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Lymphoid organ development: from ontogeny to neogenesis

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The development of lymphoid organs can be viewed as a continuum. At one end are the 'canonical' secondary lymphoid organs, including lymph nodes and spleen; at the other end are 'ectopic' or tertiary lymphoid organs, which are cellular accumulations arising during chronic inflammation by the process of lymphoid neogenesis. Secondary lymphoid organs are genetically 'preprogrammed' and 'prepatterned' during ontogeny, whereas tertiary lymphoid organs arise under environmental influences and are not restricted to specific developmental 'windows' or anatomic locations. Between these two boundaries are other types of lymphoid tissues that are less developmentally but more environmentally regulated, such as Peyer's patches, nasal-associated lymphoid tissue, bronchial-associated lymphoid tissue and inducible bronchial-associated lymphoid tissue. Their regulation, functions and potential effects are discussed here.

Secondary lymphoid organs are anatomically distinct tissues that efficiently trap and concentrate foreign antigen to initiate an adaptive immune response. These specialized lymphoid organs include the lymph nodes, spleen and mucosal-associated lymphoid tissue. The last includes the Peyer's patches, tonsils, nasal-associated lymphoid tissue and bronchial-associated lymphoid tissue. Together, secondary lymphoid organs form a highly complex and diverse system that supports the interaction between antigen-presenting cells and rare, antigen-specific lymphocytes, resulting in the stimulation of long-lived, protective immunity. Each secondary lymphoid organ is uniquely equipped to 'sample' antigens at many points throughout the body. Strategically placed lymph nodes, for example, form a protective network for detecting interstitial antigens, whereas the spleen is important for detection of and protection against blood-borne pathogens. Mucosal-associated lymphoid tissues, of course, provide critical protection at mucosal surfaces, such as the lungs, gastrointestinal tract and reproductive tract, by 'collecting' antigens directly from the local environment. Thus, their precise locations and specialized processes for monitoring tissue microenvironments bestow on secondary lymphoid organs the notable ability to protect against countless insults by pathogens and noxious antigens. Here we focus first on the lymph node, an example of a prototypic secondary lymphoid organ.

Development, structure and function of lymph nodes

Lymph node development is a highly ordered process initiated during embryogenesis and continuing, at least in the rabbit and rat, up to 3 weeks after birth[1]. Adult lymph nodes are encapsulated, bean-shaped structures containing mobile lymphocytes organized into distinct functional T cell and B cell compartments, antigen-presenting cells, lymphoid chemokines and a highly specialized network of reticular cells and fibers. Lymph nodes also have two unique vasculature systems: lymphatic vessels and high endothelial venules (HEVs; **Fig. 1**). Based on histological and molecular studies, lymph node organogenesis can be divided into five distinct stages[2]. One of the earliest stages includes the development of lymphatic vessels. Historically, two models have been used to explain 'lymphangiogenesis' during embryogenesis. The budding of lymph sacs from preexisting veins in pig embryos was first described in the early 1900s[3,4]; later, it was alternatively proposed that lymphatic vessels originate from the mesenchyme, eventually joining the vascular system[5]. The first model has gained support, resulting in something of a paradigm: lymphatic vessels arise by sprouting from venous endothelial cells after a commitment to the lymphatic lineage that is induced by an unknown signal[6,7]. Development in that way is associated with a progression from the expression of early lymphatic markers such as Lyve-1 and Prox-1 (refs. 6–8) to markers expressed later, such as podoplanin[6,9].

After lymphatic development, the lymph node anlage (or primordium) is colonized by circulating $CD45^+CD4^+CD3^-$ hematopoietic progenitor cells called lymphoid tissue–inducer cells. This unique subset of hematopoietic cells, derived from a fetal liver progenitor[10,11], is thought to provide a crucial signal for inducing lymph node and Peyer's patch organogenesis[12]. Lymphoid tissue–inducer cells accumulate in the developing lymph node, forming small clusters with resident stromal organizer cells, to initiate a cascade of intracellular and intercellular events that lead to the maturation of the primordial lymph node[2]. Notably, data obtained by treatment of pregnant mice with a fusion protein of lymphotoxin-β receptor (LT-βR) and immunoglobulin have shown that individual lymph nodes are initiated at defined and different times, with the mesenteric nodes developing earliest at embryonic day 11, and popliteal

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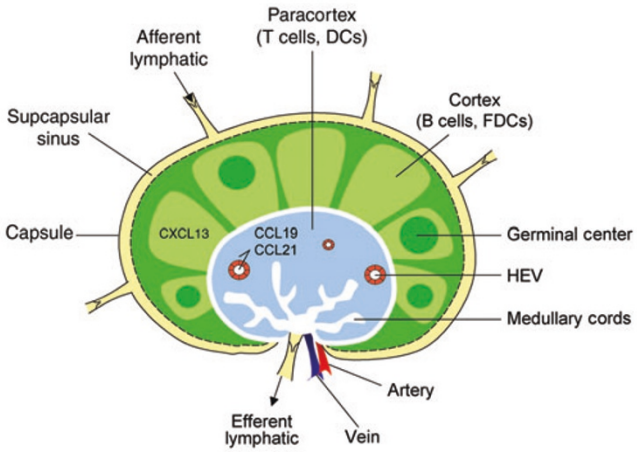

Figure 1 Lymph node structure. A fibrous capsule and an underlying subcapsular sinus surround the cellular contents of the lymph node. The lymph node can be separated into three distinct regions: cortex, paracortex and medulla. The cortex contains discrete lymphoid nodules, composed of B cells and FDCs, called 'primary follicles'. After antigen stimulation, B cells undergo intense proliferation, giving rise to secondary follicles called germinal centers. Inside the cortex lies the paracortex, which is composed of T cells and DCs. The deeper medulla consists of lymphatic tissue called medullary cords, which are separated by lymph filled spaces called medullary sinuses. The lymph node vasculature includes HEVs and lymphatic vessels. T cells and B cells circulate constantly through the lymph node by entering HEVs and exiting via efferent lymphatic vessels. DCs enter via afferent lymphatic vessels. Lymph, containing DCs and soluble antigen, enters the lymph node at several points through afferent lymphatic vessels and deposits antigens in the subcapsular sinus. Once in the lymph node, cell location is 'orchestrated' by lymphoid chemokines. CXCL13, expressed in the B cell follicles, guides B cells to them; CCL19 and CCL21, expressed in the T cell zone, 'position' T cells and DCs in the paracortex. In addition, lymphoid chemokines expressed on HEVs facilitate the recruitment of lymphocytes to lymph nodes.

lymph nodes, the latest, at embryonic day 16 (ref. 13). Prolonged interactions between lymphoid tissue–inducer and stromal-organizer cells promote the development of HEVs, which support the selective entry of naive T cells and B cells into the lymph node through the expression of vascular addressins and chemokines[14,15]. L-selectin, expressed on naive lymphocytes, interacts with peripheral node addressin (PNAd) expressed on HEVs to facilitate lymphocyte recruitment into lymph nodes[15,16]. PNAd, recognized by the monoclonal antibody MECA 79, is a shared carbohydrate-based determinant found on several scaffold proteins, including GlyCAM-1, CD34, and podocalyxin[17–19]. Optimal binding of L-selectin to PNAd requires three post-translational modifications: sialylation, fucosylation and sulfation. The last two modifications are mediated by the coordinated activity of fucosyl transferases (FucTIV and FucTVII) and at least one sulfotransferase (HEC-GlcNAc6ST, also called LSST, HEC-6ST and GlcNAcST-2)[20–23]. In addition to PNAd, HEVs can express another vascular addressin called MAdCAM-1. Immediately after birth, all HEVs express MAdCAM-1, the ligand for the integrin $\alpha_4\beta_7$ (ref. 24), which is rapidly replaced by PNAd in mouse peripheral lymph nodes[25]; thus, PNAd functions as the main L-selectin ligand that contributes to the 'mature' peripheral lymph node HEV phenotype. In contrast to peripheral lymph nodes, mucosal lymph nodes contain HEVs that maintain expression of MAdCAM-1 in addition to PNAd[25].

Tumor necrosis factor family in lymph node organogenesis

The immediate tumor necrosis factor–lymphotoxin (TNF-LT) family includes three proteins: TNF, LT-α and LT-β[26]. These cytokines are members of a 'superfamily' of proteins comprising at least 19 ligands and 29 receptors that have many biological activities associated with cancer, infectious disease, allergy, autoimmunity, transplantation biology and lymphoid organ development[27]. Individually, TNF (a type II transmembrane homotrimeric complex that can be released from cell surfaces through the activity of TNF convertase) and LT-α (a soluble homotrimeric complex) can bind to the TNF receptors TNFRI and TNFRII. In contrast, LT-β (a membrane-bound protein) must form a functional heterotrimeric complex with LT-$\alpha_1\beta_2$ to bind its receptor, LT-βR[28–31]. The involvement of additional cytokines and transcription factors, including TRANCE, LIGHT, IL-7, Id2 and RORγ, in this process has been summarized before[2]. Here we concentrate on LT-α and LT-β, in part because of their well documented activities in lymphoid neogenesis (discussed below).

Several lines of evidence demonstrate that LT-α and LT-β have crucial, nonredundant functions in lymphoid organ development. Mice deficient in LT-α (*Lta*$^{-/-}$ mice), for example, lack all lymph nodes and Peyer's patches and have highly disorganized spleen and nasal-associated lymphoid tissue[32–35]. Transgenic expression of LT-α under control of the rat insulin gene promoter element (RIP–LT-α) partially restores lymphoid organ development and function to *Lta*$^{-/-}$ mice[36]. In contrast, LT-β-deficient (*Ltb*$^{-/-}$) mice lack all peripheral lymph nodes and Peyer's patches but retain mucosal, cervical and sacral lymph nodes and have less prominent splenic and nasal-associated lymphoid tissue defects than those of *Lta*$^{-/-}$ mice[34,37–40]. It is becoming increasingly apparent that LT-$\alpha_1\beta_2$ signaling through LT-βR controls secondary lymphoid organ development through the regulation of lymphoid chemokine and HEV gene expression[41–43]. The mechanisms by which LT-α regulates this developmental process are less well defined, given that LT-α, as the LT-α3 homotrimer or in complex with LT-β, can signal through TNFR or LT-βR, respectively.

Structure and function of lymph nodes

The lymph node cortex contains densely packed B cells and follicular dendritic cells (FDCs) arranged into discrete clusters called primary follicles, whereas the paracortex is composed of a less dense accumulation of T cells and dendritic cells (DCs). Lymphocytes enter the lymph node by extravasation across HEVs; soluble antigen and DCs enter via afferent lymphatic vessels at multiple sites along the capsule[44]. Filtered lymph and cells leave the lymph node via a single efferent lymphatic vessel for later delivery to the venous blood.

The recruitment and positioning of lymphocytes and DCs in the lymph node is orchestrated by the compartmentalized expression of lymphoid chemokines (also called homeostatic chemokines)[45]. The lymphoid chemokine family includes three ligands, CCL19, CCL21 and CXCL13, and two receptors, CCR7 and CXCR5. CCL19 (also called ELC) and CCL21 (also called SLC) are constitutively expressed by T cell–zone stromal cells and share the chemokine receptor CCR7, which orchestrates the homing of naive and central memory T cells and DCs to the T cell compartment[46]. In addition, CCL21 is expressed by lymph node HEVs and lymphatic vessel endothelium in nonlymphoid tissues. Lymphatic expression of CCL21 is thought to participate in the emigration of mature DCs out of the peripheral tissues and into afferent lymphatic vessels[47]. The chemokine CXCL13 (also called BLC) is constitutively expressed by follicular stromal cells and is required for the homing of CXCR5$^+$ B cells and a small subset of T cells to the follicular compartment[48]. All of

Table 1 Lymphoid neogenesis in autoimmunity

Disease	Affected tissue	TLO characteristics	Reference
Human			
Rheumatoid arthritis	Synovial membrane	T cells and B cells, plasma cells, GCs, FDCs, CXCL13, CCL21, HEVs (PNAd, HEC-6ST)	88,91,98, 114–119
Sjögren syndrome	Salivary glands	T cells and B cells, plasma cells, GCs, FDCs, CCL21, CXCL12, CXCL13, HEVs (PNAd)	101,120–122
Myasthenia gravis	Thymus	T cells and B cells, GCs, FDCs	100,123
Hashimoto thyroiditis	Thyroid	T cells and B cells, GCs, FDCs, CCL21, CXCL13, CXCL12, plasma cells, HEVs (PNAd)	124–127
Grave disease	Thymus	T and B cells, GCs, FDCs, CCL21, CXCL13, CXCL12, HEVs (PNAd)	125,126
Multiple sclerosis	Brain	Lymphatic capillaries, B cell follicles, GCs, CCL19, CCL21	128–131
Ulcerative colitis	Colon	CXCL13	132
Inflammatory bowel disease (Crohn disease)	Bowel	T cell–B cell compartments, lymphatic vessels, HEVs (HECA-452)	95,127
Rodent			
NOD mouse (prediabetic)	Pancreas, salivary gland	T cell–B cell compartments, HEVs (MAdCAM-1, PNAd, HEC-6ST) CCL21	87,133–135
AKR mouse	Thymus	HEVs (PNAd, HEC-6ST)	22
EAE mouse	Brain	HEVs, CCL19, CCL21, CXCL13, BAFF, FDCs	136–138
BB rat	Thyroid	T cell–B cell compartments, DCs	139
Autoimmune gastritis, mouse	Stomach	HEVs, CXCL13	140

TLO, tertiary lymphoid organ; GC, germinal center; NOD, nonobese diabetic; EAE, experimental autoimmune encephalomyelitis; BAFF, B cell–activating factor.

these chemokines are regulated by TNF-LT family members[42]. The regulation of CXCL12 (also called SDF-1), which is also expressed in lymph nodes, is less well understood[49].

Structural features of lymph nodes enhance interactions between antigen-presenting cells and the few lymphocytes specific for any given antigen. Such features make lymph nodes optimal sites for inducing adaptive immune responses. Antigen presentation in lymph nodes, the cornerstone of adaptive immunity, is mediated by two highly specialized cells: DCs and FDCs. DCs are the quintessential antigen-presenting cells, as they present antigens as peptides in the context of major histocompatibility complex class I and class II to prime T cells. FDCs, in contrast, prime lymphocytes by means of unprocessed antigens in the form of antigen-antibody immune complexes, which help to shape the B cell responses. Antigen challenge induces the formation of germinal centers in primary B cell follicles after extensive B cell proliferation. In germinal centers, FDCs present antigen-antibody immune complexes to activated B cells, which is thought to be pivotal for the generation of antigen-specific B cell clones. Such clones express high-affinity antigen receptors through a combination of somatic hypermutation of the immunoglobulin variable gene and affinity-based selection, after which the maturing B cells undergo terminal differentiation into antibody-secreting plasma cells.

Shaping of the T cell repertoire and removal of self-reactive T cells occur in the thymus. However, some self-reactive T cells escape the thymus and its central tolerance mechanisms and enter the periphery[50]. Thus, additional mechanisms serve as mediators of peripheral tolerance and act to minimize self-reactivity. In steady-state conditions, lymph nodes can provide a niche for the generation of peripheral tolerance as DCs constitutively sample self antigen, migrate to draining lymph nodes and tolerize T cells[51–54]. Because most self antigen–bearing DCs in lymph nodes are immature[55] and have low expression of costimulatory molecules, they function to regulate potential self-reactive T cell activity by inducing anergy or clonal deletion or by causing the population expansion of regulatory T cells[55–59].

Plasticity of secondary lymphoid organs

It is understood that the developmental program for lymph nodes is generally turned 'off' soon after birth[1,60] and in normal physiological conditions cannot be turned 'on' in the adult. However, studies in rabbits, sheep and rodents have demonstrated remodeling during acute inflammation, characterized by considerable changes in lymph flow, lymph content, blood flow and HEV differentiation. In general, the flow of afferent lymph, as well as its cell constituents, increases soon after the initial inflammatory insult and gradually returns to normal[61–64]. Similarly, efferent lymph flow increases immediately after immunization, peaking at 24–48 hours, although lymphocytes are temporarily absent from the efferent lymph[61,63–66]. Lymph node remodeling during inflammation is also characterized by an increase in blood flow and lymphocyte migration. Changes in cell distribution in the lymph node are regulated in part by the fibroblast reticular network by signaling through TNFR and LT-βR[67]. Changes in the lymph node include an increase in the number and dimensions of HEVs[68–73]; the expression of many HEV-specific genes is downregulated soon after the inflammatory insult[74,75]. After antigen clearance, the lymph node returns to its steady-state morphology (**Fig. 2**). The plasticity of some secondary lymphoid organs is also apparent from the observation that intraperitoneal injection of LT-βR–immunoglobulin into adult mice reduces the expression of several HEV genes and lymph node cellularity[43].

Several lymphoid tissues are more plastic and more susceptible to antigen exposure than are lymph nodes. Some are in set locations with a capsule and distinct organization, whereas others are less fixed. The number of Peyer's patches can increase after immunization, and their lymphoid cell content decreases with aging[76]. Nasal-associated lymphoid tissue, actually a pair of lymphoid organs above the soft palate in the mouse and rat and functionally analogous to tonsils and adenoids in humans[77], is hypocellular at birth and undergoes considerable changes at weaning. These include the expression of LT-α and LT-β, increases in cellularity, T cell and B cell compartmentalization, lymphoid chemokine expression and HEV maturation[34,35,78,79]. In fact, fully developed nasal-associated lymphoid tissue is not apparent until 6 weeks after

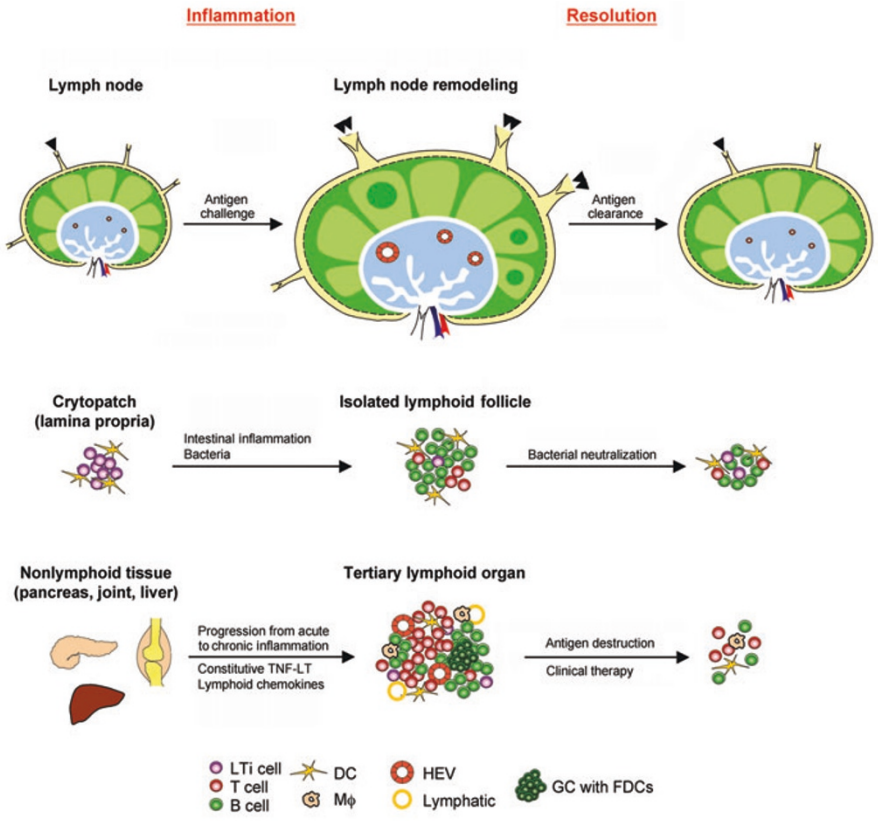

Figure 2 Plasticity in secondary and tertiary lymphoid organ development. Lymph node development is initiated during ontogeny and continues with HEV maturation and cellular population for a short time after birth, depending on the species, giving rise to mature lymph nodes at precise anatomical locations. Once established, lymph nodes cannot be ablated, although these tissues undergo substantial remodeling after inflammatory insult, including increase in size, cellularity and lymph and blood flow and HEV morphology. Unlike the development of lymph nodes, the development of many mucosal tissues can be regulated in the adult by environmental stimuli. Presented here is the development of inductive intestinal lymphoid tissue called isolated lymphoid follicles that have been proposed to arise from a rudimentary lymphoid tissue called crytopatches. These crytopatches, found in the intestinal lamina propria at birth, are small clusters of lymphoid tissue–inducer (LTi) cells and DCs that are thought to provide an organizing center for isolated lymphoid follicle development. After intestinal colonization by gut flora or inflammation, crytopatches have been proposed to develop into isolated lymphoid follicles that consist in part of a large B cell follicle and few T cells. The plasticity of these isolated lymphoid follicles is demonstrated by the observation that such lymphoid tissues resolve, for example, after removal of bacterial stimuli by antibiotic treatment. The most plastic tissue of the lymphoid organs is the tertiary lymphoid organ that can arise at anytime and at nearly any anatomical location in the adult. Tertiary lymphoid organs develop in nonlymphoid tissues (such as pancreas, liver and joints) after the switch from acute to chronic inflammation. Prolonged cytokine production and/or lymphoid chemokine expression is sufficient to induce lymphoid neogenesis. Notably, tertiary lymphoid organ development is reversible if the inflammation-inducing agent is cleared or after therapeutic treatment. GC, germinal center.

birth. Although its development is consistent with the importance of an environmental stimulus, it remains to be determined whether development of this tissue is regulated by a preset developmental program, by environmental stimuli or by a combination of both.

Bronchial-associated lymphoid tissue is a less organized but more environmentally regulated tissue than Peyer's patches or nasal-associated lymphoid tissue. Bronchial-associated lymphoid tissue can simulate immune responses even in splenectomized *Lta*$^{-/-}$ mice, which lack lymph nodes and Peyer's patches[80]. An inducible form of the bronchial-associated lymphoid tissue has been described[81] that develops in *Lta*$^{-/-}$ mice after infection with influenza virus. Inducible bronchial-associated lymphoid tissue has many characteristics of 'canonical' secondary lymphoid organs, including expression of CXCL13 and CCL21, T cell and B cell compartmentalization and the presence of FDCs and PNAd^{+} HEVs. An additional inducible lymphoid tissue has been described in the adult gut. Clusters of lymphoid tissue–inducer cells and DCs in the lamina propria, called 'cryptopatches', resemble primordial lymph nodes[11,76,82,83]. After exposure to microbes or during autoimmunity, cryptopatches give rise to so-called 'inducible lymphoid follicles', which are presumed to have great plasticity because they resolve completely after microbe removal or after treatment with cytokine inhibitors (**Fig. 2**).

The inducibility and plasticity of lymphoid organ development are further demonstrated by two observations. First, injection of lymphoid tissue–inducer cells restores Peyer's patch development in neonatal CXCR5-deficient mice (*Blr*$^{-/-}$ mice)[84]. Second, intradermal injection of lymph node cells derived from the mucosal lymph nodes of newborn mice induces the formation of lymph node–like structures, although only in newborn recipients[60]. Similarly, a notable example of the plasticity of lymphoid organs is the *de novo* formation of ectopic lymphoid tissue in nonlymphoid organs during chronic inflammation, the so-called 'tertiary lymphoid organs'.

Tertiary lymphoid organs

Tertiary lymphoid organs, also called tertiary lymphoid tissues, are ectopic accumulations of lymphoid cells that arise in chronic inflammation through a process called lymphoid neogenesis (or lymphoid neo-organogenesis)[85]. Notably, unlike canonical secondary lymphoid organs, or even the inducible lymphoid organs such as Peyer's patches, which develop in specified locations, tertiary lymphoid organs arise in the adult in random, typically nonlymphoid locations. Such tissues have been described in autoimmunity (**Table 1**), microbial infection and chronic allograft rejection (**Table 2**) and even in the endometrium during the menstrual cycle[86]. In addition, tertiary lymphoid organs have been induced in several transgenic models characterized by expression of inflammatory cytokines or lymphoid chemokines driven by tissue-specific promoters (**Table 3**). Those mouse models, in addition to serving as models of autoimmunity, have provided invaluable insight into the regulation of secondary lymphoid organ development.

Tertiary lymphoid organs have considerable morphological, cellular, chemokine and vasculature similarity to secondary lymphoid organs, particularly to lymph nodes. Notably, specialized HEVs have been detected in tertiary lymphoid organs that express the enzymes and scaffold proteins necessary to generate functional PNAd[41,87,88]. Lymphatic vessels have also been noted in tertiary lymphoid organs, although it is not apparent if they function as afferent and/or efferent vessels[89]. Although not every secondary lymphoid organ characteristic has been

described for each tertiary lymphoid organ, it is apparent that tertiary lymphoid organs have the antigen-presenting and antigen-responding cells needed to generate an immune response.

From chronic inflammation to organized lymphoid tissues

Among the prevailing issues in the field of lymphoid neogenesis is how tertiary lymphoid organs arise in chronic inflammation. Studies over the past 15 years addressing the cellular and molecular requirements for lymph node development have provided a paradigm for understanding the development of tertiary lymphoid organs in chronic inflammation. That paradigm proposes that the same processes and molecules governing lymph node development are also the basis of tertiary lymphoid organ development[85]. The physiological events that precipitate the *de novo* formation of tertiary lymphoid organs, however, remain unclear. How does a chronic inflammatory lesion develop into a highly organized lymphoid tissue? Experimental data obtained using knockout and transgenic mice and clinical observations indicate that cooperative activities of TNF-LT family members and the lymphoid chemokines are central to this process. However, the identity of the TNF-LT–responsive stromal cell in tertiary lymphoid organ development is unknown.

Inflammation is a localized response to tissue injury, irritation or infection often characterized by tissue damage. Acute inflammation is an early innate immune response that is generally short-lived and self-limiting. However, in some situations acute inflammation transitions to a chronic inflammatory response that is long-lived and self-perpetuating (**Fig. 2**). In conditions of constitutive cytokine and/or chemokine expression, tertiary lymphoid organs arise, but the precise signals that initiate their development are unknown. With the integration of studies of lymphoid neogenesis in human pathologies and in animal models, it is becoming apparent that at least three critical events promote tertiary lymphoid organ formation: inflammatory cytokine expression (such as TNF-LT); lymphoid chemokine production by stromal cells; and HEV development. One issue is whether lymphoid tissue–inducer cells are necessary for lymphoid neogenesis. A study using RIP-BLC mice (which express CXCL13 under control of the rat insulin promoter and have pancreatic tertiary lymphoid organs) has demonstrated lymphoid tissue–inducer cells in the pancreas before the accumulation of conventional lymphocytes[90]. The inductive function of the lymphoid tissue–inducer cells in lymphoid neogenesis in this model is unknown; furthermore, it remains to be determined whether lymphoid tissue–inducer cells are a general feature of tertiary lymphoid organs. An additional issue is whether lymphatic vessels are generally associated with tertiary lymphoid organs as they are with secondary lymphoid organs.

Lymphangiogenesis in tertiary lymphoid organs

One notable observation in the field of lymphoid neogenesis has been the detection of lymphangiogenesis in tertiary lymphoid organs. Transplanted human kidneys undergoing chronic rejection show a substantial increase in lymphatic vessel density in and surrounding infiltrates reminiscent of tertiary lymphoid organs[89]. Additionally, increased expression of the lymphangiogenic factor VEGF-C is detectable in the synovium of joints of rheumatoid arthritis patients[91]. Lymphatic vessels have also been noted in murine models of corneal transplantation, suture-induced corneal inflammation and *Mycoplasma pulmonis*–induced airway inflammation[92–94]. Lymphangiogenesis has even been noted in human pathologies, including inflammatory bowel disease, Crohn disease and ulcerative colitis[95].

The process of lymphangiogenesis during inflammation remains poorly understood. It is unclear whether newly formed lymphatic vessels bud from existing lymphatics or from veins. A mechanism has been suggested by which lymphangiogenesis occurs during inflammation: $CD11b^{+}CD11c^{-}$ macrophages (in a mouse corneal transplant model) seem to differentiate into lymphatic endothelial cells[92]. Obviously the function of macrophages during lymphatic vessel development must be elucidated. For example, do macrophages differentiate to lymphatic endothelial cells or integrate with lymphatics; alternatively, do macrophages induce lymphatic development through the secretion of lymphangiogenic molecules such as VEGF-C? Do cytokines or chemokines present during inflammation promote lymphangiogenesis directly or through the stimulation of macrophages or other cells? The possibility that lymphangiogenesis is an integral aspect of lymphoid neogenesis thus suggests that lymphatic vessels might be a new therapeutic target in autoimmunity.

Tertiary lymphoid organs: beneficial and harmful

The ectopic accumulation of lymphoid cells has been considered the '*sine qua non*' of destructive inflammation. Indeed, some of the tertiary lymphoid organs described in **Table 1** are accompanied by tissue damage. However, the functional properties of tertiary lymphoid organs and their involvement in immunity are the subject of intense investigation. In microbial infection, tertiary lymphoid organs seem to develop to sequester pathogens and to prevent their access to other parts of the body. Although local antigen presentation in the tertiary lymphoid organ itself probably functions to prevent bacteremia or viremia, their

Table 2 Lymphoid neogenesis in chronic inflammatory pathologies

Organism (disease)	Affected tissue	TLO characteristics	Reference
Infectious disease			
Borrelia burgdorferi (lyme disease)	Joints	T cells and B cells but not compartmentalized, HEVs, FDCs	141,142
Borrelia burgdorferi (neuroborreliosis)	Central nervous system–cerebrospinal fluid	CXCL13	143,144
Hepatitis C virus	Liver	T cell–B cell compartments, MAdCAM-1	110,145
Helicobacter pylori	Gastric mucosa	CXCL13, HEVs (MAdCAM-1, PNAd)	146–148
Helicobacter spp	Liver	T cell–B cell compartments, naive T cells, HEVs (PNAd), CCL21, CXCL13	149
Propriobacterium acnes	Liver	CCL21, $CD11c^{+}$ DCs	150
Bartonella henselae (cat scratch disease)	Granuloma	CXCL13	151
Graft rejection			
Organ			
Mouse heart		T cell–B cell compartments, PNAd	152
Human kidney		Lymphatic vessels	89
Rat aorta		HEV	153
Human heart		Germinal centers	153
Human kidney		Germinal centers	153
Other			
Human artery	Atherosclerosis	HEVs ($HECA\text{-}452^{+}$), FDCs, organized B cell follicles	154
Human uterus	Menstrual cycle	T cell–B cell compartments	86

Table 3 Lymphoid neogenesis in transgenic mice

Transgene	Organ	TLO characteristics	Reference
RIP-TNF (LT-α)	Pancreas, kidney	T cell–B cell compartments, L-sel$^+$ T cells, HEVs (MAdCAM-1, ablumenal PNAd), FDCs, DCs, plasma cells with IgG and IgM anti-SRBC, CCL21, CXCL13, prion accumulation	85,96,108,155
RIP–LT-α. RIP–LT-β	Pancreas, kidney	T cell–B cell compartments, L-sel$^+$ T cells, CCL19, CCL21, CXCL13 FDCs, DCs, HEVs (MAdCAM-1, lumenal PNAd, HEC-6ST)	41
RIP-SLC (CCL21)	Pancreas	T cell–B cell compartments, naive T cells, DCs, HEVs (MAdCAM-1, PNAd), FDCs, prion accumulation	96,156,157
RIP-CCL21a and RIP-CCL21b	Pancreas	T cell–B cell compartments, DCs, HEVs (MAdCAM-1, PNAd)	158
RIP-BLC (CXCL13)	Pancreas	T cell–B cell compartments, DCs (no FDCs), HEVs (PNAd, HEC-6ST, MAdCAM-1) CCL21	87,157
RIP-CCL19 (ELC)	Pancreas	Small infiltrates, T cell–B cell compartments, HEVs (PNAd)	157
RIP-CXCL12 (SDF-1)	Pancreas	Small infiltrates, mainly naive B cells, few T cells, DCs, plasma cells	157
Alb–LT-αβ	Liver	B cells, FDCs, IgD$^+$ and IgG1$^+$ cells, DCs, PNA$^+$ clusters, prion accumulation	96
TG-CCL21	Thyroid	T cell–B cell compartments, CD62L$^+$ T cells, CD11c$^+$ DCs, HEVs (PNAd)	159

Ig, immunoglobulin; anti-, antibody to; SRBC, sheep red blood cell; Alb–LT-αβ, albumin promoter–LT-αβ; PNA, peanut agglutinin; TG-CCL21, thyroglobulin promoter–CCL21.

propensity to develop into lymphomas and to serve as sites of prion accumulation are obvious manifestations of possible detrimental functions[96]. Furthermore, the development of tertiary lymphoid organs in autoimmunity may perpetuate disease.

Understanding of the induction of immune responses in tertiary lymphoid organs has been derived mainly from studies of B cell differentiation in their germinal centers. Emerging data from both human and mouse studies have provided circumstantial evidence supporting the idea that tertiary lymphoid organs are permissive microenvironments for the induction of antigen-specific humoral immune responses. Extensive immunohistochemical analyses of tertiary lymphoid organs in autoimmunity and other chronic inflammatory states have established the presence of germinal centers and FDC networks in these tissues. Moreover, several groups have demonstrated that tertiary lymphoid organ germinal centers can support B cell differentiation. The earliest evidence regarding competent germinal center reactions was provided by the results of microdissection of discrete lymphocytic foci and subsequent DNA sequence analysis of germinal center B cells from the inflamed synovial tissue of patients with rheumatoid arthritis. Analysis of the κ–light chain of synovial B cells has demonstrated a restricted number of variable-region κ-gene rearrangements, a result consistent with oligoclonal B cell expansion in the synovial tissue[97]. Extensive DNA analysis of synovial germinal center B cells has shown that these cells undergo somatic hypermutation in tertiary lymphoid organ germinal centers, as demonstrated by the stepwise accumulation of somatic mutations in the immunoglobulin variable region[98]. Furthermore, it has been shown that synovial B cells have a limited number of heavy- and light-chain gene rearrangements, consistent with local clonal expansion of these cells[98]. Those early studies in rheumatoid arthritis provided the impetus to investigate B cell differentiation in other autoimmune diseases. Indeed, molecular analysis of tertiary lymphoid organ germinal centers from the salivary glands of patients with primary Sjögren syndrome or the thymi of patients with myasthenia gravis has demonstrated oligoclonal B cell proliferation in these tissues in addition to somatic hypermutation of immunoglobulin variable genes[99–101]. Those studies collectively indicate that tertiary lymphoid organ germinal centers in many autoimmune pathologies can support antigen-driven clonal expansion and extensive diversification.

Another important hallmark of antigen-driven B cell responses is the terminal differentiation of activated B cells into immunoglobulin-secreting plasma cells. Plasma cells have been detected in tertiary lymphoid organs associated with germinal centers in rheumatoid arthritis and Sjögren syndrome and in RIP–LT-α–transgenic mouse kidneys after immunization with sheep red blood cells[85,102]; analysis of plasma cells in RIP–LT-α kidney tertiary lymphoid organs has shown that they had also undergone isotype switching. Although the presence of plasma cells in tertiary lymphoid organs is consistent with local antigen presentation, it remains unclear whether these cells develop in the tertiary lymphoid organs themselves or have migrated from canonical secondary lymphoid organs. Nonetheless, those studies collectively indicate that lymphoid organs in several human pathologies and animal models can support antigen-driven B cell differentiation characterized by somatic hypermutation of immunoglobulin variable genes, affinity maturation, isotype switching and terminal differentiation into antibody-secreting plasma cells.

Although it is becoming increasingly apparent that tertiary lymphoid organs can support the induction of humoral immune responses, to our knowledge no conclusive evidence exists directly demonstrating that tertiary lymphoid organ DCs can internalize, process and present antigens to prime naive T cells in the local tertiary lymphoid organ microenvironment. Circumstantial evidence suggesting that this is the case includes isotype-switched plasma cells in the RIP–LT-α tertiary lymphoid organ[85], T cell epitope spreading in the central nervous system during experimental autoimmune encephalomyelitis[103] and the restricted T cell receptor repertoire in a melanoma-associated tertiary lymphoid organ[104]. One of the principal challenges in addressing this issue is making a distinction between T cell priming in draining lymph nodes versus priming in the tertiary lymphoid organ microenvironment. It is also unknown whether induction of peripheral tolerance occurs in tertiary lymphoid organs as it does in secondary lymphoid organs.

Determinant or epitope spreading is a phenomenon that arises in several autoimmune diseases when epitopes other than the inducing epitope become the chief targets of an ongoing immune response. It is believed to occur after the tissue damage induced by the initiating autoreactive T cells and is therefore believed to be the result of the presentation of new antigens[105]. This type of autoreactivity can spread 'intramolecularly' (to epitopes in the original immunizing antigen) or 'intermolecularly' (when responses to separate unrelated antigens are seen). The physical location where antigen presentation occurs to generate epitope spreading is of critical importance to the possible development of treatments for autoimmune diseases that are perpetuated by this mechanism. For

example, are 'neo-antigens' transported to secondary lymphoid organs and do they then prime naive T cells, or are 'neo-antigens' retained in the tertiary lymphoid organ itself? Two models of central nervous system inflammation support the idea that T cell priming and epitope spreading occur in tertiary lymphoid organs. First, in relapsing experimental autoimmune encephalomyelitis induced by proteolipid protein amino acids 139–151, adoptively transferred T cells specific for another proteolipid protein epitope proliferate only in the central nervous system and not in the draining lymph nodes[103]. Second, intermolecular epitope spreading has been noted in mice infected with Theiler murine encephalomyelitis virus in which reactivity to central nervous system antigens is detected[103]. Those data provide strong evidence that tertiary lymphoid organs can be important sites for epitope spreading and are of crucial importance when considering strategies to prevent the progression of diseases such as multiple sclerosis.

The central nervous system is the site of prion diseases such as scrapie, Cruetzfeldt-Jakob disease and bovine spongiform encephalopathy. However, prions accumulate in secondary lymphoid organs even before the onset of clinical signs[106]. It has been noted that several different tertiary lymphoid organs can serve as additional sites of prion accumulation. Those include the kidney in spontaneous autoimmunity (NZB × NZW mouse) or the kidney, liver or pancreas in lymphoid neogenesis induced by chronic expression of LT-α at those sites[96] (**Table 3**). In fact, mice with tertiary lymphoid organs of the kidney excrete prions in the urine[107], suggesting a previously unsuspected mode of transmission of prion infectivity. Those observations demonstrate that tertiary lymphoid organs function as new reservoirs for prion accumulation, allowing the invasion of tissues that are otherwise resistant.

Lymphoid neogenesis can also have many functions in cancer. Tertiary lymphoid organs can be detrimental by inducing tumor formation or facilitating metastasis; however, tertiary lymphoid organs can also be beneficial by providing a microenvironment for local presentation of tumor antigens and induction of antitumor immunity. There are many examples in which tertiary lymphoid organs can 'progress to' (develop into) lymphomas, including Hashimoto thyroiditis, rheumatoid arthritis, Sjögren syndrome and celiac disease[108]. The development of lymphomas in those situations is most likely associated with continual B cell proliferation. Lymphoid neogenesis in gastritis induced by *Helicobacter pylori* can progress to adenocarcinoma or mucosal-associated lymphoid tissue lymphoma, although the latter often regresses after antibiotic treatment[109]. Similarly, in hepatitis C virus infection, chronic inflammation can also lead to progression of tertiary lymphoid organs into hepatocellular carcinoma[110]. It seems likely that tertiary lymphoid organs establish a supportive tumor microenvironment through the provision of growth factors and angiogenic signals in those conditions. Tertiary lymphoid organ lymphatic vessels may also serve as conduits for tumor cell metastasis. Alternatively, targeting tertiary lymphoid organs to established solid tumors might represent a new therapy for cancer treatment. As proof of principle, LT-α has been targeted with a tumor-specific antibody to murine melanomas, and the development of tumor-associated tertiary lymphoid organs and concomitant tumor regression have been demonstrated[104]; analysis of the T cell receptor repertoire of tertiary lymphoid organ cells has suggested that specific T cell responses are induced in the tumor. Additionally, it has been shown that constitutive expression of the ligand LIGHT by a fibrosarcoma induces tumor-associated tertiary lymphoid organ formation and subsequent tumor regression[111]. Naive T cells enter the tumor site and undergo extensive proliferation; there is minimal proliferation in the draining lymph nodes and spleen.

Plasticity of tertiary lymphoid organs

Tertiary lymphoid organs are the most plastic of the lymphoid tissues, as is apparent from many studies. They can be induced to develop by a variety of stimuli. Moreover, it is becoming apparent that they can be 'turned off' (or that they resolve) after removal of the initial stimulus or after therapeutic intervention; the destruction of islet of Langerhans β-cells in type I diabetes mellitus is an example of such a situation in which removal of the antigen stimulus is accompanied by tertiary lymphoid organ resolution. Also, antibiotic treatment results in the resolution of tertiary lymphoid organs and even mucosal-associated lymphoid tissue lymphomas[109]. Finally, treatment with LT-βR–immunoglobulin has been shown to resolve some established tertiary lymphoid organs; for example, reversing insulitis and protecting against diabetes in non-obese diabetic mice[112]. Such treatment can also 'turn off' established tertiary lymphoid organs in mouse collagen-induced arthritis[113].

Concluding remarks

The lymphoid system, once considered fixed in development and location, has been shown through the work of many studies to be very plastic. Secondary lymphoid organs, in discrete locations, expand and contract in response to the environment, whereas tertiary lymphoid organs arise in nonlymphoid organs (such as pancreas, liver, brain, joints and intestine) to generate local, often temporary, immune responses. The full degree of plasticity of the expansive lymphoid system is apparent, given that lymphoid organs exist throughout the body. Many careful observations have already provided insight into the nature of developmental regulation and clues for medical interventions. Yet many questions remain, including the following: What are the signals that activate the organizer cells in embryonic development? Are lymphatic vessels initiators of this program? How does lymphoid neogenesis overcome and 'usurp' that program? What drives acute inflammation to become chronic inflammation? The answers to those questions and many others should allow more precise manipulation of the immune system with the aim of conquering immune-induced pathologies and providing prophylaxes and therapeutics for pathologies as diverse as cancer, cardiovascular diseases and spongiform encephalopathies.

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The authors declare that they have no competing financial interests.

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