Published in final edited form as:

Semin Immunol. 2008 February ; 20(1): 26–42.

Ectopic lymphoid tissues and local immunity

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Abstract

Ectopic or tertiary lymphoid tissues develop at sites of inflammation or infection in peripheral, non-lymphoid organs. These tissues are architecturally similar to conventional secondary lymphoid organs, with separated B and T cell areas, specialized populations of dendritic cells, well-differentiated stromal cells and high endothelial venules. Ectopic lymphoid tissues are often associated with the local pathology that results from chronic infection or chronic inflammation. However, there are also examples in which ectopic lymphoid tissues appear to contribute to local protective immune responses. Here we review how ectopic lymphoid structures develop and function in the context of local immunity and pathology.

Keywords

ectopic lymphoid tissue; germinal center; BALT; inflammation; autoimmunity

1. Introduction

Secondary lymphoid organs are an integral part of the immune system and are important sites of immune activation. Encapsulated lymph nodes (LNs) are found along lymphatic vessels that drain regional tissues, while mucosal lymphoid organs, including Peyer's patches and Nasal Associated Lymphoid Tissue (NALT) are not encapsulated and are found directly beneath the mucosal epithelium. Despite their structural differences, lymphoid organs are organized in a similar fashion, with B cell follicles, separated T cell areas, multiple populations of dendritic cells and specialized stromal cells. Each secondary lymphoid organ has the ability to recruit naïve lymphocytes from the blood as well as activated antigen presenting cells from the surrounding tissues or mucosal surfaces. Moreover, the specific architecture of each secondary lymphoid organ has evolved to maximize encounters between antigen, antigen presenting cells and lymphocytes in order to efficiently generate rapid and robust adaptive immune responses (reviewed in [1–5]).

The development of LNs as well as many of the mucosal lymphoid organs is pre-programmed and does not require pathogen-induced inflammation or any type of adaptive immune response (reviewed in [6,7]). In contrast, tertiary lymphoid tissues, also known as ectopic lymphoid tissues, develop in adults in response to chronic inflammation, chronic infection or autoimmunity (previously reviewed in [8]). These tissues can develop in nearly every organ in the body and do not appear in predictable sites. Moreover, these tissues are not encapsulated

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like LNs, nor are they typically sub-mucosal tissues like Peyer's patches or NALT. Instead they are often embedded in non-lymphoid organs at sites of inflammation or infection.

Many of the cellular and molecular signals that control the development of secondary lymphoid organs have been identified, and not surprisingly, many of these signals are also involved in the development of ectopic lymphoid tissues (reviewed in [6,8]). However, the development of ectopic follicles is not identical that of secondary lymphoid organs and it is not well understood what triggers their formation. Here we propose that secondary and tertiary lymphoid tissues are part of a spectrum of lymphoid tissues that ranges from highly organized encapsulated tissues, which develop at specific sites according to pre-programmed developmental pathways, to granulomas and ectopic follicles, which develop only after local inflammation or infection at a variety of sites in multiple tissues. All of these lymphoid tissues participate in immune responses to some degree and while ectopic follicles have been primarily associated with pathology and detrimental autoimmune responses, they are also likely to participate in protective immune responses to local infections.

2. Current model of secondary lymphoid organ development

The development of lymphoid organs has received considerable attention since the discovery that mice lacking molecules in the lymphotoxin (LT) signaling pathway do not develop LNs and Peyer's patches [9–13]. It is now generally accepted that Lymphoid Tissue inducer (LTi) cells are instrumental for the initiation of lymphoid organ development [14,15]. LTi cells are derived from common lymphoid progenitors in fetal liver [16] and can be distinguished from other lymphocytes based on their surface phenotype ($CD3^-CD4^+CD45^{+}IL-7R^{+}c-Kit^{+}$) [17]. Not all LTi cells are functionally equivalent, as the IL-7R is important for the expansion and differentiation of LTi cells at mucosal sites, such as Peyer's patches [18,19], whereas signaling through RANK is important for the differentiation and survival of LTi cells that home to LNs [20]. LTi cells also express the chemokine receptors, CCR7 and CXCR5 [21], which promote their homing to sites of future lymphoid organ development and lead to their interactions with local mesenchymal $VCAM^{+}ICAM^{+}$ LN organizer cells [19,22,23]. LTi cells express $LT\alpha\beta$ and signal through the $LT\beta R$ on LN organizer cells. In turn, the responding mesenchymal cells produce homeostatic chemokines, including CCL19, CCL21 and CXCL13, which accelerate the recruitment of LTi cells as well as other lymphocytes and promote the development of adult lymphoid architecture [23–27]. The expression of homeostatic chemokines by stromal cells and the expression of $LT\alpha\beta$ on recirculating lymphocytes, particularly B cells [28,29], are maintained by positive feedback, in which LT controls chemokine expression by stromal cells and chemokine signaling on lymphocytes maintains surface expression of $LT\alpha\beta$ on lymphocytes [30]. Thus, the disruption of any part of this loop leads to impaired lymphoid organ development and architecture [21,30,31].

LT also controls the differentiation of stromal cells and high endothelial venules (HEVs) [28, 32–34] and is important for the expression of various adhesion molecules on vascular endothelial cells and stromal cells. For example, the expression of MAdCAM, PNA^d, VCAM-1 and ICAM-1 is controlled, in part, by the activities of $LT\alpha$, $LT\alpha\beta$ and TNF signaling through LTR and TNFR1 [35–37]. Similar pathways control the expression of the sulfotransferases that generate the PNA^d epitope as well as the expression of GlyCAM and MAdCAM, which are common protein substrates for post-translational sulfation [37–39]. Thus, LT signaling is essential for numerous aspects of secondary lymphoid organ development, lymphoid architecture and the proper recirculation of lymphocytes.

3. Transgenic models of tertiary lymphoid organogenesis

Much of what we know about the formation of tertiary lymphoid tissues comes from transgenic mice in which various chemokines and cytokines are expressed at ectopic sites. For example,

LT α expressed in the pancreas under the control of the rat insulin promoter leads to inflammatory lesions in the kidney and pancreas [40]. These areas are organized like secondary lymphoid tissues with separated T and B cell zones, specialized stromal cells and HEVs. These ectopic tissues also appear to be functional, as they respond to antigen, form germinal centers and promote isotype switching in B cells [40]. However, on their own, they do not lead to diabetes. LT α expression in the pancreas also promotes the expression of CXCL13 and CCL21, which appear to organize B and T cells zones. In this model, the expression of CXCL13 and CCL21 is dependent on TNFR1, and not LT β , suggesting that signals through the TNFR1 rather than the LT β R are sufficient for at least the minimal development of this tertiary lymphoid tissue [41].

Although LT α alone can clearly initiate tertiary lymphoid organogenesis, the simultaneous expression of LT α and LT β much more efficiently promotes the formation of ectopic lymphoid tissues [39]. Mice that express both LT α and LT β in the pancreas have much larger infiltrates in the pancreatic islets than those in LT α single transgenic mice [39]. Separation into T and B cell areas is more distinct and large FDC networks are visible in all B cell areas. In addition, CCL21, CCL19 and CXCL13 are expressed at higher levels in the islets of LT $\alpha\beta$ double transgenic mice than in the islets of LT α single transgenic mice. Moreover, PNAd is strongly expressed on the luminal surface of HEV in LT $\alpha\beta$ double transgenic mice, whereas it is primarily expressed on the abluminal surface of HEVs in LT α single transgenic mice [39]. These data highlight distinct roles of LT α and LT $\alpha\beta$ in lymphoid organogenesis [39].

In addition to LT $\alpha\beta$, LIGHT also signals through the LT β R and plays a role in the expression of homeostatic chemokines and the formation of tertiary lymphoid structures in some situations [42–44]. LIGHT expression is induced in the islets of older NOD mice concomitantly with the appearance of ectopic lymphoid tissues and the development of diabetes [45]. Blockade of LT β R signaling disrupts the organization of tertiary structures and prevents diabetes. Conversely the transgenic expression of LIGHT in the pancreas of NOD mice promotes the rapid formation of ectopic follicles and results in accelerated onset of diabetes [45]. Thus, the expression of LT α , LT β and LIGHT can all contribute to the formation of tertiary lymphoid structures via signaling through TNFR1 and LT β R [45].

As mentioned above, the expression of LT α , LT β and the expression of homeostatic chemokines are linked via a positive feedback loop that is critical for the development of ectopic lymphoid tissues [30]. Mice expressing CXCL13 in the pancreas develop tertiary lymphoid tissues that contain B and T cell zones, HEVs, stromal cells and CCL21 [46]. Development of these features is dependent on B lymphocytes and LT $\alpha\beta$ [46]. The expression of CCL21 in the pancreas also promotes the development of tertiary lymphoid tissues in an LT β R-dependent manner [26,47,48]. In contrast, the ectopic expression of CCL19 in pancreatic islets only leads to small infiltrates composed of lymphocytes and dendritic cells with HEVs and stromal cells [26]. Similarly, the ectopic expression of CXCL12 in the pancreas promotes the accumulation of small infiltrates that contain relatively few T cells, but enriched in dendritic cells, B cells, and plasma cells [26]. Thus, the homeostatic chemokines, CXCL12, CXCL13, CCL19 and CCL21, are not equal in their ability to promote tertiary lymphoid organogenesis. In part, this may be explained by their differential ability to promote LT $\alpha\beta$ expression on lymphocytes. CXCL13 triggers expression of LT $\alpha\beta$ on B cells, while CCL19 and CCL21, but not CXCL12 promote LT $\alpha\beta$ expression on CD4 T cells [26]. Interestingly, LT $\alpha\beta$ can also be induced on naive T cells by IL-4 and IL-7, suggesting that there may be multiple ways to engage the chemokine/lymphotoxin loop and to trigger the formation of tertiary lymphoid tissues [26].

The pancreas is not the only organ in which the ectopic expression of cytokines and chemokines leads to the development of tertiary lymphoid organogenesis. Mice expressing LT α via the rat insulin promoter also develop tertiary lymphoid tissues in the kidney [40]. Moreover, mice

expressing CCL21 under control of the thyroglobulin promoter develop organized lymphoid tissues in the thyroid [49–51]. These tissues have separated B and T cell areas as well as HEVs and lymphatic vessels surrounding the follicles [50]. Although HEVs expressing PNAd are frequently observed in the thyroid tissue, lymphocyte recruitment is independent of L-selectin and LT α , but does require CCR7 [49]. On the other hand, the ability to form segregated B and T cell areas in the thyroid, express PNAd and develop lymphatic vessels is dependent on LT β R signaling and LT α expression as well as on the activities of lymphocytes [50]. Interestingly, LT-expressing CD4 T cells rather than B cells are important for the initiation of lymphangiogenesis in this model [50]. In contrast, the transgenic over-expression of CCL21 in the skin does not lead to the development of tertiary lymphoid tissues [47], even though CCL21 is highly expressed at this site. Although the numbers of B cells, T cells and Langerhans cells in the skin are unchanged by the CCL21 transgene, the Langerhans cells are retained in the skin when activated by topically applied FITC [47]. Thus, the CCL21 transgene is functional, even though it does not promote the formation of ectopic lymphoid tissue. This implies that there are differences in the mechanisms that lead to the development of ectopic lymphoid tissues in different organs, which probably reflects cell types and vascular beds that are present in each organ.

Although many studies focus on the role of LT α , LT $\alpha\beta$ and homeostatic chemokines in the development of tertiary lymphoid tissues, the transgenic over-expression of cytokines that are not obviously part of the lymphotoxin/homeostatic chemokine axis can also promote the development of tertiary lymphoid organs. For example, the transgenic co-expression of IL-6 and IL-6R under the Class I and β -actin promoters leads to the development of organized inducible Bronchus-Associated Lymphoid Tissue (iBALT) in the peri-bronchial and perivascular regions of the lung [52]. Similar to its expression in ectopic lymphoid tissues that develop in other transgenic models, CXCL13 is expressed in the B cell follicles of iBALT. However, the signaling pathways that link IL-6 and CXCL13 are not known [52]. As in pancreatic islets and thyroid tissue, the IL-6-induced formation of ectopic lymphoid tissue in the lung does not seem to lead to local pathology or autoimmunity. However, this is not always the case. For example, the over-expression of IL-5 in the respiratory epithelium also promotes the development of organized iBALT [53]. Unlike the chemokine or LT-dependent development of tertiary lymphoid tissues in the pancreas and thyroid, which do not lead to diabetes or thyroiditis, the IL-5-dependent development of BALT in the lung leads to goblet cell hyperplasia, epithelial hypertrophy, focal collagen deposition and accumulation of eosinophils in peribronchial areas and in the airway lumen - all signs of lung pathology [53]. Moreover, IL-5 transgenic mice exhibit airway hyperresponsiveness to methacholine in the absence of antigen challenge [53]. Thus, in some cases, the transgene-induced formation of tertiary lymphoid tissues is accompanied by pathology. This is probably related to the nature of the transgene and whether it is a homeostatic cytokine/chemokine, like LT α , LT β , CXCL13 and CCL21 or whether it is an effector cytokine, like IL-5.

4. The role of LT α i cells in the formation of ectopic lymphoid tissues

Although the organogenesis of LNs and Peyer's patches clearly requires LT α i cells [54–57], it is less clear whether LT α i cells are required for the formation of ectopic follicles. In transgenic mice that express CXCL13 in the pancreas, CD4⁺CD3[−] cells are the first hematopoietic cell types recruited to the islets very early after birth [46], suggesting that LT α i cells may be involved in the development of the tertiary lymphoid tissues that will eventually develop in this location. However, it is unclear whether this is due to a coincidence in timing or due to a causal relationship between LT α i cells and the development of tertiary lymphoid tissues. More direct evidence for the involvement of LT α i cells in the formation of ectopic lymphoid tissues comes from the generation of transgenic mice that over-express IL-7 [58]. These mice have abnormally high numbers of LT α i cells and develop a much higher number of LNs and Peyer's

patches than normal mice [58]. They also develop ectopic lymphoid tissues in pancreas and salivary gland. Interestingly, IL-7 by itself is not sufficient to drive the development of these ectopic lymphoid tissues, since IL-7 transgenic mice crossed to $ROR\gamma^{-/-}$ mice, which lack LTi cells [54], do not develop any secondary lymphoid organs or ectopic lymphoid tissues [58]. Thus, in this model, the development of ectopic follicles is dependent on LTi cells.

Importantly, IL-7 is also implicated in the formation of ectopic follicles in patients with rheumatoid arthritis, who have high concentrations of IL-7 in serum and the inflamed synovium of affected joints [59,60]. While IL-7 in these conditions may be contributing to ectopic follicle formation by promoting the survival, expansion or differentiation of LTi cells, IL-7 is also likely to de-regulate T and B cell development, longevity and function [61–63]. As a result, the precise role of IL-7 in the development of ectopic lymphoid tissues in these patients remains difficult to determine.

If LTi cells are required for the development of ectopic lymphoid follicles, then there may be a developmental window during which tertiary lymphoid tissue development is dependent on the availability of LTi cells. This is clearly the case for the development of conventional secondary lymphoid organs, which occurs during a developmental window that varies depending on the particular lymphoid organ [64]. For example, mesenteric LNs develop first (around day E9–E10), followed by brachial (day E13), axillary (day E15) inguinal (day E16) and popliteal LNs (day E17)[64]. Mucosal lymphoid organs, such as Peyer's patches and NALT appear to develop last, as disruption of lymphotoxin signaling just before birth blocks Peyer's patch formation [64] while the development of NALT can be recovered in neonatal $Id2^{-/-}$ mice by the administration of LTi cells [56]. There is also evidence that the formation of ectopic lymphoid tissues also occurs during a defined developmental window shortly after birth. For example, the transfer of cells from disrupted neonatal LNs to ectopic sites, such as the skin, triggers the de novo development of lymphoid tissues [65,66]. However, while the ectopic administration of LTi cells to *neonatal mice* results in lymphoid tissues with segregated B and T cell areas, follicular dendritic cells (FDCs) and HEVs, the administration of LTi cells to ectopic sites in *adult mice* results in generation of disorganized accumulations of lymphocytes without FDCs or HEVs [65,66]. Thus, it appears that interactions between LTi cells and immature mesenchymal cells during a neonatal period is important for the proper formation of ectopic lymphoid tissues under some circumstances.

On the other hand, recent studies using mice that express CCL21 in the thyroid suggest that LTi cells are not required for the development of ectopic lymphoid tissues [67]. Instead, the first cells that appear in the thyroid are $CD3^{+}CD4^{+}$ T cells, which interact with local dendritic cells. Subsequently, PNA^{+} HEVs appear, homeostatic and inflammatory chemokines are produced and lymphocytes and dendritic cells are recruited and organized into tertiary lymphoid tissue [67]. Importantly, when CCL21 transgenic mice are crossed to $Id2^{-/-}$ mice, which lack LTi cells [57], tertiary lymphoid tissues still form in the thyroid, despite the absence of conventional secondary lymphoid organs [67]. These data suggest that the formation of tertiary lymphoid structures in adults does not require $Id2$ -dependent LTi cells, but depends on a program initiated by mature $CD3^{+}CD4^{+}$ T cells.

One reason that the development of ectopic lymphoid tissues may not require LTi cells is that these tissues typically form in adults in response to chronic inflammation or infection. Since adults have a wide variety of circulating mature lymphocytes that express $LT\alpha\beta$, particularly when activated [68,69], the role of LTi cells may be performed by activated lymphocytes. In fact, the presence of LTi cells in adults remains a controversial point [70]. Some investigators have found $CD4^{+}CD3^{-}$ cells in adults and demonstrated that they provide help to follicular T cells in germinal centers [71]. Unlike embryonic LTi cells, adult $CD4^{+}CD3^{-}$ cells express high levels of OX40L and CD30L [72]. However, $CD4^{+}CD3^{-}$ cells in adults also express high levels

of LT $\alpha\beta$ and TNF. Moreover, the adoptive transfer of normal CD4⁺CD3⁻ cells from either embryonic or adult tissues into LT $\alpha^{-/-}$ mice leads to the segregation of B and T cell areas and the expression of CCL21 [73]. Despite these data however, the precise lineage relationship between embryonic LTi cells and adult CD4⁺CD3⁻ cells is unclear and any potential role of these cells in the formation of ectopic follicles is unknown.

Another complication in the analysis of LTi cells and their role in the development of ectopic lymphoid tissues is that there are multiple populations of LTi cells. For example, ROR γ -dependent LTi cells are not required for NALT development [74], whereas Id2-dependent LTi cells are essential for NALT development [56]. This may reflect a different tissue tropism of various populations of LTi cells or may reflect changing differentiation of LTi cells over time during embryogenesis. Given that NALT is one of the last secondary lymphoid organs to develop after birth [56], the LTi cells that home to NALT may have fundamentally different properties. If so, then it is possible that ectopic follicles may also require a particular subset of LTi cells or that ectopic follicles developing in different organs require different LTi cells. Thus, investigators should use caution when concluding that LTi cells are not required for ectopic lymphoid tissue development based only on the use of ROR $\gamma^{-/-}$ or Id2 $^{-/-}$ mice. In the meantime, the role of LTi cells in the development of ectopic follicles remains an open and important question.

5. The role of local stromal cells in the formation of ectopic follicles

The counterparts to LTi cells in the development of secondary lymphoid organs are lymph node organizer cells. These cells are mesenchymal in origin and form the stromal cell matrix of the developing lymphoid organ [19,22,23,75]. Just as there are multiple populations of LTi cells, there are also multiple types of LN organizer cells, with different expression patterns of adhesion molecules, cytokines and receptors [76]. In fact, different lymphoid tissue organizer cells are found at sites of mesenteric LN and Peyer's patch development [76]. This may explain why different lymphoid organs have different requirements of their development. For example, IL-7R is essential for Peyer's patch development [18,19,21], while RANK is required for LN development [20]. Although an organizer cell type that promotes the development of ectopic follicles has not yet been identified, it is clear that stromal cells are present in ectopic follicles [77], even though their precursors remain undefined. It is possible that local fibroblasts differentiate into stromal cells in situ due to interactions with LT $\alpha\beta$ expressing lymphocytes [78]. However, it is also possible that circulating fibrocytes may be recruited by homeostatic chemokines to sites of ectopic follicle development from distal sites like the bone marrow [79,80]. This has clearly been shown in lung fibrosis [79], but has not been rigorously tested in the formation of ectopic follicles.

6. Autoimmunity and the development of lymphoid follicles

Tertiary lymphoid organs often develop in autoimmune diseases and tend to appear in the organs that are under autoimmune attack, such the joints and lungs of rheumatoid arthritis patients [38,81–84], the salivary glands in Sjogren's syndrome [85–87], the thyroid in Hashimoto's thyroiditis or Grave's disease [51,88,89], the pancreas in diabetes [90,91] and the central nervous system in multiple sclerosis [92–95] (Table 1). In many cases, the formation of well-developed ectopic follicles in these sites correlates with increased severity of disease and the local production of auto-antibodies. For example, patients with rheumatoid arthritis can be divided into those with diffuse lymphoid aggregates in their synovium, those with B and T cell aggregates and those with highly developed germinal centers, FDCs and segregated B and T cell areas [59]. Those with the most developed ectopic follicles have more severe symptoms of disease [83]. However, other studies suggest that lymphoid neogenesis is not necessarily related to arthritis activity or severity, but does correlate with local autoantibody

production and higher titers of anti-nuclear antibodies [81]. Increased local production of autoantibodies is also observed in patients with pulmonary manifestations of rheumatoid arthritis [82] and in patients with Sjogren's syndrome [87].

Increasing size and complexity of tertiary lymphoid tissues in autoimmune tissues also correlates with dramatically increased local expression of homeostatic cytokines and chemokines, including LT α , LT β , CXCL13 and CCL21 [59,82,96], CCL20 [97] and CXCL12 [81]. Tissues with germinal centers tend to express much higher levels of LT α , CXCL13 and CCL21 than those with diffuse lymphoid infiltrates [59]. In fact, the expression level of CXCL13 and LT β in synovial biopsies is highly predictive of the presence of germinal centers [59]. The expression of CXCL13 and LT β also correlates with the presence of highly organized secondary B cell follicles in patients with pulmonary disease associated with rheumatoid arthritis [82] and in patients with Sjogren's syndrome. [86].

CXCL13 is typically expressed within the B cell follicle in a reticular pattern consistent with FDCs [82]. Although CXCL13 expression is often associated with FDCs in mice, fibroblast-like synoviocytes also express homeostatic and inflammatory chemokines when stimulated by LT $\alpha\beta$, even though these cells do not differentiate into FDCs - at least in vitro [78]. Moreover, CXCL13 is also expressed by activated monocytes or macrophages, which may be located within FDC networks or even in B cell aggregates that lack FDCs [98,99]. Interestingly, CXCL13 and CCL21 can also be expressed in inflamed tissues that do not yet have organized lymphoid tissue [96], suggesting that these chemokines are induced by inflammation prior to the formation of segregated T and B cell areas, FDCs and germinal centers. This early expression of homeostatic chemokines may be independent of LT $\alpha\beta$ and TNF, as seen in influenza infected lungs [100]. However, the pathways that lead to LT $\alpha\beta$ -independent CXCL13 expression remain undefined.

Although B cells are known to be a potent source of surface LT $\alpha\beta$ expression [30,68] and are important for maintaining the organization of B cell follicles and FDC networks in conventional lymphoid organs [28,101], B cells are also critical for T cell activation in rheumatoid arthritis [102]. Adoptive transfer experiments using human/mouse chimeras show that activation of CD4 T cells in rheumatoid synovium is strictly dependent on B cells and that non-B cell antigen-presenting cells can not maintain T cell activation on their own [102]. CD8 T cells are also activated in the ectopic lymphoid tissues in the joints of arthritis patients and often share common TCR V regions [103,104], suggesting that they are responding to particular antigens. Depletion of CD8 T cells in human/mouse chimeras causes loss of FDCs, disintegration of germinal centers and reductions in autoantibody secretion. Moreover, the expression of LT $\alpha\beta$ is reduced in the absence of CD8 T cells, which express CD40 ligand [103,104]. A similar role for CD8 cells is observed in the development and function of ectopic follicles in transgenic mice that express the lymphocytic choriomeningitis virus glycoprotein in pancreatic islets [91]. These mice develop islet-associated lymphoid tissues and autoimmune diabetes upon immunization with dendritic cells that display the CTL epitope from the lymphocytic choriomeningitis virus glycoprotein [91]. Thus, there are surprising interactions between B cells and both CD4 and CD8 T cells in the induction and maintenance of tertiary lymphoid tissues in autoimmune disease.

B-cell follicles are also detected in the meninges of patients with secondary progressive multiple sclerosis, but not in patients with primary progressive multiple sclerosis [95]. These tertiary lymphoid tissues contain B and T cells, plasma cells and FDCs [95]. The presence of germinal centers along with memory B cells that express hyper-mutated immunoglobulin V genes corresponds to high expression of LT α and CXCL13 [105]. Those patients with follicles are more likely to experience onset of multiple sclerosis at a younger age, irreversible disability,

more pronounced demyelination, microglia activation and loss of neurites in the cerebral cortex [94].

Experimental autoimmune encephalomyelitis (EAE) is widely used as an animal model of multiple sclerosis in which the availability of cytokines and chemokines can be experimentally manipulated. Many features of the mouse model are similar to those in human disease, such as the local upregulation of LT β and LT β R [93], CXCL13 [106,107] and the two CCR7 ligands, CCL19 and CCL21 [92]. CXCL13 co-localizes with FDCs [107], whereas CCL21 is expressed by endothelial cells and CCL19 is expressed by astrocytes and microglia. [92,93] Consistent with the postulated role of the LT/homeostatic chemokine axis in the maintenance and function of tertiary lymphoid tissues in EAE and multiple sclerosis, the blockade of the LT β R and HVEM signaling pathways using LT β R-Ig disrupts the structure of tertiary lymphoid tissues, reduces B and T cell accumulation, decreases the local expression of CXCL10 and CXCL13 and reduces the clinical symptoms of EAE in affected mice [93]. Surprisingly, disease attenuation in this model is due to the blockade of LT β R rather than HVEM [108]. However, neither the treatment with LT β R-Ig nor the loss of LT α prevents EAE in a model that uses pertussis toxin as an adjuvant [43,108]. CXCL13^{-/-} mice also have reduced clinical symptoms of EAE [106], which may be partly due to poor lymphocyte priming, since CXCL13^{-/-} mice lack numerous LNs and have poorly organized secondary lymphoid organs [30]. However, CXCL13 also appears to be important for the effector phase of the disease as the adoptive transfer of effector cells from normal mice to CXCL13^{-/-} recipients results in attenuated disease [106]. Thus, the maintenance and function of tertiary lymphoid tissues via the LT/homeostatic chemokine axis appears to be a part of the disease process in EAE and multiple sclerosis.

Homeostatic chemokines and molecules in the LT signaling pathway also correlate with the development of ectopic lymphoid tissues in numerous other autoimmune diseases. For example, CXCL13 and LT β are highly expressed in the B cell follicles that develop in the stomachs of mice that have autoimmune gastritis [109]. The number and size of the cell clusters correlate with the age of the mice and the serum autoantibody titer [109]. LT $\alpha\beta$, CCL21, CXCL12 and CXCL13 are increased in thyroids from autoimmune patients [88], and CXCL12 and CXCL13 are significantly higher in autoimmune glands with ectopic secondary lymphoid follicles than in those without follicles [50,88,89]. However, despite clear demonstrations that tertiary lymphoid follicles are immunologically active in patients with these diseases, it is difficult to determine whether the formation of ectopic follicles leads to disease or whether autoimmunity promotes the development of ectopic follicles.

7. Infection often elicits the formation of ectopic lymphoid follicles

The development of ectopic lymphoid tissues probably evolved as a response to local infections, as numerous infectious agents trigger the development of tertiary lymphoid tissues in a variety of non-lymphoid organs (Table 2). For example, infection of the liver triggers the formation of ectopic lymphoid tissue in the portal area known as Portal tract-Associated Lymphoid Tissue or PALT [110–112]. Liver biopsies from patients with chronic hepatitis C infection exhibit PALT, with B cell follicles, FDCs and germinal centers surrounded by a T cell zone [113]. Germinal center B cells in the ectopic follicles of hepatitis infected patients exhibit antigen-driven oligoclonal expansions [114], which are associated with extrahepatic manifestations of hepatitis [112] and a high level of viral replication [113]. Infection with bacteria, such as *Propionibacterium acnes*, also promotes the development of PALT [111]. Interestingly, the granulomas that surround the bacteria and PALT are spatially separated. Inflammatory chemokines, such as MIP1 α , are expressed in granulomas, whereas homeostatic chemokines, like CCL21 are expressed in PALT [115]. Anti-CCL21 antibody reduces the PALT expansion while exacerbating granuloma formation [115], suggesting that ectopic

lymphoid follicles may result from the accumulation of dendritic cells that are activated locally and congregate at sites of lymphatic drainage [116].

Infection with *Helicobacter pylori* also triggers the development of tertiary lymphoid tissues in the stomach. CXCL13 is highly expressed in these areas, possibly by FDCs [117]. In contrast, CCL21 is expressed on endothelial cells in and around ectopic follicles [117]. PNAd is also expressed on HEVs, which increase in number during the course of disease. Lymphoid aggregates in gastric biopsies are nearly always associated with chronic active gastritis and concurrent *Helicobacter pylori* infection [118,119]. Moreover, eradication of *Helicobacter pylori* is associated with the disappearance of lymphoid follicles and HEV-like vessels in the gastric mucosa [120]. Interestingly, HSP60 is often expressed on FDCs in gastric MALT lymphoma tissues [121] and auto-antibodies that react with the HSP60 antigen are often increased in MALT lymphomas that result from *Helicobacter pylori* infection [122], suggesting that MALT lymphoma may be driven by antigen receptor stimulation.

In another example, infection with *Borrelia burgdorferi* often leads to Lyme arthritis [123], which like rheumatoid arthritis, is associated with the formation of tertiary lymphoid tissues in the joints. Although bacteria are sometimes visible surrounding the ectopic follicles, they are relatively few in number [123], and synovial inflammation may persist after the apparent eradication of the infection using antibiotics [124]. Interestingly, ectopic follicles in synovial biopsies from Lyme arthritis patients exhibit clonal clusters of isotype-switched B cells that have sequentially accumulated mutations in their V regions, suggesting antigen driven selection [125]. In fact, many of these B cells recognize cytokeratin 10, which is present in the synovial endothelium [126]. Furthermore, synovial B cells recognize both cytokeratin 10 and surface protein A of *Borrelia burgdorferi* [126]. Similar antigen driven B cell expansions are seen in the skin of *Borrelia burgdorferi*-infected patients, who often develop lymphocytoma cutis [127,128]. Thus, as with *Helicobacter pylori* infection, the formation of ectopic follicles in response to *Borrelia* infection is associated with both autoimmunity and malignant transformation of B cells.

The lung is also a common site for the formation of tertiary lymphoid tissue, which is sometimes known as inducible Bronchus Associated Lymphoid Tissue (iBALT). iBALT forms in patients with a wide variety of diseases, including chronic obstructive pulmonary disease (COPD) [129,130], AIDS [131–133], pulmonary fibrosis [79], hypersensitivity pneumonitis [82] and even lung cancer [82] (Table 2). However, iBALT formation is mostly linked with chronic or acute lung infection and the involvement of opportunistic infectious agents cannot be ruled out in many of the cases listed above. For example, infection of the lung with *Mycobacterium tuberculosis* promotes the development of iBALT in areas surrounding granuloma formation [134,135]. Germinal centers are also observed in the lungs of *Mycobacterium tuberculosis*-infected mice [136], suggesting that B cells are responding to local antigens. Consistent with the idea that B cells play some role in local resistance to infection, B cell-deficient mice exhibit exacerbated immunopathology and enhanced susceptibility to *Mycobacterium tuberculosis* [136].

iBALT formation is also triggered by acute infection with influenza [4,77,100,137] or murine γ -herpesvirus-68 in mice (Figure 1). In these cases, iBALT consists of B cell follicles with germinal centers and FDCs surrounded or interspersed with T cell areas that have CD11c⁺ DCs, PNAd-expressing HEVs and lymphatic vessels [77,100]. Like other ectopic lymphoid tissues, iBALT appears to participate in lymphocyte priming [100], recruitment of effector cells and maintenance of local memory cells [137]. Surprisingly, mice that lack all conventional lymphoid organs, but retain iBALT, survive higher inoculating doses of influenza than normal mice [100]. Thus, iBALT promotes local protective immunity in a way that reduces immunopathology.

As in many tertiary lymphoid organs, CXCL13 is expressed on reticular cells in the B cell follicles of iBALT and CCL21 is expressed on HEVs that co-express PNAd [77,100]. Both CXCL13 and CCL21 are inducibly expressed in the lung after infection and interestingly, the influenza-induced expression of these chemokines is independent of both LT α and TNF [100]. Despite the LT α -independence of CXCL13 and CCL21 expression, iBALT is not organized properly in either LT $\alpha^{-/-}$ or LT β R $^{-/-}$ mice [11]. This may be because the differentiation of HEVs or stromal cells remains dependent on LT α or that the expression of homeostatic chemokines is controlled differently in the lung during acute infection than it is during iBALT homeostasis after infection is resolved. Determining what controls chemokine expression during acute infection is probably one key to understanding how the formation of tertiary lymphoid tissues is initiated.

Despite the clear evidence that CXCL13 is highly expressed in iBALT as well as in spontaneously occurring ectopic lymphoid follicles in other tissues [77,100], CXCL13 is not necessary for the formation or the function of ectopic follicles in the lung [77]. For example, the basic structure of iBALT, with separated B and T cell areas, germinal centers and HEVs still forms in CXCL13 $^{-/-}$ mice after influenza infection. The only apparent defect in these mice is that the B cell areas of iBALT do not form true follicles - the B cells are loosely aggregated and CD21 $^{+}$ FDCs are not observed [77]. However, these areas are functional and promote the normal expansion of influenza-specific B and T cells and lead to the normal production of influenza-specific IgG [77]. In contrast, the CCR7 ligands, CCL19 and CCL21 are very important for the formation and function of iBALT [77]. Both B and T cell responses are impaired in *plt/plt* mice, which lack CCL19 and CCL21, even though B cell follicle formation in iBALT appears intact [77]. These defects are even more pronounced in mice triply deficient in CXCL13, CCL19 and CCL21. Thus, the expression of homeostatic chemokines in pulmonary ectopic lymphoid tissues is important for both their development and function.

LT α is also important for the function of iBALT. LT $\alpha^{-/-}$ mice exhibit impaired immune responses to influenza [138], particularly when splenectomized [100]. Although CXCL13 and CCL21 are expressed in the lungs of these mice and B and T cells accumulate in the lungs, organized follicles are not formed, FDCs do not appear, PNAd $^{+}$ HEVs do not develop and the number of dendritic cells in the lungs is reduced. Nevertheless, T and B cell responses to influenza and murine γ -herpesvirus-68 can eventually be generated in these mice - albeit after a significant delay [138,139]. These data argue that the organization of ectopic lymphoid tissues makes local immune responses more efficient, but is not absolutely necessary. Even so, LT is important for more than just the organization of lymphoid tissues (reviewed in [43,140], including the homeostatic proliferation and survival of dendritic cells [69,141] and T cell priming [69]. Thus, impaired immune responses in the absence of LT are likely due to multiple factors, in addition to poor tertiary lymphoid organ development.

8. Some mucosal lymphoid tissues have properties of both conventional secondary lymphoid organs and tertiary lymphoid tissues

The studies cited above demonstrate that tertiary lymphoid tissues can form in numerous sites in a wide variety of non-lymphoid organs given the appropriate stimulation by pathogens or autoantigens. In contrast, LNs and Peyer's patches form at predetermined sites in the absence of stimulation via pathogens or antigens. However, there are other types of lymphoid tissues that are formed at predetermined locations, but require some level of microbial stimulation for their development. For example, isolated lymphoid follicles (ILFs) are small clusters of lymphocytes that can be observed throughout the length of the anti-mesenteric wall of the mouse small intestine [142]. They are composed of a large B cell area, usually including a germinal center, and contain relatively few T cells. These tissues are clearly mucosal in nature, as the epithelium overlying the follicles contains M cells and the B cells within the follicles

often switch to IgA [143]. Like conventional lymphoid organs, ILFs are absent in $LT\alpha^{-/-}$ and *aly/aly* mice [142]. However, they reappear in adults after in utero treatment with LT β R-Ig, suggesting that their development is similar to that of Nasal Associated Lymphoid Tissue (NALT), which develops in adult $LT\alpha^{-/-}$ mice that have been reconstituted with normal bone marrow [74]. Although the maintenance of ILFs absolutely requires LT α and signaling through the LT β R and TNFR1 [144], mice that were treated in utero with LT β R-Ig and allowed to recover as adults have enlarged ILFs [145]. This is similar to the exaggerated formation of iBALT in mice that lack conventional lymphoid organs [77,100]. On the other hand, treatment of adult mice with LT β R-Ig abolishes ILFs, even when the mice are immunized [146]. These results suggest that, like the formation of ectopic lymphoid follicles, the organogenesis of ILFs is not restricted to embryonic or neonatal development, even though LT signaling is important for maintaining the architecture of these tissues.

Another similarity of ILFs with ectopic lymphoid tissues is that germ free mice develop very few and atrophied ILF [144]. Other investigators find that ILFs (also known as Solitary Intestinal Lymphoid Tissue - SILT) are present in normal numbers in germfree mice, but are very small and immature and lack identifiable B cell follicles [147,148]. Moreover, c-kit⁺ cells outnumber B220⁺ cells in the ILFs of germfree mice. However, ILFs return to normal upon restoration of intestinal flora [144]. Importantly, infection with the enteric pathogen, *Salmonella enterica*, triggers the formation of inflammatory foci in the ILFs and promotes ILF maturation, but does not increase the number of ILFs [149]. These data argue that the location and number of ILFs is determined developmentally and cannot be altered by inflammation or infection. However, the maturation and organization of ILFs is dependent on microbial stimuli and can even be enhanced by infection with pathogenic bacteria.

Given that B cells are the predominant cell type in mature ILFs, it makes sense that they also play an important role in the development and maturation of ILFs. Interestingly, although LT α is required on B cells for the maturation of ILFs [150], B cells do not have to be antigen specific. In fact, B cells expressing a lysozyme-specific antigen receptor were incorporated into ILFs and expressed high levels of LT on their surface [150]. These B cells also induced high levels of CXCL13 in the ILFs [150]. In contrast, T cell derived LT is not necessary for ILF formation [150]. Consistent with the idea that mucosal phenotype B cells are important for ILF differentiation and function, both immature and mature ILFs are abolished in $CCR6^{-/-}$ mice [151]. When B cells are selectively deficient in CCR6, only a few immature ILFs are formed and no mature ILFs develop [151]. Finally, both ILFs and Peyer's patches are hyperplastic in $AID^{-/-}$ mice [152] due to the inability of $AID^{-/-}$ B cells to terminally differentiate [153]. Together, these data argue that although B cells do not require BCR signaling to promote ILF development, B cell stimulation does lead to ILF maturation.

9. Relationship between the formation of tertiary lymphoid tissues and tumor progression and regression

Given the apparent immune function of ectopic lymphoid tissues in response to infectious disease, it seems that the development of functional tertiary lymphoid tissues surrounding tumors could be beneficial for tumor antigen recognition and tumor regression. In fact, tumor cell lines that are transfected with LT α promote the formation of tertiary lymphoid tissues, with B cell follicles, dendritic cells, HEVs and FDCs surrounding the tumors [154]. Interestingly, these LT α -expressing tumor lines still promote the development of HEVs and FDCs in the absence of B and T cells, suggesting that LT α by itself is directly promoting the differentiation of these cells. CD4⁺CD3⁻ cells also infiltrate the tumor site, although it is unclear whether these cells are LTi cells or dendritic cells, or even whether they are required for the development of tertiary lymphoid tissue [154]. The engagement of the LT signaling pathway using an anti-tumor antibody fused to LT α also elicits the formation of tertiary lymphoid tissues surrounding

the tumor [155] and promotes the activation and differentiation of tumor specific T cells - ultimately leading to tumor regression. Importantly, immune activation in this model occurs locally at the tumor site, as it still occurs in the absence of conventional LNs [156]. Thus, the development of tertiary lymphoid organs surrounding established tumors is clearly beneficial under some circumstances.

Activation of the LT signaling pathway is not the only way to achieve the formation of ectopic follicles and local T cell activation, since the local administration of CCL21-expressing dendritic cells also results in tumor-specific T cell activation and differentiation as well as the inhibition of tumor growth [157,158]. Targeting LIGHT to tumors also leads to tumor regression. For example, the inoculation of a LIGHT-expressing adenovirus directly into tumors elicits tumor-specific CD8 T cell responses and promotes tumor regression [159]. Although CCL21 expression in the tumor environment is increased by LIGHT signaling [159], immune activation seems to correlate more with direct T cell activation and recruitment, rather than the development of ectopic lymphoid tissues [160]. Consistent with this idea, the inoculation of primary tumors with LIGHT adenovirus also leads to the regression of metastases at distal sites [160]. Similar results are found using an attenuated strain of *Salmonella typhimurium* that expresses LIGHT [161], and an agonistic anti-LT β R monoclonal antibody [162], although the role of ectopic lymphoid tissues is not directly addressed in these experiments.

Given that both LIGHT and LT $\alpha\beta$ bind to the LT β R [163] and that LIGHT can functionally replace LT α in some circumstances [42], it makes sense that these two molecules might exert similar anti-tumor effects. However, LIGHT also binds another receptor, HVEM [163]. LIGHT is expressed by dendritic cells and is important for T cell costimulation [164] and normal CD8 T cell responses to antigen [44,165]. Furthermore, HVEM signaling, rather than LT β R signaling, seems to be most important for T cell costimulation [166]. In part, this is due to the ability of HVEM signals to prevent apoptosis in T cells [166]. In contrast, LT β R signaling on DCs is important for T cell costimulation via dendritic cell activation [69]. Thus, LIGHT and LT $\alpha\beta$ may promote local anti-tumor responses through mechanisms that are partly overlapping and partly distinct.

In addition to the therapeutically induced development of ectopic lymphoid tissues at sites of tumor growth, there are also examples of spontaneous ectopic follicles forming around some, but not the majority of tumors [82] (Figure 2). However, it is not clear whether these tissues are beneficial or promote tumor regression. For example, tertiary lymphoid tissues that develop around tumors could be responding to tumor antigens and inflammation and providing a beneficial effect. Alternatively, they could be developing in response to the local expression of homeostatic cytokines or chemokines and helping to promote tolerance to tumor antigens. In addition, there are examples in which the formation of ectopic follicles is actually associated with tumor development. For example, gastric carcinoma and MALT lymphoma develop at sites of *Helicobacter pylori* infection in the gastric mucosa of humans [167–169]. Similar effects are seen in the skin of *Borrelia burgdorferi*-infected patients, who often develop lymphocytoma cutis [127,128]. Although the malignant transformation of B cells may be driven by antigen receptor stimulation in some cases [121,122], the stromal cell microenvironment of ectopic and even conventional lymphoid tissues also plays an important role in the maintenance of some tumors [170]. These stromal cells seem to depend on TNF and LT $\alpha\beta$ to differentiate and to support malignant B-cell survival [170]. As a result, the development of ectopic lymphoid tissue surrounding some tumors may promote tumorigenesis rather than facilitate anti-tumor immunity.

10. The role of Tregs in the formation of ectopic lymphoid follicles

Given that T regulatory cells (Tregs) are reduced in numbers or functionally impaired in many autoimmune diseases [171–175], it is not surprising that they might play a role in either the formation or maintenance of ectopic lymphoid tissues. However, it is difficult to distinguish the role of Tregs in the prevention of autoimmunity from their role in tertiary lymphoid organ development. For example, the impaired homing of Tregs to LNs is at least partly responsible for the formation of iBALT in *CCR7^{-/-}* mice [176]. *CCR7^{-/-}* mice have very few Tregs in their LNs and also develop iBALT shortly after birth [176]. Furthermore, the development of iBALT is prevented by the adoptive transfer of *CCR7^{+/+}* Tregs to *CCR7^{-/-}* recipients. Surprisingly, *CCR7^{-/-}* mice actually have higher numbers of Tregs in peripheral tissues, including the lung, suggesting that it is the activity of Tregs in the LNs rather than in the lungs that prevents iBALT formation [176].

If poor Treg activity or low Treg numbers results in the formation of ectopic lymphoid tissues, then we should see evidence of this in other autoimmune systems. However, NOD mice have normal numbers of Tregs [177] and still develop ectopic lymphoid tissues in the pancreas and salivary glands [90] as well as the lung [178]. Conversely, if the loss of *CCR7* leads to reduced Treg activity, then *CCR7* mice should manifest some aspects of autoimmunity. Although this has not been explicitly reported, it is clear that *CCR7^{-/-}* mice have impaired tolerance induction to oral and inhaled antigens [179–181]. This is due in part to impaired migration of dendritic cells from the site of antigen deposition to the draining LNs [179,180] and may or may not have anything to do with the impaired Treg migration to the LNs.

Interestingly, other studies find that *plt/plt* mice, which are unable to express the ligands for *CCR7* – CCL19 and CCL21 [182,183], do not spontaneously form iBALT [77]. Moreover, while these mice do form iBALT after influenza infection, the iBALT is not as functional and exhibits impaired germinal center formation and poor T cell activation [77]. This makes sense in the light of the expression of CCL21 on HEVs [77] and the expression of CCL19 in the T cell areas of ectopic lymphoid tissues. Interestingly, the number of Tregs in the lungs of influenza-infected *plt/plt* mice is dramatically increased and seems to play a role in dampening immune responsiveness to influenza (Rangel-Moreno unpublished). Thus, *CCR7* signaling appears to play multiple and contradictory roles in the formation and function of ectopic lymphoid tissues.

11. Lymphocyte recirculation and the acquisition of antigen in ectopic follicles

CCR7 also plays a critical role in lymphocyte recirculation through secondary lymphoid organs [184] as well as tertiary lymphoid tissues. For example, PNAd-expressing HEVs that coexpress CCL21 are often observed in tertiary lymphoid tissues [49,77], suggesting that *CCR7* and L-selectin may be the primary means that lymphocytes use to gain entry to these tissues from the blood. In fact, CCL21 is shown to trigger integrin-dependent adhesion of T cells to venules and to promote extravasation in tertiary lymphoid tissues [185]. Moreover, HEVs in areas of iBALT express relatively high levels of VCAM-1 and PNAd and lymphocyte homing to iBALT is dependent on L-selectin/PNAd, $\alpha 4\beta 1$ /VCAM and LFA-1 pathways [178]. On the other hand, entry of lymphocytes to ectopic lymphoid tissue in the thyroid is independent of L-selectin, even though it requires *CCR7* [49]. These data point out that homing to ectopic lymphoid tissues does not follow the same rules as homing to conventional secondary lymphoid organs and needs to be further explored.

CCR7 is also important for the recovery of central memory cells from peripheral non-lymphoid tissues [186,187]. Although effector and memory T cells are attracted to non-lymphoid organs

and sites of inflammation via inflammatory chemokines, those that express CCR7 can return to the circulation by entering lymphatic vessels, which often express CCL21 [186,187]. In the absence of CCL21 or CCR7, these cells are unable to return and may simply pile up in peripheral tissues. If enough T and B cells are stuck in these sites, they may begin to spontaneously form organized lymphoid tissues. This possibility is proposed by some investigators to explain the development of ectopic lymphoid tissues in CCR7^{-/-} mice [188]. Although there are numerous immune abnormalities, such as altered Treg function or incipient autoimmunity in CCR7^{-/-} mice, the poor recovery of activated B and T cells from peripheral non-lymphoid organs may play an important role in the development of ectopic lymphoid tissues in these animals.

A similar mechanism is proposed to explain the formation of tertiary lymphoid tissues at sites of inflammation or infection in normal individuals. In this model, infection or inflammation leads to rerouting of the lymphatic drainage towards newly formed tertiary lymphoid tissues [116]. As a result, the developing lymphoid tissue is maintained by the continuous influx of antigen and APCs, which leads to sustained local immune activation. Although there is no definitive evidence to support this model, it brings up the question of how tertiary lymphoid tissues acquire antigen. In cases where ectopic follicles are formed in response to autoantigens, such as in diabetes or rheumatoid arthritis, antigens could be expressed directly in the follicle - perhaps by dendritic cells. However, in cases where ectopic follicles are formed in response to infection, it is less clear how antigen may be acquired. When ectopic lymphoid tissues form around sites of infection, such as in granulomas, antigen may be present directly at the site of lymphoid tissue formation. However, there is often a spatial distinction between the granulomas that surround the bacteria and the tertiary lymphoid tissues [111,134,135,189]. In *Propionibacterium acnes* infection of the liver, dendritic cell precursors first migrate to the granuloma in response to MIP1 α , where they mature and acquire antigen, and then migrate to the portal area in response to CCL21 [111,189]. This implies that there must either be lymphatic drainage between the granuloma and PALT or that dendritic cells directly migrate through the tissue. In addition, areas of iBALT in the lung seem to respond to exogenous inhaled antigens [190,191], suggesting that there is a mechanism to get antigen from the airways, across the respiratory epithelium and into iBALT. This may involve M cells [192], although the majority of iBALT areas seem to lack a true dome epithelium [4]. Thus, it is possible that tertiary lymphoid tissues such as iBALT develop afferent lymphatics that bring in antigens and dendritic cells from distal parts of the tissues. Lymphatic vessels surrounding the B cell areas of ectopic lymphoid tissues are found in both mouse and human tissues [50,82]. These lymphatics surround the B cell follicle and co-localize with the T cell area [50,82] (Figure 3). However, it is not clear whether these are afferent lymphatics that would bring antigens into the follicle or whether they are efferent lymphatics that would drain away from the follicle to downstream LNs. Understanding the lymphatic drainage of tertiary lymphoid tissues is another important issue that needs to be resolved.

12. Concluding remarks

The development of ectopic or tertiary lymphoid tissues at sites of inflammation or infection is an important part of local immune responses. Depending on the type of immune responses, the development of these tissues can lead to detrimental or pathological immune responses (autoimmunity) or may help resolve local infection. In this respect, one might view ectopic lymphoid tissues much like other local lymphoid tissues that serve to initiate and amplify immune responses. However, there are hints that ectopic follicles may be controlling or imprinting particular characteristics on locally generated immune responses independently of the activities of conventional lymphoid organs. This means that it is even more important to understand the cellular and molecular mechanisms responsible for ectopic follicle development

and to determine how these follicles function in both pathologic and protective immune responses.

Acknowledgements

This work was supported by the Trudeau Institute, NIH grants HL69409 and AI072689 and by the Sandler Program for Asthma Research.

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Figure 1. Ectopic lymphoid tissue develops in the lungs of mice after viral infection

Mice were infected with murine γ -herpesvirus-68 and allowed to clear the lytic phase of the infection. A. Although the majority of the inflammatory response in the lung has resolved, areas of pulmonary lymphoid tissue remain next to major airways. B. The boxed area in panel A contains B220⁺ B cell areas (red), and CD3⁺ T cell areas (green). C. A magnification of the B cell areas shows infiltrating T cells. D. In a serial section of panel B, it is clear that some of the B cell follicles in iBALT (green) have CD21⁺ FDCs (red).

Figure 2. Ectopic lymphoid tissues spontaneously develop next to some tumors

A. This panel shows an H&E stained section of a lung biopsy from a patient with bronchioalveolar carcinoma. Numerous ectopic follicles (blue) can be observed. B and C. Some of the B cells in ectopic follicles express Proliferating Cell Nuclear Antigen (PCNA), suggesting that they are activated B cells that could contribute to germinal centers.

Figure 3. Lymphangiogenesis and chemokine expression in ectopic lymphoid tissue

Lungs from naïve mice (A) or mice that had recovered from influenza infection (B) were probed with antibodies to B cells (red) or lymphatic vessels (green). C. CCL21 (red) is expressed on vascular endothelium surrounding B cell follicles (green) in iBALT. D. CXCL13 (red) is expressed in a reticular pattern in the B cell follicle (green) of iBALT.

Table 1

Development of ectopic lymphoid follicles in inflammatory diseases.

Disease	Tissues affected	Species	References
Arthritis	Joints, Lung	Human	[82,83,96,98,103,104]
Sjogren's syndrome	Lung, Salivary gland	Human	[82,87]
Diabetes	Pancreas	Human, Mouse	[45,91]
Wegener's granulomatosis	Lung	Human	[193]
Multiple sclerosis/EAE	CNS	Human, Mouse	[94,95,105,107]
Thyroiditis	Thyroid	Human, Mouse	[88,89,101]
Hypersensitivity Pneumonitis	Lung	Human	[190,191]
COPD	lung	Human, Mouse	[129,130,194]
Autoimmune gastritis	Gut	Human	[109]
Idiopathic pulmonary fibrosis	Lung	Human	[195]
Atherosclerosis	Arteries	Human	[196,197]
Inflammatory bowel disease	Gut	Human	[98,185,198,199]

Table 2

Development of ectopic lymphoid tissues in infectious diseases.

Infectious Disease	Tissues affected	Species	References
Influenza	Lung	Mouse	[77,100,137]
HIV and complications	Lung, Vagina, Brain	Human, Monkey	[131–133,200]
Hepatitis C	Liver	Human	[114,201–203]
<i>Helicobacter pylori</i>	Stomach	Human, Mouse, Pig	[110,204–207]
<i>Propionibacterium acnes</i>	Liver	Mouse	[111]
<i>Mycobacterium tuberculosis</i>	Lung	Mouse, human	[134–136,208]
<i>Borrelia burgdorferi</i>	Skin	Human	[127,209,210]
<i>Klebsiella pneumoniae</i>	Lung	Human	[190]
<i>Pseudomonas aeruginosa</i>	Lung	Human	[190]
<i>Haemophilus influenzae</i>	Lung	Human	[190]
Other infection	Lung	Human	[211,212]