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Role of Lymphatic Vessels in Tumor Immunity: Passive Conduits or Active Participants?

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Abstract Research in lymphatic biology and cancer immunology may soon intersect as emerging evidence implicates the lymphatics in the progression of chronic inflammation and autoimmunity as well as in tumor metastasis and immune escape. Like the blood vasculature, the lymphatic system comprises a highly dynamic conduit system that regulates fluid homeostasis, antigen transport and immune cell trafficking, which all play important roles in the progression and resolution of inflammation, autoimmune diseases, and cancer. This review presents emerging evidence that lymphatic vessels are active modulators of immunity, perhaps fine-tuning the response to adjust the balance between peripheral tolerance and immunity. This suggests that the tumor-associated lymphatic vessels and draining lymph node may be important in tumor immunity which in turn governs metastasis.

Keywords Lymph node · Lymphangiogenesis · Inflammation · Autoimmunity · Cancer

Abbreviations

APC	antigen-presenting cell
CCL19/21	C-C chemokine ligand 19/21
CCR7	C-C chemokine receptor 7
CXCL13	C-X-C Chemokine ligand 13
DC	dendritic cell
FRC	fibroblastic reticular cell
LN	lymph node
TLO	tertiary lymphoid organ
T_{Reg} cell	regulatory T cell
VEGF	vascular endothelial growth factor

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Introduction

The lymphatic system is an extensive network of vessels that function to regulate tissue fluid homeostasis, immune cell trafficking and transport of dietary lipids [1]. Lymphatic vessels bring peripheral antigens and antigen presenting cells (APCs) like dendritic cells (DCs) to lymph nodes (LNs) where adaptive immunity can be initiated. This occurs either from lymph-borne antigen capture by B cells and lymph node resident APCs [2–4], or by the more classical route of peripherally-activated DC migration to the LN and activation of resident T cells [5]. Lymph nodes are also important centers for the maintenance of tolerance to self-antigens [4, 6–9].

For such functions, lymphatic vessels are traditionally considered to be passive conduits that deliver peripheral antigens and cells to the LN. However, new evidence is emerging that lymphatic vessels play very active roles in directing the initial steps of the immune response and sensing and responding to subtle changes in the peripheral microenvironment that may in turn alter LN functions in immunity and tolerance. Furthermore, evidence is emerging that indicates a coupling between lymphatic vessel function and LN function in tolerance and immunity.

Adult lymphangiogenesis occurs during inflammation and wound healing [1, 10], but is also strongly associated with chronic inflammation, autoimmunity, and allograft rejection [11–16] as well as with tumor invasion and metastasis when initiated both around the tumor and in the draining LN [17–21]. These are all associated with different immunological outcomes: from Th1 and Th2 inflammatory responses to immune escape and tolerance. Thus, the specific and functional roles of lymphatics and lymphangiogenesis in regulating the host immune response remain obscure.

Lymphatics pervade the interlobular connective tissue of the mammary gland during lactating periods and drain into collecting lymphatics, which run along mammary ducts [22–24]. During lactation the number of interlobular lymphatics and interendothelial gaps increase to promote transport [22]. Additionally, the lymphatics are the key route for LN and distal metastases, have altered flow patterns during cancer and increased levels of vascular endothelial growth factor C (VEGF-C) and its receptor VEGFR-3 lead to poor overall prognosis [25–27]. In this way, the lymphatics are active components of both normal tissue function and disease in the mammary gland; however, little is known about the dynamics of their function in this microenvironment.

In this review, we discuss recent insights into the immunological relationships between lymphatic vessels, their functional regulation, and the draining LN, with broad application to the biology of the mammary gland and tumors. We introduce new perspectives on how lymphatic vessels may help fine-tune the immune response, and present new perspectives on the potential role of tumor-associated lymphatics on tumor immunity and tolerance.

Lymphatic Physiology and Neogenesis

Lymphatic vessels are found in all vascularized tissues, with the exception of bone marrow and the central nervous system. Interstitial fluid drains into lymphatic capillaries (also known as initial lymphatics and terminal lymphatics) that are blind-ended vessels with a discontinuous basement membrane that lack pericytes. The interendothelial adhesions are maintained by discontinuous, “button-like” junctions serving to both freely drain interstitial proteins and also facilitate immune cell transmigration [28–31]. These overlapping cell-cell junctions serve as primary valves to prevent backflow from the lymphatic vessel into the tissue [32] and secondary valves prevent backflow within the vessel [33]. These capillaries drain into precollecting and collecting vessels that have continuous, “zipper-like” interendothelial junctions [30] and are surrounded by smooth muscle. Collecting vessels are organized into contractile segments called lymphangions, separated by bileaflet valves, that create the driving force for unidirectional lymph propulsion [30, 31, 34–36]. Collecting lymphatic vessels that carry lymph to and from the lymph nodes are referred to as afferent and efferent lymphatic vessels, respectively. Lymph typically passes through several lymph nodes before collecting in the thoracic duct where it is returned to the blood via connection with the great veins of the neck. Thus, the lymphatic vascular hierarchy is adapted to specifically promote the entrance of APCs and antigen-rich lymph into blind-ended capillaries and drive continuous, one-way movement of antigen and cells towards the draining LNs.

The initial lymphatic vasculature can be subdivided into the initial lymphatic capillaries and the precollectors, which link the capillaries to the collecting vessels. Interestingly, these segments express different levels of podoplanin that is associated with differential chemokine expression [37]. Lymphatic capillaries express high levels of podoplanin as well as the chemokine (C-C motif) ligand 21 (CCL21), which may bind podoplanin and which attracts activated ($CCR7^+$) antigen-presenting cells; the coexpression of podoplanin and CCL21 may have important implications for their specific function. On the other hand, precollectors express lower levels of podoplanin and secrete CCL27, which recruits memory $CCR10^+$ T cells. In this way, different immune cell types enter lymphatic capillaries at specialized sites [37].

Enhanced vascular permeability and leakage is observed during tissue injury and certain types of inflammation, thereby increasing the fluid load on the draining lymphatics. Both the increase of interstitial pressure and flow, as well as the change in cytokines and inflammatory mediators, stand to influence this drainage, yet the latter is only beginning to be explored [38, 39]. Transmural flow itself can activate the lymphatic endothelium, increasing fluid and solute permeability and uptake as well as upregulating adhesion molecules required for immune cell transmigration [40]. These changes can be observed at very low fluid flows of 0.1-1dyn/cm^2, which is well-correlated to the slow interstitial flows observed in normal and inflamed tissue. Flow also enhances the expression of CCL21 both in lymphatic vessels [40] and in the lymph node [41]. In addition to activating lymphatic endothelium, lymphatic drainage also facilitates interstitial flow, directed towards the lymphatics. This directional flow promotes cell homing by biasing pericellular autocrine chemokine gradients thereby driving DC and tumor cell chemotaxis towards draining lymphatics [42, 43]. Thus, lymphatics are dynamic sensors of acute biomechanical changes that accompany the onset of tissue injury and inflammation and are inherently coupled to immune cell trafficking and antigen transport to and within the draining LN [44].

In addition to regulating function, fluid flow is also an important regulator of lymphatic morphogenesis or lymphangiogenesis, and has been shown to drive lymphatic capillary organization in dermal wound healing models [45, 46] as well as in vitro culture models [47–49]. Without flow, as in the case of lymphedema, lymphatic endothelium becomes hyperplastic (i.e. the diameter of lymphatic capillaries increases while their density remains unchanged) [50]. In general, lymphatic hyperplasia and lymphangiogenesis have not been carefully distinguished in the literature, even though these are likely to have different effects on tissue fluid clearance and drainage to the lymph node[1, 28, 51]. Molecular mediators of lymphangiogenesis are well described in several recent reviews [1, 52, 53] and will not be discussed in detail here. Briefly, lymphangiogenesis is mainly associated with vascular endothelial growth factor (VEGF)-C and -D, VEGFR-3 ligands, and contribution from other factors such as VEGF-A and co-receptors VEGFR-2 and neuropilin-2 have also been reported. Blocking VEGFR-3 in adult mice, alone or in combination with VEGFR-2 blockade, can specifically and completely inhibit lymphangiogenesis [1, 52, 54, 55], and has been a useful tool to determine the effects of lymphangiogenesis on graft rejection, autoimmunity, and cancer metastasis, as described later.

Secondary Lymphoid Organs

Transport of lymph to the draining LN is achieved in such a way as to optimize the delivery of pathogenic signals, antigens, and immune cells to promote antigen capture by B cells and DCs, and facilitate T cell education and priming [5, 51] (Fig. 1). Lymph enters the subcapsular sinus of the draining LN through afferent vessels and moves through the medullary sinus via a network of conduits, formed by follicular dendritic cells in the B cell zone and fibroblastic reticular cells (FRCs) in the T cell zone, prior to leaving the LN via efferent vessels [56, 57]. FRCs express podoplanin (gp38) and the CCR7 ligands, CCL21 and CCL19; CCL21 is readily immobilized into the proteoglycan components of the extracellular matrix to form solid-phase gradients upon which DCs and T cells migrate [58, 59].

FRCs bundle collagen fibers to form 10–20 μm conduits that are in close proximity to an extensive network of DCs that directly sample antigen carried by the lymph for presentation to naïve T cells [60]. These conduits direct antigen and APCs towards high endothelial venules, a specialized vasculature that promotes the delivery of naïve T cells into the LN. This site of interaction between extravasating lymphocytes and mature APCs is important for initiating T cell-specific immunity.

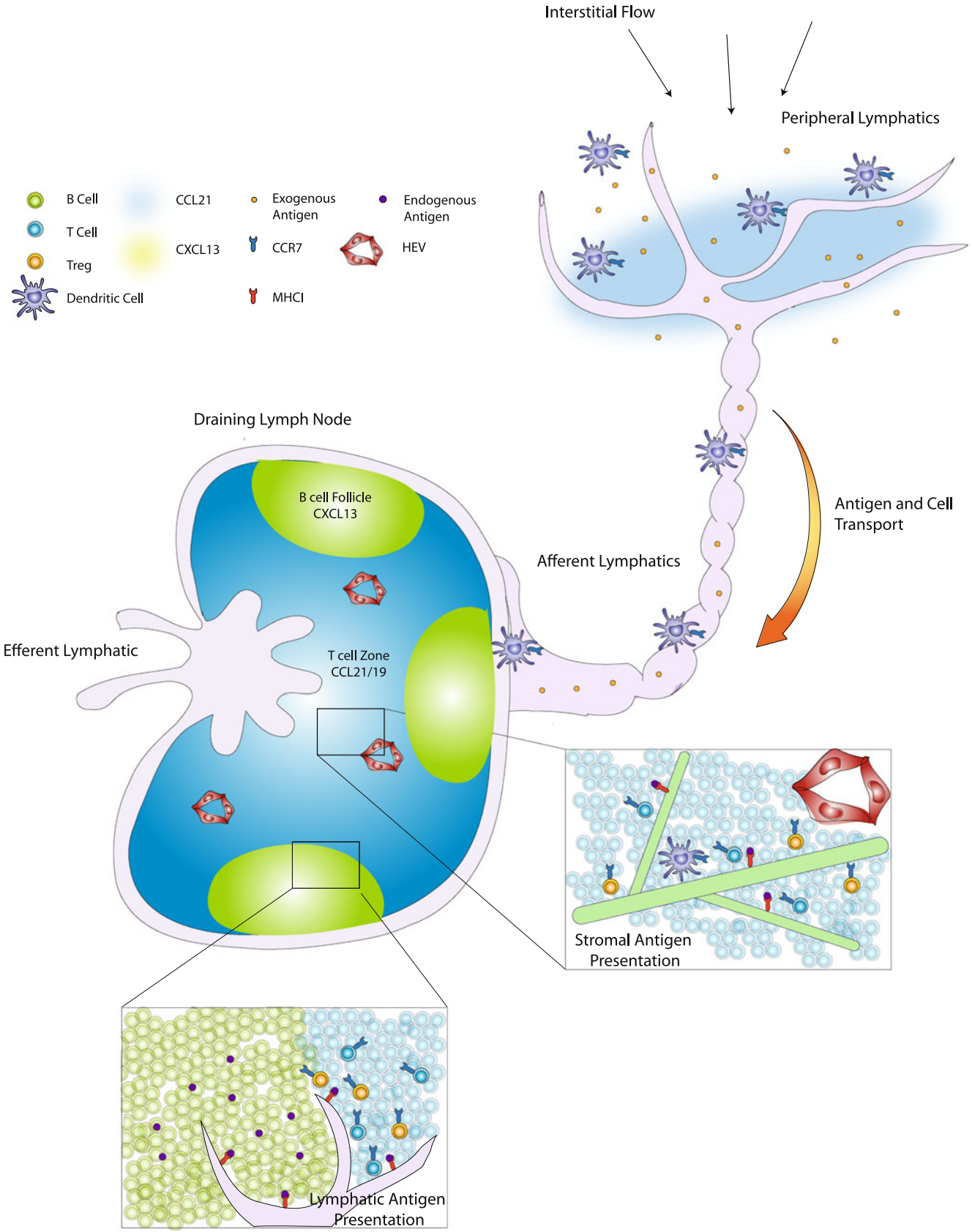
Fluid flow through this FRC/collagen network is required for proper 3D organization in vitro [41]. During acute inflammation or injury, lymph flow through the LN can be increased, dramatically enhancing CCL21 expression by FRCs [41] and accelerating the rate of APC and antigen delivery to the draining LN. Occlusion of the afferent lymphatic vessel, and consequent blockage of lymph flow into the LN, results in alterations in high endothelial venules (flattening of lumen and decreased luminal peripheral node addressin expression) and a subsequent decrease in lymphocyte extravasation from the blood [61]. Thus, the drainage function of peripheral lymphatic vessels is critical not only for peripheral tissue homeostasis and fluid balance, but also for proper LN organization and function.

Lymph node development and organization is accomplished mainly by the differential expression of CCL21 and CXCL13 in the T and B cell zones, respectively [62–65]. CCL21 and CXCL13 are ligands for CCR7 and CXCR5 (expressed by B cells), respectively, and are secreted by mesenchymal cells in the early developing lymph node to drive lymphoid tissue formation through the attraction of $CD3\varepsilon^{-}CD4^{+}IL\text{-}7R\alpha^{hi}$ $CCR7^{+}$ and $CXCR5^{+}$ lymphoid tissue inducer cells [62, 66]. The 3D organization of the lymph node is also driven and maintained by these cytokines; FRCs of the T cell zone express CCL21 and CCL19, which guide the interactions between $CCR7^{+}$ T cells and APCs, while follicular dendritic cells express CXCL13 to define and maintain zones of $CXCR5^{+}$ B cells [67]. Mice deficient in both CXCR5 and CCR7 lack peripheral lymph nodes and exhibit abnormal architectures of the deep mesenteric lymph nodes and the spleen [66].

LN architecture is conserved across anatomical locations, but the immune responses induced in different LNs can differ [68] as the homing capacity of T cells is dependent upon the draining LN in which they are activated. For example, T cells activated in the mesenteric lymph node express CCR9 and $\alpha_4\beta_7$ integrin and specifically home to the intestinal mucosa, whereas T cells activated by peripheral LN home to skin [68]. This specificity is unique to the LN stroma and cannot be replicated by resident DCs; for example, DCs extracted from the mesenteric LN can stimulate T cells in vitro to home to the gut, but when injected subcutaneously they instead direct T cell homing to the skin [69]. Therefore, the induction of specific and directed immune responses is attributable to innate functions of the LN stromal compartments.

Lymphoid Organs in Peripheral Tolerance

The compartmentalized expression of CCL19 and CCL21 in the paracortex of the LN is not only critical for the


Interstitial Flow
Peripheral Lymphatics
B Cell
T Cell
Treg
Dendritic Cell
CCL21
CXCL13
Exogenous Antigen
CCR7
MHCI
Endogenous Antigen
HEV
Draining Lymph Node
B cell Follicle
CXCL13
T cell Zone
CCL21/19
Efferent Lymphatic
Afferent Lymphatics
Antigen and Cell Transport
Stromal Antigen Presentation
Lymphatic Antigen Presentation

◀ **Figure 1** Lymphatic-mediated antigen and cell transport to the draining lymph node primes the resident stroma and lymphatic endothelial cells for antigen presentation. Peripheral lymphatics collect soluble antigen and secrete CCL21 to attract activated dendritic cells (DCs), which use lymphatics for transport to the draining lymph node (LN). Lymph is drained through the subcapsular lymphatics and through the fibroblastic reticular cell conduit network bathing the LN in antigens and peripherally expressed inflammatory cytokines. Lymph exits the LN through efferent lymphatic vessels and passes through several lymph nodes prior to recycling back into the blood vasculature. Peripherally activated DCs migrate along the $CCL21^{+}gp38^{+}$ stromal conduit networks to educate naïve $CCR7^{+}$ T cells. Resident immature DCs also lie close to this stromal network and sample antigen draining through the LN. $CCR7^{+}$ regulatory T (T_{Reg}) cells similarly require this $CCL21^{+}$ stroma to migrate through the LN and become activated while efficient $CD8^{+}$ T cell responses can occur outside of the LN. FRCs endogenously express peripheral antigen on MHC class I molecules to promote tolerance through $CD8^{+}$ T cell deletion. Similarly, $CCL21^{+}gp38^{+}LYVE1^{+}$ lymphatic endothelial cells express endogenous antigen for T cell deletion. Thus, the tolerance-maintaining functions of the draining lymph node are dependent upon peripheral tissue drainage and local non-hematopoeitic cell populations

correct positioning of APCs with T cells, but also required for its tolerance-maintaining functions [62]. Mice lacking CCR7 show impaired ability to maintain tolerance to peripheral antigens as they develop signs of autoimmunity [70, 71]: they exhibit lymphocyte infiltration in peripheral organs, elevated levels of circulating antibodies towards tissue-specific antigens, IgG deposition around the renal glomeruli, and increased susceptibility to inducible diabetes, and they can spontaneously develop chronic autoimmune renal disease [70]. These mice, as well as those lacking CCL19 and CCL21 (*plt* mice), which lack recognizable T cells zones within all LNs [72–76], can still mount strong cellular immune responses [6].

This is consistent with the mounting evidence that although B cell responses require their residence in functional lymph nodes, T cell immunity apparently does not, and T cells can be activated in the spleen or even liver when lymph nodes are absent or dysfunctional (reviewed in [77]). For this reason, proper LN function may be more indispensible for peripheral tolerance than cellular immunity. Unlike $CD8^{+}$ effector cells, $FoxP3^{+}$ T_{Reg} cells require LN occupancy and CCR7 signaling for their activation and function [78–80]. T_{Reg} cells sequentially migrate between peripheral tissues and LNs to both inhibit DC migration from the periphery as well as prevent effector T cell migration, activation and proliferation [81]. In the inflamed peripheral tissues, T_{Reg} cells are activated, secrete transforming growth factor β and interleukin 10 (IL-10) and then migrate to the draining LN in a CCR7-dependent manner [81]. Upon reaching the LN, T_{Reg} cells preferentially migrate to the paracortex where they interact with $CD8\alpha^{+}$ DCs and tissue derived $CD11b^{-}CD8\alpha^{-}$ DCs [82]. $CD8\alpha^{+}$ DCs, which are critical for peripheral cross-tolerance to soluble antigen, cluster with T_{Reg} cells in the paracortical area of the LN [60, 82]. The role of CCR7 in coordinating these interactions is again highlighted by studies in $CCR7^{-/-}$ mice, which exhibit impaired T_{Reg} cell positioning and loss of their ability to promote tolerance and prevent autoimmune pathologies [83, 84]. In addition, both $CCR7^{+}MHCII^{+}CD86^{+}$ DCs as well as $CCR7^{+}$ T_{Reg} cells are required for the optimal induction of a tolerance response, implying that CCR7 critically functions to bring these two cell types together within the context of the LN stroma [84].

The tolerance-maintaining functions of the LN are also facilitated by stromal cells, which express adhesion molecules and chemokines that shape cell migration routes, fluid distribution and tissue-specific immune responses (Fig. 1). In LN transplantation experiments, where the hematopoietic cells of the LN were completely replaced by host cells while the stromal compartments remained donor derived, the specific T cell homing responses induced were those of the original location, rather than the transplanted location [9, 69, 85]. Additionally, while removal of the cervical LN blocked the induction of musosal tolerance, rescue could be achieved by transplanting a donor cervical LN but not mesenteric or peripheral LNs [86]. The stromal cells maintain peripheral tolerance functions through the constitutive expression of relevant peripheral tissue antigens presented on MHC class I molecules for $CD8^{+}$ T cell deletion [87]. This mirrors the central tolerance maintaining functions of medullary thymic epithelial cells, which exhibit promiscuous gene expression and thereby express antigen from all tissues of the body to deactivate self-reactive T cells through deletion (recessive tolerance) and T_{Reg} cell induction (dominant tolerance) [88]. Thus, the LN stromal cells hold intrinsic, tissue-specific capabilities that likely play similar roles in peripheral tolerance as thymic epithelial cells play in central tolerance [7, 89–91].

Recently, it has been shown that lymphatic endothelial cells in the LN also present endogenous antigen on MHC class I molecules (Fig. 1), and this tolerance function is independent of the autoimmune regulator *Aire* [8]. Specifically, peripheral LNs draining the skin were shown to express the antigens tyrosinase and melanocytic differentiation antigen, leading to deletional tolerance of autoreactive $CD8^{+}$ T cells. Thus, lymphatic vessels themselves are likely to be important players in maintaining tolerance to self-antigens, particularly after tissue injury. This is achieved both through their control over peripheral drainage, which provides constant antigen sampling to the largely immature APC population that resides in the draining LN [44], as well as their ability to directly present endogenous antigens for deletional tolerance [8].

Together, these recent findings provide a new perspective on the role of LN lymphangiogenesis, which occurs in

LNs draining inflamed [14, 16, 92], immunized [14, 93], and tumor-bearing tissues prior to metastasis [18, 19]. LN lymphangiogenesis leads to increased DC trafficking from the periphery and is dependent on LN resident B cells [14]. Both VEGF-C and VEGF-A promote LN lymphangiogenesis when secreted by lymphoid tissue inducer cells and B cells respectively [92, 94]. In addition, VEGF-A produced by chronically-inflamed tissue induces lymphangiogenesis in the draining LN which, taken with lymphangiogenesis at the periphery, may enhance the immune response through trafficking of macrophages and dendritic cells [14, 16]. The lymph node itself, therefore, acts as an early downstream signal to promote peripheral changes in immune cell trafficking and lymphatic expansion. In light of the new tolerance-maintaining functions of LN lymphatic endothelium described above [8], it is intriguing to consider the possibility that lymphangiogenesis in the LN draining inflamed or tumor-bearing tissue might contribute to tumor tolerance, if these newly formed lymphatic vessels also express antigen on MHC class I molecules.

Tertiary Lymphoid Organs

While secondary lymphoid tissues can be remodeled during acute inflammation to alter lymph flow, lymph content, blood flow and high endothelial cell differentiation [63, 95], de novo lymphoid tissue formation occurs during states of chronic inflammation. These tertiary lymphoid organs (TLOs) are characterized by lymphocyte infiltrates, B cell follicles and defective fluid drainage [96]. TLOs have been observed in a variety of autoimmune disorders including autoimmune thyroid disease, rheumatoid arthritis and Crohn's disease [62, 97] (Fig. 2). The defining characteristic of autoimmune-related TLOs are their germinal centers that produce auto-antibodies [96]. Furthermore, as B cells stimulate lymphangiogenesis (mentioned earlier), such TLOs are often also associated with lymphangiogenesis. In renal interstitial disease, for example, lymphangiogenesis is stimulated around the newly formed follicles and contributes to the formation of intrarenal lymphoid follicle-like structures [98]. The crosstalk between developing B cell follicles and the lymphangiogenesis that it induces may play an important role in promoting mature follicle formation and remains an interesting question to address.

Just as with LNs, TLO formation can be induced through overexpression of CCL21, CCL19 and CXCL13 in a tissue-specific manner [65, 99, 100]. Furthermore, they may accumulate antigen and APCs, bypassing the LN and therefore restricting normal LN function [62, 101]. Such circumvention of the normal LN may prevent the induction of an appropriate immune response to foreign antigen or, since the LN has important tolerance-maintaining functions described earlier, result in autoimmunity (Fig. 2). Indeed, the role of peripheral TLO neogenesis as either a pathology to target therapeutically or an important protective mechanism of immunity remains controversial. For example, while TLOs have been shown to sequester pathogen and prevent its systemic spread during bacterial infection, they may also contribute to lymphoma development, prion accumulation and autoimmunity [62]. Importantly, the way that TLOs affect lymphatic drainage to bypass relevant LN and thereby circumvent their tolerance-maintaining function is poorly understood. Further research into this question will likely lead to therapeutic strategies for autoimmunity and has potential to strongly impact cancer research, where increased lymphatic drainage seems to promote tumor growth (implying an inhibition of anti-tumor immunity).

Pathological Lymphangiogenesis

Chronic Inflammation

Lymphangiogenesis has long been associated with chronic inflammation. Inflammatory lymphangiogenesis can be driven by immune cell-released VEGF-C, and inflammatory stimuli promote lymphatic endothelial cell susceptibility to VEGF-C through the upregulation of VEGFR3 and Prox-1 [102]. Tissue necrosis factor α, as well as newly recruited macrophages and granulocytes, can enhance the local expression of VEGF-C within inflamed tissue to promote lymphangiogenesis [103]. Another pro-inflammatory cytokine, interleukin (IL)-1β, promotes lymphangiogenesis by stimulating VEGF-A, VEGF-C, and VEGF-D [104]. In addition to the inflamed tissue, lymphangiogenesis also occurs in the LN draining this tissue [14, 16, 51].

Inflammation-associated lymphangiogenesis may be important for the clearance of immune infiltrates; for example, inhibiting lymphangiogenesis by VEGFR-3 blockage exacerbated pulmonary edema caused by chronic *myocplasma pulmonis* infection and prevented the resolution of inflammation [103]. On the other hand, in cases of chronic inflammation, the extensive lymphatic remodeling that occurs can have detrimental affects on normal immune function [1, 105, 106]. Hyperplastic lymphatics in chronic diseases such as psoriasis, inflammatory bowel disease, chronically-inflamed skin disease and rheumatoid arthritis have negative implications for disease resolution. For these conditions, treatment with anti-VEGF antibodies can, in some cases, promote disease resolution [106–109]. Furthermore, while vascular angiogenesis can be reversed in chronic inflammation, lymphangiogenic structures seem to persist [103, 110]. This implies that while the blood

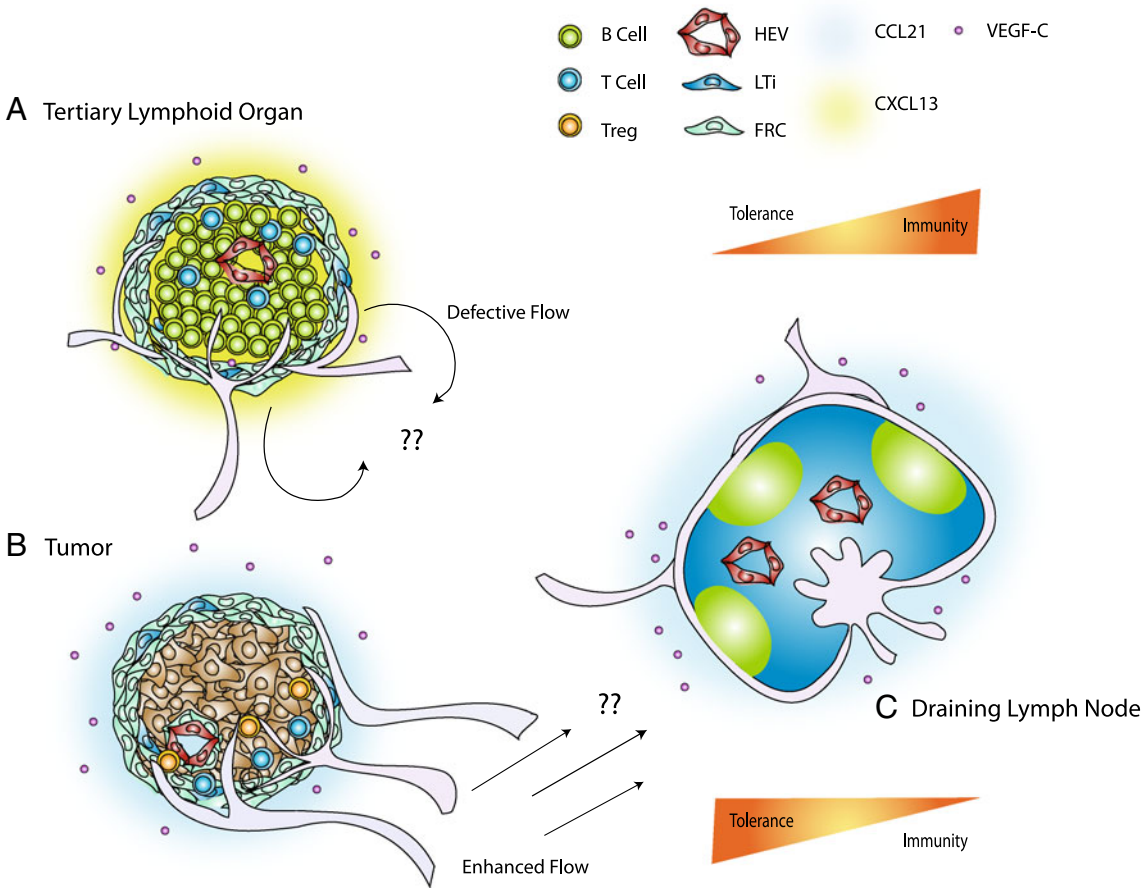


Figure 2 Peripheral lymphoid organogenesis alters fluid flow to the draining lymph node and can shift the balance between immunity and tolerance. **a** Tertiary lymphoid organs (TLOs) can develop in response to chronic inflammation and is characterized by immune cell infiltrates, lymphangiogenesis and altered fluid flow patterns. **b** Additionally, melanomas can develop stromal features reminiscent of the paracortex of the lymph node. Both the lymphoid stromal transformation that occurs in the peritumoral space as well as that surrounding immune cell infiltrates in TLOs exhibit a $gp38^{+}ER\text{-}TR7^{+}$ stromal network, high endothelial venule-like $PNAd^{+}CD31^{+}$ blood vessels, and $gp38^{+}LYVE1^{+}$ lymphatics. A distinguishing feature between the two structures is the absence of B cell follicles and CXCL13 within the melanoma environment, which is predominated by T cells and CCL21. B cell germinal centers within TLOs are largely responsible for autoantibody production and autoimmunity in chronic inflammation and graft rejection. Additionally, it has been suggested that TLO-associated lymphatic vessels reroute lymph flow from the draining lymph node [101], whereas the enhanced flow that results from lymphangiogenesis in the tumor environment may promote constant soluble antigen presentation that may be a requirement for tolerance maintenance by the draining lymph node. The tolerance-maintaining functions of the draining LN are thereby critically linked to the functionality of peripheral lymphatics, the pattern of drainage and inflammatory state of the tissue

vasculature requires both growth and maintenance cues the lymphatic vasculature may not.

Chronic Graft Rejection

Dysfunctional lymphatics and changes in lymphatic drainage and immune cell trafficking can initiate organ-specific autoimmunity. Chronic graft rejection correlates with increased lymphatic density within the grafted tissue and these lymphatic vessels are significantly enriched in areas of immune cell infiltrates [12, 111]. In models of corneal implantation, lymphatic ingrowth is significantly associated with poor graft survival and rejection [11, 112–114]. Blocking lymphangiogenesis using anti-VEGFR-3 antibodies promote the acceptance of grafted tissue [104], although it is difficult to generalize this to other tissues that are normally not alymphatic like the cornea. For bone implants, lymphangiogenesis at the bone-implant interface can promote host destruction of the tissue as well as the formation of distal granulomas, neoplasia and lymphoma [15].

Cancer

Lymphatic dissemination of solid tumors involves the directional homing of tumor cells to primed and expanding

lymphatic vessels [1, 115]. Expression of the lymphangiogenic factors VEGF-C and -D is significantly correlated with lymphangiogenesis (tumoral and nodal) and LN metastasis in a variety of primary tumors including thyroid, prostate, gastric, colorectal, lung and breast in both human and animal models [17, 20, 116, 117]. Although VEGF-C is well-correlated with cancer metastasis, the requirement for tumor lymphangiogenesis is controversial, and there is evidence that VEGF-C can promote metastasis in the absence of tumor lymphangiogenesis [118–123]. VEGF-C can also attract macrophages [124] that can alter the tumor microenvironment to promote invasion. Furthermore, some tumor cells also express VEGFR-3 and thus may benefit from autocrine signaling of VEGF-C or –D [121, 125–129]. Such autocrine signaling could help tumor cells home to lymphatics by guiding them in the direction of flow [42]. Finally, in addition to driving lymphangiogenesis, tumor VEGF-C also upregulates the expression of CCL21 by lymphatic endothelium to further promote lymphatic invasion via CCR7 expression [121]. Clearly, VEGF-C in the tumor microenvironment promotes tumor progression and invasion, but since VEGF-C can play so many different roles in the tumor microenvironment it has been difficult to dissect out the specific contributions of tumor lymphangiogenesis to these processes.

Tumor association with the lymphatic system may not only affect the local microenvironment, but also the host immune response to the tumor. As mentioned, VEGF-C and CCL21 between tumors and lymphatic endothelium display significant cross-talk [121], and the upregulation of CCL21 in the tumor microenvironment may impart certain features of lymphoid neogenesis to the tumor. B16-F10 murine melanoma cells, like many other cancer cells, express low levels of CCL21 [42]. When these cells were engineered to knockdown endogenous CCL21, they were rejected (as evidenced by tumor-antigen specific $CD8^+$ T cells and tumor regression), while control and CCL21-overexpressing tumors recruited lymphoid tissue inducer cells, formed lymphoid-like stroma, and were infiltrated with T_{Reg} cells [130]. Unlike in autoimmune-associated TLOs, these tumors did not express CXCL13 or generate B cell follicles, but mimicked features of the T cell zone stroma of the LN, which are again important in peripheral tolerance. Therefore, while the LN contains many different features that collectively orchestrate an immune response, it is likely that TLOs and tumors only recapitulate certain features of the LN—germinal center formation and paracortical stromal mimicry, respectively—to skew the immune response towards one extreme (autoantibody formation, as in the case of most TLOs) or the other (immunological tolerance, as in the case of tumors).

These recent findings raise the interesting possibility that peritumoral lymphangiogenesis may affect host immunity (Fig. 2). First, as mentioned, VEGF-C drives an upregulation of CCL21 in the local lymphatic vessels [121], and this may drive stromal changes that promote a switch from immunogenic to T_{Reg} cell education [130]. Second, the lymphatic endothelium itself may express tumor antigen to delete $CD8^+$ effector cells, as it does in the lymph node with endogenous peripheral tissue antigen [8]. Finally, the increased drainage it induces to the draining LN [131–133] could activate the tolerance-maintaining functions of the lymph node by upregulating CCL21 [41] and bathing the LN with tumor antigen.

Future Directions

Lymphatic vessels and lymphatic drainage are emerging players in our understanding of the balance between immunity and tolerance. The presence of lymphatics draining mammary tissues and their active regulation during lactation, infection and cancer highlights their importance in mammary gland biology and cancer; however, few studies have addressed this specific topic in breast tissue. Additionally, the extent to which lymphatic vessels participate in programming the immune response—for example, by modulating lymph flow and immune cell trafficking, by expressing endogenous antigen for T cell modulation, or by adapting various immune cell functions—remains incompletely understood.

While individual pieces of the puzzle have been identified, the overall picture of lymphatic function in immunity is only beginning to emerge. Recent evidence demonstrates that LN-resident lymphatic endothelium can present endogenous antigens, yet its overall importance in maintaining peripheral tolerance to those tissues that it specifically drains is unknown; whether this mechanism can extend to peripheral lymphatics such as those that are expanded in the tumor microenvironment remains an interesting question that is yet to be answered. Additionally, recent work demonstrates the importance of lipid transport from perilymphatic adipose depots by trafficking APCs, thereby modulating their ability to present antigen and stimulate and immune response [134]. The relevance of the dynamic adipose microenvironment in the breast to lymphatic function and immunity remains an open question.

In general, lymphatic sensitivity to the dynamic microenvironment under steady state and diseased conditions remains poorly understood. Much more research on the interface between lymphatic biology and immunology is needed to elucidate the importance of this underappreciated component of immunity. In particular, we believe that expounding the differences between lymphangiogenesis and TLO formation (or lymphoid stromal mimicry) in cancer vs. autoimmunity will provide novel therapeutic targets for cancer immunotherapy.

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