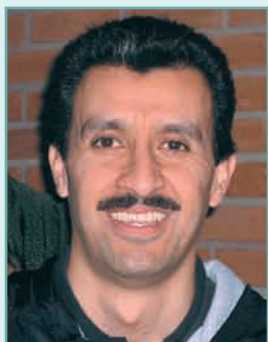


Lymphotoxin (LT) was originally described as a soluble factor that induces the cytolysis of fibroblast targets in the absence of antigen, similar to tumor necrosis factor (TNF)(1,2). The functional similarity between LT and TNF is not coincidental as LT and TNF are members of the structurally and genetically related TNF family of cytokines (3). Both LT and TNF are expressed as soluble homotrimers (4) and signal through the TNFR1 (p55) as well as TNFR2 (p75)(5). However,



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LT is also expressed on the cell surface as a membrane bound heterotrimer composed of LT $\alpha$  and LT $\beta$  subunits (6). Unlike soluble LT $\alpha$  or TNF, membrane-bound LT $\alpha\beta$  signals through the LT $\beta$ R (7). Thus, while LT and TNF are structurally related and have overlapping receptor specificity and biological activities (8), LT and TNF play distinct roles in the immune system.

### ● *Lymphotoxin signaling and lymph node development.*

The identification of a receptor specific for membrane-bound LT $\alpha\beta$  demonstrated that LT had the ability to mediate biological effects distinct from those mediated by TNF. However, the differences between LT and TNF were not entirely appreciated until the generation of LT $\alpha^{-/-}$  mice. Unlike TNF $^{-/-}$  mice, LT $\alpha^{-/-}$  mice lack Peyer's patches and most lymph nodes (LNs), except for rudimentary mesenteric LNs that occasionally appear in some mice (9,10). Since LN development is also aberrant in LT $\beta^{-/-}$  mice as well as LT $\beta$ R $^{-/-}$  mice (11,12), but not in mice deficient in TNFR1 and TNFR2 (13,14), it is clear that the surface form of LT $\alpha\beta$  must be primarily responsible for the induction of LN development. However, since LT $\beta^{-/-}$  mice retain

cervical and mesenteric LNs (11), the soluble form of LT $\alpha$  must contribute to LN formation, either by signaling through a receptor other than TNFR1 or TNFR2, or by synergizing with surface LT $\alpha\beta$ . Furthermore, since all LNs are absent from LT $\beta$ R $^{-/-}$  mice (12), it is likely that another TNF family member, such as LIGHT (15,16), acts through LT $\beta$ R to facilitate the develop-



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ment of cervical and mesenteric LNs.

In fact, TNF, LT $\alpha$ , LT $\beta$  and LIGHT all contribute to LN development. For example, although mice treated in utero with soluble LT $\beta$ R (sLT $\beta$ R) retain mesenteric and cervical LNs (17,18), mice treated in utero with both sLT $\beta$ R and sTNFR1 are unable to develop these LNs (19). Furthermore, mice treated with sLT $\beta$ R and anti-TNF also lack all LNs (19), indicating that TNF signal-

ing through TNFR1 plays some role in mesenteric LN development. As discussed above, LIGHT also contributes to mesenteric LN development. Although both LIGHT $^{-/-}$  and LT $\beta^{-/-}$  mice have mesenteric LNs (16), a portion of LIGHT-LT $\beta^{-/-}$  mice lack mesenteric LN (16), suggesting that LIGHT and LT $\beta$  cooperate to induce mesenteric LN formation. Furthermore, since all LT $\beta$ R $^{-/-}$  mice lack mesenteric LN (12), these results also suggest that there may be another ligand for the LT $\beta$ R that facilitates mesenteric LN development. Thus, although LT $\alpha\beta$  signaling through the LT $\beta$ R is the major pathway through which LN development is initiated, other TNF family members can contribute to LN development as well.

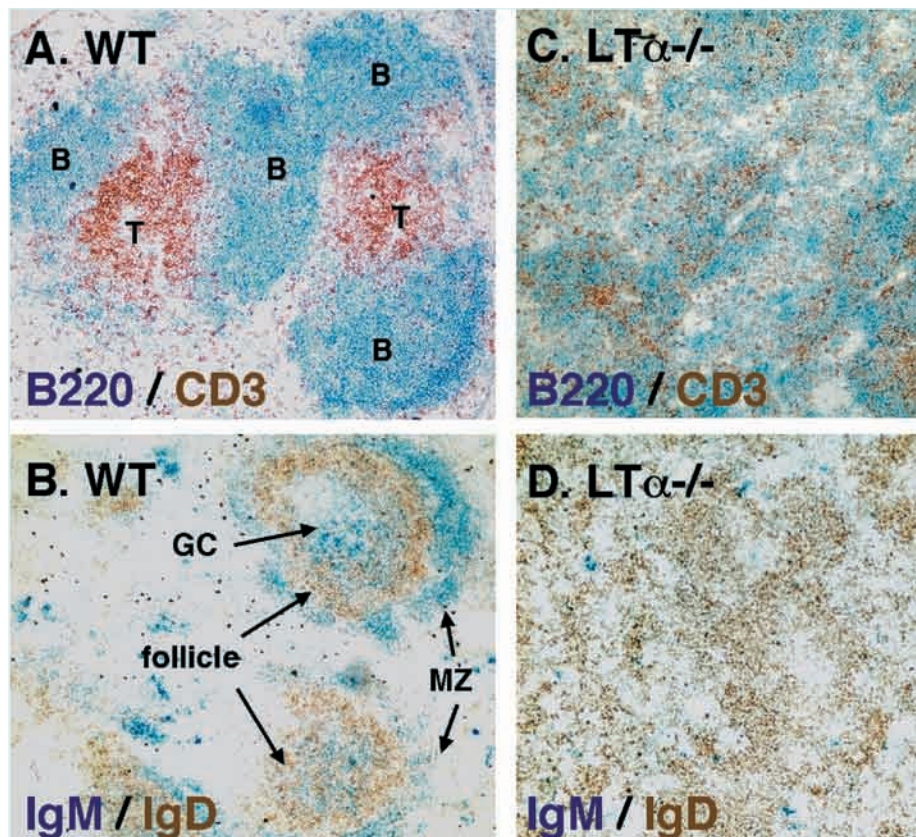
Most TNFR family members are coupled to the NF $\kappa$ B signaling pathway (20) and not surprisingly, some molecules important for these pathways are involved in LT $\beta$ R signaling and lymphoid organogenesis. For example, LT $\beta$ R ligation induces the canonical pathway of NF $\kappa$ B activation that involves

IKK $\beta$  and IKK $\gamma$ /NEMO and leads to the phosphorylation of I $\kappa$ B and the translocation of p50/RelA or p52/RelA



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complexes to the nucleus (21). In turn, these complexes control the expression of inflammatory genes such as VCAM-



**Figure 1: Lymphotoxin is necessary for the organization of lymphocytes in lymphoid tissues.** A Organized B cell follicles (blue) and T cell areas (brown) are found in sections of WT spleens. B B cells are organized into germinal centers (GC), marginal zones (MZ) and follicles in the spleens of WT mice. C The B cells (blue) and T cells (brown) are disorganized in the spleens of  $LT\alpha^{-/-}$  mice. D Germinal centers, marginal zones and follicles are absent in the spleens of  $LT\alpha^{-/-}$  mice.

1, MIP1 $\beta$  and MIP2 (21). This pathway also promotes an increased production of the NF- $\kappa$ B2/p100 precursor (21). However, LT $\beta$ R signaling also induces a second pathway that leads to the sequential activation of NIK and IKK $\alpha$  and leads to the processing of p100 to p52 (21-23). In association with RelB, p52 translocates to the nucleus and activates transcription of molecules involved in secondary lymphoid organogenesis and homeostasis (21), such as the homeostatic chemokines SLC and BLC. It appears that both pathways are important in lymphoid organ development, as mice homozygous for a natural mutation in NIK (alymphoplasia or *aly/aly* mice) (24,25) as well as TNFR1-RelA $^{-/-}$  mice lack LNs and Peyer's patches (26). Furthermore, RelB is essential for Peyer's patch organogenesis and aspects of lymphoid architecture in the spleen (27-29). These results partly explain why LT $\beta$ R and NIK signaling pathways are essential for lymphoid organ development and also explain why signaling through TNFR1 can facilitate LN development under some circumstances.

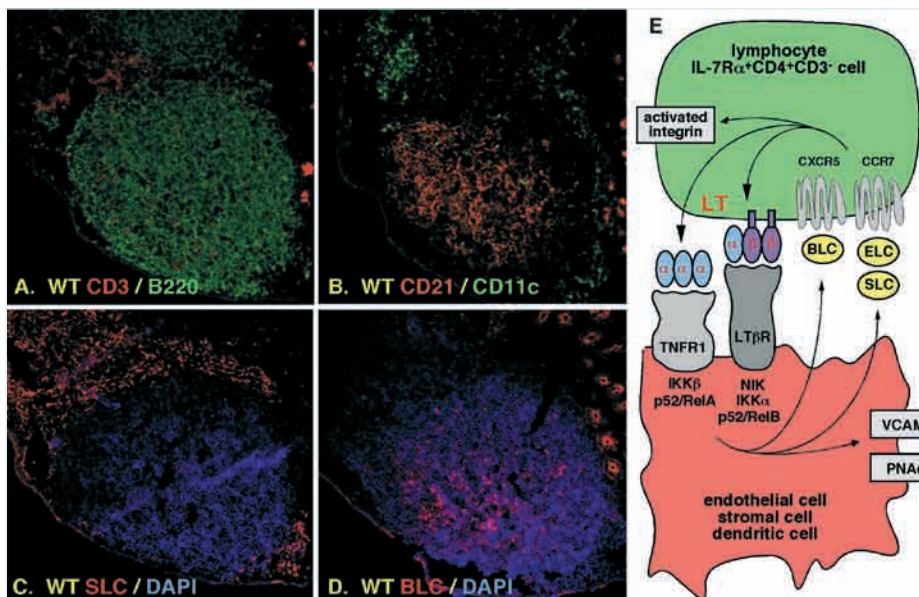
#### ● Cellular interactions in lymphoid organ development.

The cellular interactions that initiate lymphoid organ development have also been characterized, particularly with regards to Peyer's patch development. The earliest visible step in Peyer's patch organogenesis is the appearance of clusters of VCAM and ICAM expressing mesenchymal cells on the antimesenteric side of the small intestine around day E15 of embryonic development (30). The expression of VCAM and ICAM on mesenchymal cell clusters is induced by IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells (31), which express surface LT $\alpha\beta$  (32) and provide a signal to LT $\beta$ R bearing mesenchymal cells (33). IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells also express the chemokine receptor, CXCR5 (33,34), as well as the integrins,  $\alpha$ 4 $\beta$ 7 and  $\alpha$ 4 $\beta$ 1 (34,35), which respectively bind the chemokine, BLC, and the homing molecules, MAdCAM and VCAM. Since BLC and MAdCAM are locally expressed in the Peyer's patch organizing center (33), these molecules likely promote the recruitment of IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells. Although IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells and VCAM $^{+}$ ICAM $^{+}$  mesenchymal cells are initially arranged in homog-

enous clusters, they begin to segregate into follicular structure around day E18 (36). At this time, CD11c $^{+}$  cells become apparent in the developing Peyer's patches and are also segregated into follicular structures (36). Interestingly, the IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells can differentiate into NK as well as CD11c $^{+}$  DCs (32,35), suggesting that these precursors are differentiating in situ. Importantly, the induction of the Peyer's patch organizing center and the initial follicular formation occurs independently of B and T cells (36). Once follicular formation is initiated, however, B and T lymphocytes begin to populate the developing Peyer's patch around birth and are segregated into the appropriate locations within the Peyer's patch. Similar events are thought to take place during LN development (35).

The pivotal role of IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells in lymphoid organ development was demonstrated in mice deficient for the transcription factor, Id2 (37), and in mice deficient for the nuclear hormone receptor, ROR $\gamma$  (38). Both Id2 $^{-/-}$  mice and ROR $\gamma^{-/-}$  mice fail to develop IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells and subsequently lack LNs and Peyer's patches (37,38). Furthermore, the injection of purified IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells from normal mice into neonatal CXCR5 $^{-/-}$  recipients confirmed that these cells could induce the development of Peyer's patches (34). Similarly, the injection of IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells into neonatal Id2 $^{-/-}$  mice induces the development of Nasal Associated Lymphoid Tissue (NALT) (39). Thus, IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells appear to be essential for lymphoid organ development.

Although Peyer's patch organogenesis serves as an instructive model for the organogenesis of all lymphoid organs, the molecular requirements for the development of Peyer's patches and other lymphoid organs are not identical. For example, isolated lymphoid follicles (ILF) (40) as well as colonic patches (41) are structurally similar to Peyer's patches. However, treatment of mice in utero with sLT $\beta$ R or anti-IL-7R blocks Peyer's patch development, but not the development of ILF (40). In contrast, treatment with sLT $\beta$ R in utero completely blocked the formation of colonic patches (41), even after immunization. Interestingly, ILF are absent from LT $\alpha^{-/-}$  and *aly/aly* mice (40) suggesting that LT signaling is



**Figure 2:** The localized expression of BLC, SLC and ELC organizes lymphocytes within lymphoid organs. **A** In WT Peyer's patches B cells (green) are located in follicles, while T cells (red) are located in the interfollicular regions. **B** In serial sections of WT Peyer's patches, follicular dendritic cells (red) are located in the B cell areas, while interdigitating dendritic cells (green) are found in the T cell areas. **C** SLC is found primarily in the T cell areas on stromal cells and on HEVs, but not in the B cell area. **D** In contrast, BLC is produced by stromal cells such as FDCs within the B cell follicles. **E** The expression of these chemokines is controlled by a positive feedback loop involving lymphotoxin. Mature lymphocytes as well as the IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells that initiate lymph node development during embryogenesis both express lymphotoxin, which signals through TNFR1 or LTβR expressed on stromal cells and some populations of dendritic cells. These receptors trigger different aspects of the NFκB signaling cascade, which culminate in the expression of homeostatic chemokines as well as homing molecules, such as VCAM and PNAc. In turn, the chemokines attract receptor-bearing lymphocytes, induce integrin activation and trigger the increased production of lymphotoxin.

as LNs and Peyer's patches. However, it is unclear why the presence of IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells is necessary for the development of NALT when LT signaling is not necessary for NALT development. Furthermore, RORγ<sup>-/-</sup> mice also lack IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells as well as LNs and Peyer's patches, but retain NALT (47). Although it is not clear why NALT development requires IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells in Id2<sup>-/-</sup> mice but not in RORγ<sup>-/-</sup> mice, we do know that Id2<sup>-/-</sup> mice lack NK cells as well as multiple populations of dendritic cells (37,48-50). Thus, these additional cell types may contribute to NALT development in RORγ<sup>-/-</sup> mice in the absence of IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells. Regardless, the unusual aspects of NALT development clearly indicate that the model of Peyer's patch organogenesis cannot be directly applied to the development of all lymphoid organs.

#### ● Role of LT in homeostatic chemokine expression and lymphoid architecture.

In addition to LN development, LT expression is required for proper lymphoid architecture in the spleen as well as the appropriate homing and segregation of B and T cells within secondary lymphoid organs (9,10). Thus, the spleens of LTα<sup>-/-</sup> mice lack organized B and T cell areas (9,10), marginal zones (10,51) and germinal centers (51,52). In addition, follicular dendritic cells (FDCs) are absent from mice deficient in LT signaling (53-55), and the numbers of dendritic cells (56) and gp38-expressing stromal cells (57) are reduced in the spleens of LTα<sup>-/-</sup> mice. Furthermore, the expression of the addressins, PNAc and MAdCAM, is dependent on LT signaling (19,58). Interestingly, soluble LTα and membrane bound LTαβ are responsible for different aspects of lymphoid architecture. For example, the defects in B and T cell separation are less severe in the spleens of LTβ<sup>-/-</sup> (11) than they are in the spleens of LTα<sup>-/-</sup> mice (10) and LTβR<sup>-/-</sup> mice (12), suggesting that LTα signaling through TNFR1 as well as LTαβ signaling through LTβR is important for lymphocyte organization. In contrast, the presence of DCs in the spleen as well as the expression of MAdCAM and PNAc on high endothelial venules (HEVs) and in marginal zone sinuses is controlled by surface LTαβ and the LTβR but not by TNF, LTα or TNFR1 (18,56). Finally,

important for their formation, possibly after embryonic development. Thus, colonic patches and Peyer's patches appear to have similar requirements for their development, while ILFs utilize different mechanisms for their formation. Furthermore, although IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells are thought to initiate the development of both Peyer's patches and LNs (42), the requirement for molecules in the IL-7 signaling pathway (IL-7, IL-7Rα, IL-2Rγ and JAK3) is most evident in Peyer's patch development and not in LN development (43). In contrast, LN development absolutely requires the activity of the TNF family member TRANCE and its associated signaling molecules (TRAF6 and RANK) (44-46), while Peyer's patch and NALT development is TRANCE-independent (44,47). Since both TRANCE and IL-7 trigger the proliferation of IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells and induce the expression of LTαβ on their surface (32,43,44), the differential requirement for the TRANCE and IL-7 in LN and Peyer's patch development suggests that TRANCE is exclusively expressed at sites of LN

development, while IL-7 is exclusively expressed in the developing Peyer's patch. Thus, even though specific aspects of lymphoid organ development differ depending on the location of a particular lymphoid organ, the overall developmental pathway of most lymphoid organs is similar.

Interestingly, unlike the development of LNs and Peyer's patches, the development of NALT is not dependent on the LT signaling pathway (39,47). NALT is present in LTα<sup>-/-</sup>, LTβ<sup>-/-</sup>, TNF-LTα<sup>-/-</sup>, LTβR<sup>-/-</sup> and TNFR1<sup>-/-</sup> mice (39,47), demonstrating that neither TNF nor LT signaling are required for NALT development. However, the structure of NALT is severely compromised in LTα<sup>-/-</sup>, TNF-LTα<sup>-/-</sup> and LTβR<sup>-/-</sup> mice (47), demonstrating that LT signaling is important for the proper architecture of NALT. In addition, *aly/aly* mice also have rudimentary NALT structures (39), even though these animals do not have other LNs or Peyer's patches (25). In contrast, NALT is absent from Id2<sup>-/-</sup> mice (37,39), consistent with the role of IL-7Rα<sup>+</sup>CD4<sup>+</sup>CD3<sup>-</sup> cells in the development of NALT as well

the differentiation of FDCs requires TNF, LT $\alpha$  and LT $\beta$  (53,55,59,60). Thus, various aspects of lymphoid architecture are controlled by the overlapping, yet distinct, activities of soluble LT $\alpha$ , membrane LT $\alpha\beta$  and TNF.

Unlike the defects in LN organogenesis, which are developmental in nature and cannot be reversed upon restoration of LT expressing cells to LT $\alpha^{-/-}$  mice, many of the defects in splenic architecture can be reversed upon reconstitution of LT $\alpha^{-/-}$  mice with normal bone marrow (BM) (51,53,55,59). Conversely, the reconstitution of normal mice with LT $\alpha^{-/-}$  cells (61,62), or the blockade of LT signaling *in vivo* by treating mice with soluble LT $\beta$ R (17,18), results in the loss of lymphoid organization in the spleen. An exception to this general rule is the differentiation of gp38-expressing stromal cells of the spleen, since the reconstitution of adult LT $\alpha^{-/-}$  mice with normal BM does not restore normal numbers of this population (57). Therefore, the differentiation of these cells is fixed during development. In fact, the development of these cells requires LT $\alpha$  signaling during late gestation and within the first few days after birth (57). Thus, while the formation of LNs and gp38-expressing stromal cells is dependent on the expression of LT during a discreet developmental window, the maintenance of most lymphoid organization in the spleen is dependent on the constitutive expression of LT.

LT does not directly control the organization of lymphocytes within lymphoid tissues. Instead, LT signaling induces the expression of several chemokines, including BLC (CXCL13), SLC (CCL21) and ELC (CCL19) (63). These "homeostatic" chemokines control the steady state recruitment and positioning of naïve B and T cells as well as activated APCs in lymphoid organs (64-66). Expression of BLC, SLC and ELC is regulated in a positive feedback loop by LT. Thus, LT induces the expression of BLC, SLC and ELC by stromal and dendritic cells (63, 67). In turn, BLC, SLC and ELC recruit lymphocytes and induce their increased expression of LT. This positive feedback loop controls not only splenic architecture, but regulates LN development as well (67). In fact, the phenotypes of mice lacking BLC (67) or its receptor (CXCR5) (68) are similar to that of

LT $\beta^{-/-}$  mice (disrupted splenic architecture and loss of some LNs). These ideas are dramatically illustrated in transgenic mice that express either LT $\alpha$  or BLC in the pancreas (69-72). These transgenic mice develop ectopic LN like structures in the pancreas, complete with T cell areas, B cell follicles, FDCs and HEVs expressing lymph node homing receptors (69-72).

Again, the role of chemokine expression in the development of lymphoid organs is best characterized in Peyer's patch development. Low levels of BLC are expressed in Peyer's patch anlage during development (33). This low level expression attracts CXCR5-bearing IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells to the Peyer's patch anlage and activates  $\alpha 4\beta 1$  integrin in order to facilitate adhesion to the local stroma (34). Once IL-7R $\alpha^{+}$ CD4 $^{+}$ CD3 $^{-}$  cells have arrived at the future sites of Peyer's patch genesis, they provide surface LT $\alpha\beta$  (32), which induces the expression of higher levels of chemokines as well as adhesion molecules on the mesenchymal cells (33,63,73). These newly expressed molecules then recruit lymphocytes, which provide a sustained source of surface LT and induce the differentiation of FDCs (53). Thus, the interplay of LT, TNF and the homeostatic chemokines induces and maintains LNs and Peyer's patches.

### ● Role of Lymphotoxin in humoral immune responses.

Given the absence of lymph nodes and Peyer's patches in LT $\alpha^{-/-}$  mice as well as the lack of FDCs, organized B cell follicles and germinal centers in their spleens, it is not surprising that mice deficient in LT signaling generally make poor humoral immune responses (9,11,12,58,74,75). These animals have defects in isotype switching (59) affinity maturation (12,52,76), generation of B cell memory (77) and antibody production to both T dependent (9,11,12) and T independent type II antigens (74). Most of the defects in B cell responsiveness can be reversed by the transplantation of normal BM into LT $\alpha^{-/-}$  mice (51,53,55,59). To illustrate this, the reconstitution of LT $\alpha^{-/-}$  mice or LT $\alpha$ -TNF $^{-/-}$  mice with normal BM restores the ability of B cells to form germinal centers in the spleen, isotype switch in response to T dependent antigens and become memory cells with high affinity antigen receptors (51,53,55,59,77,78).

Since the restoration of humoral responsiveness correlates with restored lymphoid architecture within the spleen (51,53,55,59,77,78), many investigators suggested that humoral immune responses are dependent on the normal structure of lymphoid organs. However, LT and normal lymphoid architecture are not essential for all antibody responses. In fact, IgM responses are relatively normal in LT $\alpha^{-/-}$  mice (78) and in some cases are actually enhanced relative to those in WT mice (79). This suggests that a major defect in LT $\alpha^{-/-}$  mice is the inability to isotype switch rather than an inability of B cells to differentiate into antibody secreting plasma cells. Furthermore, depending on the type of antigen/adjuvant used, IgG responses in LT $\beta$ R $^{-/-}$  mice can be nearly normal (12). Thus, normal lymphoid architecture in the spleen is not always necessary for robust antibody responses. Likewise, these data indicate that antibody responses are not always dependent on the presence of LNs or Peyer's patches.

Interestingly, LT $\alpha^{-/-}$  mice also have reduced levels of secretory IgA in the gut (80,81). To test whether the lack of secretory IgA in LT $\alpha^{-/-}$  mice was due to absence of Peyer's patches, mice were treated with sLT $\beta$ R in utero, which blocks the development of Peyer's patches, but does not alter the development or organization of spleen and mesenteric LNs (82). Despite the lack of Peyer's patches, these mice produced normal levels of IgA in the gut (82). Furthermore, LT $\alpha^{-/-}$  mice reconstituted with WT cells produced normal levels of secretory IgA, despite their complete lack of LNs and Peyer's patches (80). Conversely, IgA production in the gut is normal in WT mice reconstituted with LT $\alpha^{-/-}$  BM, demonstrating that LT $\alpha$  itself is not necessary to induce isotype switching to IgA (80). Finally, experiments in which a section of small intestine from RAG $^{-/-}$  mice was transplanted into LT $\alpha^{-/-}$  mice elegantly demonstrated that IgA production in the gut was dependent on the LT $\alpha$ -induced differentiation of non-hematopoietic cells in the lamina propria (80). In turn, these cells produce SLC and BLC, which recruit B cells to the lamina propria. Once there, the newly recruited B cells differentiate into IgA secreting cells *in situ* in the absence of LN and Peyer's patches (83). Thus, LT signaling promotes antibody responses in the

gut as well as the spleen by inducing the local expression of chemokines that recruit B cells to an environment suitable for their differentiation and antibody secretion. It is not yet clear whether the ILFs identified in anti-mesenteric side of the intestine are the sites at which IgA responses develop (40).

### ● Role of lymphotoxin in cellular immune responses.

In addition to defects in B cell responses, mice deficient in components of the LT signaling pathway are susceptible to a variety of intracellular bacteria, fungi and parasites. For example, TNF- $LT\alpha^{-/-}$  mice are more susceptible to *C. albicans* (84-86), *C. neoformans* (87), *L. major* (88), *E. multilocularis* (89) and *M. bovis* BCG (90, 91). In addition, LT-deficient mice are more susceptible to a number of viral infections. For example, *aly/aly* mice do not make detectable primary CD8 responses to vesicular stomatitis virus (VSV) or lymphocytic choriomeningitis virus (LCMV) and make a reduced CD8 response to vaccinia virus (VV)(75, 92). Similarly,  $LT\alpha^{-/-}$  mice make severely impaired cellular immune responses to LCMV (93) and cannot clear herpes simplex virus (HSV)(94). However,  $LT\alpha^{-/-}$  mice can clear murine gammaherpes virus 68 (MHV-68)(95) and influenza (79), albeit with delayed kinetics. Likewise,  $LT\alpha$ -TNF $^{-/-}$  mice can clear VV and LCMV after a significant delay (58). These last results demonstrate that  $LT\alpha^{-/-}$  mice can mount protective antiviral CD8 responses, but that the process is inefficient.

The impaired immunity of mice lacking functional LT could be due to the absence of LNs, the lack of homeostatic chemokine expression, poor APC/macrophage activation, the inability to prime T cells or some combination of all of these factors. Since  $LT\alpha^{-/-}$  T cells are not thought to have intrinsic defects in activation (10,96), many of the studies discussed above attributed delayed or absent T cell responses to inefficient T cell priming due lack of lymph nodes and a functional spleen in mice deficient in LT signaling. In contrast, the susceptibility of TNF- $LT\alpha^{-/-}$  mice to intracellular bacteria, fungi and parasites is primarily attributed to an inability to properly recruit and activate macrophages and neutrophils. To determine which aspects of LT signaling are important

for resistance to infectious agents, investigators have again used reciprocal BM chimeras to independently explore the roles of LT and LNs in cellular immune responses. For example, although  $LT\alpha^{-/-}$  and  $LT\beta^{-/-}$  mice exhibit impaired CD8 T cell responses to LCMV (93,96), immune responses and viral clearance are restored in  $LT\alpha^{-/-}$  and  $LT\beta^{-/-}$  mice that have been reconstituted with normal BM (93,96). Similar experiments demonstrated that the transfer of normal BM to  $LT\alpha^{-/-}$  recipients restored their ability to make normal B and T cell responses to influenza (47). In fact, numerous studies show that the reconstitution of  $LT\alpha^{-/-}$  mice with normal bone marrow restores the ability of these animals to make normal immune responses (47,51,59,62,80,93). These results suggest that LNs are not obligatory for protective immune responses, and that an organized spleen, albeit with reduced T cell areas (57), is sufficient to generate immunity in reconstituted  $LT\alpha^{-/-}$  mice.

The opposite approach was used to characterize the roles of  $LT\alpha$  and  $LT\beta$  in the response to *M. tuberculosis* (97). In this study, investigators transferred either  $LT\alpha^{-/-}$  or  $LT\beta^{-/-}$  hematopoietic cells into RAG $^{-/-}$  hosts. Interestingly, although a comparable T cell response was observed in all animals, the mice that had received  $LT\alpha^{-/-}$  cells were susceptible to disease, while mice receiving normal cells or  $LT\beta^{-/-}$  cells were resistant to *M. tuberculosis* (97). The mice receiving  $LT\alpha^{-/-}$  cells generated abnormal granulomas with few activated macrophages (97). Thus,  $LT\alpha$  expression was necessary for resistance, despite the presence of LNs. However, it is not yet clear whether the inability of  $LT\alpha^{-/-}$  cells to clear mycobacteria is due to lack of chemokine expression and poor cell recruitment to the granuloma or the inability to appropriately activate macrophages (97).

Unlike the studies described above, the response to some infectious agents is absolutely dependent on LNs. For example,  $LT\beta^{-/-}$  mice are susceptible to leishmaniasis and die within 8 to 14 weeks of infection due to delayed and reduced T and B cell responses (88). However, normal mice reconstituted with  $LT\beta^{-/-}$  cells were able to control the infection and survive (88), indicating that  $LT\beta$  is not necessary for resistance. In contrast,  $LT\beta^{-/-}$  mice

reconstituted with WT cells exhibited signs of disease and died after 6-9 weeks, despite the normal expression of LT (88). Similar types of BM chimeras were used to demonstrate that contact hypersensitivity to the hapten DNFB is also dependent on the presence of LNs and independent of LT expression (98). Thus, the presence of LNs and not LT is essential for these immune responses. Furthermore, the spleen was unable to compensate for the lack of LNs in these immune responses, despite the presence of normal hematopoietic cells.

### ● $LT\alpha$ mediated pathology.

Although the expression of LT is essential for the clearance of some pathogens, its expression can also lead to pathology or even autoimmunity. For example, although TNF-  $LT\alpha^{-/-}$  mice are more susceptible to *S. aureus* infection, they also do not acquire *S. aureus*-induced arthritis (99). Likewise, the loss of TNF and LT has been shown to reduce the severity of experimental autoimmune encephalomyelitis (EAE)(100-102). One study suggested that  $LT\alpha$  (but not  $LT\beta$ ) played a direct role in EAE (100), while other studies showed that TNF was more important than LT (103). These reports were later reconciled by a study showing that TNF was primarily responsible for inducing pathology and disease, while  $LT\alpha$  was responsible for inducing LN development, which provided an environment for efficient autoimmune T cell priming (102). Thus, the activities of TNF and LT can contribute to pathology in different ways.

LT has also been shown to play a role in the development of autoimmune diabetes by inducing the formation of lymphoid tissues in the pancreas that promote the recruitment and activation of lymphocytes (104,105). Moreover, studies with  $LT\alpha$  transgenic mice have shown that the induction of homeostatic chemokine expression in the pancreas is an important component of LT-mediated pathology (72). In fact, the induction of homeostatic chemokine expression and lymphoid neogenesis has been demonstrated in a variety of chronically inflamed non-lymphoid tissues and is thought to contribute to autoimmune and inflammatory diseases, including rheumatoid arthritis (106,107), Hashimoto's thyroiditis (108), myasthenia gravis (109,110) and *H. pylori* induced gastritis (111). For instance, Sjogren's

syndrome is an autoimmune disease characterized by the presence of inflammatory infiltrates that form ectopic organized lymphoid tissues within the salivary glands. It is now believed that the local production of BLC in the salivary gland plays an important role in the maintenance of the chronic inflammatory process (112). BLC has also been detected in *H. pylori* induced gastric mucosa-associated lymphoid tissue (111) and in dendritic cells from aged BWF1 mice suffering from murine systemic lupus erythematosus (113). In another study, SLC was expressed in the liver of mice infected with *P. acnes* and was shown to be responsible for granuloma formation in the portal tract-associated lymphoid tissue (PALT) (114). Although the link between LT expression, chemokine expression and disease has not been directly demonstrated in all the examples above, new mouse models are being developed, in which the role of LT in disease can be distinguished from the roles of LNs and chemokines (67,115,116).

In summary, LT is involved in immune responses to infectious disease at multiple levels. At the most basic, LT is instrumental for the development of LNs and Peyer's patches, tissues in which primary immune responses are initiated. Furthermore, LT is necessary for the expression of chemokines, which organize lymphoid organs. LT is also necessary for the differentiation of FDCs and gp38 stromal cells of the spleen. Finally, LT acts as an effector molecule during the acute stages of disease similarly to TNF, by activating macrophages and providing anti-viral and anti-microbial resistance to multiple TNFR1-expressing cell types. Thus,

the role of LT in infectious disease is surprisingly complex. We expect that future studies will further define the interrelated roles of LT, homeostatic chemokines and lymphoid organs in resistance to infectious disease.

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