Immune Network
DOI 10.4110/in.2010.10.4.115
pISSN 1598-2629 eISSN 2092-6685
REVIEW ARTICLE

Heterogeneity of IL-22-producing Lymphoid Tissue Inducer-like Cells in Human and Mouse

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Lymphoid tissue inducer (LTi) cells have been characterized in mouse as a key cell when secondary lymphoid tissues are organized during development and memory T cells are formed after birth. In addition to their involvement in adaptive immune responses, recent studies show that they contribute to innate immune responses by producing large amount of interleukin (IL)-22 against microbial attack. Here, we compare IL-22-producing LTi and LTi-like cells in human and mouse and discuss their heterogeneity in different tissues.
[Immune Network 2010;10(4):115-119]

INTRODUCTION

Since a lymphoid tissue inducer (LTi) cell was found in mouse lymphoid tissues (1), many groups have studied on its functions. Firstly, it has a key role in secondary lymphoid tissue development through the lymphotoxin-$\alpha 1\beta 2$ expression during ontogeny (1-5), and provides survival signals to memory CD4 T cells through the OX40-ligand (OX40L) expression in adulthood (6-8). Secondly, they are also involved in ectopic lymphoid tissue development where lymphocytes infiltrate and organize lymphoid tissue like structure (9-11). In addition, thymic LTi cells promote the expression of AIRE (for autoimmune regulator), which is a transcription factor regulating the expression of self-tissue-restricted antigens on thymic medullary epithelial cells (12). Although LTi cells were actively studied in mouse during the last decade, the human counterpart was just recently identified in fetal mesentery and postnatal tonsils and reported to express large amount of IL-22 (13-15). Coincidently IL-22-producing LTi-like cells were reported in mouse gut (16,17), and referred as 'LTi-like' not 'LTi' cells because these cells express both a natural killer (NK) marker, NKp46 and a LTi characteristic marker, transcription factor retinoid-related orphan receptor (ROR) γt whose expression is critical for their development (18,19).

IL-22 is a Th17 cytokine and IL-22-producing cells are involved in innate immune responses against microbial attack (20,21). Here, the similarity and difference of mouse splenic and mucosal IL-22-expressing LTi and LTi-like cells and human IL-22-expressing LTi and LTi-like cells are discussed based on studies published within two years. In addition, the gene expression patterns of human LTi cells isolated from different secondary lymphoid tissues are compared with mouse splenic LTi cells.

SIMILARITY AND DIVERSITY OF IL-22-PRODUCING LTi-LIKE AND NK CELLS IN HUMAN AND MOUSE

'Conventional' LTi cells found in mouse lymph nodes and spleen are lineage$^-$CD4$^+$CD127$^+$CD117$^+$ (3,22,23) and OX40L$^+$ in adulthood (23,24). They do not express molecules related to cytotoxicity such as interferon (IFN)-γ and granzyme, and do depend on RORγt expression for their development (18,22,23). These characteristics are distinct from those of NK cells (13). In addition, NK subsets are heterogeneous, and develop through four stages according to their expression of CD56, CD34, CD117 and CD94 (25,26). The phenotype of immature NK cells in stage 3 are similar to that of LTi cells; they are CD56$^-$CD127$^+$CD117$^+$CD94$^-$ (13).

Received on August 11, 2010. Accepted on August 16, 2010.

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Keywords: LTi, OX40L, IL-22, NK-22

Takatori et al. showed that adult mouse splenic LTi-like cells express IL-22 and IL-17 after injection of zymosan in vivo (27), and Luci et al. showed that NKp46^{+}RORγt^{+}CD3^{-} cells in mouse gut constitutively express IL-22 transcripts and produce IL-22 rapidly after stimulation (17). In addition, Sanos et al. showed that RORγt^{+}NKp46^{+}NK1.1^{int} cells in mouse gut produce IL-22 and their emergence is dependent on RORγt expression (16). Because IL-22-producing cells described by Luci et al. and Sanos et al. express NKp46, they assumed that the cells could be derived from LTi cells. Recently, Buonocore et al. showed that Thy1^{+}SCA1^{+}RORC^{+} IL-23R^{+}CD4^{-}CD117^{-} cells in mouse colon enhance IL-22 expression after IL-23 stimulation and the cell number is increased about 10 times more than other leukocytes by intestinal inflammation (28). The difference between these cells and conventional LTi cells is CD117 expression.

Several groups reported IL-22-expressing cells in human mucosa-associated lymphatic tissues. Cella et al. reported NK-22 cells which are IL-22-expressing NKp44^{+} cells found in human tonsil and Peyer's patches (29) and redefined them as CD56^{+}NKp44^{+}CCR6^{+}CD103^{-} (30). NK-22 cells stimulated by IL-23 and IL-1β increase not only IL-22 but also IFN-γ expression. They also identified NK-22 cells in mouse gut; ~40% of CD3^{-}CD19^{-}NKp46^{+} NK1.1^{-} cells express IL-22 af-

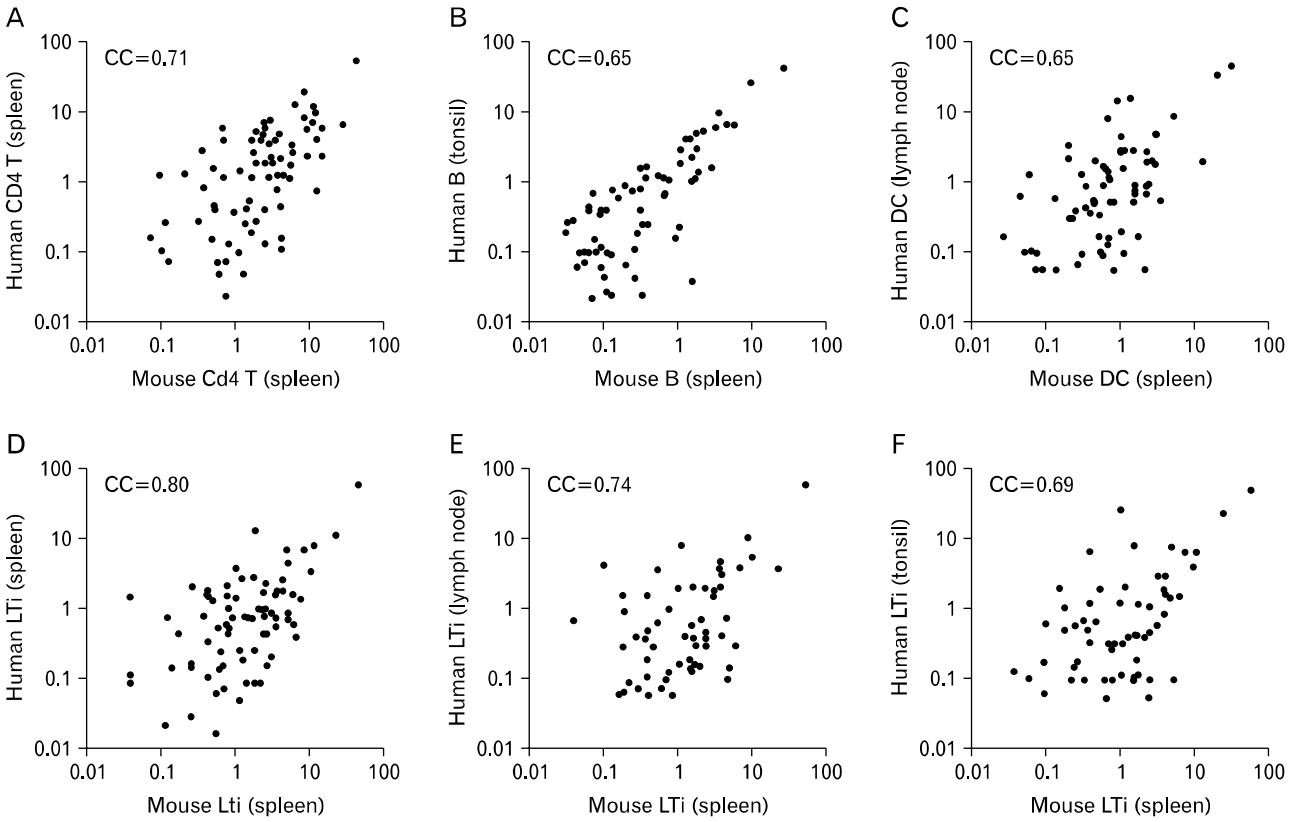

Figure 1. Correlation between the gene expression patterns of human and mouse cell populations. (A) Mouse splenic Th1 primed cells in vitro for 6 days in the presence of IL-4 and anti-IL-12 and human tonsilar CD45RO^{+} memory CD4 T cells. (B) Mouse splenic B cells and human tonsilar B cells. (C) Mouse splenic CD11c^{+} dendritic cells (DC) and human lymph node CD11c^{+} DC. (D-F) Mouse splenic LTi cells and human LTi cells isolated from spleen (D), lymph node (E), and tonsil (F). Over 0.01% expression of individual *mRNAs* of β-actin signals were plotted. X- and Y- axes show mRNA expression levels relative to the β-actin signal (β-actin signal=100%, log scale). Each plot compares two cell types, one on each axis (as labeled). CC=correlation coefficient. Analyzed 90 genes are: LT-α, TNF-α, LT-β, OX40L, CD40L, FASL, CD70, CD30L, 4-1BBL, TRANCE, TWEAK, APRIL, BAFF, LIGHT, TL1, TNFR1, TNFR2, LTβR, OX40, CD40, FAS, CD30, 4-1BB, RANK, TWEAKR, BAFFR, HVEM, GITR, DR3, CCR7, CXCR3, CXCR5, Bcl-2, Bcl-6, Bcl-XL, ROR-γ, GATA3, Foxp3, Myd-88, TLR2, TLR3, TLR4, TLR5, TLR7, TLR9, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, TSLP, IL-9, IL-10, IL-12p35, IL-12p40, IL-13, IL-15, IL-18, IFN-α1, IFN-β, IFN-γ, TGF-β1, IL-2Rα, IL-2Rβ, IL-2Rγ, IL-4Rα, IL-7Rα, IL-10Rα, IL-10Rβ, IL-12Rβ1, IL-12Rβ2, IFNγR1, IFNγR2, CD80, CD86, DC-SIGN, CathepsinS, integrin alpha x, CTLA4, ICOS, ICOSL, β-2m, T-bet, CD4, CD74, perforin, and granzymeB.

ter IL-23 stimulation (30). Crellin et al. showed that $CD56^+$ $CD127^+$ cells are similar to NK-22 cells and $CD56^-CD127^+$ cells are LTi cells, and both express IL-22 and proliferate in the presence of IL-15 (15). Our group reported that $CD3^-$ $CD117^+CD56^-OX40L^+$ cells in tonsil are very similar to LTi cells (31), and $OX40L^+$ cells in this population express large amount of IL-22 (submitted).

In summary, several IL-22 expressing subsets have been reported in human and mouse. In human NK-22 cells by Cella et al. were identified in NK subsets, and the IL-22-producing cells by the others were studied after excluding NK cells. Cella et al. supposed that NK-22 may be derived from LTi cells. Many groups studying on IL-22 expression in mouse gut showed that IL-22-expressing cells are $NKp46^+$ (16,17,29, 30). The common factor of all IL-22-producing cells is RORγt expression which is critical for LTi development (18,32) and not expressed in conventional NK cells leading to the IL-22-producing cells are related to LTi cells.

HETEROGENEITY OF HUMAN LTi CELLS IN DIFFERENT TISSUES

As mentioned above, not only LTi cells but also other IL-22-producing cells are heterogeneous. Lane's group previously reported that mouse LTi cells are heterogeneous according to their chemokine receptor expression and exist as $CD4^+$ and $CD4^-$ cells indicating different functions in particular organs (22). In comparison, most human LTi cells which have been reported are $CD4^-$ (13,14). We therefore speculated that LTi cells in different tissues may express different molecules. To answer the question, we isolated $CD3^-CD117^+$ $OX40L^+$ cells in human spleen, lymph node, and tonsil, and compared 90-immune related gene expression patterns to mouse LTi cells isolated from spleen (Fig. 1). The gene expression was normalized to β-actin signals.

Firstly, the correlation between cells of different types in human and mouse was compared. Correlation coefficient (CC) between human CD4 memory T cells and mouse Th1 primed cells showed 0.71, between human and mouse B cells was 0.65, and between human and mouse dendritic cells (DCs) was 0.65 (Fig. 1A-C). Next, gene expression patterns of mouse splenic LTi cells were compared with human LTi cells isolated from different tissues; spleen (CC=0.80), lymph node (CC=0.74) and tonsil (CC=0.69) (Fig. 1D-F). Human splenic LTi cells showed the strongest correlation as mouse LTi cells were isolated from spleen. We further analyzed genes which are expressed over 10 times more in each tissue than genes expressed in mouse LTi cells; human splenic and lymph node LTi cells expressed higher levels of mRNA for BAFF (for B cell activating factor belonging to the tumor necrosis factor family) and Toll-like receptor 9, and tonsilar LTi cells expressed higher levels of mRNA for CD70, OX40, and GITR (glucocorticoid induced tumor necrosis factor gamily related gene). This indicates that LTi cells in specific tissues have different functions. This finding encourages us to investigate other functions of LTi cells in different tissues and diverse immune circumstances. In particular, their involvement in diseases such as cancer and autoimmunity has not been studied yet and may provide new insights for understanding human immune system.

CONCLUSION

LTi cells, LTi-like cells producing IL-22 in spleen and mucosa-associated lymphatic tissue and NK-22 cells produce large amount of IL-22 by IL-23 stimulation in bacterial infection. In comparison of LTi cells which do not express NK markers, LTi-like and NK-22 cells express NK cell marker(s). However, all of the cells are dependent on RORγt expression for their development indicating they may share a certain developmental stage with LTi cells. In addition, several differences of gene expression patterns by LTi cells in different tissues suggest their heterogeneity and various functions.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (NRF-2009-0073480).

CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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