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Cellular Interactions in Lymph Node Development

Tom Cupedo* and Reina E. Mebius†‡

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.

The successful formation of lymph nodes depends on interactions between lymphotixin (LT)αβR²-expressing organizing cells and LTααββ-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 LTßR 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

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2 Abbreviations used in this paper: LT, lymphotixin; 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.
more, it has been shown that TRANCE-R triggering leads to enhanced expression of LTαβ, on LTβi cells (20).

In the absence of the chemokine receptor CXCR5, LTβi cells in mesenteric lymph nodes were shown to lack the activated form of β 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 β integrin, allowing intimate interaction with stromal cells.

Several genes are involved in functioning of LTβi 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 LTβi 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 LTβi cells for lymph node formation.

**FIGURE 2. Requirements for the generation of functional lymphoid tissue inducers and organizers.** For the generation of LTβi cells, which are derived from IL-7R-expressing fetal liver precursors, expression of RORγ and Id2 by LTβi cells is mandatory. Also, in the absence of TRANCE-R signaling, the number of LTβi cells is markedly reduced. Within the lymph node anlage, LTβi cells mediate triggering of the LTβR on stromal cells through expression of LTαβ, which is induced by ligation of either the TRANCE-R and/or IL-7R. Functional LTβi cells need to express CXCR5 as well as CCR7, to initiate expression of MadCAM-1, ICAM-1, and VCAM-1 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 LTβi and stromal organizers are listed.

**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 pathways 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 LTβi 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 LTβi 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 LTβi 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 lymphatic sacs, the lymphatic vessels grow out, and by E15.5, the lymphatic vasculature is complete (28). At the location of the lymphatic vessels, connective tissue protrudes into these lymphatic 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 for the differentiation of mesenchymal cells toward lymph node organizer cells (33–37). Regarding the PDGF family of growth factors, it is noteworthy that stromal organizer cells present early in PP anlagen are induced to express LTβR leading 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 subset 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 LTα−/− 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
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 induce 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 patholog- ogy 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 signalizing via TNFRs.

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