7$^+$ GCs	Not determined	117
Experimental autoimmune encephalomyelitis (chronic-relapsing form)	Central nervous system	Intrameningeal B-cell follicles with FDCs and GCs	Not determined	51
Allergic disease				
Airway antigenic challenge	Lungs	B-cell follicles with FDCs and PNA$^+$ GCs	IgE production	105
Infectious disease				
Helicobacter pylori-induced gastritis	Stomach	B-cell follicles with GCs; T-cell areas with CD62L^{hi} naive T cells and MADCAM1$^+$PNAD$^+$ HEVs	Weak correlation with *H. pylori*-specific antibody responses	50,126
Propionibacterium acnes-induced granulomatous liver disease	Liver	B-cell follicles with FDCs without GCs; T-cell areas with PNAD$^+$ HEVs	Not determined	48
Influenza A virus	Lungs	B-cell follicles with FDCs and GCs; T-cell areas with CCL21$^+$PNAD$^+$ HEVs	Influenza-virus-specific CD8$^+$ T cells and antibodies	68
Transplantation				
Allograft rejection	Heart	B-cell follicles; T-cell areas with PNAD$^+$HEVs	Not determined	110
Ectopic expression of:				
Lymphotoxin-α	Pancreatic islets, kidneys	B-cell follicles with or without FDCs; T-cell areas with CCL21$^+$MADCAM1$^+$PNAD$^+$ HEVs	Production of antibodies to foreign antigen	28–34
	Tumours		T-cell responses against tumour antigens	32,33
Lymphotoxin-αβ	Pancreatic islets	B-cell follicles with FDCs; T-cell areas with CD62L^{hi} naive T cells, CD11c$^+$ DCs and CCL21$^+$HEC-GlcNAc6ST$^+$MADCAM1$^+$PNAD$^+$ HEVs	Not determined	31
LIGHT	Tumours	Infiltrating CD62L^{hi} naive T cells and CCL21$^+$MADCAM1$^+$ stromal cells	Intratumoral expansion and activation of tumour-antigen-specific T cells	34
CCL19	Pancreatic islets	Non-organized B- and T-cell infiltrates; MADCAM1$^+$PNAD$^+$ HEVs	Not determined	38
CCL21	Pancreatic islets, thyroid gland	B-cell follicles without FDCs; T-cell areas with CD11c$^+$ DCs, MADCAM1$^+$PNAD$^+$ HEVs and stromal cells	Not determined	35,36,38,39
CXCL12	Pancreatic islets	B-cell, plasma-cell and DC infiltrates	Not determined	38
CXCL13	Pancreatic islets	B-cell aggregates without FDCs; T-cell areas with stromal cells and MADCAM1$^+$PNAD$^+$ HEVs	Not determined	37

*Autoimmune-prone biobreeding (BB) rats were used in these experiments. CCL, CC-chemokine ligand; CD62L, CD62 ligand; CXCL, CXC-chemokine ligand; DC, dendritic cell; FDC, follicular dendritic cell; GC, germinal centre; HEC-GlcNAc6ST, high endothelial cell *N*-acetylglucosamine 6-*O*-sulphotransferase; HEV, high endothelial venule; MADCAM1, mucosal addressin cell-adhesion molecule 1; NOD, non-obese diabetic; PNA, peanut agglutinin; PNAD, peripheral node addessin; T_H1, T helper 1.

factor BAFF in autoimmune lesions[90–92]. Consistent with this idea, low apoptosis frequency and increased levels of the anti-apoptotic protein BCL-2 have been shown in ectopic GC B cells in Sjogren's syndrome and myasthenia gravis[90,93].

Most importantly, production of tissue-specific, disease-relevant autoantibodies by ectopic GC plasma cells has been shown in myasthenia gravis[61], Sjogren's syndrome[16] and Hashimoto's thyroiditis[15] (TABLE 1). In some studies, a correlation has been found between ectopic GC formation and autoantibody serum levels, which has raised the possibility that TLOs substantially contribute to the disease process[15,44,80]. GC-plasma-cell-derived autoantibodies, together with local complement synthesis and activation, might be the direct effectors of tissue destruction. Additional mechanisms by which B cells that are generated in ectopic GCs might contribute to the inflammatory process include: production of pro-inflammatory cytokines, T-cell activation through presentation of autoantigens and co-stimulation[82,94], and formation of anti-idiotypic networks[95].

Together, these studies provide strong evidence that functional GC reactions take place within TLOs and can be considered a common feature of autoimmune diseases. Much less is known about the role of TLOs in sustaining autoimmune T-cell responses. A recent study carried out in two mouse models of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-induced demyelinating disease, has shown that activation of T cells to endogenous myelin peptides (epitope spreading) occurs in the inflamed CNS, which indicates that local T-cell priming occurs[96]. However, further work is needed to find out whether naive T cells are activated within ectopic T-cell areas and to establish a relationship with GC reactions.

Features of ectopic lymphoid tissue have also been detected in animal models of autoimmunity, such as EAE and insulitis that spontaneously develops in non-obese diabetic (NOD) mice, with the most organized structures generally being associated with the chronic disease phases[30,47,51] (TABLE 2). These animal models are useful tools for identifying the factors that regulate lymphoid tissue organization in inflammatory conditions and for testing the efficacy of therapies targeted to TLOs.

Infectious diseases. Although lymphoid neogenesis probably evolved as part of a strategy to contain chronic local infection[13], so far it has been well documented in only a few chronic infectious diseases. Among them, *H. pylori*-induced gastritis shows a high degree of TLO organization and a large number of locally generated IgA-producing plasma cells specific for *H. pylori*-derived antigens[40,59,97]. In contrast to the gut, the gastric mucosa does not normally contain MALT, and the increased frequency of lymphoid neogenesis at this site represents a high-risk condition for the generation of MALT-type gastric lymphoma[40]. Lymphoid neogenesis also seems to be a common event in chronic hepatitis C virus (HCV) infection[98,99]. Clonally expanded and somatically hypermutated B cells have been observed in the liver of patients with chronic HCV infection[99,100], which indicates that ectopic GCs might elicit antiviral immune responses. Interestingly, the HCV core protein has been shown to bind to the cytoplasmic tail of LTβR[101], and it has been suggested that this interaction could result in perturbation of immune function, perhaps due to mimicking of $LT\alpha_1\beta_2$-induced signalling, and induction of lymphoid neogenesis. This scenario could explain the frequent association of HCV infection with autoimmune diseases.

In Lyme disease — a systemic illness caused by infection with the tick-borne spirochete *Borrelia burgdorferi* — chronic arthritis develops after the infectious stage in a large proportion of patients and some of these patients develop well-organized GCs in the joint synovium[102]. Although there is evidence that an antigen-driven B-cell response occurs in the joints[103], it is not yet known whether a few residual spirochetes or self-antigens are the antigenic stimuli for such a chronic inflammatory response. Several infectious diseases of the CNS are characterized by the presence of clonally related and somatically hypermutated B cells in the CSF and ocular fluid and by intrathecal production of antibodies against the causative agent[82]. Currently, however, there is a lack of anatomical evidence of GC formation in the infected CNS.

Other diseases. Data on lymphoid neogenesis in various other unrelated diseases, such as allergic lung disease[104,105], atherosclerosis[106,107] and cancer[108,109], are limited, but they highlight the fact that TLO formation extends beyond an autoimmune or infectious aetiology. Molecular analysis of the B-cell response in ectopic GCs in breast cancer confirms that these are sites for antigen-specific B-cell expansion and maturation[108,109]. Interestingly, recent work has provided evidence of lymphoid neogenesis in an animal model of allograft rejection, which establishes an association between chronic rejection and lymphoid tissue organization[110]. More thorough analyses of diseased tissues are warranted to establish whether lymphoid neogenesis might indeed be a relevant pathophysiological component of a larger range of chronic inflammatory diseases.

Pathophysiological significance

TLO formation is now recognized as a common feature of many chronic inflammatory diseases. So, what is the advantage of building such complex structures outside the canonical lymphoid organs and in such diverse pathological conditions? An efficient immune response is usually mounted when antigen enters SLOs in sufficient quantity and for a sufficient length of time to encounter and stimulate rare lymphocytes that bear the appropriate receptor[111]. It can be envisaged that when the immune system fails to mount an efficient response against an infectious agent or tumour, it might be more advantageous to move all of the crucial elements of the immune response, namely antigen-presenting cells and lymphocytes, to the site of antigen deposition. This would create the optimal conditions for an effective immune response, as more antigen would be available

Anti-idiotypic network
A peripheral immunoregulatory mechanism by which anti-idiotypic T cells and antibodies recognize idiotypic determinants residing within the variable or hypervariable regions (CDR2 and CDR3) of T-cell receptors or antibodies. Such a regulatory network is thought to have an important role in the regulation of autoimmune responses.

and antigen-specific T cells and antibodies would readily carry out their effector functions *in situ*. Moreover, a current hypothesis predicts that in an inflammatory context — in an infected or damaged tissue — it is more likely for an antigen to be recognized as 'dangerous' and to elicit an immune response[112].

In some cases, the stimulus that triggers lymphoid neogenesis in infected tissues is the causative agent itself, which maintains a positive-feedback loop involving activation of innate immune cells and stromal cells, synthesis of inflammatory mediators, and continued lymphocyte recruitment and activation. In this case, lymphoid neogenesis might have a protective function by inducing an immune response against the infectious agent, unless it contributes to breaking immune tolerance against self-antigens[113]. Potential mechanisms of induction of B- and T-cell-mediated autoimmunity by microbial antigens include structural similarity to an autoantigen epitope (molecular mimicry) and activation of autoreactive lymphocytes favoured by the inflammatory setting (bystander activation)[113].

By contrast, in autoimmune diseases, once an autoimmune response is initiated, it is likely that lymphoid neogenesis in the target tissue will be driven by tissue antigen released by the destructive inflammatory process, independently of the inducing mechanism. The outcome of TLO formation in this case would be inevitably detrimental by promoting the autoimmune response. An important question is: are TLOs only involved in generating immune responses to a restricted number of self-antigens within the target tissue, or are they also able to recognize exogenous antigens (such as during an infectious episode) and participate in a protective immune response? This possibility is supported by the observation that ectopic B-cell follicles could respond to immunization with an exogenous antigen by production of specific antibodies[28,114]. The surprising finding that systemic infections increase the incidence of multiple sclerosis relapses in the absence of detectable blood–brain-barrier disruption and immune-cell recruitment to the CNS, indicates that the 'exacerbating' event might be sustained by a local immunopathological process[115].

Modulating TLO formation for immunotherapy

Given the many shared features of SLOs and TLOs, modulation of the signalling pathways that regulate lymphoid tissue organization can be thought of as a useful approach to counteract or promote lymphoid neogenesis. Disruption of established TLOs or prevention of TLO formation could be advantageous in autoimmune diseases and all inflammatory conditions in which TLOs are suspected to have adverse effects (such as transplant rejection and allergic diseases). Conversely, promoting TLO formation at sites of infection or tumour growth could facilitate the eradication of infectious agents and tumours, but this approach could increase the risk of autoimmunity[67].

TLO-associated molecules and pathways that might be blocked for therapeutic intervention include: adhesion molecules (such as MADCAM1 and PNAD), and lymphoid chemokines (such as CCL21), to suppress lymphocyte migration and compartmentalization; the $LT\alpha_1\beta_2$–LTβR pathway or downstream chemokine-driven signalling (such as CXCL13) to inhibit lymphoid organization and the formation of FDC networks; FDC signalling molecules that promote B-cell survival and proliferation in GCs (such as BAFF and IL-15), to counteract dysregulated B-cell homeostatic processes; and the CD40–CD40L and CD86–CD28 pathways to block B-cell–T-cell interactions that are required for the activation and/or maturation of B cells.

Several tools, including neutralizing antibodies and decoy receptors, that interfere with these signalling pathways are now available and can be tested in animal models. Among these, the LTβR–immunoglobulin fusion protein, which acts as a decoy receptor and blocks the $LT\alpha_1\beta_2$–LTβR pathway, suppresses pathogenic immune responses in experimental autoimmune disease, and this approach is being tested in preclinical trials[26]. Notably, in NOD mice, treatment with LTβR–immunoglobulin fusion protein at a late stage of disease reverses insulitis and causes disassembly of already formed pancreatic lymphoid aggregates[116]. Of the lymphoid chemokines, only the blockade of CXCL13 has been evaluated in an experimental therapeutic setting, and this has been shown to be effective in reducing the severity of collagen-induced arthritis in mice and GC formation in splenic and synovial tissues[117]. Injection of TACI (transmembrane activator and CAML (calcium-modulating cyclophilin ligand) interactor)–immunoglobulin fusion protein, which blocks binding of BAFF to its three receptors (TACI, BCMA (B-cell maturation antigen) and BAFF receptor), inhibits the production of collagen-specific antibodies and disease progression in a mouse model of rheumatoid arthritis[118].

However, it can be foreseen that the use of systemic compounds that target the lymphoid tissue could have serious limitations in chronic inflammatory diseases, owing to a generalized suppressive activity on SLOs and other microanatomical tissue compartments, such as MALT. For example, the treatment of adult mice and non-human primates with LTβR–immunoglobulin results in the collapse of FDC networks and the splenic marginal zone[26,119], whereas TACI–immunoglobulin impairs B-cell maturation[118]. Because TLOs are highly localized in the diseased tissue, a more suitable strategy would be to provide a local supply of the interfering agent, for example by using a gene-therapy approach. This strategy should also overcome the problem of reaching sequestered sites, such as the CNS. In multiple sclerosis, the intrathecal humoral immune response is not affected by any of the approved systemic immunomodulatory or immunosuppressive treatments, or by the most extensive immunoablative approaches, such as B-cell-depleting therapy with the CD20-specific monoclonal antibody rituximab[120] and haematopoietic stem-cell transplantation[121]. In recent experiments, we have shown that intrathecal delivery of LTβR–immunoglobulin to mice that have established relapsing–remitting EAE transiently inhibits the formation of intrameningeal

B-cell follicles and significantly delays clinical relapses (S. Columba-Cabezas, B. Serafini and F.A., unpublished observations). Based on these findings, we propose that CNS-tissue-restricted delivery of compounds that interfere with TLO formation might be used in combination with other systemic immunotherapeutics to block, more effectively, tissue-compartmentalized immunopathological processes.

Concluding remarks

During the past decade, lymphoid neogenesis has moved from a histopathological curiosity to an important pathophysiological mechanism that can be either detrimental, in the case of autoimmune disease, or beneficial, in the case of infectious and neoplastic diseases. Despite considerable progress in our understanding of the factors that promote TLO formation, many fundamental questions remain to be answered. The first and most general of such questions concerns the differences, if any, between the immune response generated in TLOs and the response generated in canonical SLOs. To answer this general question, we probably need to solve many others, such as what are the crucial signals that are generated during immune activation? How is the expression of certain essential mediators, such as lymphoid chemokines, regulated in an inflamed environment and to what extent do they contribute to the selective accumulation of lymphocytes that give rise to the ectopic lymphoid tissue? Which antigens stimulate ectopic GC reactions and which B-cell subsets (naive and/or memory B cells) are involved? Despite a few exceptions, the identity of the activating antigens (most probably multiple) in autoimmune diseases remains unknown. In this context, the use of genetic engineering with antibody-synthesis techniques to generate recombinant antibody fragments from individual B cells isolated from ectopic GCs will help to characterize the B-cell specificities in the diseased tissue and the pathogenic relevance of locally produced antibodies. Another unresolved issue is whether stabilization of ectopic GCs in chronic inflammatory diseases is due to continuous antigenic stimulation as well as to dysregulated homeostatic mechanisms to which the surrounding inflamed tissue might contribute. If so, what are the crucial checkpoints in the B-cell response that are subverted in lymphoid neogenesis? Answers to these questions could allow us to design improved treatments for the increasing number of chronic inflammatory diseases in which lymphoid neogenesis seems to be involved.

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