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Lymphoid tissue inducer — a divergent member of the ILC family

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Abstract

Innate lymphoid cells (ILCs) that are capable of producing effector cytokines reminiscent of CD4⁺ T helper (Th) cells during infections and tissue inflammations have drawn much attention in the immunology field in recent years. Within the ILCs, the lymphoid tissue inducer (LTi) cells that play a critical role in lymphoid organogenesis were identified long before the establishment of the ILC concept. LTi cells, developed and functioning mainly at the fetal stage, and LTi-like cells, presumably generated during the adulthood, are regarded as a subset of type 3 ILCs (ILC3s) because they express the ILC3 lineage-defining transcription factor ROR γ t, and like other ILC3s, can produce an ILC3 signature cytokine IL-22 and initiate protective immune responses against extracellular bacteria. However, LTi/LTi-like cells have a unique gene expression pattern, and they develop from a progenitor that is distinct from the progenitor of all other ILCs and the progenitor of conventional natural killer (cNK) cells. There are also several other unique features of LTi/LTi-like cells comparing to non-LTi ILC3s. In addition to their classical function in lymphoid organogenesis, LTi/LTi-like cells also have specialized functions in association with the adaptive immune system, which include their effects on T and B cell development, activation and function. In this review, we summarize these specific features of LTi/LTi-like cells and propose that these cells should be considered as a separated innate lymphoid lineage in parallel with other non-LTi ILCs and cNK cells.

Graphical Abstract

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Conflicts of interest

The authors declare no competing financial interests.

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1. Introduction

Our knowledge on the innate immune system has greatly expanded in the past few years due to the identification and defining of several new innate lymphoid populations, now known as innate lymphoid cells (ILCs)¹. These new members of the innate immune system are featured by their functional similarity to CD4⁺ T helper (Th) cells in the adaptive immune system². Based on their lineage-defining transcription factor (also called master regulator) expression and signature cytokine production, mature ILCs can be divided into three major subsets (ILC1s, ILC2s and ILC3s) mirroring distinct CD4⁺ T effector cells (Th1, Th2 and Th17 cells). ILC2s express the Th2 cell master regulator GATA-3, and secrete IL-5 and IL-13, but low levels of IL-4^{3, 4, 5, 6}. ILC3s express the Th17/Th22 cell master regulator RORγt and their effector cytokines, IL-22, IL-17A, and IL-17F^{7, 8, 9}.

The defining of ILC1s was established later than other ILC subsets. The conventional natural killer (cNK) cells had been considered as an ILC1 subset¹, however, the most recent view in the field prefers to classify cNK cells as the innate counterpart of cytotoxic CD8⁺ T cells since they both exhibit cytolytic activities and express transcription factor Eomes^{2, 10}. Up to date, several non-NK ILC1s residing in various tissues have been discovered by different research groups^{11, 12, 13}. Both ILC1s and cNK cells express the Th1 master regulator T-bet and several NK cell surface markers such as NK1.1 and NKp46 in C57BL/6 mice. However, Eomes expression may distinguish cNK cells from ILC1s¹³. Similar to Th1 cells, ILC1s do not express Eomes, but they can secrete effector cytokine IFN-γ and TNF-α upon stimulation by IL-12, IL-15, and/or IL-18^{11, 12, 13}.

All these ILC subsets and cNK cells constitutively express an important transcriptional regulator, inhibitor of DNA-binding protein 2 (Id2), which is also required for an overall fate determination of innate lymphoid cell lineage through antagonizing the functions of E-box proteins^{13, 14, 15}. Recent studies have also found that these terminally developed ILC subsets may retain certain plasticity to convert to each other^{16, 17, 18}, a phenomenon that has been well described for differentiated CD4⁺ T helper cell subsets^{19, 20, 21}.

Compared to ILC1s and ILC2s, ILC3s are much more complicated since they can be further divided into two distinct lineages, lymphoid tissue inducer (LTi) or LTi-like cells, and the

remaining ILC3s some of which express natural cytotoxicity receptors (NCRs)²². LTi cells, named after their function in organogenesis of secondary lymphoid structures at fetal stage, are the ILC population that was first discovered^{23, 24, 25, 26, 27, 28}. In the adult stage, there is also a group of cells that are phenotypically similar to LTi cells but unable to facilitate the generation of secondary lymphoid organs^{29, 30, 31, 32}. Thus, they are referred to as LTi-like cells. LTi and LTi-like cells are similar in their gene expression profiles, except that LTi-like cells express the T cell survival molecules OX40L^{33, 34, 35} and CD30L³⁶ (Figure 1). In mice, LTi cells or LTi-like cells can be distinguished from other ILC3s by their expression of a chemokine receptor, CCR6²². A proportion of LTi/LTi-like cells can also express CD4, and these cells are considered as the most mature LTi cells. However, a gene expression comparison did not reveal substantial differences between the CD4⁺ and CD4⁻ LTi-like cells³⁷.

The CCR6⁻ ILC3s belong to another lineage²²; some mouse CCR6⁻ ILC3s express Nkp46⁷. The development of Nkp46-expressing ILC3s depends on the master regulator Tbet^{22, 38, 39}. Some of these Nkp46⁺ ILC3s can further convert into ILC1s after losing RORγt expression^{16, 22, 40}. In human, however, it is still unclear how to distinguish LTi/LTi-like cells from the NCR⁺ ILC3s since all the human RORγt⁺ ILC3s reported so far express CCR6⁴¹. ILC3s in human are divided into different subsets mainly based on CD56 and Nkp44 expression^{41, 42, 43, 44}. The CD56⁻ human ILC3s, regardless of Nkp44 expression, are most likely the counterpart of the CCR6⁺ mouse LTi/LTi-like cells⁴¹. Effector cytokine production is also somewhat different between human and mouse ILC3 subsets. While both ILC3 lineages in mice are capable of producing IL-22, the IL-22 production is restricted to the Nkp44⁺ populations, CD56⁺Nkp44⁺ ILC3s in particular, in human⁴². Thus, the human ILC3s may have a greater diversity. In this review, we will discuss LTi/LTi-like cells mainly based on mice studies, if not specifically mentioned.

Although LTi/LTi-like cells express the ILC3 master regulator RORγt²⁸ and effector cytokines IL-22, IL-17A and IL-17F⁹, their development is distinct from all other ILCs and cNK cells^{45, 46, 47}, indicating that this population is a divergent member of the ILC family. Here, we summarize the current knowledge on LTi/LTi-like cells with respect to their characterization, development, and particular functions hoping to introduce a novel view on the innate lymphoid system.

2. Developmental difference between LTi cells and other ILCs

LTi cells were discovered much earlier than other ILCs²³. Thus, how they developed had already been studied through in *vitro* and in *vivo* analyses even before the establishment of ILC concept^{24, 48, 49, 50, 51, 52, 53}. However, only after the discovery of several common innate lymphoid cell progenitors^{45, 46}, we started to have a comprehensive understanding of the innate lymphoid lineages generation including the development of LTi cells. The major argument for LTi/LTi-like cells being divergent from other ILCs is that they develop through distinct pathways.

The development of all innate lymphocytes is affected by deficiency of certain transcriptional regulators such as Id2, GATA-3⁴⁷, TCF-1^{54, 55, 56}, TOX^{57, 58}, and Nfil-3^{59, 60},

indicating that a common ILC progenitor may exist. Indeed, an Id2-expressing common helper-like innate lymphoid progenitor (ChILP) population was discovered; these cells are able to generate all ILC subsets including LTi cells⁴⁶. However, ChILP cells are not a homogeneous population as some of them can transiently express PLZF and these cells can generate all other ILCs except for LTi cells⁴⁵. Although a unique progenitor for LTi-like cells in adult bone marrow has not been identified, it is clear that LTi progenitors in fetal liver express ROR γ t and integrin $\alpha_4\beta_7$ ⁶¹. A single cell gene expression analysis of LTi progenitors and PLZF⁺ ILC progenitors in the fetal liver confirmed that these were two distinct lineages with different transcriptional regulatory network⁶² (Figure 2). Thus, although in adult stage, LTi-like cells and NCR⁺ ILC3s show a great similarity and redundancy at their immune effector functions⁶³, their development is quite different.

Even after development, some ILC subsets still exhibit certain degree of plasticity under some circumstances. For example, the NCR⁺ ILC3s can further develop into ROR γ t[−] ILC1s⁴⁶, and conversely, some ILC1s are able to convert back to the NCR⁺ ILC3s¹⁶. In patient with chronic obstructive pulmonary disease (COPD), the ILC2s in inflamed lungs can convert into ILC1s which may contribute to the pathogenesis of the disease^{17, 18}. However, the lineage conversion between LTi cells and other ILCs has not been reported (Figure 2). This is probably because the “boundary” between LTi lineage and other ILC lineages is much harder to cross than the “boundary” between non-LTi ILCs.

3. Molecular features of LTi/LTi-like cells

Although LTi cells and NCR⁺ ILC3s show great redundancy during immune responses, they also exhibit remarkable differences in gene expression and cellular function. Using a T-bet and ROR γ t double reporter mouse strain, we have isolated highly pure CCR6⁺T-bet[−] LTi/LTi-like cells and T-bet⁺NKp46⁺ ILC3s, and compared their gene expression differences through RNA-Seq analysis⁶⁴. The result has revealed several classes of genes that are differently expressed by these two lineages. The genes that highly enriched in LTi/LTi-like cells include transcription regulators such as Egr2, Id1 and Id3, cytokines, chemokines and growth factors such as IL-17F, CCL21, Egf17, BMP-2, antigen presenting molecules such as CD83 and H2-Oa, and other surface molecules such as CCR6, CXCR5, DLL1, Ly6C, Nrp-1, PD-1, and S1PR1⁶⁴. Many of these LTi/LTi-like cell-specific genes have already been reported to be associated with the unique functions of LTi cells and the rest of genes may also indicate other special functions of LTi/LTi-like cells which need further investigation.

As discussed above, the majority of LTi/LTi-like cells express CCR6, which can distinguish these cells from other ROR γ t⁺ ILC3s in mice²². CCL20, the chemokine ligand for CCR6, is constitutively expressed in the gut. Although *Ccr6* deficiency does not alter the development and expansion of LTi cells in the gut, it seems to affect the production of effector cytokine IL-22. However, two studies from different labs using *Ccr6*-deficient mice showed opposite results. Sawa *et al.* observed an increase in IL-22 production by LTi cells in *Ccr6*-deficient mice⁶⁵, while Lin and colleagues reported a decreased IL-22 production by *Ccr6*^{−/−} ILC3s⁶⁶. It is not clear why this contradiction occurs. One possibility is that the housing conditions of the animal facilities may affect IL-22 production by LTi cells.

Besides CCR6, there are also certain functionally related molecules uniquely expressed by LTi cells. The neurotrophic factor receptor RET is a tyrosine kinase receptor that is expressed mainly by the nervous system, kidney and hematopoietic progenitors to sense the environmental cues such as glial-derived neurotrophic factor (GDNF) family ligands (GFL)⁶⁷. Interestingly, ILC3s in gut lamina propria also express RET⁶⁸. We and others found that RET⁺ cells were highly enriched in the LTi/LTi-like population but not in the NCR⁺ ILC3s. In response to the neurotrophic factors from glial cells in the gut, the RET⁺ ILC3s produce more IL-22 through the p38 MAPK-ERK AKT cascade and STAT3 activation to control the activity of epithelial cells. Thus, RET expression on ILC3s forms a glial-ILC3-epithelial cell axis to orchestrate the homeostasis of the gut⁶⁸.

4. The role of LTi/LTi-like cells in lymphoid structure generation

The naming of LTi cells is because of their critical role in secondary lymphoid organogenesis during the fetal stage⁶⁹. In the fetal liver, all innate lymphocyte progenitors including that for LTi cells express integrin $\alpha_4\beta_7$ ^{46, 70, 71}, which allows migration of LTi cells towards the lymph nodes or the Peyer's patch anlagen through interacting with MAdCAM-1 expressed by high endothelial venules (HEVs)^{72, 73}. At the sites of lymph node or the Peyer's patch anlagen, a group of specialized stromal cells called lymphoid tissue organizer (LTo) cells^{50, 74} produce chemokine CXCL13, which recruits/attracts LTi cells that selectively express its receptor CXCR5^{50, 75, 76}, resulting in the formation of an initial hematopoietic cell cluster. LTi cells then start to express lymphotoxin $\alpha_1\beta_2$ (LT $\alpha_1\beta_2$), which will engage with its receptor LT β R on stromal cells^{48, 77, 78, 79}. The signals through LT β R activate the classical and an alternative NF- κ B signaling in stromal cells, leading to their further maturation and the expression of several genes related with secondary lymphoid structure organization such as TNF-related activation-induced cytokine (TRANCE)⁴⁹, homeostatic chemokine CCL19, CCL21, and CXCL13⁸⁰, as well as adhesion molecules MAdCAM-1, VCAM-1, and ICAM-1^{81, 82}. The IL-7R and TRANCE feedback on LTi cells to enhance their LT $\alpha_1\beta_2$ expression, and thus sustained the activation of stromal cells⁴⁸. The homeostatic chemokines recruit more lymphocytes including LTi cells and can also function in maintaining the LT $\alpha_1\beta_2$ expression on LTi cells^{80, 81, 83}. The adhesion molecules will also attract/retain LTi cells to expand the initial cluster. Once B and T cells start to be recruited to the developing lymph nodes or Peyer's patches, they will take over the role of LTi cells in expressing LT $\alpha_1\beta_2$ (Figure 3). The importance of the molecules mentioned above during this process has been demonstrated by the causative impairment in lymphoid structure generation in mice with deficiency in these molecules^{49, 52, 75, 84, 85}.

5. LTi/LTi-like cells in association with T cells

5.1 Effect of thymic LTi cells on T cell development

At the embryo stage, LTi cells appear in the thymus starting from E14 and sustain throughout the adulthood³¹. These thymic LTi cells are phenotypically similar to LTi cells in other lymphoid tissues. These LTi cells also express TRANCE as other LTi cells, which enables the interaction between LTi cells and epithelial cells in thymic medulla that express the TRANCE receptor, receptor activator of NF- κ B (RANK). The signaling initiated by

TRANCE/RNAK interaction promotes the expression of AIRE in medullary epithelial cells³¹, resulting in subsequent expression of self-tissue-restricted antigens that are required for negative selection during T cell development^{86, 87}. Thus, the thymic LTi cells are involved in regulating the central tolerance of T cells to self-antigens through its interaction with medullary epithelial cells in the thymus (Figure 4).

5.2 LTi effect on CD4⁺ T cell memory maintenance

The initial knowledge about the function of LTi-like cells in facilitating memory CD4⁺ T cell maintenance is from the observation that memory cells do not survive well in *Rorc*^{-/-} mice in which LTi-like cells are absent⁸⁸. Further investigation confirmed that LTi-like cells in lymphoid organs are sufficient to mediate the survival of CD4⁺ memory T cells but not for the CD8⁺ memory T cells⁸⁸. LTi-like cells in lymphoid organs locate at the sites of memory T cell recirculation. LTi-like cells in adult stage express two additional T cell survival molecules, OX40L and CD30L, comparing to LTi cells in fetal stage^{32, 89}. While the memory CD4⁺ T cells express their receptors OX40 and CD30 on the surface. Thus, the interaction between LTi and memory CD4⁺ T cells will transduce the survival signaling to T cells and facilitate the maintenance of the CD4⁺ T cell memory (Figure 4).

5.3 Suppression of commensal antigen specific T cells by LTi cells

Besides the involvement in the central tolerance establishment during T cell development, LTi/LTi-like cells were also found to suppress the commensal antigen-specific T effector cells to maintain the homeostasis of gut environment. This role of LTi/LTi-like cells was initially realized from the phenomenon that *Rorc*-deficient mice harbored more effector/memory-like CD4⁺ T cells as well as elevated levels of commensal bacteria-specific IgG in serum, indicating that the commensal bacteria are involved in this abnormal activation of CD4⁺ T cells⁹⁰. In consistent with this finding, antibiotic administration restores the percentage of effector/memory-like CD4⁺ T cells and commensal bacteria-specific IgG level in the *Rorc*-deficient mice to levels comparable to those in wild type mice. Further investigation indicates that expression of major histocompatibility complex-II (MHC-II) on LTi cells is required for limiting the effector/memory CD4⁺ T cell number, and conditional depletion of the MHC-II from ILC3s recapitulates the phenotype in the *Rorc*-deficient mice⁹¹. Similar as antigen presenting cells (APCs), MHC-II-expressing LTi/LTi-like cells are capable of presenting antigens to CD4⁺ T cells. However, unlike classical APCs, LTi/LTi-like cells do not express co-stimulatory molecules CD40, CD80 and CD86. Thus, such an antigen presentation by LTi/LTi-like cells in the absence of co-stimulation can induce T cell death and thus limit CD4⁺ T cell responses⁹⁰ (Figure 4). Since LTi cells are abundant in intestinal lamina propria, such tolerance mechanism is crucial in maintaining local gut homeostasis. Dysregulated MHC-II expression on LTi cells is found to be linked to patients with inflammatory bowel diseases such as pediatric Crohn's disease.

6. LTi/LTi-like cells and B cell responses

Increasing evidence indicates that ILC3s are involved in facilitating the antibody production by B cells in either a T cell-dependent or -independent manner^{92, 93}. Though it is not fully

clarified in these studies, from cell location or functional gene expression, these ILC3s are presumably LTi/LTi-like cells.

6.1 LTi/LTi-like cells facilitate systemic antibody production

The majority of ILC3s in lymphoid organs such as spleen and lymph nodes are CCR6⁺ LTi/LTi-like cells. Similar to the gut-resident LTi/LTi-like cells, the ILC3s in the lymphoid organs can also internalize antigens and present antigen peptides to CD4⁺ T cells, which triggering the T cell dependent antibody production. Different from the situation in the gut, the ILC3s in lymphoid organs start expressing co-stimulatory molecules CD80 and CD86 in the presence of IL-1 β , and producing cytokines such as IL-2, IFN- γ and TNF- α towards fully activate T cells⁹³ (Figure 4).

Besides, LTi/LTi-like cells can also promote T cell independent antibody generation against carbohydrate antigens⁹². LTi/LTi-like cells locate at the marginal zone and perifollicular zone of spleen and lymph nodes⁹⁴, and can activate the marginal reticular cells (MRC) through the production of TNF and lymphotoxin⁹⁴. They can also be induced to express BAFF, CD40L, DLL1 and APRIL to promote B cells to produce antibodies against T-independent antigens⁹². ILC3s in lymphoid organs can also indirectly help B cell responses by secreting GM-CSF which in turn acts on neutrophils to express BAFF and APRIL⁹² (Figure 4).

Furthermore, LTi/LTi-like cells may facilitate memory B cell responses owing to their role in promoting the T cell memory. The expression of OX40L and CD30L on the surface of LTi/LTi-like cells provides a survival signal to CD4⁺ T cells and thus enhances the generation of memory T cells including memory T follicular helper (Tfh) cells. As a result, in a secondary antibody response, B cells can respond and mature more quickly to generate high-affinity antibodies⁹⁵ (Figure 4).

6.2 LTi/LTi-like cells promote mucosal antibody responses

IgA produced by B cells in mucosal tissues such as the intestine mucosa is critical for controlling the composition of commensal bacteria. This IgA production happens in either a T cell-dependent manner mainly in Peyer's patches or a T cell-independent manner in the lamina propria or the isolated lymphoid follicles (ILFs) of the gut^{96, 97}. In the lamina propria, ILC3s secrete soluble LT α_3 which promotes the homing of CD40L-expressing T cells to the gut and thus induce IgA production by B cells.

As we mentioned earlier, LTi/LTi-like cells also express transmembrane LT $\alpha_1\beta_2$ as well as TNF. These molecules are capable of activating the scavenger cells (SCs) in the gut to release CXCL13, CCL19 and CCL20 that are important for recruiting B cells and dendritic cells⁹⁷. Then the interaction between dendritic cells and LTi/LTi-like cells, in addition to the stimulation of dendritic cells by microbial TLR ligands, will induce the generation of active TGF- β 1, which is required for the IgA production^{97, 98}. LTi/LTi-like cells can also promote SCs, dendritic cells and macrophages to produce BAFF through LT $\alpha_1\beta_2$, TRANCE, and LIGHT⁹⁷.

7. Concluding remarks

LTi cell population is the first identified innate lymphoid cells. They have been considered as a subgroup within the T helper-like ILC family due to their similarity to the NCR⁺ ILC3s in terms of their immune functions. However, several clues also indicate that LTi cells have their unique features that may have been overlooked. Obviously, LTi cells are associated with the secondary lymphoid organogenesis. Moreover, as we discussed above, LTi cells are also associated with the adaptive immune system in multiple aspects, including facilitating the central tolerance during the generation of T cells, repressing the activation of commensal antigen-specific CD4⁺ T effector cells in the gut, promoting the survival of CD4⁺ memory T cells, and supporting the production of T-dependent or -independent antibodies in lymphoid or mucosal tissues. Thus, regarding their roles in modulating adaptive immune system, it is possible that LTi cells and the generation of adaptive immune system during evolution may have a close relationship. All innate lymphocytes are supposed to originate from a common progenitor. Some recent studies indeed support this hypothesis. However, unlike the functional similarity between LTi and other ILC3s, the lineage development of LTi is nevertheless distinct from all other ILC subsets, as well as the cytotoxic conventional NK cells. Taking together, we should consider LTi cells as the third major group of innate lymphocytes, in parallel with cNK cells and T helper-like ILCs that are derived from the PLZF⁺ progenitors.

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Biographies

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Summary

1. Lymphoid tissue inducers (LTi cells) are developmentally distinct from other innate lymphoid cells (ILCs).
2. LTi cells have a unique transcriptome which is associated with their specialized functions.
3. LTi cells are associated with the adaptive immune system through multiple mechanisms.
4. LTi cells represent a third innate lymphocyte population which is in parallel with NK cells and other non-LTi ILCs.

Figure 1. Development and differences of fetal and adult LTi cells

In the fetal stage, LTi cells are developed from their progenitors in the fetal liver. LTi progenitor cells are featured by their high expression of integrin $\alpha_4\beta_7$, IL-7R α and ROR γ t. Other molecules such as CCR6, TRANCE and CXCR5 are also expressed by these progenitors. The progenitor cells then migrate to other organs such as fetal spleen, small intestine and thymus, where they further mature and start to express lymphotoxin $\alpha_1\beta_2$ (LT $\alpha_1\beta_2$) and IL-22. In the adult stage, LTi cells (or LTi-like cells) are developed from the progenitors in the bone marrow. These progenitors still express high levels of integrin $\alpha_4\beta_7$ and IL-7R α , but relatively low levels of ROR γ t. Gene expression in mature adult LTi-like cells are very similar to that in fetal LTi cells, except that adult LTi cells additionally express OX40L and CD30L.

Figure 2. Similarities and differences between the development of innate and adaptive lymphoid system

The development of innate lymphocytes and T cells shows certain similarities. At mature stage, $CD4^{+}$ T cells and ILCs produce similar sets of effector cytokines, while $CD8^{+}$ T cells and cNK cells exhibit cytotoxic activities. In the innate lymphoid system, the development of LTi cells is distinct from other ILCs and cNK cells though they all generated from an early innate lymphocyte progenitor (EILP). The non-LTi ILCs are generated from an PLZF-expressing common progenitor, ILCP, while LTi cells developed from their own progenitor which depends on the master transcription factor $ROR\gamma t$. At mature stage, while non-LTi ILCs show plasticity under certain circumstances similar to what have been observed in differentiated $CD4^{+}$ T cells, LTi cells are relatively stable.

Figure 3. Role of LT α cells in secondary lymphoid structure organogenesis

In the fetal stage, LT α cells will enter the lymph nodes or Peyer's patches anlagen through the interaction between integrin $\alpha_4\beta_7$ and MAdCAM-1 on the HEVs. CXCL13 secreted by LTo cells will also help to recruit LT α cells through interact with CXCR5 on them. Then LT α cells start to express lymphotoxin $\alpha_1\beta_2$ (LT $\alpha_1\beta_2$) and engage with the stromal cells expressing its receptor LT β R. This interaction will stimulate the stromal cells to upregulate TRANCE, CXCL13, CCL19, CCL21, MAdCAM-1, VCAM-1, and ICAM-1, which feedback on LT $\alpha_1\beta_2$ expression and recruit more LT α as well as T and B cells in to the anlagen site.

Figure 4. Interactions between LT_i cell and T or B cells in the adaptive immune system

LT_i cells can affect development and functions of T and B cells in the adaptive immune system through multiple mechanisms. They interact with the mTECs through RANK/ TRANCE interaction and thus induce the expression of AIRE, which is critical for T cell central tolerance. LT_i cells can also directly regulate the effector T cell responses through antigen presentation by MHC-II. The adult LT_i-like cells specifically express OX40L and CD30L, which may promote the survival of memory CD4⁺ T cells. The same mechanism may be responsible for the survival of memory follicular T helper (Tfh) cells and maintenance of memory antibody responses. In addition, LT_i cells may promote antibody

production by B cells through both T-dependent and -independent manners. Antigen presentation and cytokine secretion to Tfh cells from LT_i cells can enhance B cell responses. Alternatively, LT_i cells may directly activate B cell through BAFF, CD40L, DLL1 and APRIL expression. TNF, lymphotoxin (LT), and GM-CSF expression by LT_i can also activate B cells through marginal reticular cells (MRC) and neutrophils.

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