THE JOURNAL OF IMMUNOLOGY

Cellular Interactions in Lymph Node Development

Tom Cupedo and Reina E. Mebius

This information is current as of April 29, 2014.

References	This article **cites 63 articles**, 27 of which you can access for free at: http://www.jimmunol.org/content/174/1/21.full#ref-list-1
Subscriptions	Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscriptions
Permissions	Submit copyright permission requests at: http://www.aai.org/ji/copyright.html
Email Alerts	Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/cgi/alerts/etoc

BRIEF REVIEWS

Cellular Interactions in Lymph Node Development

Tom Cupedo* and Reina E. Mebius[1][†]

The organized accumulation of lymphocytes is a biological phenomenon used to optimize both homeostatic immune surveillance, as well as chronic responses to pathogenic stimuli. During embryonic development, circulating hemopoietic cells gather at predestined sites throughout the body, where they are subsequently arranged in T and B cell-specific areas characteristic of secondary lymphoid organs. In contrast, the body seems to harbor a limited second set of selected sites that support formation of organized lymphoid aggregates. However, these are only revealed at times of local, chronic inflammation, when so-called tertiary lymphoid structures appear. Once thought of as two distinct phenomena, recent insights suggest that highly similar networks of paracrine interactions regulate the formation of both secondary and tertiary lymphoid structures. This review will focus on these cellular interactions between organizing and inducing cell populations leading to the formation of lymph nodes or organized inflammatory infiltrates. The Journal of Immunology, 2005, 174: 21–25.

FIGURE 1. Representative examples of developing peripheral lymph nodes at E16.5. *A* and *B*, $CD45^+CD4^+$ LTi cells colocalize with MAdCAM-$1^+ICAM-1^+$ organizer cells. *C* and *D*, Organizer cells also coexpress ICAM-1 and VCAM-1.

T he successful formation of lymph nodes depends on interactions between lymphotoxin $(LT)\beta R^2$-expressing organizing cells and $LT\alpha_1\beta_2$-expressing inducer cells (1–4). The inducer cells are believed to be hemopoietic cells that express the IL-7Rα chain and CD4, but lack CD3. These $CD45^+CD4^+CD3^-$ lymphoid tissue inducer (LTi) cells are among the earliest cells to be found within the embryonic lymph node anlagen (1, 2, 5), and were shown to be the inducer cells for Peyer's patches (PPs) and the nasal-associated lymphoid tissue (Fig. 1) (6, 7). Ligation of the LTβR on stromal organizers by LTi cells leads to the initiation of two sequential NF-κB signaling pathways, initiating the expression of adhesion molecules like ICAM-1, VCAM-1, and mucosal addressin cell adhesion molecule (MAdCAM)-1, and the production of homeostatic chemokines such CXCL13, CCL19, and CCL21 (8–10). As a result, circulating hemopoietic cells will subsequently be attracted to, and retained within, these lymph node anlagen (Fig. 1).

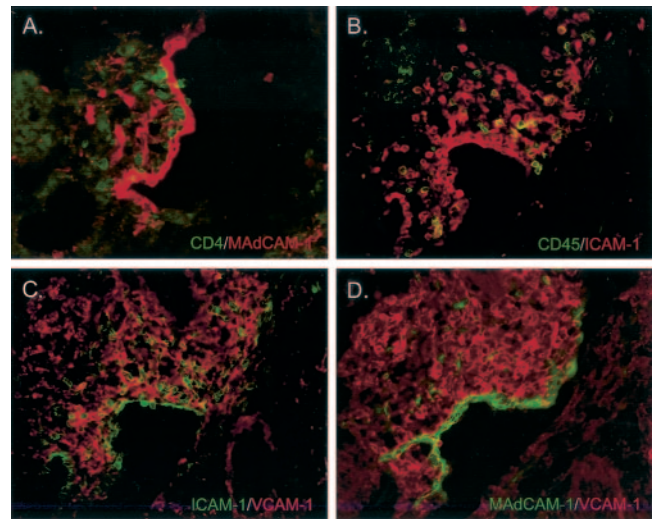Many of the gene products involved in lymph node genesis were identified through serendipitous observations made in gene-targeted mice that were generated for different reasons, and most genes are involved in processes like cellular differentiation and inflammation. This growing list of genes involved in lymph node formation can be divided into genes that influence formation or function of LTi cells, or those that affect the $VCAM-1^+ICAM-1^+$ stromal organizers.

Generation of functional LTi cells

Among the genes influencing formation of LTi cells, defective functioning of the nuclear retinoid orphan receptor (ROR)γ (11, 12), the negative regulator of basic helix-loop-helix (bHLH) protein signaling Id2 (13), and the TNF family member TNF-related activation-induced cytokine (TRANCE; also known as receptor activator of NF-κB ligand, osteoclast differentiation factor, or osteoprotegerin ligand) (14–18) all lead to either the absence (RORγ and Id2) or severe reduction (TRANCE) of LTi cells, resulting in aborted lymph node formation (Fig. 2). Whereas the instrumental role of these molecules in formation or accumulation of the LTi cells in lymph node anlagen is eminent, the exact mechanism by which they influence LTi cells remains largely unknown. The importance

*Department of Cell Biology and Histology, Academic Medical Center of the University of Amsterdam, and †Department of Molecular Cell Biology and Immunology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands

[1] Address correspondence and reprint requests to Dr. Reina E. Mebius, Department of Molecular Cell Biology and Immunology, Vrije Universiteit Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands. E-mail address: r.mebius@vumc.nl

[2] Abbreviations used in this paper: LT, lymphotoxin; LTi, lymphoid tissue inducer; MAdCAM, mucosal addressin cell adhesion molecule; PP, Peyer's patch; ROR, retinoid orphan receptor; bHLH, basic helix-loop-helix; TRANCE, TNF-related activation-induced cytokine; E, embryonic day; PDGF, platelet-derived growth factor.
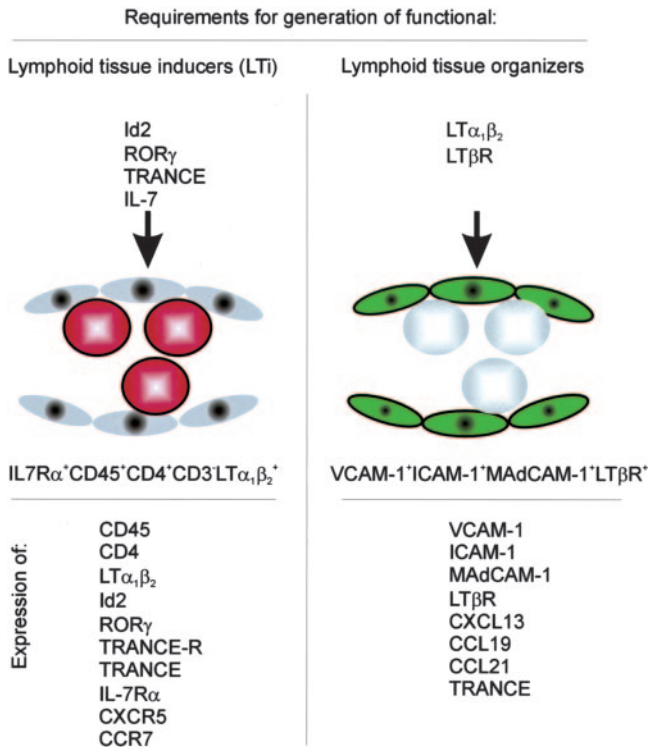
Requirements for generation of functional:

Lymphoid tissue inducers (LTi)	Lymphoid tissue organizers

Id2
RORγ
TRANCE
IL-7

LTα₁β₂
LTβR

$IL7R\alpha^+CD45^+CD4^+CD3^-LT\alpha_1\beta_2^+$

$VCAM-1^+ICAM-1^+MAdCAM-1^+LT\beta R^+$

Expression of:

CD45	VCAM-1
CD4	ICAM-1
LTα₁β₂	MAdCAM-1
Id2	LTβR
RORγ	CXCL13
TRANCE-R	CCL19
TRANCE	CCL21
IL-7Rα	TRANCE
CXCR5	
CCR7	

FIGURE 2. Requirements for the generation of functional lymphoid tissue inducers and organizers. For the generation of LTi cells, which are derived from IL-7R-expressing fetal liver precursors, expression of RORγ and Id2 by LTi cells is mandatory. Also, in the absence of TRANCE-R signaling, the number of LTi cells in the lymph node anlagen is severely reduced. Within the lymph node anlage, LTi cells mediate triggering of the LTβR on stromal cells through expression of LTα₁β₂, which is induced by ligation of either the TRANCE-R and/or IL-7R. Functional LTi cells need to express CXCR5 as well as CCR7, to respond to CXCL13, CCL19, and CCL21, chemokines involved in lymph node formation. To generate functional lymphoid tissue organizers, LTβR signaling leads to expression of MAdCAM-1, ICAM-1, and VCAM-1 on these cells, as well as the production of the chemokines CXCL13, CCL19, and CCL21. Other molecules expressed by LTi and stromal organizers are listed.

of Id2 implies the involvement of bHLH proteins, yet to date, no bHLH protein has been linked to negatively regulate development of LTi cells or lymph node formation. The role of RORγ is even more elusive, mainly as a result of the fact that signaling routes for this orphan receptor are largely unknown. However, the recent identification of the chromatin remodeler Mi-2β as a natural RORγ signaling repressor is likely to fuel research into the signaling events evoked by this nuclear receptor (19). In contrast to the phenotype of Id2 or RORγ mutant mice, deficient TRANCE-R signaling does not lead to an absence, but to a severe reduction of LTi cells in lymph node anlagen (16). The fact that these mice fail to develop most lymph nodes shows that a critical number of LTi cells is required to initiate lymph node formation. This is supported by the fact that, upon transgenic overexpression of TRANCE in hemopoietic cells of the TRANCE$^{-/-}$ mice, numbers of LTi cells increase, resulting in the formation of several lymph nodes (16). During embryonic development, TRANCE expression is found on LTi cells, but most notably on stromal cells within the developing lymph nodes (2, 16). Local production of TRANCE, together with the findings that PPs develop normally in the absence of TRANCE, implies that TRANCE acts locally by either attracting or differentiating LTi cells. Further-

more, it has been shown that TRANCE-R triggering leads to enhanced expression of LTα₁β₂ on LTi cells (20).

In the absence of the chemokine receptor CXCR5, LTi cells in mesenteric lymph nodes were shown to lack the activated form of β₁ integrin (6). According to the model presented by the authors, the function of CXCL13 in lymph node development is not restricted to the induction of chemotactic activity and additionally involves the generation of the activated form of β₁ integrin, allowing intimate interaction with stromal cells.

Several genes are involved in functioning of LTi cells, rather than development of these cells. Most of these genes encode proteins that are involved in ligation of the LTβR on stromal cells. Signaling via the IL-7R or via the TRANCE-R are two ways by which LTi cells can initiate surface LT expression (20). Defects in either one of these pathways, as well as absence of surface LT (21), results in severely defective lymph node development (Fig. 2). Despite the fact that TRANCE-R and IL-7R signaling seem to have a redundant function, their in vivo roles might, however, be very distinct. Mice lacking TRANCE-R signaling develop all PPs, while lacking most lymph nodes (14, 16). In contrast, IL-7R signaling-deficient mice lack all PPs, and develop only mesenteric and brachial nodes consistently (22–25), whereas other peripheral lymph nodes develop with variable incidence (25). This suggests that, although TRANCE can substitute for IL-7 in the development of several lymph nodes, the signals delivered by TRANCE cannot be replaced by IL-7R ligation, again suggesting an important role for TRANCE in the generation of LTi cells for lymph node formation.

Generation of functional organizer cells

Disturbed lymph node development by mutations influencing the stromal organizers is almost exclusively affecting LTβR signaling (Fig. 2). Upon LTβR ligation, two sequential signaling paths are initiated (10). The first signals via RelA, p50, and IκBα, and initiates expression of adhesion molecules such as VCAM-1 (10). Expression of these adhesion molecules facilitates the interaction of stromal organizers with LT$^+$ LTi cells, assuring sustained triggering of the LTβR by these cells. As a consequence of this prolonged signaling, the p100 precursor will be formed, leading to a second NF-κB pathway via NF-κB-inducing kinase, IκB kinase α, and RelB (8, 10). This signaling will lead to induction of homeostatic chemokines such as CXCL13, CCL21, and CCL19, which in turn will mediate the clustering of LTi cells (8, 9). In this way, a positive-feedback loop is instigated, which ensures the correct generation of lymph nodes. Defects in any component of these two pathways leads to the disruption of lymph node formation (reviewed in Refs. 26 and 27), most likely due to the inability of the lymph node anlage to attract and retain LTi cells and subsequently additional hemopoietic cells (Fig. 2).

The earliest events

At this point, not much is known about the earliest events of lymph node organogenesis. Lymph nodes are described to develop at locations where lymph sacs form. Lymph sacs are the earliest developments of the lymphatic vasculature that appear around embryonic day (E)10.5 by budding of endothelial cells that originated from the large vasculature in mice (28–31). For endothelial cell budding, as well as for the differentiation of these cells toward lymphatic endothelial cells, expression of the

homeobox gene *Prox-1* is required (28, 32). From the lymph sacs, the lymphatic vessels grow out, and by E15.5, the lymphatic vasculature is complete (28). At the location of the lymph sacs, connective tissue protrudes into these lymph sacs, forming the very first anlagen of the lymph nodes. At this moment, differentiation of mesenchymal cells into specialized cells that can initiate the formation of lymph nodes is expected, and one can assume that components involved in the specification of mesenchymal cells during the organogenesis or morphogenesis of other organs are involved here as well. As such, the platelet-derived growth factor (PDGF), fibroblast growth factor, as well as TGFβ (super)family of growth factors, which are crucial for the differentiation of mesenchymal cells important for organogenesis during embryonic development (33–37), are likely to be involved in the earliest phases of lymphoid organ formation. Regarding the PDGF family of growth factors, it is noteworthy that stromal organizer cells present early in PP anlagen have been described to express both PDGFRα as well as PDGFRβ (38). However, no molecules have been identified that direct the early specification of mesenchymal cells into specific lymph node organizer cells. Nevertheless, by drawing parallels to the formation of other organs, one can envision that signaling of these growth factors through their specific receptors may be involved in determining the locations of future lymph node development by inducing essential molecules on mesenchymal cells.

The mesenchymal specification of lymph node organizer cells is expected to occur independent from LTβR signaling, because LTβR-expressing stromal cells are present in normal numbers in rudimentary mesenteric lymph nodes from LT$\alpha^{-/-}$ mice at day of birth, although they lack expression of VCAM-1 (39). Therefore, the stromal cell differentiation toward lymph node organizer cells would at least consist of two separate lineage determination steps (Fig. 3). First, the mesenchymal cells differentiate toward stromal cells that produce TRANCE (2), and perhaps at this point are also induced to express LTβR and TNFR. Second, triggering of LTβR leads to the up-regulation of adhesion molecules,

such as VCAM-1, and the production of chemokines. Upon production of these molecules, stromal organizer cells mediate attraction and retention of hemopoietic cells, resulting in accumulation and clustering of cells. Other factors might be expressed or produced by these stromal cells, such as factors that mediate cell cycle arrest of LTi cells, described to lack expression of Ki67 during embryogenesis (5).

Differences between lymph nodes

Development of the various lymph nodes seems to depend on different requirements. In the absence of TRANCE, cervical lymph nodes can occasionally develop while no other lymph nodes are present (16). Also, when LTβ is missing, cervical and mesenteric lymph nodes can form (40, 41). Finally, in the absence of either CXCL13 or IL-7Rα, certain peripheral lymph nodes are present (22–25, 42), whereas these fail to form when mice are deficient for both CXCL13 and IL-7Rα (25, 43). This indicates that each set of lymph nodes develops in accordance with its own subtle interplay of various molecules, with a varying dependence on each individual component.

It can be envisioned that these variations can be traced back to differences in the stromal compartment of each lymph node set, as we recently showed for peripheral vs mesenteric neonatal lymph nodes (2). In line with this, stromal cells within cervical lymph nodes could differ slightly from stromal cells in other lymph nodes, which were shown to produce TRANCE (2). The stromal cells within cervical lymph nodes might therefore produce factors that mimic TRANCE, facilitating the generation of the cervical lymph nodes in the absence of this TNF family member.

The formation of mesenteric and cervical lymph nodes in LTβ-deficient mice could also be explained by differential representation of stromal subsets (40, 41). Because these subsets express different levels of LTβR (2), variations in other TNFRs, like TNFR1, are not unlikely. Signaling through these receptors can potentially lead to the induction of chemokines and adhesion molecules required for the accumulation of hemopoietic cells. The ligands that can form in the absence of LTβ such as LTα, TNF-α, or LIGHT homotrimers, or perhaps heterotrimers formed with these molecules, might induce stromal cells in mesenteric and cervical lymph nodes to participate in lymph node formation. This threshold will not be surpassed in other lymph node anlagen. Because only mesenteric lymph nodes are occasionally present in LT$\alpha^{-/-}$ mice, the ligands that can form in the absence of LTα are very inefficient in inducing the required components for lymph node formation. Also, blockade of both LTβR and TNFR signaling in utero resulted in a lack of all lymph nodes, whereas mesenteric nodes were still formed when only LTβR was blocked (3, 4). All these data indicate that these receptors are differentially represented on stromal cells of the various lymph nodes.

In the absence of CXCL13, some peripheral lymph nodes fail to develop (42). Again, the stromal subsets, differentially represented in each pair of lymph nodes, might produce other chemokines that can compensate for the lack of CXCL13. Depending on the relative contribution of the stromal subsets, this compensation may not always occur. Indeed, in the additional absence of the chemokines CCL19 and CCL21, CXCL13-deficient mice fail to form any peripheral lymph nodes, whereas mesenteric lymph nodes develop (25). Similarly, in the absence of both CXCR5 as well as CCR7, receptors for the chemokines
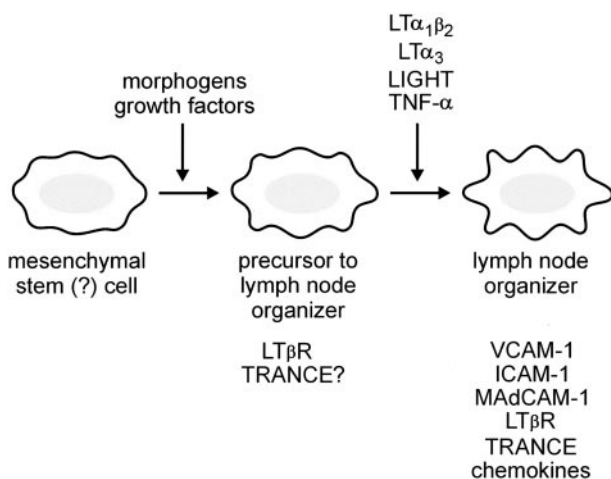

FIGURE 3. Hypothetical model for differentiation of organizer cells. Early in the specifications of mesenchymal cells, morphogens could play an important role in organogenesis by initiating the earliest differentiation toward organizer cells. It is unknown which molecules are involved and how many separate developmental steps can be distinguished. In the final differentiation step, LTβR triggering by LT$\alpha_1\beta_2$ leads to up-regulation of adhesion molecules and chemokines. Additional involvement of LIGHT, TNF-α, and LTα_3 can be expected.

CXCL13 and for the chemokines CCL19 and CCL21, respectively, a cooperative function of CXCR5 and CCR7 during lymph node formation was shown (43).

That the stromal cellular component exhibits subtle differences among the different lymph nodes could also result in a functional difference in adult life. For instance, this is indicated by the differential expression of peripheral lymph node addressin and MAdCAM-1 on high endothelial venules in peripheral vs mucosal lymph nodes (44, 45). This differential expression might be directed by the stromal cells within the lymph nodes (46). Furthermore, it has been shown that cervical lymph nodes that drain the nose mucosa have a specialized microenvironment that mediates immune tolerance when harmless Ags encounter the nose mucosa (47). Perhaps the differential requirements for development result in functional differences within the microenvironment of lymph nodes at distinct anatomical locations in adult life.

Stromal compartments in inflammatory lesions

The organizer cells identified in lymphoid organ formation have many of the characteristics of myofibroblasts that are associated with inflammation. Myofibroblasts are characterized by expression of α-smooth muscle cell actin, vimentin, and desmin, among other markers (48). They produce both cytokines and chemokines, while they also secrete extracellular matrix components. When activated, myofibroblasts express the adhesion molecules VCAM-1 and ICAM-1 (49–51). As such, the organizer cells identified in developing lymph nodes could be viewed as myofibroblasts: they produce chemokines and express ICAM-1 and VCAM-1 upon LTβR triggering. Furthermore, in adult lymph nodes, the expression of vimentin has been observed in reticular fibroblasts that colocalize with many extracellular matrix components (52, 53). Therefore, it is highly likely that the capacity of organizer cells to attract hemopoietic cells and orchestrate their spatial positioning during lymphoid organogenesis is a role that myofibroblasts fulfill in inflammatory lesions. In view of that, inflammatory lesions could be abridged to inducer and organizer cells, similar to the basic mechanisms of lymphoid organ development (54). In this scenario, the inducer cells would be hyperactivated lymphocytes that induce and subsequently continuously trigger myofibroblasts. In fact, rheumatoid fibroblast-like synoviocytes have been shown to overexpress the chemokine CXCL12, mediating the migration and accumulation of CXCR4-expressing T cells (49, 50, 55, 56). Moreover, the production of homeostatic chemokines has been reported in several autoimmune disorders (57–64). Also during inflammatory bowel disease, a central role for intestinal fibroblasts in attracting and retaining immune cells during inflammation was postulated (49, 50, 56).

Concluding remarks

It can be envisioned that various subsets of organizing stromal cells are also present within inflammatory lesions, similar to developing lymph nodes, and that this diversity accounts for the heterogeneity of tertiary lymphoid structure formation during autoimmune diseases. Comprehension of the basic concepts of lymph node genesis will shed new light on the organ-specific requirement for formation of organized inflammatory infiltrates, and might contribute to the understanding of these structures. Questions remain to be answered as to why tertiary lymphoid structures only form in a limited set of organs, and most important of all, whether they are an active part of pathology or whether formation of these structures is beneficial for disease outcome. Finally, revealing these basic components of disease might clarify why only a certain fraction of patients respond to therapies aimed at blocking signaling via TNFRs.

References

1. Mebius, R. E., P. Rennert, and I. L. Weissman. 1997. Developing lymph nodes collect CD4⁺CD3⁻ LTβ⁺ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity* 7:493.
2. Cupedo, T., M. F. R. Vondenhoff, E. J. Heeregrave, A. E. de Weerd, W. Jansen, D. G. Jackson, G. Kraal, and R. E. Mebius. 2004. Presumptive lymph node organizers are differentially represented in developing mesenteric and peripheral nodes. *J. Immunol.* 173:2968.
3. Rennert, P. D., J. L. Browning, R. Mebius, F. Mackay, and P. S. Hochman. 1996. Surface lymphotoxin α/β complex is required for the development of peripheral lymphoid organs. *J. Exp. Med.* 184:1999.
4. Rennert, P. D., D. James, F. Mackay, J. L. Browning, and P. S. Hochman. 1998. Lymph node genesis is induced by signaling through the lymphotoxin β receptor. *Immunity* 9:71.
5. Eberl, G., S. Marmon, M. J. Sunshine, P. D. Rennert, Y. Choi, and D. R. Littman. 2004. An essential function for the nuclear receptor RORγt in the generation of fetal lymphoid tissue inducer cells. *Nat. Immunol.* 5:64.
6. Finke, D., H. Acha-Orbea, A. Mattis, M. Lipp, and J. Kraehenbuhl. 2002. CD4⁺CD3⁻ cells induce Peyer's patch development: role of α₄β₁ integrin activation by CXCR5. *Immunity* 17:363.
7. Fukuyama, S., T. Hiroi, Y. Yokota, P. D. Rennert, M. Yanagita, N. Kinoshita, S. Terawaki, T. Shikina, M. Yamamoto, Y. Kurono, and H. Kiyono. 2002. Initiation of NALT organogenesis is independent of the IL-7R, LTβR, and NIK signaling pathways but requires the *Id2* gene and CD3⁻CD4⁺CD45⁺ cells. *Immunity* 17:31.
8. Dejardin, E., N. M. Droin, M. Delhase, E. Haas, Y. Cao, C. Makris, Z.-W. Li, M. Karin, C. F. Ware, and D. R. Green. 2002. The lymphotoxin-β receptor induces different patterns of gene expression via two NF-κB pathways. *Immunity* 17:525.
9. Ngo, V. N., H. Korner, M. D. Gunn, K. N. Schmidt, D. S. Riminton, M. D. Cooper, J. L. Browning, J. D. Sedgwick, and J. G. Cyster. 1999. Lymphotoxin α/β and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. *J. Exp. Med.* 189:403.
10. Muller, J. R., and U. Siebenlist. 2003. Lymphotoxin β receptor induces sequential activation of distinct NF-κB factors via separate signaling pathways. *J. Biol. Chem.* 278:12006.
11. Sun, Z., D. Unutmaz, Y. R. Zou, M. J. Sunshine, A. Pierani, S. Brenner-Morton, R. E. Mebius, and D. R. Littman. 2000. Requirement for RORγ in thymocyte survival and lymphoid organ development. *Science* 288:2369.
12. Kurebayashi, S., E. Ueda, M. Sakaue, D. D. Patel, A. Medvedev, F. Zhang, and A. M. Jetten. 2000. Retinoid-related orphan receptor γ (RORγ) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. *Proc. Natl. Acad. Sci. USA* 97:10132.
13. Yokota, Y., A. Mansouri, S. Mori, S. Sugawara, S. Adachi, S. Nishikawa, and P. Gruss. 1999. Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature* 397:702.
14. Dougall, W. C., M. Glaccum, K. Charrier, K. Rohrbach, K. Brasel, T. De Smedt, E. Daro, J. Smith, M. E. Tometsko, C. R. Maliszewski, et al. 1999. RANK is essential for osteoclast and lymph node development. *Genes Dev.* 13:2412.
15. Kong, Y. Y., H. Yoshida, I. Sarosi, H. L. Tan, E. Timms, C. Capparelli, S. Morony, A. J. Oliveira-dos-Santos, G. Van, A. Itie, et al. 1999. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 397:315.
16. Kim, D., R. E. Mebius, J. D. MacMicking, S. Jung, T. Cupedo, Y. Castellanos, J. Rho, B. R. Wong, R. Josien, N. Kim, et al. 2000. Regulation of peripheral lymph node genesis by the tumor necrosis factor family member TRANCE. *J. Exp. Med.* 192:1467.
17. Yasuda, H., N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, et al. 1998. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl. Acad. Sci. USA* 95:3597.
18. Lacey, D. L., E. Timms, H. L. Tan, M. J. Kelley, C. R. Dunstan, T. Burgess, R. Elliott, A. Colombero, G. Elliott, S. Scully, et al. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93:165.
19. Johnson, D. R., J. M. Lovett, M. Hirsch, F. Xia, and J. D. Chen. 2004. NuRD complex component Mi-2β binds to and represses RORγ-mediated transcriptional activation. *Biochem. Biophys. Res. Commun.* 318:714.
20. Yoshida, H., A. Naito, J. Inoue, M. Satoh, S. M. Santee-Cooper, C. F. Ware, A. Togawa, and S. Nishikawa. 2002. Different cytokines induce surface lymphotoxin-αβ on IL-7 receptor-α cells that differentially engender lymph nodes and Peyer's patches. *Immunity* 17:823.
21. De Togni, P., J. Goellner, N. H. Ruddle, P. R. Streeter, A. Fick, S. Mariathasan, S. C. Smith, R. Carlson, L. P. Shornick, J. Strauss-Schoenberger, et al. 1994. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science* 264:703.
22. Park, S. Y., K. Saijo, T. Takahashi, M. Osawa, H. Arase, N. Hirayama, K. Miyake, H. Nakauchi, T. Shirasawa, and T. Saito. 1995. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity* 3:771.

23. Cao, X., E. W. Shores, J. Hu-Li, M. R. Anver, B. L. Kelsall, S. M. Russell, J. Drago, M. Noguchi, A. Grinberg, E. T. Bloom, et al. 1995. Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. *Immunity* 2:223.

24. Adachi, S., H. Yoshida, K. Honda, K. Maki, K. Saijo, K. Ikuta, T. Saito, and S. I. Nishikawa. 1998. Essential role of IL-7 receptor α in the formation of Peyer's patch anlage. *Int. Immunol.* 10:1.

25. Luther, S. A., K. M. Ansel, and J. G. Cyster. 2003. Overlapping roles of CXCL13, interleukin 7 receptor α, and CCR7 ligands in lymph node development. *J. Exp. Med.* 197:1191.

26. Mebius, R. E. 2003. Organogenesis of lymphoid tissues. *Nat. Rev. Immunol.* 3:292.

27. Weih, F., and J. Caamano. 2003. Regulation of secondary lymphoid organ development by the nuclear factor-κB signal transduction pathway. *Immunol. Rev.* 195:91.

28. Wigle, J. T., and G. Oliver. 1999. Prox1 function is required for the development of the murine lymphatic system. *Cell* 98:769.

29. Sabin, F. R. 1902. On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic ducts in the pig. *Am. J. Anat.* 1:367.

30. Eikelenboom, P., J. J. Nassy, J. Post, J. C. Versteeg, and H. L. Langevoort. 1978. The histogenesis of lymph nodes in rat and rabbit. *Anat. Rec.* 190:201.

31. Bailey, R. P., and L. Weiss. 1975. Ontogeny of human fetal lymph nodes. *Am. J. Anat.* 142:15.

32. Wigle, J. T., N. Harvey, M. Detmar, I. Lagutina, G. Grosveld, M. D. Gunn, D. G. Jackson, and G. Oliver. 2002. An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. *EMBO J.* 21:1505.

33. de Caestecker, M. 2004. The transforming growth factor-β superfamily of receptors. *Cytokine Growth Factor Rev.* 15:1.

34. Mummery, C. L. 2001. Transforming growth factor β and mouse development. *Microsc. Res. Tech.* 52:374.

35. Ornitz, D. M., and N. Itoh. 2001. Fibroblast growth factors. *Genome Biol.* 2:RE-VIEWS3005.

36. Coumoul, X., and C. X. Deng. 2003. Roles of FGF receptors in mammalian development and congenital diseases. *Birth Defects Res. Part C Embryo Today* 69:286.

37. Hoch, R. V., and P. Soriano. 2003. Roles of PDGF in animal development. *Development* 130:4769.

38. Honda, K., H. Nakano, H. Yoshida, S. Nishikawa, P. Rennert, K. Ikuta, M. Tamechika, K. Yamaguchi, T. Fukumoto, T. Chiba, and S. I. Nishikawa. 2001. Molecular basis for hematopoietic/mesenchymal interaction during initiation of Peyer's patch organogenesis. *J. Exp. Med.* 193:621.

39. Cupedo, T., W. Jansen, G. Kraal, and R.E. Mebius. Induction of secondary and tertiary lymphoid structures in the skin. *Immunity. In press.*

40. Koni, P. A., R. Sacca, P. Lawton, J. L. Browning, N. H. Ruddle, and R. A. Flavell. 1997. Distinct roles in lymphoid organogenesis for lymphotoxins α and β revealed in lymphotoxin β-deficient mice. *Immunity* 6:491.

41. Alimzhanov, M. B., D. V. Kuprash, M. H. Kosco-Vilbois, A. Luz, R. L. Turetskaya, A. Tarakhovsky, K. Rajewsky, S. A. Nedospasov, and K. Pfeffer. 1997. Abnormal development of secondary lymphoid tissues in lymphotoxin β-deficient mice. *Proc. Natl. Acad. Sci. USA* 94:9302.

42. Ansel, K. M., V. N. Ngo, P. L. Hyman, S. A. Luther, R. Förster, J. Sedgwick, J. L. Browning, M. Lipp, and J. G. Cyster. 2000. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* 406:309.

43. Ohl, L., G. Henning, S. Krautwald, M. Lipp, S. Hardtke, G. Bernhardt, O. Pabst, and R. Forster. 2003. Cooperating mechanisms of CXCR5 and CCR7 in development and organization of secondary lymphoid organs. *J. Exp. Med.* 197:1199.

44. Streeter, P. R., E. L. Berg, B. T. Rouse, R. F. Bargatze, and E. C. Butcher. 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature* 331:41.

45. Streeter, P. R., B. T. Rouse, and E. C. Butcher. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107:1853.

46. Mebius, R. E., P. R. Streeter, S. Michie, E. C. Butcher, and I. L. Weissman. 1996. A developmental switch in lymphocyte homing receptor and endothelial vascular addressin expression regulates lymphocyte homing and permits CD4+CD3− cells to colonize lymph nodes. *Proc. Natl. Acad. Sci. USA* 93:11019.

47. Wolvers, D. A., C. J. Coenen-de Roo, R. E. Mebius, M. J. van der Cammen, F. Tirion, A. M. Miltenburg, and G. Kraal. 1999. Intranasally induced immunological tolerance is determined by characteristics of the draining lymph nodes: studies with OVA and human cartilage gp-39. *J. Immunol.* 162:1994.

48. Powell, D. W., R. C. Mifflin, J. D. Valentich, S. E. Crowe, J. I. Saada, and A. B. West. 1999. Myofibroblasts. I. Paracrine cells important in health and disease. *Am. J. Physiol.* 277:C1.

49. Musso, A., T. P. Condon, G. A. West, C. De La Motte, S. A. Strong, A. D. Levine, C. F. Bennett, and C. Fiocchi. 1999. Regulation of ICAM-1-mediated fibroblast-T cell reciprocal interaction: implications for modulation of gut inflammation. *Gastroenterology* 117:546.

50. Pang, G., L. Couch, R. Batey, R. Clancy, and A. Cripps. 1994. GM-CSF, IL-1α, IL-1β, IL-6, IL-8, IL-10, ICAM-1 and VCAM-1 gene expression and cytokine production in human duodenal fibroblasts stimulated with lipopolysaccharide, IL-1α and TNF-α. *Clin. Exp. Immunol.* 96:437.

51. Hellerbrand, C., S. C. Wang, H. Tsukamoto, D. A. Brenner, and R. A. Rippe. 1996. Expression of intracellular adhesion molecule 1 by activated hepatic stellate cells. *Hepatology* 24:670.

52. Kaldjian, E. P., J. E. Gretz, A. O. Anderson, Y. Shi, and S. Shaw. 2001. Spatial and molecular organization of lymph node T cell cortex: a labyrinthine cavity bounded by an epithelium-like monolayer of fibroblastic reticular cells anchored to basement membrane-like extracellular matrix. *Int. Immunol.* 13:1243.

53. Di Sciullo, G., T. Donahue, M. Schachner, and S. A. Bogen. 1998. L1 antibodies block lymph node fibroblastic reticular matrix remodeling in vivo. *J. Exp. Med.* 187:1953.

54. Nishikawa, S. I., H. Hashi, K. Honda, S. Fraser, and H. Yoshida. 2000. Inflammation, a prototype for organogenesis of the lymphopoietic/hematopoietic system. *Curr. Opin. Immunol.* 12:342.

55. Bradfield, P. F., N. Amft, E. Vernon-Wilson, A. E. Exley, G. Parsonage, G. E. Rainger, G. B. Nash, A. M. Thomas, D. L. Simmons, M. Salmon, and C. D. Buckley. 2003. Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor 1 (CXCL12), which supports distinct patterns and rates of CD4+ and CD8+ T cell migration within synovial tissue. *Arthritis Rheum.* 48:2472.

56. Rogler, G., C. M. Gelbmann, D. Vogl, M. Brunner, J. Scholmerich, W. Falk, T. Andus, and K. Brand. 2001. Differential activation of cytokine secretion in primary human colonic fibroblast/myofibroblast cultures. *Scand. J. Gastroenterol.* 36:389.

57. Salomonsson, S., P. Larsson, P. Tengner, E. Mellquist, P. Hjelmstrom, and M. Wahren-Herlenius. 2002. Expression of the B cell-attracting chemokine CXCL13 in the target organ and autoantibody production in ectopic lymphoid tissue in the chronic inflammatory disease Sjogren's syndrome. *Scand. J. Immunol.* 55:336.

58. Luther, S. A., A. Bidgol, D. C. Hargreaves, A. Schmidt, Y. Xu, J. Paniyadi, M. Matloubian, and J. G. Cyster. 2002. Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. *J. Immunol.* 169:424.

59. Takemura, S., A. Braun, C. Crowson, P. J. Kurtin, R. H. Cofield, W. M. O'Fallon, J. J. Goronzy, and C. M. Weyand. 2001. Lymphoid neogenesis in rheumatoid synovitis. *J. Immunol.* 167:1072.

60. Shi, K., K. Hayashida, M. Kaneko, J. Hashimoto, T. Tomita, P. E. Lipsky, H. Yoshikawa, and T. Ochi. 2001. Lymphoid chemokine B cell-attracting chemokine-1 (CXCL13) is expressed in germinal center of ectopic lymphoid follicles within the synovium of chronic arthritis patients. *J. Immunol.* 166:650.

61. Weninger, W., H. S. Carlsen, M. Goodarzi, F. Moazed, M. A. Crowley, E. S. Baekkevold, L. L. Cavanagh, and U. H. von Andrian. 2003. Naive T cell recruitment to nonlymphoid tissues: a role for endothelium-expressed CC chemokine ligand 21 in autoimmune disease and lymphoid neogenesis. *J. Immunol.* 170:4638.

62. Page, G., S. Lebecque, and P. Miossec. 2002. Anatomic localization of immature and mature dendritic cells in an ectopic lymphoid organ: correlation with selective chemokine expression in rheumatoid synovium. *J. Immunol.* 168:5333.

63. Hjelmstrom, P., J. Fjell, T. Nakagawa, R. Sacca, C. A. Cuff, and N. H. Ruddle. 2000. Lymphoid tissue homing chemokines are expressed in chronic inflammation. *Am. J. Pathol.* 156:1133.

64. Amft, N., S. J. Curnow, D. Scheel-Toellner, A. Devadas, J. Oates, J. Crocker, J. Hamburger, J. Ainsworth, J. Mathews, M. Salmon, et al. 2001. Ectopic expression of the B cell-attracting chemokine BCA-1 (CXCL13) on endothelial cells and within lymphoid follicles contributes to the establishment of germinal center-like structures in Sjogren's syndrome. *Arthritis Rheum.* 44:2633.