

Involvement of lymphoid inducer cells in the development of secondary and tertiary lymphoid structure

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During development lymphoid tissue inducer (LTi) cells are the first hematopoietic cells to enter the secondary lymphoid anlagen and induce lymphoid tissue neogenesis. LTi cells induce lymphoid tissue neogenesis by expressing a wide range of proteins that are associated with lymphoid organogenesis. Among these proteins, membrane-bound lymphotoxin (LT) $\alpha 1\beta 2$ has been identified as a critical component to this process. LT $\alpha 1\beta 2$ interacts with the LT β -receptor on stromal cells and this interaction induces up-regulation of adhesion molecules and production of chemokines that are necessary for the attraction, retention and organization of other cell types. Constitutive expression of LT $\alpha 1\beta 2$ in adult LTi cells can result in the formation of a lymphoid-like structure called tertiary lymphoid tissue. In this review, we summarize the function of fetal and adult LTi cells and their involvement in secondary and tertiary lymphoid tissue development in murine models. [BMB reports 2009; 42(4): 189-193]

INTRODUCTION

A population of CD45⁺ hematopoietic cells that express CD4 but not the T cell marker CD3 or dendritic cell marker CD11c are among the first cells to colonize developing lymph nodes (1). These so-called lymphoid tissue inducer (LTi) cells, which are also the first cells to enter the site of Peyer's patch formation, express many molecules associated with lymphoid organogenesis including CD127 (IL-7R α), CD132 (the common cytokine receptor γ chain), CXCR5, TNF-related activation-induced cytokine (TRANCE, TNFSF11), receptor activator of nuclear factor kappa B (RANK, TNFRSF11A), inhibitor of differentiation 2 (Id2, helix-loop-helix protein) and retinoid-related orphan receptor (ROR) γ (1-6). In addition to these proteins, LTi cells also express surface lymphotoxin (LT) $\alpha 1\beta 2$, which has led to the proposition that they are responsible for initiating

lymphoid tissue development. There are now several lines of evidence that support it; adoptive transfer of purified fetal LTi cells into neonatal CXCR5-deficient mice was shown to restore Peyer's patch formation (2), and intradermal injection of neonatal LTi cells induced lymph node-like structures (7). In addition, mice deficient in ROR γ t completely lacked LTi cells and this correlated with an absence of lymph nodes and Peyer's patches (8).

Since adult LTi cells express LT $\alpha 1\beta 2$, they may retain their potential to induce lymphoid tissue development at sites where lymphocytes infiltrate during prolonged inflammation. In support of the notion that LT $\alpha 1\beta 2$ in adult LTi cells play a role in tertiary lymphoid tissue formation, a study demonstrated that ectopic expression of LT α and LT β in the pancreas was sufficient to induce inflammation, which resulted in the development of organized infiltrates (9). Furthermore, it has been reported that early (3-4 weeks of age) administration of an LT β R-Ig fusion protein into experimental animal models, which had infiltrating lymphocytes in the pancreas, prevented the development of pancreatic inflammatory infiltrates (10).

In this review, we will discuss the mechanism of lymphoid tissue induction by fetal LTi cells and the involvement of adult LTi cells in ectopic or tertiary lymphoid development in chronically inflamed tissues.

Induction of lymphoid tissue by LTi cells

The role of LTi cells in the initiation of lymphoid organogenesis involves a complex series of molecular interactions, where many of the genes that have been implicated in lymph node and Peyer's patch formation appear to be necessary (1-6). In the earliest stages of lymph node and Peyer's patch formation, LTi cells can be found in close proximity to VCAM-1 and ICAM-1-expressing stromal cells, which also express LT β R (5, 7, 11). LT β R signaling leads to the up-regulation of VCAM-1 (12), and so it is likely that the LTi cells, through expression of their surface LT $\alpha 1\beta 2$, activate the stromal cells to express this adhesion molecule, which then allows them to be retained in the developing organs (Fig. 1).

Importantly, ligation of LT β R on stromal cells also induces the production of the lymphoid chemokines CXCL13 (B zone chemokine), CCL21 and CCL19 (T zone chemokines) (12, 13).

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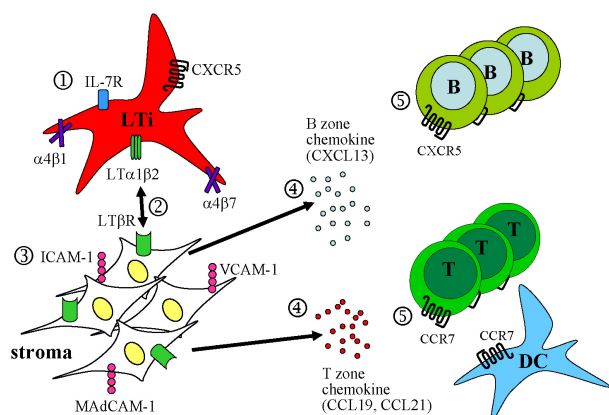


Fig. 1. Involvement of lymphoid tissue inducer (LTi) cells in secondary lymphoid tissue development. ① Signals through IL-7R up-regulate LT α 1 β 2 expression on LTi cells. ② Binding of LT β R on stromal cells with LT α 1 β , activates them leading to ③ up-regulation of adhesion molecules (ICAM-1 and VCAM-1), and ④ secretion of homeostatic chemokines. ⑤ CXCL13 (B zone chemokine) recruits B cells expressing CXCR5 to form B cell area and CCL19 and CCL21 (T zone chemokines) recruit T and dendritic cells expressing CCR7 to organize T cell area. LTi cells are further recruited by CXCL13 and adhere to stromal cells leading to the formation of LTi cell clusters and the amplification of LT β R signaling. LTi, lymphoid tissue inducer; IL-7R, interleukin-7 receptor, LT α 1 β 2, lymphotoxin alpha1 beta2; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; DC, dendritic cell.

CXCL13 initiates an important positive feedback loop by further recruitment of LTi cells, which express the CXCR5 receptor. Not only do LTi cells chemotax in response to CXCL13 (13) but CXCR5-signaling has also been shown to activate the ligand for the VCAM-1, α 4 β 1 integrin, which is expressed by LTi cells and is required for their adhesion to stromal cells (2). This results in the formation of characteristic LTi cell clusters and the amplification of LT β R signaling. This sequence of interactions is crucially dependent on IL-7R signaling, which promotes the up-regulation of LT α 1 β 2 on LTi cells (13, 14). TRANCE signalling has also been shown to contribute to LT α 1 β 2 expression in LTi cells (5) and is critical for lymph node development. This is supported by the observation that mice deficient in TRANCE did not have lymph nodes (6).

Sufficient clustering of LTi cells and production of lymphoid chemokines by activated stromal cells leads to the differentiation of high endothelial venules and accumulation of lymphoid cells within the developing structures. The subsequent organization of lymphocytes into B and T cell areas is mediated by the lymphoid chemokines, whose production is crucially dependent on lymphotoxin, which is also up-regulated in B cells in response to CXCL13, and TNF α (3).

Maintenance of lymphoid structure by LTi cells

It is important to clarify the roles of adult LTi cells in the immune system since the adult equivalent of fetal LTi cells were found in the mouse spleen (15). Adult LTi cells are localized mainly at the interface between the B and T cell areas and within the B follicle, a location that suggests it plays a role in T cell-dependent B cell responses.

The phenotypic difference between adult and fetal LTi cells is the expression of OX40-ligand (L) and CD30L, which provides survival signals to activated CD4 T cells (15-19). When these signals from LTi cells are not triggered, memory T cell-dependent responses are impaired due to an increased susceptibility to apoptosis (16, 19). The gradual increase of OX40L and CD30L expression has been detected after birth and were shown to reach normal levels at the time the mice were weaned (20).

Using the same mechanism as fetal LTi cells, the adult LTi cells, through the expression of LT α 1 β 2, activate LT β R-expressing stromal cells to produce homeostatic lymphoid chemokines, which are crucial for maintaining an organized architecture (21). Using LT α -deficient mice, Fu et al. showed that without these signals, lymphoid B and T cell areas were disorganized and activated CD4 T cells failed to survive, which resulted in impaired memory T cell-dependent responses (22). Adoptive transfer of purified adult LTi cells into LT α deficient mice was found to reconstitute lymphoid B:T structure (21). Therefore, these combined results strongly support the idea that signals from adult LTi cells through LT α are vital to maintaining the organized architecture of the lymphoid, and this is linked with another function of LTi cells; to provide survival signals to CD4 T cells through OX40L and CD30L within B follicles in lymphoid tissues.

Formation of tertiary lymphoid tissue

In certain chronically inflamed tissues, such as the synovium and salivary glands in rheumatoid arthritis and Sjögren's syndrome, respectively, infiltrating cells have been found to form lymph node-like structures with discrete T and B cell areas and in some instances, germinal centers and high endothelial venules (23). This phenomenon is known as lymphoid neogenesis and is believed to play an important pathogenic role in unwanted immune responses (24).

Several pieces of evidence suggest that the development of 'tertiary' or 'ectopic' lymphoid tissues is dependent upon similar molecular interactions to those required for the generation of lymph nodes and Peyer's patches. First, transgenic expression of CXCL13 (B zone chemokine) in a murine peripheral tissue, namely the pancreatic islets, was sufficient to generate organized lymphoid structures in a lymphotoxin-dependent manner (25). Furthermore, this chemokine has been detected in the inflammatory lesions of rheumatoid arthritis (26)

and Sjögren's syndrome (27), and CCL21 (T zone chemokine) has been detected in the pancreata of non-obese diabetic (NOD) mice (28).

TNF family members have also been strongly implicated in lymphoid neogenesis. Ectopic expression of $LT\alpha$ in the pancreatic islets induces tertiary lymphoid tissue formation (29) and NOD mice that constitutively express $TNF\alpha$ in the pancreatic islets display a more rapid accumulation of inflammatory cells (30). Furthermore, $LT\alpha$ and $LT\beta$ transcripts have been identified in rheumatoid arthritis synovium and were present at increased levels in samples containing highly organized infiltrates with ectopic germinal centers (26). Indeed, it has been suggested that inflammation is the evolutionary predecessor of organized lymphoid tissue, which would explain the crucial involvement of pro-inflammatory TNF family cytokines in secondary lymphoid tissue organogenesis (31, 32).

LTi cells in murine models of inflammation

In some instances of prolonged inflammation, such as in certain autoimmune diseases, large numbers of lymphocytes are recruited to peripheral tissues and often form compartmentalized, lymph node-like structures.

Given the vital role of fetal LTi cells in the organogenesis of secondary lymphoid tissue, it is possible that adult LTi cells are recruited to sites of inflammation and are involved in the attraction and organization of mononuclear cells. It was also hypothesized that these cells might further be involved in the

pathogenesis of inflammation by providing survival signals to infiltrating T cells. Based on this premise, tissues from murine models of CD4 T helper cell (Th)-mediated chronic inflammation, namely pancreata from non-obese diabetic (NOD) mice (Th1 or Th17), and lungs from mice with allergen-induced airway inflammation (Th2) were examined to determine whether or not cells with the morphology and phenotype of LTi cells were present.

NOD mice spontaneously develop type I (autoimmune) diabetes, and this was preceded by the accumulation of mononuclear cells around the insulin-producing islets of Langerhans (33). NOD mice have an apparently normal constitution of splenic LTi cells, and were identified in all NOD pancreata, which were examined in $CD4^+CD3^-CD11c^-$ LTi cells (Fig. 2A), suggesting that these cells play an important role at the site of autoimmune inflammation. In some instances putative LTi cells formed membrane contacts with cells that were likely to be CD4 T cells (Fig. 2B).

Since LTi cells have been shown to promote the survival of CD4 Th2 cells by mediating OX40- and CD30-signaling (15-19), the involvement of LTi cells in allergic responses, Th2-mediated inflammation, were examined. Lungs were obtained from a murine model of allergic asthma, in which airway inflammation was induced by sensitization with an intraperitoneal infection of ovalbumin and subsequent response induction was achieved by their repeated exposure to aerosolized antigens for up to 55 days (34). In all lungs examined, both T and B lymphocytes had accumulated around the bronchioles, although the lung infiltrates were less dense than those seen in the NOD pancreas and were dominated by B cells (unpublished data). However, $CD4^+CD3^-CD11c^-$ LTi-like cells were found in all of the mice examined. Furthermore, membrane interactions between these cells and CD4 T cells were also observed.

Taken together, these studies indicate that LTi cells are involved in tertiary lymphoid tissue development and possibly provide signals to T cells.

Conclusion and Perspectives

Previous studies have demonstrated that cells with the morphology and phenotype of LTi cells were not only present in secondary lymphoid tissue but that they can also be found at sites of active inflammation. This suggests that LTi cells are capable of migrating from lymphoid tissue in response to inflammatory stimuli and are recruited to inflamed peripheral tissues. However, it is also possible that they are resident within tertiary tissues, or that they differentiate therein upon initiation of the inflammatory process.

Nevertheless, LTi cells were found within the inflammatory infiltrates in all mice examined, and their apparent interactions with T cells suggest that they play an important role at sites of chronic inflammation. If the exact nature of that role is determined, adult LTi cells could be a target to treat chronic in-

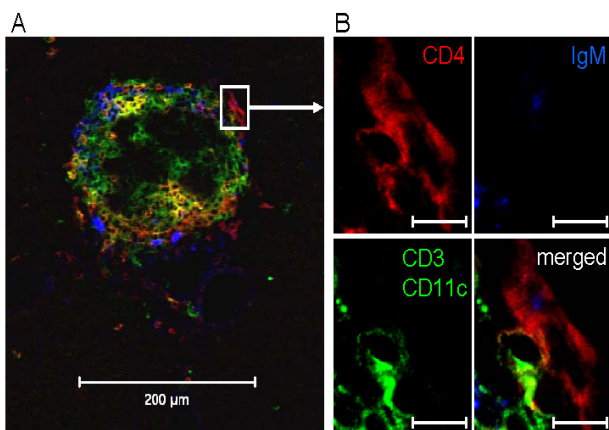


Fig. 2. Confocal images of $CD4^+CD3^-CD11c^-$ LTi cells within the pancreatic infiltrates of NOD mice. (A) Low power image of an infiltrate stained for CD4 (red), IgM (blue, indicating B cells), CD3 (green, indicating T cells), and CD11c (green, indicating dendritic cells). Area within the white box contains a $CD4^+CD3^-CD11c^-$ LTi cell interacting with a $CD4^+CD3^+$ or $CD4^+CD11c^+$ cell. Scale bar, 200 μ m. (B) Split screen high power image of area marked in (A), displaying an LTi cell making membrane contact with a $CD4^+CD3^+$ or $CD4^+CD11c^+$ cell, whose morphology suggests that it is a T cell. Colors as in (A). Scale bar, 10 μ m.

flammation and autoimmune diseases, which develop tertiary lymphoid tissues.

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