Deflating the Lymph Node

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Although the lymph node lymphatic vessel growth and development is positively regulated by B cells and macrophages, Kataru et al. (2011), in this issue of *Immunity*, show negative regulation of lymphatic vessels by interferon-γ from T cells.

Lymph nodes (LNs) can act as immunological filters that are strategically situated throughout the mammalian body to trap particles or antigens from peripheral tissue. LNs maintain active homeostasis in size and content at steady state. However, their size changes dramatically in response to changes in the local environment such as infection, cancer, and vaccination. These conditions lead to enlarged LNs followed by regression after the resolution of inflammation. Remodeling of lymphatic vessels (LVs) and blood vessels (BV) are thought to be a key step in controlling these changes. Which cells, membrane or soluble cytokines, are essential for lymphatic vessel homeostasis and dynamic changes in expansion and regression phases have not been well defined. Recent studies have reported that peripheral inflammation transiently induces robust lymphangiogenesis in draining LNs (Halin et al., 2007; Kataru et al., 2009; Liao and Ruddle, 2006). Vascular endothelial growth factors (VEGFs) are thought to mediate most of this LN lymphangiogenesis, and VEGF-A, -C, and -D are increased at the site of inflammation (Halin et al., 2007; Kataru et al., 2009). Various intranodal cellular components, such as B cells, stromal fibroblastic reticular cells, and macrophages, are also involved in the process of inflammatory LN lymphangiogenesis by secreting VEGFs (Angeli et al., 2006; Chyou et al., 2008; Kim et al., 2009). Rapidly moving T cells encounter their specific antigen inside draining LNs via LNLVs to initiate adaptive immune responses. Although T cells are one of the major cellular components and effector cells in the LN, their role in LN homeostasis or inflammatory remodeling has not been fully understood. A previous study found no role of T cells on LN enlargement or DC migration (Angeli et al., 2006). Intriguingly, Kataru et al. (2011), in this issue of *Immunity,* reveal a role for T cell-mediated negative regulation of LVs.

Kataru et al. (2011) first characterized LNLVs in athymic mice at steady state and found increased LNLVs as compared to euthymic mice, suggesting an inhibitory effect of T cells on LNLV homeostasis. However, this observation does not necessarily rule out other possibilities such as an unknown developmental defect in athymic mice or compensatory function of innate cells or B cells. Indeed, higher percentage of macrophages and dendritic cells were found in LNs of athymic mice. To address these concerns, T cells were depleted from naive wild-type (WT) mice via anti-CD3. As predicted, depletion of T cells markedly increased LNLV density. Interestingly, LNLVs regressed after T cells were restored. The gain-of-function experiment by adoptive transfer also demonstrated that either $CD4^+$ or $CD8^+$ T cells, but not B cells, were able to suppress LNLVs in athymic mice, indicating a direct impact of T cells on the LNs. The inhibitory impact of T cells is more prominent when they are activated. Thus, factors from activated T cells were speculated to suppress LNLV growth.

Interferon-γ (IFN-γ) is well known for its antiangiogenic effect (Ruegg et al., 1998). Kataru et al. (2011) hypothesized that T cell-derived IFN-γ is the LNLV-inhibitory factor. Indeed, IFN-γ treatment markedly reduced LNLV density. Furthermore, adoptive transfer of T cells from IFN-γ-deficient mice could not suppress the increased LNLV density in athymic mice. However, it is noteworthy that LNLV suppression still occurred in areas where IFN-γ-deficient T cells were heavily populated. These results together suggest that IFN-γ from T cells is a potent factor for LNLV suppression, but whether this is a direct or indirect role on the lymphatic vessels is less clear. Because very few T cells in the LNs of naive WT mice produce IFN-γ, it is intriguing that so few IFN-γ-producing T cells would have such a profound effect on LNLV suppression. Therefore, the T cell density and proximity to LNLV might be important.

Kataru et al. (2011) further tested the role of T cell-derived IFN-γ in an inflammation model induced by intradermal injection of LPS. WT and IFN-γ-deficient mice show similar LNLV increase at the expansion stage of LN upon LPS stimulation. During the resolving stage, although LNLVs markedly regressed in WT mice, the amount of LNLVs in IFN-γ-deficient mice remained elevated. To further rule out any possible indirect role of IFN-γ through innate cells or B cells, which are important for LN lymphangiogenesis, bone marrow transplantation and double adoptive transfer experiments were performed. IFN-γ receptor 1 expression on radioresistant cells (including LNLV endothelial cells) but not bone marrow-derived cells (including macrophages, DCs, and B cells) was required for LNLV regression at the resolving stage of LPS stimulation. Although these results support a direct role of T cell-derived IFN-γ on LNLV inhibition, it is still unclear whether stromal fibroblastic reticular cells and their VEGF production are involved (Chyou et al., 2008). Conditional deletion of IFN-γ receptor in the various cells would help to distinguish whether LNLV or fibroblastic reticular cells is the target. The authors further designed a series of in vitro experiments demonstrating a direct inhibition of IFN-γ on LNLV

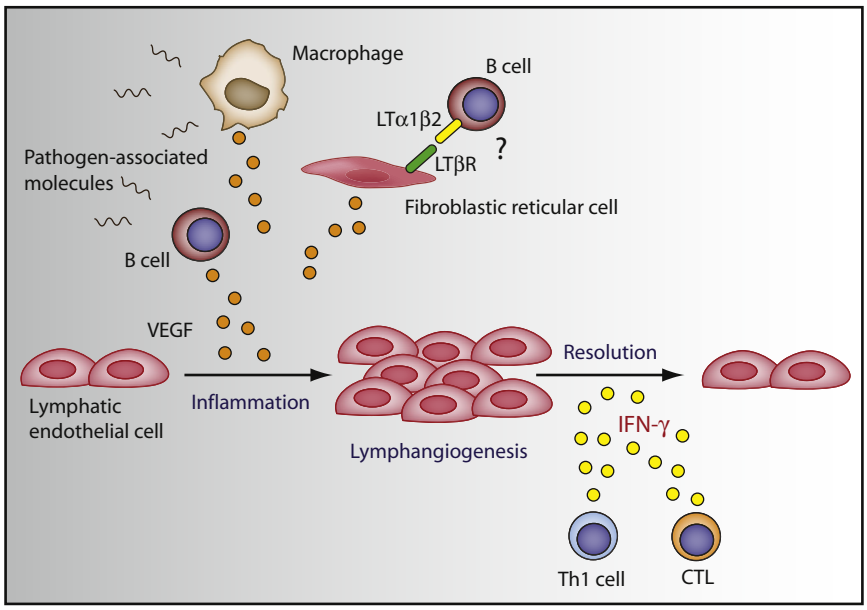

Figure 1. Dynamic Regulation of Lymphatic Vessels by Distinct Immune Cells
Upon local stimulation by pathogen-associated molecular patterns such as lipopolysaccharide or CpG DNA, B cells and innate cells such as macrophages are activated and secrete VEGF, which promotes lymphangiogenesis. We speculate that B cells also deliver lymphotoxin signaling to fibroblastic reticular cells for additional VEGF production. At a later stage of inflammation, differentiated Th1 cells and CTLs inhibit lymphatic endothelial cell growth through IFN-γ, leading to LV regression. Some unknown mechanisms may also negatively regulate VEGF production from B cells and FRCs. It is unclear whether other subsets of Th cells or other types of cells play any role for LV regression.

endothelial cell (LEC) growth. Prox-1, the master transcriptional factor of LECs, was markedly but not completely inhibited by T cell-derived IFN-γ on primary cultured LECs in vitro through the JAK1-STAT1 signaling pathway. This result suggests the presence of other T cell-derived factors on LNLV negative regulation.

What is the biological significance and implications of this work? LNLVs are used as conduits to facilitate the physical interaction between antigen-carrying DCs and their corresponding T cells (von Andrian and Mempel, 2003). An intriguing result of this study is that the elevated LV density in T cell-deficient instead of B cell-deficient LNs is associated with the increased recruitment of antigen-carrying DCs into the LNs. The biological function of LNLV regression for immune cell retention or exit is currently unknown. This IFN-γ-mediated LNLV suppression might also help to prevent excessive immune responses, which might be immunopathogenic, leading to autoimmunity or inhibiting memory T cell generation. In addition, are T cells and IFN-γ unique in this setting? NK cells migrate into LNs during early inflammation and they also produce IFN-γ (Martin-Fontecha et al., 2004). Could NK cell-derived IFN-γ also induce LNLV regression even before T cells? Although other sources of IFN-γ in the LNs are present early in the response, it could be that the LNLV does not regress because the corresponding positive signals that increase LN size are dominant at the initiation phase of the immune response. At late stages, however, the positive signals may subside, allowing for negative regulation of the LNLV by antigen-expended T cells to be dominant.

IFN-γ production from antigen-reactive T cells might indicate a well-developed T helper 1 (Th1) cell response, which is important for viral clearance, but too much IFN-γ could be detrimental to the body. From an evolutionary point of view, the host wants an optimal amount of IFN-γ in response to antigens or inflammation. If, for example, T cells are disregulated and produce too much IFN-γ, this negative-feedback mechanism controlling LNLV regression could be initiated to control the extent of inflammation- and/or antigen-specific immune responses. The current study reveals a role of T cell-derived IFN-γ on LNLV inhibition and raises many interesting questions. In addition to IFN-γ, can other cytokines play a similar role? Do IL4, IL-17, and IL-22 from other subset of T cells have similar function on LNLV? Does this effect on LNLV also apply to LN high endothelial venule (HEV) cells as well? Because HEV is the critical site where T cells migrate into LN, a negative-feedback mechanism on HEV itself would be more efficient. In addition, can effector Th1 cells control peripheral tissue vessels during their effector phase? This would be also helpful for the resolution of peripheral tissue inflammation.

LN homeostasis and remodeling is a dynamic progress involving different cellular components at various times and locations and host environmental conditions. Although B cells and some innate cells are critical for LN remodeling at the early stage of inflammation, T cells have now been found to be a critical negative-feedback regulator at the resolving stage (Figure 1). It is intriguing how this kinetic regulation occurs. One can speculate that B cells and innate cells can be activated in a nonantigen-specific way by danger signals and thus respond earlier during inflammation to produce VEGF. In addition, B cell-derived lymphotoxin could stimulate stroma fibroblastic reticular cells for additional VEGF production. As for T cells, they have to undergo antigen-specific activation and differentiation for enough IFN-γ production, which takes longer time and thus its function may display later. Perhaps an unknown negative regulation mechanism on VEGF production also exists to allow T cell-derived IFN-γ to dominate at the later stage of inflammation. Kataru et al. (2011) have extended our understanding about this process and raised a series of stimulating questions to be addressed in the future.

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Blood Relatives of Follicular Helper T Cells

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Expression of the chemokine receptor CXCR5 identifies B follicular helper T cells. In this issue of *Immunity*, Morita et al. (2011) describe a heterogeneous circulating CXCR5$^+$CD4$^+$ B cell helper population overrepresented in juvenile dermatomyositis patients.

Central memory (Tcm) and naive T cells populate T zones of secondary lymphoid organs, by virtue of expression of the chemokine receptor CCR7, L-selectin, and the integrin LFA-1. Tcm cells express IL-2 but are not necessarily polarized to T helper (Th) cell phenotypes. By contrast, effector memory T (Tem) cells are mostly polarized (Th1, Th2, and Th17 cells), secrete cytokines abundantly, have lost CCR7, and express receptors for chemokines upregulated in inflamed nonimmune parenchyma. In the past decade, B follicular helper T cells (Tfh cells) have been characterized as another type of CD4$^+$ helper T cell that specifically differentiate under the influence of the transcription factor Bcl-6.

Tfh cells exhibit effector function (help for B cells), and like other T effectors, have downregulated CCR7. Despite this, they are disproportionately located in secondary lymphoid organs rather than inflamed nonlymphoid parenchyma, but specifically within follicles rather than T zones (Campbell et al., 2003). This is due to loss of CCR7 and expression of CXCR5, the receptor for CXCL13, which is expressed constitutively by follicular stroma. CXCR5 is expressed by all mature B cells and by a small proportion of memory T cells, but the highest expression of CXCR5 is found on Tfh cells. Tfh cells secrete predominantly IL-21 and IL-4 and lesser amounts of IFN-γ and IL-17 (Yu and Vinuesa, 2010). To complicate matters further, there appears to be another population of T cells that is dependent on Bcl6, exhibits Tfh cell-like activity, but is located in extrafollicular plasma cell foci (Poholek et al., 2010). In this issue of *Immunity*, Morita et al. (2011) identify a subset of circulating CXCR5$^+$ T cells with potent B cell helper activity.

The ontogeny of Tfh cells remains unknown. It is not clear whether they differentiate from T cells shortly after priming, or whether early Th1, Th2, and/or Th17 cells can adopt follicular differentiation upon exposure to cytokines such as IL-6 and IL-21 in mice and IL-12 in humans. Their relation to CXCR5$^+$ T cells in blood also remains uncertain. These cells are absent from the blood in patients lacking ICOS, which suggests that they are related to Tfh cells (Bossaller et al., 2006). This is also supported by their abundance in mouse models characterized by excessive Tfh cell production in secondary lymphoid tissues (Simpson et al., 2010). It is possible that T cells with intermediate expression of CXCR5, shown to be potent IFN-γ and IL-17 producers (Yu and Vinuesa, 2010), could be precursors of GC Tfh cells (Figure 1). Earlier work showed that in humans, CXCR5$^+$ cells appear in the blood shortly after immunization, and CXCR5 is induced rapidly by naive and CD27$^+$ memory cells (but not CD27$^-$ Tem cells) (Schaerli et al., 2001). Compared with their tonsillar counterparts, circulating CXCR5$^+$ T cells appeared to offer little support for antibody production in coculture with autologous B cells in the absence of exogenous antigen (Schaerli et al., 2000).

Now, Morita et al. (2011) report that CXCR5$^+$ T cells from human peripheral blood provide better help to B cells than their CXCR5$^-$ counterparts. Circulating CXCR5$^+$ T cells are shown to be more effective in providing help to naive B cells at least in the presence of the superantigen staphylococcal enterotoxin B (SEB) (Morita et al., 2011). In coculture, B cells differentiate into plasmablasts and produced Ig within 6 days, in an IL-21- and ICOS-dependent manner. By contrast, CXCR5$^-$ populations are unable to induce any switched Ig and only small amounts of IgM. Furthermore, consistent with previous findings (Schaerli et al., 2001), the CXCR5$^+$ cells sampled from patients not recently vaccinated made