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The role of core TNF/LIGHT family members in lymph node homeostasis and remodeling

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Summary: Lymph nodes (LNs) maintain active homeostasis at steady state. However, in response to changes in the local environment, such as local infection, cancer, vaccination, and autoimmune disease, dramatic remodeling of LN occurs. This remodeling includes changes in size, lymph and blood flow, immune cell trafficking and cellularity, lymphatic and blood vessel growth and activation, as well as microarchitecture. Therefore, inflammatory conditions often lead to enlarged nodes; after local inflammation resolves, LNs actively regress in size and return to steady state. Remodeling of lymphatic vessels (LVs) and blood vessels (BVs) during both the expansion and regression phases are key steps in controlling LN size as well as function. The cells, membrane-associated molecules, and soluble cytokines that are essential for LV and BV homeostasis as well as dynamic changes in the expansion and regression phases have not been well defined. Understanding the underlying cellular and molecular mechanisms behind LN remodeling would help us to better control undesired immune responses (e.g. inflammation and autoimmune diseases) or promote desired responses (e.g. antitumor immunity and vaccination). In this review, we focus on how the closely related tumor necrosis factor (TNF) members: LIGHT (TNFSF14), lymphotoxin-αβ, and TNF-α contribute to the remodeling of LNs at various stages of inflammation.

Keywords: *endothelial cells, B cells, dendritic cells, cell trafficking*

Lymph nodes demonstrate plasticity

Lymph nodes (LNs) are important secondary lymphoid organs of the immune system. They are widely distributed throughout the body like sentinels to closely monitor and quickly respond to abnormal challenges in the corresponding draining tissue or organ. While LNs are genetically 'preprogrammed' and 'prepatterned' during ontogeny (1), their shape, architecture, and cellular make-up are not fixed. In fact, under various pathological conditions such as cancer, infection, and autoimmune disease, LNs are very plastic and undergo dramatic remodeling including changes in size, organization, lymph and blood flow, leukocyte trafficking, and lymphatic and blood vessels growth and function. Macroscopic LN hypertrophy or enlargement is one of the most commonly reported clinical signs of ongoing inflammation. During LN

remodeling, a series of coordinated events is set into motion: dendritic cells (DCs) carrying antigens from peripheral tissue are mobilized and migrate to draining LNs (DLNs), lymphocyte entry into DLNs is also increased, and lymphocyte egress is immediately shut down as early as within 1 h (2, 3). These steps are considered important to facilitate the increased probability of encounters between antigen-presenting cells and rare antigen-specific T and B cells. Thus, it is thought that LN remodeling favors the initiation and expansion phases of adaptive immune responses. In addition, dramatic changes in stromal cell function and number also accompany LN remodeling, steps that might play pivotal roles in regulating leukocyte trafficking (4–6).

Lymphatic vessels found within peripheral tissue are important conduit systems for leukocytes to migrate into DLNs. Blood vessels, especially high endothelial venules (HEVs), are critical channels for lymphocytes to enter DLNs from peripheral blood. Activation of cells comprising lymphatic vessels and HEVs leading to (lymph)angiogenesis and other changes quickly take place upon inflammatory insult (2, 4–8). Other LN stromal cells, such as fibroblastic reticular cells and follicular DCs (FDCs), are reported to be important for intranodal trafficking of T and B cells, respectively (9, 10).

Leukocyte and stromal cell changes during LN remodeling are a complicated interactive process involving multiple cell types and cytokines with finely regulated dynamics. Several cell types are implicated in the regulation of LN remodeling, including DCs, macrophages, mast cells and both T and B lymphocytes. Members of the tumor necrosis factor (TNF) family seem to play a critical role in the molecular mechanisms behind these processes, and we explore in detail how these family members act on each cell type to coordinately regulate LN remodeling.

TNF/LT/LIGHT pathways form a complicated cellular and molecular network

This review summarizes and discusses the current understanding of several key TNF family members, LT (lymphotoxin), LIGHT [homologous to LT, inducible expression, competes with herpes simplex virus (HSV) glycoprotein D for HSV entry mediator (HVEM), a receptor expressed on T lymphocytes], and TNF, in the context of LN remodeling. Our perspectives are also presented. *Fig.* 1 demonstrates the close TNF/LT/LIGHT core family members. As shown, many of them are widely expressed on different types of cells within different locations of the body. Furthermore, the multi-directional interaction between ligands and receptors makes the network even more complex. Several reviews have extensively discussed the complicated cellular and molecular network formed by TNF/LT/LIGHT family members and are not the focus of current review (11–13). LT β receptor (LTβR) is broadly expressed on both stromal cells and hematopoietic cells, including lymphatic endothelial cells, blood vascular endothelial cells, fibroblastic reticular cells (FRCs), FDCs (14, 15), DCs, macrophages, and mast cells. LTαβ, primarily in the form of α1β2 heterotrimer, a ligand of LTβR, is expressed at low levels on lymphoid cells, such as B and T lymphocytes, and can be upregulated upon activation. LIGHT, another ligand of LTβR, is mainly expressed on T lymphocytes and immature DCs. TNF, the prototypical TNF family member, is a strong pro-inflammatory cytokine and is broadly expressed. How this complicated cellular and molecular network orchestrates LN remodeling is currently an interesting topic in the field; studies that reveal the underlying mechanisms would shed new light on ways to control LN function, not only to dampen immune responses to regulate autoimmune diseases and uncontrolled inflammation but also enhance immune responses to promote anti-tumor immunity and vaccination efforts.

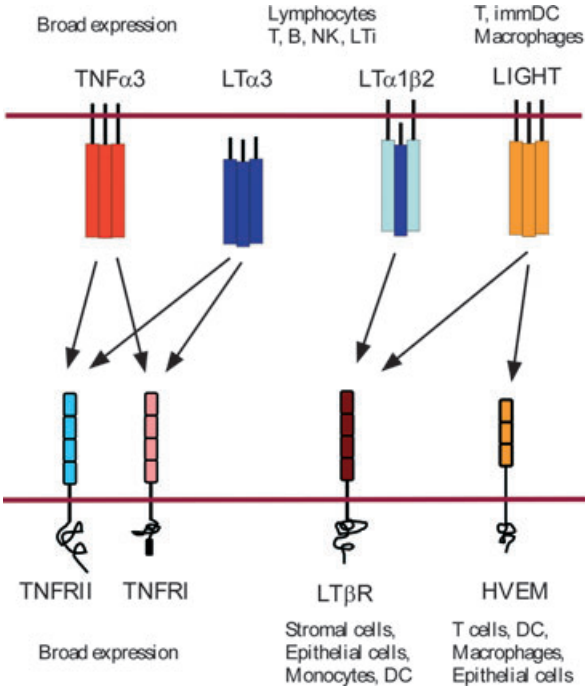

Fig. 1. Complicated cellular and molecular network of TNF/LT/LIGHT family members.

The role of LTβR in LN homeostasis and remodeling

LTβR has two ligands, LTαβ and LIGHT. The role of LTβR signaling has been well documented in lymphoid tissue organogenesis and neogenesis, most notably in tertiary

lymphoid tissue generation (1, 16, 17). It has been suggested that LTβR expressed on LN stromal cells transmits signals for their own proliferation/homeostasis and activation, such as increased expression of chemokines and adhesion molecules. These lymphoid tissue-produced chemokines and adhesion molecules can then control the migration and positioning of lymphoid tissue inducer cells and other cells that are required for the formation, development and organization of secondary lymphoid organs or tertiary lymphoid tissue (1, 17, 18). We and others showed that overexpression of lymphotoxin or LIGHT-mediated LTβR signaling in non-lymphoid tissues is sufficient to promote functional lymphoid neogenesis (1, 19). We also showed that overexpression of LIGHT in T cells can replace the role of LTαβ for the formation of some lymphoid microenvironment inside the spleen (20). Therefore, it seems that the timing and spatial effects of these molecules are more critical than the specific type of cells that express these ligands.

LTβR controls lymphatic vessel and HEV homeostasis, growth, and function

Lymphatic vessel (LV) and HEV maintain homeostasis under steady state and undergo dramatic (lymph)angiogenesis and activation during inflammation. LTβR signaling has been implicated in controlling LV and HEV during homeostasis, (lymph)angiogenesis and activation of the endothelial cells that make up the LV and HEV. In fact, short-term blockade (within 2 days) of LTβR signaling pathway using LTβR-immunoglobulin (Ig) fusion protein significantly reduces vascular endothelial cell proliferation (21). To experimentally distinguish between proliferation of HEV and LV, peripheral LN HEV endothelial cells can be marked by the expression of peripheral node addressin (PNAd), a group of sulfated glycoproteins that mediate part of the L-selectin-dependent entry of circulating cells. $PNAd^-$ cells include both lymphatic endothelial cells and endothelial cells of the arterioles and capillaries that are found both upstream and downstream of the HEV network. While both $PNAd^+$ and $PNAd^-$ endothelial cells demonstrated reduced proliferation to LTβR-Ig treatment, the $PNAd^-$ cells showed the greatest magnitude of reduction. Of note, PNAd expression level is not reduced with short-term LTβR blockade. However, prolonged treatment with LTβR-Ig dramatically reduces PNAd expression (22). Prolonged treatment with LTβR-Ig also dramatically reduces mucosal addressin cell adhesion molecule (MAdCAM) expression in those lymphoid organs, which is important because MAdCAM is an adhesion molecule primarily expressed on HEV in mucosal lymphoid organs, i.e. mucosal LN and PP. In addition, a range of genes involved in cell trafficking across the HEV are also dependent on LTβR. These genes include GlcNAc6ST-2, GlcNAc6ST-1, and FucT VII (22). Collectively, these data suggest an important role of LTβR on the homeostasis and functional status of LV and HEV under steady state conditions.

Several studies implicate the role of LTβR on LV and HEV remodeling in inflamed LNs. In a study by Liao *et al.* (14), the authors found that during oxazalone (OX) induced inflammation, PNAd and HEV related genes were inhibited by LTβR-Ig blockade, similar to what was obtained after LTβR-Ig blockade at steady state in Browning *et al.*'s work (22). Furthermore, Liao et al. (14) showed that the recovery of HEV is also dependent on LTβR signaling. In addition, the authors also found that LTβR signaling regulates the crosstalk between LV and HEV after immunization; this crosstalk function is speculated to be important for soluble factor exchange between the two vessels (14). Our own data also supports the important role of LTβR signaling in LN remodeling, as we found that systemic treatment with LTβR-Ig together with the complete Freund's adjuvant (CFA) intradermal immunization inflammation model significantly reduced DLN hypertrophy (our unpublished data).

LTβR directly and indirectly controls the function of LVs and HEVs

As mentioned previously, LTβR is expressed on both stromal cells and hematopoietic cells; LTβR signaling on stromal cells regulates expression of chemokines and adhesion molecules to maintain LN architecture as well as to attract naive lymphocytes to the inflamed LN (14, 22). Although quantitative data on LTβR-expressing FRC organization during LN expansion are lacking, qualitative data indicate that the FRC network grows with the increasing number of lymphocytes (9). These data suggest that signals from lymphocytes can regulate the FRC network. LTβR likely mediates at least some of those signals, as lack of LTβR signaling results in disorganized reticular stromal components in secondary lymphoid organs (1, 16, 22). Additionally, LTβR-expressing mast cells can indirectly affect LVs and HEVs in the LN due to LTβR-mediated activation of mast cell mediators, specifically TNF (23). Thus, the cell population or populations that require LTβR signaling for LN hypertrophy and how they respond during LN hypertrophy remain largely unclear.

LTβR and VEGF

Vascular endothelial growth factor (VEGF) is a major growth factor regulating growth of lymphatic and blood vascular endothelial cells. Interestingly, VEGF production has been

attributed to LTβR-expressing FRCs in the LN, and LTβR signaling seems both essential and sufficient for this VEGF production (21). Thus, LTβR signaling might regulate both LV and HEV homeostasis through VEGF pathway. In fact, VEGF-producing FRCs are in close proximity to both LV and HEV. VEGF blockade also reduces LV and HEV homeostasis, supporting the indirect role of LTβR on LN remodeling through FRCs affecting LV and HEV (lymph)angiogenesis. Whether VEGF regulates HEV functional status to increase lymphocytes trafficking per area remains unclear.

In the inflamed LN, VEGF remains the major regulator for LV and HEV growth. In the CFA-induced inflammation model, VEGF blockade can significantly inhibit lymphangiogenesis and angiogensis (4, 5). In Chyou *et al.*'s study (21), FRC production of VEGF in the inflamed LN is also inhibited by LTβR-Ig blockade. However, it is unclear as to what extent this mechanism of LTβR regulates LV and HEV growth under inflammation since many other sources of VEGF are identified in different studies (4, 5).

Given that there are so many potential sources of VEGF during inflammation as discussed above, it is still unclear whether LTβR can control VEGF production from macrophages, keratinocytes, or even B cells in addition to LTβR's known role on FRC. Since B cells do not express LTβR, it is unlikely LTβR directly controls VEGF production from B cells. Even if LTβR regulated VEGF production from macrophages, we argue that it does not play significant role for LN remodeling, as we found that there is no defect in LN hypertrophy after CFA immunization of $LT\beta R^{-/-}$ bone marrow chimeras of wildtype (WT) host mice (24). These data also indicate a role for LTβR on radioresistant cells to control LN remodeling. Therefore, the role of LTβR on radioresistant keratinocytes for VEGF production awaits further study using conditional knockout of LTβR in epithelial cells, such as K14-LTβR conditional knockout mice.

Angeli *et al.* found that markedly upregulated VEGF-A expression, but not VEGF-C, colocalized with B cells in CFA-induced inflamed LN. Furthermore, significant amounts of VEGF-A were detected from B cells activated for 48 h *ex vivo* with anti-CD40+IL-4 or anti-IgM+IL-4 (5). This finding contrasts with Chyou *et al.*'s finding that FRC remains the principal cells expressing VEGF mRNA in VEGF-lacZ transgenic mice. It is unclear whether the mRNA and protein level detection can explain the differences in results. Complicating the picture even further, Webster *et al.* (4) reported that recruited monocytes or LN resident cells, i.e. macrophages or mesenchymal cells, may also contribute to VEGF production. In fact, macrophages seem to be the main producer of VEGF-C, -D, and -A in skin and DLN during lipopolysaccharide (LPS) and LTA-induced inflammation (25). Depletion of macrophages or VEGF blockade reduce both dermal and DLN lymphangiogenesis (25). Furthermore, lymphangiogenesis could also be controlled remotely by inflammation in the tissue. In an oxazolone-induced delayed type hypersensitivity chronic ear inflammation model, VEGF-A is primarily produced by the inflamed ear, likely by keratinocytes, but not by DLN. Locally produced VEGF-A in the ear tissue induced significant lymphangiogenesis both in the ear and DLN; this process can be blocked by anti-VEGF-A (26). Although we have learned much from these experimental systems, the principal source of VEGF during inflammation still remains obscure. This could be due to following differences in experimental conditions among the various studies: (i) the different inflammatory stimuli and route of administration might preferentially activate different types of cells, or (ii) the different time points that were taken for the determination of lymphangiogenesis. It will be interesting to address whether LTαβ from B cells regulates VEGF creating the molecular basis for influxing cells leading to LN hypertrophy.

LTβR on endothelial cells

In addition to the potential role of LTβR on VEGF regulation, LTβR can directly regulate stromal cell function, especially those endothelial cells that form the LV and HEV. In fact, numerous studies have shown that LTβR expressed on stromal cells can directly regulate expression of molecules related to cell trafficking including adhesion molecules, chemokines, cytokines, etc. (27, 28). In terms of the role of LTβR on vascular endothelial cell activation, *in vitro* work showed that LTαβ and LIGHT can both activate cultured human umbilical vein endothelial cells (HUVECs) or human dermal microvascular endothelial cells (HDMECs) (29, 30). LTαβ and LIGHT induced the expression of classical NF-κB-dependent genes in HUVECs, including those encoding the adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). In addition, the homeostatic chemokine CXCL12 was also upregulated by LIGHT and LTαβ, but not TNF, in both HUVECs and HDMECs. These data therefore suggest that LTβR signaling on endothelial cells could directly influence their function and contribute to LN remodeling. Further *in vivo* study using endothelial cell-specific Tie-2-LTβR conditional knockout mice would help to clarify the role of direct LTβR signaling on endothelial cells in LN remodeling under inflammatory settings.

LTβR on other cells

In addition to FRCs and endothelial cells, other types of cells may be also important for LN remodeling, such as FDCs, whose function can also be directly regulated by LTβR and TNFR. FDCs form a dense network in the center of B-cell follicles in LNs and other secondary lymphoid organs. FDCs not only provide a scaffold for B cells, as during immune responses, FDC can also aggregate to support germinal center B cells for somatic hypermutation. LTβR plays a critical role on the maintenance of FDCs, since LTβR signaling blockade quickly collapses FDCs. FDCs are also the main source of CXCL13, a key chemokine dependent on LTαβ that attracts B cells into follicles. In fact, CXCL13 also upregulates LTαβ expression on B cells, thus creating a positive feedback loop (31). Given the important role of LTαβ and B cells in LN remodeling, we hypothesize that LTβR on FDCs is also important for LN remodeling. LTβR expression on FDCs may be important for LTαβ upregulation on B cells through CXCL13. Given the close proximity between B cells and LVs and HEVs, upregulated LTαβ on B cells might work on FRC for VEGF production or even directly modulate the status of LV or HEV.

Mast cells are first responders to environmental stimuli and play a key role in skin inflammation. They are one of the major producers of proinflammatory cytokines, such as TNF, IL-1β, in the skin (32). Mast cell-produced TNF is essential for *E. coli* infection-induced LN hypertrophy (33). This reaction is thought to be due to TNF-induced DC/Langerhans cell (LC) migration, which initiates LN remodeling and forms a positive feedback loop with B cells for full LN hypertrophy (4, 5). How mast cell activation itself is regulated during inflammation is not clear. It has been shown that a LTβR agonist or LIGHT can stimulate mast cells to produce various inflammatory cytokines, including TNF and interleukin-6 (23). This suggests that while mast cells promote migration of DCs/LCs, DCs/LCs themselves could also activate mast cells via LIGHT signaling, creating a positive feedback loop contributing to LN hypertrophy. DCs/LCs can also use mast cell-independent mechanisms. Further studies are required to determine whether mast cells require LTβR signaling for full activation, including TNF production, under *in vivo* inflammation conditions, and whether mast cell-derived TNF is essential for inflammation-induced LN remodeling.

LT and LIGHT are both important for LN remodeling

As mentioned previously, LTαβ and LIGHT are both LTβR ligands. The current literature suggests that LTβR engagement via LTαβ but not LIGHT is essential for normal lymphoid tissue organogenesis. Indeed, both LTαβ knockout (KO) and LTβR KO mice do not develop peripheral LNs, while LIGHT-deficient mice have normal lymphoid development and organization in LNs (34). In the presence of LTβ deficiency, LIGHT inactivation partially impair the development of mesenteric LNs, which are intact in normal LTβ-deficient mice, suggesting a potential cooperation between LIGHT and membrane LTαβ in LN organogenesis (34). Because of these LN development defects, the role of LTαβ and LTβR in LN hypertrophy has been difficult to study. However, LNs can be rescued in LTαβ KO mice by treatment with LTβR agonist antibody while mice are still in the embryonic stage (14, 22). This ability to rescue LNs in LTαβ KO mice also further supports the role of LTαβ-LTβR engagement in hypertrophy and HEV function seen previously in WT mice treated with LTβR-Ig to block LTβR signaling. Thus, LTαβ through LTβR engagement plays an essential role in regulating LN hypertrophy, while the role of LIGHT is thought to play a less significant role.

LIGHT, however, plays a role in leukocyte migration and lymphoid tissue formation if LTαβ is not available. Our data indicate that interaction between LIGHT and LTβR restores the splenic organization in LTαβ-deficient mice (35). We also found that overexpression of LIGHT within the tumor or pancreatic microenvironment was sufficient to induce neogenesis of lymphoid-like tissue (1, 36). Constitutive transgenic expression of LIGHT in the T-cell compartment leads to dramatically enlarged LNs, even without vaccination; this was initially considered to be due to a costimulatory effect of LIGHT on T cells via the HVEM receptor (37, 38), but more work should be done to determine whether engagement through LTβR also plays a role. Impressively, we recently found that LIGHT KO mice show a remarkable defect in LN hypertrophy after immunization with strong adjuvants, such as CFA (24). Thus, recent data reveal an interesting interplay for future study of how LTαβ and LIGHT can be both essential for LN hypertrophy through the same receptor, LTβR. We hypothesize that LTαβ and LIGHT can exert their functions in temporal and spatial patterns unique to each ligand-receptor interaction.

Emerging role of LIGHT in LN remodeling

LTαβ is expressed on activated T, B, and NK cells, while LIGHT is usually detected on immature DCs and T cells (15, 16, 39). However, in contrast to the common view that it is the radiosensitive DCs or T cells that express the LIGHT, data

from our recent study surprisingly show that LIGHT from a radioresistant source is essential for LN hypertrophy (24). However, the actual cellular source remains to be determined.

Peripheral DCs play a critical role for LN hypertrophy upon CFA immunization, shown by using the CD11c-DTR DC depletion system (4). Skin DCs are comprised of several subsets, including epidermal LCs and dermal Langerin-positive and -negative DCs (40). While LCs are radioresistant, both Langerin$^+$ dermal DCs and Langerin$^-$ dermal DCs are radiosensitive (40, 41). In the CD11c-DTR system, the major DC subset depleted by DT treatment is dermal DCs, while LCs are preserved due to low CD11c expression (42). Although LCs have previously been regarded as a paradigm of general DC behavior, they have been found in recent years not to comply with paradigmatic views of DC biology in terms of radioresistance, local homeostasis, and slower mobilization than expected after inflammation. Indeed, LCs were thought to quickly migrate in large numbers to the DLN during local inflammation, as a study using recombinant TNF found that exogenous TNF dramatically reduced epidermal LC number within 1 h of TNF administration (43). However, recent identification of the LC marker Langerin and subsequent experiments using Langerin-green fluorescence protein (GFP) mice demonstrated that fewer LCs actually migrate to DLNs with slower kinetics upon contact sensitivity induced inflammation (44–46). Thus, it remains unclear whether and how LCs are involved in LN hypertrophy upon immunization/infection.

To address some of these discrepancies in the literature and to determine whether LTβR regulates LN hypertrophy through LCs, we used a topical treatment of clobetasol propionate to deplete LCs in our study (47) to test whether LCs in the skin are required for LN hypertrophy. We found that LN hypertrophy was dramatically reduced after clobetasol propionate treatment. Furthermore, LIGHT expression actually increased on LCs after CFA immunization in stark contrast to conventional DC that reduce LIGHT expression after activation (39), further implicating LIGHT as a signal delivered by Langerhans cells to control LN hypertrophy. Of note, although these data are in line with our hypothesis that LC-derived LIGHT plays an important role at the early phase of LN hypertrophy, alternative explanations exist. For instance, topical clobetasol propionate treatment could lead to skin damage or have other effects on LN lymphatic vessels or other stromal cells, which may be critical for DC or lymphocyte migration. Therefore, a more conclusive answer about the role of LC-derived LIGHT in LN hypertrophy requires more specific tools, such as Langerin-DTR mice and LC-specific LIGHT KO mice.

One way LC-derived LIGHT could indirectly stimulate LN hypertrophy without migrating to the DLN is by activating other responder cells, such as mast cells, that deliver soluble factors to the DLN to stimulate lymphangiogenesis and hypertrophy. For example, mast cell and mast cell-derived TNF is required for LN hypertrophy induced by *E. coli* (33), as discussed above. Another way LC-derived LIGHT can regulate LN hypertrophy might be direct regulation of LV activation within the skin or the local inflammatory tissue in general. Indeed, in addition to its function as a costimulator for T cells, LIGHT can also function like a proinflammatory cytokine. LIGHT can activate vascular endothelial cells or other stromal cells in the skin directly through LTβR (30), thus helping to recruit more inflammatory cells and drive their migration to DLNs. We hypothesize that the role of LIGHT contribution to LN remodeling consists of functioning in the local tissue, in this case skin, at an early stage of induction of inflammation. Supporting this idea, our kinetic study using HVEM-Ig to block LIGHT signaling showed that blocking LIGHT at the early stage of CFA immunization (day 0), but not later (day 1), inhibits LN hypertrophy (24).

New views on LT in LN remodeling

How does the LTαβ pathway regulate LN remodeling? The lack of LN in the LTβ$^{-/-}$ mice makes this question experimentally difficult to answer. Although there are few lines of direct evidence to support it, current studies suggest that B-cell-derived LT plays an important role in LN remodeling. Indeed, LT is expressed on B cells, in addition to T, NK, and LTi cells (13).

B-cell-derived LTαβ plays a critical role on secondary lymphoid organogenesis, microarchitecture maintenance, and neogenesis (1, 48–50). Recently, several studies showed that B cells are essential for LN remodeling, since μMT mice showed dramatically reduced LN hypertrophy and lymphangiogenesis in the CFA-induced inflammation model (4, 5, 14). In the oxazalone (OX) immunization model, B cells are required for HEV recovery, as μMT mice have delayed HEV recovery after OX treatment. In the same model, B cells are also required for lymphangiogenesis at day 4 and day 7 post-OX treatment. However, at a later-stage post-OX treatment (day 14), lymphangiogenesis occurred even in the absence of B cells, indicating that factors in addition to those derived from B cells contribute to this later remodeling process (14). Consistent with the latter finding, B cells were not required for inflammation induced lymphangiogenesis in an independent study using the same OX inflammation

model (26). However, these authors did not check the lymphangiogenesis at time points other than day 9 after OX challenge. It is possible that temporal effect is critical, for example, a later and intranodal regulation by B-cell-derived LTαβ on LN remodeling in contrast to early regulation by LC-derived LIGHT. We hypothesize that LIGHT expression in the local inflammatory tissue triggers or amplifies the inflammation, during which innate cells are activated and proinflammatory cytokines and growth factors are secreted for local and remote control of LV and HEV growth and activation. We envision a model where this early LIGHT-mediated activation in HEV allows for more B-cell homing to the DLN as well as HEV upregulation of LTαβ; this, in turn, exerts its role through LTβR inside the DLN for further LV and HEV remodeling (*Fig. 2*).

In comparison with B-LTβ single KO mice that demonstrate defects in LN architecture, B-LT/T-LT double KO mice have additional defects in the structure of the marginal zone and in the development of FDCs in spleen (49). However, T-cell-derived LTαβ by itself plays no apparent role in secondary lymphoid organogenesis, as LN organogenesis and structure is normal in T-LTβ KO mice. Taken together, these results suggest that T-LTαβ might play a complementary but minor role to B-LTαβ during LN hypertrophy. Further study is needed to test whether this is the case.

LTi cell-derived LTαβ plays an essential role in LN organogenesis (50). Given its rare number in adult lymphoid tissues, however, its role on LN remodeling has not been actively tested until recently. In a recent study, $CD4^+$ $CD3^-$ cells that bear a phenotype like embryonic lymphoid tissue inducer (LTi) cells (52) express high levels of LTα, LTβ, and TNF and were identified to be linked with the organized B/T segregation observed in adult spleen (53). Retinoid-related orphan receptor γ $(ROR\gamma)^+$ LTi-like cells can also be observed within the cryptopatches of the adult gut, where they are postulated to promote the development of isolated lymphoid follicles in response to microbial stimulation (54). Thus, the possibility exists that an LT-expressing LTi-like cell regulates remodeling of spleen and/or mucosal associated lymphoid tissue; this is an interesting topic to pursue in the future. Overall, the temporal and spatial effects unique to each of the ligand-receptor pairs examined here demonstrate the beauty and finesse of LTαβ and LIGHT in the regulation of LN remodeling.

The role of TNF and TNFR in LN remodeling

Although the LTβR signaling pathway plays a more prominent role in secondary lymphoid organogenesis, TNF also plays an important role (13, 17). The TNF-TNFR1 system serves an important role as a sentinel cytokine produced by innate recognition pathways that are involved in promoting inflammatory

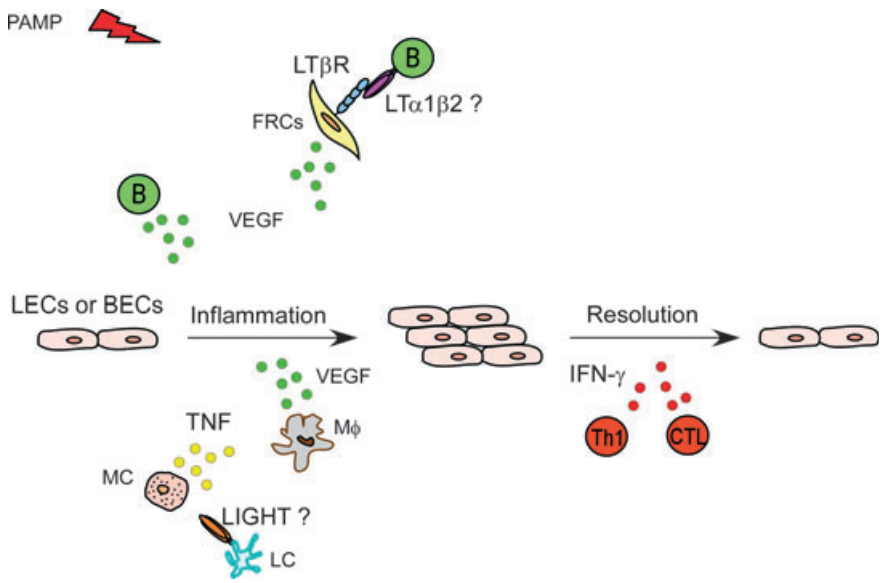

***Fig. 2.* Dynamic regulation of lymphatic vessels (LVs) and high endothelial venules (HEVs) by distinct immune cells during lymph node (LN) remodeling.** Upon local stimulation, such as pathogen-associated molecular patterns, B cells and innate cells such as macrophages are activated and produce vascular endothelial growth factor (VEGF), which promotes LV and HEV growth. The B-cell was also suggested to deliver lymphotoxin signaling to fibroblastic reticular cells for additional VEGF production. LIGHT, another ligand of LTβR, from radioresistant cells, likely Langerhans cells, was recently found to be important for LN remodeling. Langerhans cell-derived LIGHT likely stimulates mast cells to produce TNF for LV remodeling. At a later stage of inflammation, differentiated Th1 and CTLs inhibit LEC growth through IFN-γ, leading to LV regression.

processes (13). Thus, it is not surprising that TNF is also involved in inflammation-related LN remodeling. McLachlan *et al.* (33) first showed that TNF derived from mast cell is important for LN hypertrophy in an *E. coli* infection model. Kunder *et al.* (55) from the same group also revealed a novel mechanism by which mast cell secreted TNF is drained into DLN to control LN remodeling. They found that upon stimulation, mast cells quickly release stable submicrometer heparin-based particles containing TNF and other proteins (55). In our study, we also found that blocking TNF can significantly reduce LN hypertrophy in the CFA immunization model (24). More interestingly, local TNF production is dramatically reduced in LIGHT$^{-/-}$ mice, suggesting some relationship between LIGHT and TNF during local inflammation and coordination for LN remodeling (24). We hypothesize that LIGHT might be essential for mast cells to produce TNF that then promotes LN hypertrophy after CFA immunization. Thus, LIGHT might regulate local inflammation and LV remodeling by both direct and indirect pathways through LTβR or TNF, respectively.

The underlying mechanism of how TNF regulates LN remodeling is not completely understood. However, the published studies suggest a direct effect of TNF on vascular endothelial cells. Kunder *et al.* (55) found that mast cell released TNF-containing particles drained into LN via lymphatics, supporting a direct role of TNF on lymphatic endothelial cells. How can then TNF target HEV in the LN? The authors presumed that TNF can be slowly released from the particles while trafficking through the lymphatics to reach HEVs via conduits that connect the afferent lymphatics and sinus system to the HEVs (56). The direct effect of TNF on vascular endothelial cells is also supported by *in vitro* studies (29, 57), as described above.

Macrophages, DCs, and keratinocytes in the skin can also produce TNF, and their function in LN remodeling remains to be determined. Of note, adoptive transfer of activated bone marrow-derived DCs (BMDCs) induced vascular endothelial cell growth even in the absence of lymphocytes, which are the major cell population expressing LTαβ (4). It is unclear what ligand or cytokine BMDCs provide for HEV remodeling. However, VEGF seems not the factor BMDCs provide, since the VEGF production level by BMDCs is extremely low (4). Whether TNF itself could be the initiating factor by BMDCs remains to be determined in future.

Why does LN remodeling matter?

LN remodeling is frequently observed under various inflammatory conditions ranging from acute infection, to immunization, cancer, and autoimmune disease. These observations lead to some basic questions about LN biology. What is the biological role of LN remodeling? If we exploit the mechanisms underlying LN expansion and regression, can we modulate LN remodeling for clinical benefit? Even though DLNs are an important part of the immune system, it is difficult to demonstrate an essential role of DLNs because of the following reasons: (i) widely spread lymphatic vessels will reach local and distal LNs, which makes the location of essential LNs more difficult, (ii) although likely less efficient than DLNs, non-lymphoid tissues might replace some of the lymphoid tissue functions in priming T cells, and (iii) a significant number of antigen or non-antigen activated lymphocytes can likely respond to rechallenge outside lymphoid tissues, even in the absence of DLNs.

Infection

As mentioned previously, a major advantage of fully developed LN hypertrophy is that it provides a larger surface area to allow for more flow-through of and quicker access to a larger repertoire from which to screen antigen-specific lymphocytes. This would provide a significant immunological advantage upon pathogen infection. Since unchecked pathogens can replicate exponentially, a timely generation of a strong effector response would benefit the infected host. A fully developed LN hypertrophic state might facilitate the generation of an optimal effector response in terms of timing and strength. Several studies including ours seem to support this function of LN hypertrophy.

Martin-Fontecha *et al.* (6) found that the magnitude and quality of the CD4^{+} T-cell response is proportional to the number of antigen-carrying DCs that reached the lymph node. In our recent study (24), we also found that impaired LN hypertrophy in LIGHT$^{-/-}$ is associated with impaired T- and B-cell responses against immunogen, independent of costimulatory function of LIGHT. Interestingly, the defect of T- and B-cell responses become more dramatic when antigen dose is low. This further underscores the importance of optimal LN hypertrophy after pathogen invasion and more closely resembles natural infection conditions where pathogen load is usually much lower than in experimental animal models.

Tumor metastasis and tumor immunotherapy

Lymphangiogenesis within tumors has long been considered a contributing factor of tumor metastasis. Many studies provide evidence that enhanced lymphangiogensis in or around a tumor can promote tumor cell metastasis to regional LNs

(58, 59). Tumors also seem to release pro-lymphangiogenic factors that modulate regional LNs (60). In this way, the tumor cells prepare the 'soil' in the LN beforehand to render it more hospitable for secondary tumor formation (59). If so, blocking tumor and LN lymphangiogenesis might serve as a good way to prevent tumor metastasis. In fact, VEGF-targeted tumor therapy has long been investigated to achieve this goal (61).

In both preclinical and clinical settings, the benefits are at best transitory and are followed by a restoration of tumor growth and progression (62). This makes us reconsider the role of lymphangiogenesis in tumor growth/metastasis. Although the tumor-beneficial role has long been emphasized, lymphangiogenesis also promotes DC/T-cell migration. DC migration to the tumor might be helpful for tumor-associated antigen acquisition and migration to tumor DLN (TDLN), leading to better immune priming. Thus, appropriate stimulation of lymphangiogenesis could actually generate enhanced tumor immune response and promote the efficacy of tumor immunotherapy. How can we balance these two opposing effects of lymphangiogenesis? One way is to reveal in future studies the tumor or DC-specific mechanisms that control their trafficking to DLNs. We may also need to determine other ways to manipulate tumor cell trafficking or promoting anti-tumor immune response.

Perspectives

LN homeostasis and remodeling is a dynamic progress involving different cellular components at various times and locations and host environmental conditions. Previous work has been largely focused on its positive regulation, i.e. the mechanisms that promote LN homeostasis and remodeling. However, we must keep in mind that overt LN remodeling may not always be good. It could lead to unwanted, pathological immune damage to the body. Thus, negative regulation of LN remodeling at an appropriate time point is also necessary. Katatu *et al.* (63) recently extended our understanding of lymphatic vessel remodeling during inflammation, as they found that the neglected T cells in previous studies on positive LN remodeling actually play a critical role on the negative regulation of lymphatic vessels. Their study opened an interesting new direction for future study. Thus, we propose the following model (*Fig.* 2): Upon local inflammatory stimulation, B cells express higher level of LT$\alpha\beta$ that signals to fibroblastic reticular cells and vessels for VEGF production and promotes LV and HEV for more recruitments. LC-derived LIGHT likely stimulates mast cells or other cells to produce TNF and other cytokines for local LV remodeling. At later stage of inflammation, activated and differentiated T cells produce IFN-γ to dampen lymphangiogenesis thus guarding against excessive T cell response and immunopathology.

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