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The IL-7 Signaling Pathway Regulates Lymph Node Development Independent of Peripheral Lymphocytes

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Lymph node (LN) organogenesis is initiated by the interaction between hematopoietic lymphoid tissue inducer (LTi) cells and the mesenchymal organizer cells. Mice in which the IL-7 signaling pathway has been disrupted have a severe defect in LN development; however, the reasons underlying this defect are as yet unknown. In this study, we show that the overexpression of thymic stromal lymphopoietin (TSLP) increased LTi cell numbers and restored LN development in IL-7−/− and RAG2−/− γc−/− mice. The TSLP-mediated LN restoration was strictly dependent on LTi cells and independent of lymphocyte colonization. Increased LTi cell numbers in the LN anlagen of RAG2−/− γc−/− TSLP transgenic mice were associated with the restoration of organizer cells, suggesting that LTi cell number is a critical parameter for LN organogenesis. Our results shed light on the minimal cellular requirement for LN development during ontogeny. We show that the presence of LTi and organizer cells, but not of peripheral lymphocytes, is critical for LN development and persistence and further suggest that the IL-7 signaling pathway regulates LN organogenesis by controlling the size of the LTi cell pool. The Journal of Immunology, 2010, 184: 000–000.

...required for NK cell development (22). Although the reasons underlying the strong reduction in numbers of several LNs observed in adult IL-7−/− and γc−/− mice remain poorly defined, IL-7 availability also regulates the size of the pool of LTi cells in vivo (16), indicating that LN organogenesis may be defective in IL-7−/− and γc−/− mice due to low LTi cell numbers. Alternatively, LN organogenesis may occur normally, and lymphopenia prevents the maintenance of the LN anlage after birth (23).

In this study, we have investigated whether the deficiencies observed in LN development in IL-7−/− and RAG2−/− γc−/− mice result from an early intrinsic defect in LN organogenesis or from the lack of colonization by peripheral lymphocytes. We show that overexpression of TSLP increased LTi cell numbers and restored LN development in IL-7−/− and RAG2−/− γc−/− mice. LN anlagen from RAG2−/− γc−/− TSLP transgenic (Tg) mice were devoid of B, T, and NK cells, showing that colonization of the LN by peripheral lymphocytes was not required for persistence of the LN anlage. In contrast, TSLP-mediated LN restoration in IL-7−/− mice was strictly dependent on LTi cells. Together these results indicate that the LN defect in IL-7−/− and RAG2−/− γc−/− mice is a consequence of insufficient LTi cell numbers.

Materials and Methods

Mice

All of the mice were bred and maintained in our animal facility under specific pathogen-free conditions. The animal experiments received the approval of the Cantonal Veterinary Office of the city of Basel, Switzerland. C57BL/6 mice were purchased from RCC (Bingen, Switzerland). IL-7−/− (20), LTα−/− (8), RORγ−/− (28), and K14-TSLP Tg (24) mice were previously described. RAG2−/− γc−/− mice on C57BL/6 background were kindly provided by Jörg Kirberg (MPI Freiburg, Germany).

LN enumeration was performed a week after peritoneal injection of 100 μl of 1% Chicago sky blue 6B (Sigma-Aldrich, St. Louis, MO) ink in PBS. Images of LNs were captured with a stereoscopic SMZ1500 microscope coupled with a DS camera control unit DS-L1 (Nikon).
Flow cytometry

FITC–, PE-, PE/Cy7-, allotypy-cyocyanin-, or biotin-conjugated α-CD4 (GK1.5), α-CD8a (53-6.7), α-CD19 (ID3), α-NK1.1 (PK136), α-CCXCR4 (2B11), α-CXCR5 (2G8), α-CD48 (DATK32), and α-ICAM-1 (3E2) Abs were purchased from BD Biosciences. α-CD3 (145-2C11), α-CD11a (M17/ 4), α-CD11b (M170), α-CD44 (IM7), α-CD62L (MEL14), α-CD69 (HI.2F3), α-CD20 (RA3-6B2), α-Ter-119 (Ter-119), α-CD43 (R6B-8C5), and α-β2 integrin (M18/2) Abs were purchased from Biologend. α-CD11c (N418), α-CD45 (30-F11), α-CD45.1 (A20), α-CD122 (TM-b1), α-IL-7Rα (A7R34), α-VCAM-1 (429), α-Gr-1 (RB6-8C5), and α-Flt3 (A2F10) Abs were purchased from eBioScience (San Diego, CA). As secondary reagent, streptavidin–PE and streptavidin–Alexa Fluor 488 (Molecular Probes, Leiden, The Netherlands), Lyve-1 with a goat α-rabbit Alexa Fluor 488 (Molecular Probes), and Cr1 and CD31 with streptavidin–Alexa Fluor 488 (Molecular Probes).

For LTi cell number quantification, mesenteric LN (mLN) and spleen absolute cell numbers were obtained from analysis.

LT staining was performed on cell suspensions from individual organs as described previously (15). Briefly, cells were treated with α-Fc (2B11), α-CD4 (GK1.5), α-CD11b (RB6-8C5), and α-CD31 (390; eBioscience) Abs. U.K.), Lyv-1 with a goat α-rabbit Alexa Fluor 488 (Molecular Probes), and CR1 and CD31 with streptavidin–Alexa Fluor 488 (Molecular Probes).

Images were captured on a LSM 510 Meta laser scanning confocal microscope system (Carl-Zeiss, Fiedbach, Switzerland). When required, images were assembled using Adobe Illustrator CS or Adobe Photoshop CS (Adobe).

Results

TSLP Tg expression rescues LN development in IL-7−/− mice

In IL-7−/− mice, LN development is severely impaired (16), resulting in substantial reduction or absence in LNs at multiple locations. To address whether increased TSLP availability could restore LNs in IL-7−/− mice, adult IL-7−/−/K14-TSLP Tg mice and IL-7−/− littermates were i.p. injected with Chicago blue, and LNs were enumerated 7 d later. Although mLNs were found in all of the animals analyzed regardless of TSLP Tg expression, the number of LNs in mice overexpressing TSLP was substantially increased compared with that of non-Tg littermates, except for sacral and deep cervical (Fig. 1A, 1B). These results show that increased TSLP availability was sufficient to restore LN development in IL-7−/− mice.

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** Increased TSLP availability restores LN development in IL-7−/− mice. A, Quantification of LNs in IL-7−/− and IL-7−/− K14-TSLP Tg littermates. Eight to 11 mice were analyzed per group. B, Aortic region of IL-7−/− (i) and IL-7−/− K14-TSLP Tg (ii) mice. mLNs are indicated by arrow heads. C, Absolute cell number of B220+ CD19+ B cell, NK.1.1+ NK cell, and CD4+ and CD8+ T cells in the spleen of 4.5-d-old IL-7−/−, IL-7−/− K14-TSLP Tg, and WT mice. Shown are the mean values and SD from analyzing five animals per group. D, Representative FACS plots of IL-7−/−, IL-7−/− K14-TSLP Tg, and WT newborn spleens (0.5 d old). Gates indicate CD4+ IL-7Rα+ LTi cells. Numbers are mean and SD of percentages. Absolute CD4+ CD3− IL-7Rα+ LTi cell number in spleen (E) and mLN (F) of IL-7−/− (circles, n = 44), IL-7−/− K14-TSLP Tg (triangles, n = 17), and WT (diamonds, n = 40) 0.5-d-old mice. Each symbol represents LTi cell number from an individual animal. The mean values are indicated by a bar. G, LTi-Tα1/2 level expression of WT CD19+ B cells (shaded), LTi cells from newborn IL-7−/− (plain line), and IL-7−/− K14-TSLP Tg (dotted line) mice in spleen and mLN.

CD45.1+). Ten days after reconstitution, FL chimaera were analyzed for LTi cell presence.

Immunofluorescence microscopy

Eight-micrometer acetone-fixed LN sections were incubated with combinations of α-ER-T7 (ER-T7; AbD Serotec), α-PAAD (MECA-79; Pharmingen), α-Lyve-1 (RELIXtech), α-CCXCL13 (R&D Systems, Abington, U.K.), α-CCL19 (R&D Systems), biot-CR1 (8C12, Pharmingen), and biot-CCL3 (390; eBioscience). Abs. α-ER-T7 and α-PAAD were detected with goat α-rat-Cy5 (Jackson ImmunoResearch Laboratories), α-CCXCL13, and α-CCL19 with a donkey α-goat Alexa Fluor 488 (Molecular Probes, Leiden, The Netherlands). Lyve-1 with a goat α-rabbit Alexa Fluor 488 (Molecular Probes), and CR1 and CD31 with streptavidin–Alexa Fluor 488 (Molecular Probes).

For organizer cell staining, inguinal LNs (iLNs) of 2.5-d-old mice were digested with 1 mg/ml dispase (Life Technologies) together with 100 μg/ml DNase I (AppliChem) in PBS at 37°C for 20–30 min under mild agitation. Cell suspension was then filtered and stained on ice.

Adoptive transfer experiments

For fetal liver (FL) chimeras, IL-7−/− K14-TSLP Tg mice, IL-7−/− littermates, and C57BL/6 wild-type (WT) mice were lethally γ-irradiated (9 Gy) and i.v. injected with 109 total FL cells from 12.5 dpc embryos.
Because IL-7Rα is almost exclusively expressed by hematopoietic cells (30), LN restoration in IL-7−/− K14-TSLP Tg animals was likely mediated through the effect of TSLP on hematopoietic cells. Hence, to gain insight into the mechanism underlying the restoration of LN development in IL-7−/− K14-TSLP mice, we investigated in neonatal mice which hematopoietic cells were affected by TSLP Tg expression. TSLP overexpression increased the B cell compartment ∼100-fold in the spleen of 4.5-d-old IL-7−/− mice when compared with that of littermate controls (Fig. 1C). CD4 and CD8 T cell numbers were increased 18- and 20-fold, respectively, whereas NK cells were less affected (Fig. 1C). TSLP Tg expression in IL-7−/− newborn mice restored T cell numbers to WT levels and increased B cell numbers 3-fold when compared with those of WT controls (Fig. 1C). These results show that TSLP overexpression increased the size of B, T, and NK cell compartments in the periphery of newborn IL-7−/− mice. In the spleen of 0.5-d-old IL-7−/− K14-TSLP Tg mice, the percentage of CD4+ IL-7Rα+ LTi cells was 3-fold increased when compared with that of non-Tg littermates (Fig. 1D). LTi absolute cell numbers were 3.8- and 2-fold increased in the spleen and the mLN, respectively (Fig. 1E, 1F). Hence, TSLP Tg expression restored LTi cell number in the mLN of newborn IL-7−/− mice to WT values (Fig. 1F). LTi cell number in IL-7−/− spleen was 2-fold increased when compared with that of WT controls (Fig. 1E), suggesting that the failure of formation of LN anlage in IL-7−/− animals leads to the accumulation of LTi cells in the spleen.

Because LTi cell function directly relies on the expression of LTα1β2, we analyzed LTi cells from newborn IL-7−/− K14-TSLP Tg and non-Tg littermates and found that they expressed similar levels of LTα1β2 (Fig. 1G). These results show that TSLP Tg expression increases LTi cell numbers in vivo, without altering LTα1β2 expression levels.

**TSLP promotes LTi cell generation from FL precursors**

LTi cells arise from FL progenitors (31, 32). To understand the mechanism underlying the increase in LTi cell number in IL-7−/− K14-TSLP Tg mice, we investigated whether FL progenitors could give rise to LTi cells in response to TSLP. Although IL-7 favored the generation of LTi cells from FL progenitors in vitro, we failed to detect a similar activity of TSLP in various in vitro settings (data not shown). We therefore reconstituted lethally irradiated adult Ly5.2+ IL-7−/− K14-TSLP Tg mice and non-Tg littermates with lineage (CD3, CD8α, CD11c, CD19, B220, NK1.1, Ter119, Gr-1)− CD45.2+ donor cells. Numbers indicate percentages. A, Absolute LTi cell numbers in spleen of IL-7−/−, IL-7−/− K14-TSLP Tg, and WT recipients.

**TSLP-mediated LN restoration in IL-7−/− mice is LTi cell-dependent**

LN development crucially relies on the cross talk between LTi cells and LN mesenchymal cells (5, 28). To address whether LTi cells were instrumental for the restoration of LNs in IL-7−/− K14-TSLP Tg mice, IL-7−/− K14-TSLP Tg mice were backcrossed to RORγ−/− mice. Newborn IL-7−/− RORγ−/− K14-TSLP Tg mice were devoid of LTi cells (Fig. 2A), suggesting that LTi cell activity and homing were not altered by TSLP Tg expression. In 6.5-d-old WT mice, mLNs were colonized by CD45+ hematopoietic cells, of which the vast majority were CD4+ and CD8+ T cells together with CD19+ B220+ B cells (Fig. 5A). WT mLN contained a mean of 3 × 104 CD4+ T cells, 9.5 × 105 CD8+ T cells, 2.2 × 105 B cells, and 6 × 105 NK cells (Fig. 5B). In contrast, mLNs from RAG2−/− γc−/− K14-TSLP Tg and RAG2−/− γc−/− mice were devoid of T cells, and B and NK cell numbers were ≤100 (Fig. 5A, 5B). These results show that LN anlagen of RAG2−/− γc−/− K14-TSLP Tg mice contained higher LTi cell numbers than those of non-Tg littermates, while being almost devoid of lymphocytes.

**TSLP restores organizer cells and LNs in RAG2−/− γc−/− mice independent of peripheral lymphocytes**

Peripheral lymphocytes were proposed to play a role in the maintenance of the LN anlage during postnatal life (23). Because we could not rule out that restoration of peripheral lymphocytes partially contributed to the LN restoration in IL-7−/− K14-TSLP Tg mice, we generated RAG2−/− γc−/− K14-TSLP Tg mice. Consistent with our previous findings, the percentage of CD4+ IL-7Rα+ LTi cells in the spleen was 2.5-fold increased in RAG2−/− γc−/− K14-TSLP Tg newborn compared with that in non-Tg littermates (Fig. 4A). Absolute LTi cell numbers were 2.5-fold increased in the spleen and mLNs of TSLP Tg newborn mice compared with those of non-Tg littermates (Fig. 4B, 4C). LTα1β2, cytokine receptors, chemokine receptors, integrins, and adhesion molecules were expressed at similar levels by LTi cells from TSLP Tg and non-Tg littermates (Fig. 4D–G), suggesting that LTi cell activity and homing were not altered by TSLP Tg expression. In 6.5-d-old WT mice, mLNs were colonized by CD45+ hematopoietic cells, of which the vast majority were CD4+ and CD8+ T cells together with CD19+ B220+ B cells (Fig. 5A). WT mLN contained a mean of 3 × 104 CD4+ T cells, 9.5 × 105 CD8+ T cells, 2.2 × 105 B cells, and 6 × 105 NK cells (Fig. 5B). In contrast, mLNs from RAG2−/− γc−/− K14-TSLP Tg and RAG2−/− γc−/− mice were devoid of T cells, and B and NK cell numbers were ≤100 (Fig. 5A, 5B). These results show that LN anlagen of RAG2−/− γc−/− K14-TSLP Tg mice contained higher LTi cell numbers than those of non-Tg littermates, while being almost devoid of lymphocytes.

**FIGURE 2.** TSLP promotes LTi cell generation from FL precursors. Lethally irradiated adult IL-7−/−, IL-7−/− K14-TSLP Tg, and WT CD45.2+ mice were reconstituted with 10⁶.125 dpc CD45.1+ FL cells. A, Representative cytometry plots of splenocytes from FL chimera 10 d after reconstitution, previously gated on lineage (CD3, CD8α, CD11c, CD19, B220, NK1.1, Ter119, Gr-1)− CD45.2+ donor cells. Numbers indicate percentages. B, Absolute LTi cell numbers in spleen of IL-7−/−, IL-7−/− K14-TSLP Tg, and WT recipients.
Adult RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) K14-TSLP Tg and non-Tg littermates were analyzed for the presence of LNs. As expected, RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) mice had a severe defect in LN development with the frequency of inguinal, popliteal, periaortic, axillary, pancreatic, and hepatic LNs <20% of that of WT mice (Fig. 5C, 5D). TSLP overexpression fully restored inguinal, popliteal, axillary, and hepatic LN development and substantially rescued the organogenesis of periaortic LNs (Fig. 5C, 5D). The formation of brachial, superficial cervical, and deep cervical LNs was also clearly enhanced by TSLP Tg expression. Hence, RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) K14-TSLP Tg animals had almost normal LN numbers. Furthermore, the depletion of NK cells with NK1.1 Ab for the first 2 wk of life of RAG2\(^{-/-}\) mice did not prevent the normal formation of LNs (data not shown). These results show that peripheral B, T, and NK lymphocyte compartments are not required for the maintenance of LN anlagen.

TSLP overexpression increased LTI cell numbers and restored LN development in RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) mice. Because LTI cells are mandatory for the formation of organizer cell clusters (5), the increase in LITi cell number may have a direct effect on the organizer compartment. To test this hypothesis, we analyzed iLN anlagen from RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) K14-TSLP Tg and non-Tg newborn mice. Consistent with the absence of peripheral lymphocytes, iLNs from both RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) and RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) K14-TSLP Tg animals contained few CD45^+ cells (Fig. 5E). Within the CD45^+ fraction, a homogenous VCAM-1^+ ICAM-1^+ organizer population, which was absent from non-Tg littermates, was present in TSLP Tg animals (Fig. 5E). These results suggest that the TSLP-mediated increase in LITi cell numbers is instrumental for the restoration of organizer cells in newborn RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) mice and later for the restoration of LNs in adult mice.

Aberrant lymphocytes compromises LN architecture but not the presence of chemokine-producing stromal compartments and high endothelial venules

The LN microenvironment is composed of different stromal populations, which constitute the LN framework, regulate lymphocyte segregation, and actively participate in adaptive immunity (33–35). The contribution of lymphocytes to LN architecture and microenvironment remains elusive. To address these issues, we compared the iLNs from WT and RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) K14-TSLP Tg mice.

The LN reticular network can be visualized by staining with an Ab specific for the ER-TR7 Ag (34). Structures such as capsule, high endothelial venules (HEVs), cortex, and medulla were readily detectable in iLNs from both WT and RAG2\(^{-/-}\) \(\gamma_c^{-/-}\) K14-TSLP
upper quadrants subcapsular sinus (Fig. 6A). In the absence of peripheral lymphocytes (Fig. 6B), iLN anlagen from RAG2−/− mice contained CXCL13+ or CCL19+ stroma and PNA+ HEVs and displayed a similar ER-TR7+ reticular architecture as RAG2−/− K14-TSLP Tg animals (Supplemental Fig. 1). These results show that there are mesenchymal and endothelial compartments within the LN microenvironment, such as HEVs and chemokine-expressing stroma, which develop normally in the absence of lymphocytes and, in contrast, that other cells, such as FDCs, are critically dependent on the presence of lymphocytes. In the small iLN anlagen that were present in some RAG2−/− mice, PNA−, CR1−, CXCL13−, and CCL19-expressing cells were undetectable (Supplemental Fig. 1).

Discussion
This study aimed at understanding the role of the IL-7 pathway in LN organogenesis. Our results show that LN development occurs independent of peripheral lymphocytes and suggest that the IL-7 signaling pathway controls LN development by regulating the size of the LTi cell pool. We show that TSLP Tg expression increases the size of the LTi cell pool and restores LN development in IL-7−/− mice. These results extend our previous finding on the overlapping activity of TSLP and IL-7 in adult lymphopoiesis (24) and suggest that the low TSLP availability in vivo limits LN formation in IL-7−/− mice independent of peripheral lymphocytes. These results are in contrast to a previous study where the injection of high numbers of adult lymphocytes in the first week after birth restored LN
development in RAG2−/−γc−/− mice, suggesting that peripheral lymphocytes could play a role in maintaining the LN anlage in postnatal life (23). These findings may be explained by the fact that adult lymphocytes express LTα1β2 (40) and are therefore able to restore LN anlagen.

LTi cells are central to the TSLP-mediated LN restoration in IL-7−/− mice, as demonstrated by the fact that IL-7−/−RORγ−/− K14-TSLP Tg mice lack all LNs. TSLP Tg expression increases LTi cell numbers and restores organizer cells in newborn RAG2−/−γc−/− mice, suggesting that the increase in LTi cell number is essential for the presence of organizer cells. These data are in line with the proposal (5, 41, 42) that there is a numerical threshold of LTi cells that is required to effectively “instruct” LN stroma. Indeed, there are several lines of evidence indicating that low LTi cell numbers fail to induce proper LN development. The maintenance of a fetal LN transplanted under the kidney capsule of an adult mouse is dependent on LTi cell numbers (42). A 15.5 dpc LN fails to persist unless exogenous LTi cells are added to the graft, whereas 17.5 dpc LN, containing more LTi cells, persists without LTi addition (42). Furthermore, mice in which LTi cell recruitment to the LN anlage is impaired (15, 43–45) are devoid of LNs. Collectively, these data suggest that LN development in IL-7−/− and RAG2−/−γc−/− animals is defective due to insufficient numbers of LTi cells.

Interestingly, several cellular compartments typically found in the LN microenvironment, such as chemokine-expressing stromal cells and PNAd+ HEVs, develop in the lymphocyte-free LNs of RAG2−/−γc−/− K14-TSLP Tg mice. Within the B cell follicle of WT LNs, a substantial fraction of the CXCL13-expressing cells do not display a FDC phenotype (37). Our results suggest that these CXCL13-producing cells differentiate independent of lymphocyte

FIGURE 6. Visualization of LN architecture and stromal compartments. iLNs from 6-wk-old RAG2−/−γc−/− K14-TSLP Tg mouse and from WT control were stained for the fibroblastic reticular cell marker ER-TR7 (A, B). B, B cell zone; CA, capsule; T, T cell zone. iLN sections were stained with ER-TR7 together with CR1 (C, D), CXCL13 (E, F), CCL19 (G, H), or Lyve-1 (I, K) or stained for CD31 in combination with PNAd (J, L).
presence, potentially in response to LT signals initially provided by LTi cells (12), whereas lymphocytes are required for proper LN organization and for correct HEV positioning, likely because they shape the LN architecture by interacting with reticular fibroblastic cells (35). In line with the requirement of B cells for FDC generation (46), FDC networks were absent in iLNs of RAG2−/− γc−/−K14-TSLP Tg mice. Therefore, our results show that some of the mesenchymal and endothelial compartments, which constitute the LN microenvironment, are independent of peripheral lymphocytes, whereas others critically depend on lymphocyte presence. In contrast to RAG2−/− γc−/−K14-TSLP Tg mice, rudimentary LN anlagen in RAG2−/− γc−/− mice were devoid of PNA+ HEVs and chemokine-expressing stroma. These results suggest that the severely reduced number of LTi cells was insufficient to promote maturation of the LN anlage in RAG2−/− γc−/− mice.

Altogether, our results show that lymphocyte colonization is not required for persistence of the LN anlage and suggest that the IL-7 signaling pathway regulates LN organogenesis by controlling LTi cell numbers.

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Disclosures

The authors have no financial conflicts of interest.

References

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