

Roles of Embryonic and Adult Lymphoid Tissue Inducer Cells in Primary and Secondary Lymphoid Tissues

Mi-Yeon Kim

Department of Bioinformatics and Life Science, Soongsil University, Seoul, Korea.

The nomenclature "embryonic lymphoid tissue inducer (LTi) cell" reflects the fundamental role of the cell in secondary lymphoid tissue organization. In addition, it is equally important in primary lymphoid tissue development as it regulates central tolerance to self-antigens in the thymus. An adult LTi cell constitutively expresses two sets of tumor necrosis factor (TNF) family members, whereas its embryonic counterpart expresses only one. The first set is lymphotoxin (LT) α , LT β , and TNF α , which are essential for the secondary lymphoid organogenesis during embryogenesis and for maintaining an organized secondary lymphoid structure during adulthood. The second set is OX40- and CD30-ligands, which are critical for memory T cell generation. Adult LTi cells regulate adaptive immune responses by providing LT β R signals to stromal cells to maintain secondary lymphoid tissue structure, and determine adaptive immune responses by providing OX40 and CD30 survival signals to activated T cells in memory T cell generation. Along with the consideration of the roles of embryonic LTi cells in primary and secondary lymphoid tissues, this review highlights the roles of adult LTi cells in secondary lymphoid tissue function.

Key Words: Lymphoid tissue inducer cell, secondary lymphoid tissue, thymus

ROLE OF LTi CELLS IN THE DEVELOPMENT OF SECONDARY LYMPHOID TISSUES

One of the earliest colonizing cells in lymphoid tissues is the lymphoid tissue inducer (LTi) cell, expressing CD4 and CD45 but not lineage markers

including CD3 (T cell marker), CD11c (dendritic cell marker), B220 (B and plasmacytoid dendritic cell marker) or macrophage cell marker.¹ LTi cells are a unique subset of the liver derived haematopoietic cells found to colonize fetal secondary lymphoid organs early in embryonic day 13 (E13) in mice.^{1,2} Since they are found in recombinate activating gene (RAG)-deficient or T cell deficient mice, they do not require receptor rearrangement.^{3,4}

LTi cells express a set of TNF family members; lymphotoxin (LT) α , LT β , and TNF α , and the first two molecules are upregulated via IL-7 signaling. LT α 1 β 2-expressing LTi cells interact with vascular cell adhesion molecule (VCAM)-1⁺ stromal cells that express LT β R, which is pivotal for the development of the secondary lymphoid tissues.⁵⁻⁸ In addition to LT α 1 β 2 expression, LTi cells express many of the molecules that are implicated in lymphoid-tissue formation, including TNF-related activation-induced cytokine (TRANCE), receptor activator of nuclear factor kappa B (RANK), IL-7R α , CD132, CXCR5, and retinoid-related orphan receptor gamma (ROR γ).^{3,9-14} Involvement of these genes in lymphoid tissue development has been demonstrated by studies using gene-knockout mice: mice deficient for LT α , LT β R, or ROR γ lack lymph nodes and Peyer's patches,^{9,15-20} mice deficient for IL-7R α , CD132, or CXCR5 show impaired development of lymph nodes and Peyer's patches,^{3,12,14,21} and TRANCE-deficient mice have no lymph nodes and a significant reduction in LTi cells. These results indicate that LTi cells are crucial for the development of lymph nodes and Peyer's patches.¹¹

Further studies directly support the idea that LTi cells can give the inductive signal for secondary

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Reprint address: requests to Dr. Mi-Yeon Kim, Department of Bioinformatics and Life Science, Soongsil University, 511 Sangdo-dong, Dongjak-gu, Seoul 156-743, Korea. Tel: 82-2-828-7192, Fax: 82-2-824-4383, E-mail: kimmy@ssu.ac.kr

lymphoid development. An adoptive transfer experiment of fetal splenic LTi cells into neonatal CXCR5-deficient mice, which lack Peyer's patches, has shown restoration of Peyer's patch formation.⁷ Intradermal injection of the neonatal embryonic LTi cells induced an ectopic lymphoid-like structure,²² and IL-7 transgenic mice have a significantly increased number of LTi cells, which are sufficient to form more Peyer's patches, ectopic lymph nodes and cecal patches.²³

Taken together, the published evidence supports the idea that LTi cells are pivotal for the development of secondary and tertiary lymphoid tissues.

ROLE OF LTi CELLS IN THYMUS

We have recently reported another role of LTi cells: thymic regulation of central tolerance to self.²⁴ LTi cells in the thymus are detected from E14 onwards and throughout adulthood, and show the same phenotype as those found in secondary lymphoid tissues. Thymic LTi cells interact with RANK⁺ medullary epithelial cells through TRANCE, which is critical for secondary lymphoid tissue development.¹¹ TRANCE signals by LTi cells to RANK, the receptor for TRANCE on medullary epithelial cells, promote the expression of Aire, which regulates the expression of self-tissue-restricted antigens on thymic medullary epithelial cells. Thymic medulla is the place for negative selection of immature thymocytes and expresses self-tissue-restricted antigens to eliminate reactive thymocytes to self-antigens.^{24,25} Aire-deficient mice lose the expression of self-tissue-restricted antigens and develop multi-organ autoimmunity.²⁶ Aire expression on medullary epithelial cells in thymus is, therefore, critical for central tolerance to self-antigens, and is regulated by LTi cells.

ROLE OF LTi CELLS AFTER BIRTH

Compared with the intensive studies of embryonic LTi cells in lymphoid tissue organogenesis, a few studies of adult LTi cells have been reported.²⁷⁻³¹ Embryonic LTi cells are found from E13 in mouse spleen, and induce lymphoid tissue formation during embryogenesis as mentioned

earlier. We have found the adult equivalent cells which share the same phenotype as embryonic LTi cells; positive for CD4, CD45, IL-7R α , and TRANCE, and negative for lineage markers.^{29,31} Adult LTi cells are mainly found in B cell area and the interface between B and T cell areas, and some of them are found in T cell area in spleen.²⁹ Here, we discuss the genetic relationship between embryonic and adult LTi cells, and the role of adult LTi cells in secondary lymphoid tissues and memory T cell generation.

Genetic relationship between embryonic and adult LTi cells

Immunity related gene array assays have shown that adult LTi cells have strong correlation with embryonic LTi cells (correlation coefficient (CC) = 0.86) compared with the correlation with T cells (CC = 0.63), B cells (CC = 0.65), dendritic cells (CC = 0.68), or natural killer cells (CC = 0.66).²⁷ Not only embryonic but also adult LTi cells express the genes, such as LT α , LT β , TNF α , TRANCE, RANK, IL-7R α , CD132, and CXCR5, which are important for lymphoid tissue development. The remarkable difference between embryonic and adult LTi cells is the expression of TNF family members, OX40-ligand (CD252, TNFRSF4) and CD30-ligand (CD153, TNFRSF8), which are important for memory T cell generation.^{29,32} LTi cells develop the expression of OX40-ligand and CD30-ligand a week after birth and show gradual upregulation of these molecules to the adult levels by weaning time.³⁰ The absence of T cell survival molecules on LTi cells in the neonatal period may explain the phenomenon that exposure to antigens, usually self-antigens in the first week of life, induces peripheral tolerance rather than immunity.

Studies dissecting the molecules which regulate the gene expression showed that OX40-ligand expression is regulated by TL1A (TNFSF15) via its receptor, DR3 (TNFRSF25) on LTi cells,²⁷ and CD30-ligand expression is by IL-7 via IL-7R.³⁰ Compared with DR3 signaling, which rapidly upregulates OX40-ligand expression in 24-hour culture with TL1A, IL-7R signaling induced slow upregulation of CD30-ligand after 5-day culture with IL-7.³⁰ In support of the idea that embryonic LTi cells can upregulate these ligands, we have shown that an

adoptive transfer of embryonic LTi cells into an adult mouse expressed the levels of these ligands comparable to the adult host.²⁷ These data, taken in conjunction with previous studies strongly suggest that embryonic LTi cells can be differentiated into an adult type of LTi cells.

Maintaining secondary lymphoid structure

The expression of LT α 1 β 2 on embryonic LTi cells provides LT β R signals to VCAM-1⁺ stromal cells to promote homeostatic chemokine expression for segregated T cell and B cell area formation in secondary lymphoid tissues.^{17,19,33,34} The high levels of expression of LT α 1 β 2 on adult LTi cells have recently been used to explain the extended function of embryonic LTi cells in secondary lymphoid tissue organization.²⁸ Lymphoid structure organized during embryogenesis requires continuous signals to maintain its organization via the expression of homeostatic chemokines, which recruit T and B lymphocytes to their appropriate location in the lymphoid tissues. Just as embryonic LTi cells do, adult LTi cells provide LT β R signals to stromal cells, leading to the secretion of the chemokines. This is evidenced by an adoptive transfer experiment in which adult LTi cells transferred into a LT α -deficient mouse, which lacked both separation of B and T cell areas and homeostatic chemokine expression, were able to partially reconstitute the splenic structure.²⁸ Furthermore, the reconstituted LT α -deficient spleen showed upregulation of the homeostatic T zone chemokine expression, supporting the proposal that LT α signals from adult LTi cells are sufficient for maintaining the segregation of T and B cell areas by their interaction with the VCAM-1⁺ stromal cells that secrete homeostatic chemokines.

Providing survival signals to generate memory T cells

The key difference between embryonic and adult LTi cells is the expression of T cell survival molecules, OX40-ligand and CD30-ligand^{29,32} as mentioned above. By the time when mice are weaned, OX40-ligand and CD30-ligand expression has reached their adult levels.³⁰ It has been reported that activated T cells receive survival signals

through OX40 and consequently upregulate antiapoptotic molecules like Bcl-2 and Bcl-X_L.³⁵ Genetic studies with OX40 and CD30 double knockout mice have shown that these molecules are critical for memory T cell generation.^{29,32} The double knockout mice were not able to generate memory T cells and failed to make secondary antibody responses when they were exposed to the same antigen for the second time. The analysis of OX40 or CD30 single knockout mice, which showed impaired levels of secondary antibody production compared to wild type mice, but higher levels than the double knockout mice, demonstrated that both OX40 and CD30 contribute to T cell survival and memory responses.³⁶

CONCLUSION

By providing LT β R signals to stromal cells, embryonic and adult LTi cells contribute to secondary lymphoid tissue organization and provide an efficient microenvironment for adaptive immune responses. The highly structured architecture of secondary lymphoid tissues provides better opportunities for efficient cognitive interaction between activated T and B cells and allows the formation of germinal centers, which produce high affinity antibodies. In germinal centers, adult LTi cells provide OX40 and CD30 survival signals to T cells when antigen is scarce.

In addition to their roles in secondary lymphoid tissues, LTi cells are also involved in the primary lymphoid tissue development by regulating thymic medullary epithelial cells to express self-tissue-restricted antigens. The expression of self-antigens is vital for negative selection, leading to central tolerance to self. In addition, the lack of OX40-ligand and CD30-ligand expression on LTi cells during neonatal life may eliminate T cells, which are activated on peripheral self-antigens, by failing to provide the survival signals to the self-activated T cells in secondary lymphoid tissues. This may lead to tolerance rather than immunity.

In summary, LTi cells contribute to primary and secondary lymphoid tissue organization by providing signals to medullary epithelial cells in thymus and to stromal cells in lymph nodes and spleen, respectively.

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