Lymphatic Function and Immune Regulation in Health and Disease

Shan Liao, PhD and Timothy P. Padera, PhD

LYMPHATIC VESSELS ARE PRESENT throughout the body, except in the central nervous system and bone marrow. The lymph nodes (LNs), spleen, and other secondary lymphoid organs (e.g., Peyer's patches) are strategically located in anatomical sites where they collect foreign antigen and antigen presenting cells to activate antigen-specific lymphocytes efficiently.¹ In the peripheral tissues, specialized lymphatic capillaries—called initial lymphatic vessels—allow soluble materials and cells to enter the lymphatic system easily. The collected fluid and cells form lymph, which is transported by smooth muscle-invested collecting lymphatic vessels to the draining lymph node. The lymph node provides a highly organized microarchitecture that supports optimal immune surveillance. The “filtered” lymph fluid, as well as naïve and activated lymphocytes, exit the lymph node via efferent lymphatic vessels. Lymphatic capillaries, collecting lymphatic vessels, and lymph nodes together provide protective immunity for the body. Disruptions of lymphatic function compromise immune function and result in lymphedema. In this review, we will summarize the current knowledge of how lymphatic function is altered in inflammatory states, cancer, and infection.

Lymphatic Vessels

Blood capillaries consist of endothelial cells that are connected by intercellular tight junctions and surrounded by a continuous basement membrane and pericytes. In contrast, lymphatic capillaries are composed of endothelial cells supported by a thin, discontinuous basement membrane with sparse pericyte associations. Capillary lymphatic endothelial cells (LECs) are linked to each other with button-like intercellular junctions that make lymphatic capillaries highly permeable to interstitial fluid and its contents.² These oak leaf-shaped LECs have a unique microarchitecture, in which overlapping flaps of adjacent cells form primary valve structures. These primary valve structures are critical for the formation of lymph. When tissue pressure increases above that in the lymphatic capillary, the primary valves open to allow lymph to enter freely into the lymphatic vessel.^{3,4} These primary valves also provide large enough gaps for dendritic cells

(DCs) to enter the vessel without the need for integrin adhesion or pericellular proteolysis.⁵ Antigen presenting cells, mostly DCs, migrate to lymphatic capillaries using local chemokine CCL21 gradients produced by LECs and interstitial flow.^{6–9} When activated, DCs enter the lymphatic capillaries and interact with LECs while migrating towards the lymph node.¹⁰

Lymphatic capillaries merge into collecting lymphatic vessels. The LECs in collecting lymphatic vessels are tightly connected in a continuous “zipper-like” junction pattern.² Collecting lymphatic vessels are invested in smooth muscle cells (SMCs) and have intraluminal lymphatic valves; the vessel segment between two such valves is known as a lymphangion. Intraluminal lymphatic valves maintain unidirectional lymph flow by preventing flow back toward the periphery. Smooth muscle cells are well organized along the lymphangion, but are scattered sparsely around the valve. As such, lymphangions are muscular pumping units that propel lymph through the lymphatic system. In physiological conditions, both active and passive forces drive lymph flow.^{11,12} Passive forces include arterial vasomotion, venous pressure, skeletal muscle motion, or drainage by gravity. In many circumstances, a more active mechanism—the autonomous SMC-mediated contractions of lymphatics vessels—is needed to drive lymph through lymph nodes toward the blood circulation.^{13–15} These inherent lymphatic contractions are vital components in the maintenance of both fluid homeostasis and the transport of lymphocytes. Numerous factors have been shown to regulate lymphatic contractility, including endothelial cell-derived nitric oxide (NO) and certain neurotransmitters.

NO is produced by three isoforms of nitric oxide synthase (NOS)—endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). eNOS is expressed by LECs, but it is not expressed uniformly along collecting lymphatic vessels; instead, NO production is higher near the intraluminal valve relative to the tubular portions.^{16–19} During phasic pumping, NO is also produced at specific times,^{18,19} which is thought to be critical for sustained lymphatic contractions.^{20,21} Spatial and temporal gradients are created by the rapid decay of NO *in vivo*. During the systolic phase of a contraction, NO is

E. L. Steele Laboratory, Department of Radiation Oncology, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts.

Funding support: The authors are supported by NIH R00CA137167, NIH DP2OD008780, NIH R21AI097745 and NCI Federal Share/Proton Beam Income (TPP), Charles King Trust Fellowship and NIH K99HL111343 (SL).

released near the valves in a process that is hypothesized to be activated by lymph flow.¹⁸ NO, acting on the SMCs investing the collecting lymphatic vessel, triggers vessel opening and allows for lymphangion filling. The relaxation signal dissipates as NO decays, which leads to the start of the next contraction. eNOS blockade decreases lymph fluid velocity and increases the pumping frequency of mesenteric lymphatic vessels.^{14,16,22} *In vivo*, lymphatic contractions are much weaker in eNOS^{-/-} mice, but there is a somewhat paradoxical increase in lymphatic diameter. This observation suggests other cellular, metabolic, and/or nervous system control pathways act in concert with NO on SMCs.^{14,23–25}

The endothelium of collecting lymphatic vessels is generally thought to prevent transvascular cell or fluid transport. However, this notion of a passive endothelium may not be accurate. There is evidence that DCs interact with LECs in initial lymphatic vessels.^{10,26} Furthermore, there are many ongoing investigations trying to understand if the permeability of collecting lymphatic vessels may represent an additional avenue for antigen collection. Collecting lymphatic vessels have been difficult to study *in vivo*, which in turn has limited our understanding of their biology, molecular regulation, and function.

Lymph Nodes

Lymph transported through collecting lymphatic vessels is concentrated in the draining lymph node, which is the key location where adaptive immune responses are initiated. The unique lymph node microenvironment is organized to provide optimal conditions for naïve lymphocytes—which enter the lymph node through high endothelial venules (HEVs)—to interact with antigen and DCs for immune surveillance. The cell contents in the incoming afferent lymph are mostly memory T cells and DCs. The outgoing efferent lymph is composed of a greater number of B cells, together with T cells that have surveyed antigens in that particular lymph node. Myeloid cells usually turn over locally without exiting the lymph node.

Lymphatic vessels surround the lymph node, thereby forming the subcapsular and medullary sinuses. Lymphatic vessels are also found in the hilum, and facilitate the outflow of lymph from the lymph node. Macrophages are most often found in these lymphatic-rich areas. Blood vessels enter and exit the lymph node from the hilum. They run through the medulla, and they branch within and are distributed throughout the cortex. In the paracortex, blood vessels specialize into HEVs, which facilitate lymphocyte homing into LNs from the blood.^{27,28} The L-selectin expressing naïve lymphocytes tether with peripheral node addressin (PNAd) on HEVs, followed by chemokine activation and tight binding of integrins. This facilitates the diapedesis of lymphocytes for transendothelial migration.^{29–33} Lymph node stromal cells, which include endothelial cells, fibroblastic reticular cells (FRCs), follicular stromal cells, and marginal reticular cells, express chemokines to orchestrate cell compartmentalization. HEVs and fibroblastic reticular cells express CCL19 and CCL21 to attract CCR7-expressing T cells and DCs. Therefore, activated DCs and T cells are efficiently concentrated in the paracortical zone to facilitate antigen-specific T cell activation.^{34,35} Follicular stromal cells, follicular DCs, and marginal reticular cells each express CXCL13, which guides CXCR5-

expressing B cell homing to B cell follicles located in the cortex of the lymph node. Sphingosine-1-phosphate, a lipid signaling molecule expressed—along with its receptor—by LECs, facilitates the egress of lymphocytes from LNs into efferent lymphatic vessels.^{36,37}

Functional lymphatic vessels are required for the maintenance of the LN micro-architecture.^{11,38–40} Severing afferent lymphatic vessels produces dramatic effects in HEVs. These manifest as gross morphological changes, reduced lymphocyte adherence to the vessels,^{39–41} altered expression of several HEV genes,^{39,40,42} and a decrease in the uptake of ³⁵S-sulfate (a functional marker of PNAd modification).^{43,44} Why lymphatic function is required to maintain the normal LN microenvironment is not clear, but several facts suggest an answer. The majority of HEVs are located in the paracortical area, which is separate from the lymphatic vessels. However, a reticular network within the lymph node known as the conduit system enables lymph factors to reach HEVs quickly.^{28,45,46} Low molecular weight materials (below 70 kD) move rapidly via the conduit system and reach the wall of HEVs within minutes. Lymph-borne chemokines likely adopt this route to regulate HEV function and rapidly change lymphocyte HEV transmigration.^{47–49} DCs and macrophages are also important in maintaining the normal HEV phenotype while regulating lymphocyte entry into the lymph node.^{50,51} These activities are each dependent on the entry of lymph into the node. Thus, when lymphatic function is disrupted, abnormal lymph node function would be predicted.

A critical element of immune function is the efficient interaction between activated APCs and naïve T cells. For this to occur, free antigen or the APCs themselves must localize to the T cell zone of the lymph node. Small, free antigen that enters the lymph node moves rapidly along the conduit system where it is taken up and processed by resident DCs.^{46–47} However, large antigens and microbial particles cannot penetrate deep into the LN via the conduit system. These particles enter the LN sinus and are sampled by macrophages and B cells.^{52–54} These lymphatic-associated macrophages prevent pathogens from exiting the lymph node without being properly screened.⁵⁵ In addition to free antigen entering the node, DCs from the peripheral tissue can migrate to LNs and initiate T-cell responses. This mechanism occurs much more slowly than the presentation of free antigen by resident DCs.³⁵

The stimulated tissue-migrating DCs have increased expression of co-stimulatory molecules and CCR7. CCL19 and CCL21 produced by HEVs and FRCs support CCR7-dependent migration of activated DCs towards the T cell zone in the draining LN.^{6,56} The migrating tissue DCs play a different role in initiating T cell responses than do resident DCs of the lymph node. However, both are required for priming CD4 T cells.⁵⁷ Combining all of these mechanisms, the lymph node provides a unique “filter” structure to capture antigen or migrating DCs in the lymph node. The LN ultimately serves as a site for activation of lymphocytes that attack foreign antigens and restrict systemic spread of infections.

A fine-tuned immune response generates an immune reaction to foreign antigens while preventing overt reactions to self-antigens. Infection and inflammation cause extensive host cell damage and release large quantities of self-antigens. Not all self-antigens have access to the thymus during the development of the T cell repertoire. Thus, autoreactive T cells that escape from central tolerance need to be silenced in the

periphery.⁵⁸ During normal conditions, LNs function as a niche for generating peripheral tolerance as DCs continuously sample self-antigens prior to migrating to the draining lymph node.^{59–65} Most self-antigen-bearing DCs in lymph nodes are immature.⁶⁶ These cells express low levels of costimulatory molecules and limit self-reactive T cell response by inducing anergy, clonal deletion, and/or the expansion of regulatory T cells.^{59,60,66–68} In addition, nonhematopoietic stromal cells in the lymph node (e.g., LECs, FRCs) also promote tolerance by presenting self-antigens and depleting self-reactive CD8 T cells via clonal deletion.^{69–71}

Lymphatics in Inflammation

Inflammation causes lymphangiogenesis and expansion of the lymphatic network (Fig. 1). However, changes to lymphatic drainage to the lymph node during lymphangiogenesis appear to depend on the antigen and the site of immunization. Drainage is reduced in response to inflammation caused by oxazolone skin painting and lipopolysaccharide in peritoneal models. However, drainage increases when complete Freund's adjuvant is applied to the mouse foot pad.^{38,72,73} The contradiction between the expansion of the lymphatic network and the reduction of lymphatic drainage in some models may be explained by the regulation of collecting lymphatic vessel function during inflammation. Accumulated macrophages can also promote lymphangiogenesis by expressing multiple lymphangiogenic growth factors such as VEGF and VEGF-C.^{74–76} B cells also enhance lymphangiogenesis, but T cells appear to inhibit lymphangiogenesis during inflammation.^{38,72,77,78}

Temporally and spatially dependent production of NO by activated eNOS in LECs is required for the contraction of collecting lymphatic vessels under physiological conditions, while NO produced by iNOS during inflammation attenuates lymphatic contraction strength by overwhelming these critical NO gradients. Induction of cutaneous inflammation in mice by oxazolone skin painting results in infiltration of iNOS-expressing, CD11b⁺Gr-1⁺ myeloid cells from the bone marrow, resulting in locally elevated levels of NO. iNOS depletion in these cells allows the temporal and spatial NO gradients to be maintained by eNOS and prevents the attenuation of lymphatic contractions. These experiments underscore a mechanism by which immune cell infiltrates alter lymph flow.^{22,23} iNOS-mediated suppression of lymphatic contraction and dilation of lymphatic vessels is independent of cell type. *Ex vivo* activation of macrophages suppresses lymphatic contraction *in vivo* and *ex vivo* at a level similar to that seen in iNOS-expressing bone marrow derived cells stimulated by oxazolone.^{23,79} More importantly, the interruption of lymphatic contraction during acute inflammation is associated with the prevention of self-antigen induced autoimmunity.²³ Collecting lymphatic vessels and lymph nodes are embedded in adipose tissue. Accumulation of fat tissue and macrophage infiltration are associated with the progression of inflammation, and both may contribute to the suppression of collecting lymphatic vessel contractility.

Lymph nodes appear to be more tolerogenic to new antigens during inflammation. The draining LN structure undergoes dramatic changes during local inflammation, including angiogenesis, lymphangiogenesis, decreased expression of genes contributing to PNAd, and decreased

FIG. 1. Alterations to the lymphatic system in pathological conditions. (A) The normal lymphatic system consists of lymphatic capillaries that form lymph, collecting lymphatic vessels that transport lymph, and lymph nodes that organize immune responses. Each of these structures has its own unique microarchitecture that contributes to the maintenance of proper immune function. (B) During inflammation, lymphangiogenesis is seen in the lymphatic capillaries as well as in the local draining lymph node. Furthermore, a robust infiltrate of myeloid-derived cells accumulate around the collecting lymphatic vessels, which is correlated with suppression of lymphatic contractions and weakened immune response. (C) During tumor growth, lymphangiogenesis is also seen in the lymph node, along with architectural changes associated with creating an environment receptive to tumor metastases. The ability of tumors to alter collecting lymphatic vessel contraction is not known. (D) Little is known about the response of lymphatic vessels to microorganisms or viral infections. However, there is a strong clinical link between infectious disease and lymphedema. This area needs much greater scrutiny to identify new opportunities to treat infections that are difficult to clear.

chemokine production of CCL21 and CXCL13.^{38,39,42,77,80–82} These temporary changes in the LN hinder naïve T-cell and DC access to the node, and thus limit their interactions. The ability of effector cells to leave the LN is also enhanced, thus preventing pathological immune-mediated damage in the LN. One can speculate that these changes in the lymph node are intended to maintain self-tolerance during a non-infectious inflammatory process. However, these changes in the lymph node may also prevent an immune response to subsequent bacterial or viral infection. It has been shown that disruption of lymphatic vessels impairs fluid drainage and the body's ability to activate an immune response to a pathogen, which leads to persistent infection.^{38,83–87} During clinical lymphedema, immune cell accumulation and impaired immune response are also frequently observed.⁸⁸

Chronic inflammation can lead to the formation of tertiary lymphoid organs (TLOs), which recapitulate key features of LNs. The formation of TLOs is associated with lymphangiogenesis induced by chronic allograft rejection, autoimmune reactions, or microbial infections. A substantial increase in lymphatic vessel density associated with immune cell infiltrates can be found in human kidney transplants that are rejected.⁸⁹ Additionally, corneal graft rejection can be predicted by the presence of lymphangiogenesis.⁹⁰ In rheumatoid arthritis patients, the lymphangiogenic factor VEGF-C is increased in the synovium.⁹¹ Lymphangiogenesis is also associated with autoimmune conditions in the gastrointestinal tract. In addition to lymphangiogenesis, collecting lymphatic contraction is found to be reduced in models of inflammatory bowel disease.⁹² Factors such as macrophages, mast cells, iNOS, and prostaglandins contribute to this suppression of lymphatic contraction.⁹² Other inflammatory cytokines and growth factors produced during inflammation may also affect collecting lymphatic function. The role of cytokines, chemokines, and growth factors in lymphatic contraction remains an area of research focus.

Lymphatics in Cancer Progression

Cancer progression shares many features of wound healing and inflammatory conditions, including angiogenesis,^{38,72,77} lymphangiogenesis,^{93,94} and immune cell recruitment^{95,96} (Fig. 1). Tumor-induced lymphangiogenesis provides an expanded lymphatic network that enhances molecular and cell delivery to the draining LN.⁹⁷ In fact, lymphangiogenesis correlates with increased lymph node metastasis and poor prognosis of cancer patients.^{88,98–101} However, the role of lymphangiogenesis in immune function during cancer progression is not well understood.

Upon cytotoxic treatment—such as radiation and chemotherapy—large numbers of tumor cells die through apoptotic pathways. Rather than triggering an anti-tumor immune response, these apoptotic cells in general induce immune tolerance to their associated antigens.^{102,103} However, vaccination with an injection of lethally-irradiated tumor cells induces CD8⁺ cytotoxic T cell activation and prevents subsequent syngeneic tumor growth.¹⁰⁴ The injected apoptotic cells travel directly to the lymph node and are picked up by macrophages in the lymph node sinus. The macrophages then cross-present tumor antigen to DCs or directly present antigen to cytotoxic T cells, activating a response.¹⁰⁴ These contradic-

tory results from cytotoxic treatment and vaccination suggest that the route or kinetics of clearance of material from apoptotic cells may determine the ultimate immune response.

Lymphangiogenesis occurs within the tumor-draining LN prior to tumor seeding⁹⁴ and is thought to be preparing the “soil” for metastatic cells (“the seed”). The growth of metastatic cancer cells in LNs is a critical event in disease progression that profoundly impacts patient prognosis and treatment decisions.^{105,106} The poor prognosis for patients with lymph node metastasis may be due to immune tolerance caused by the primary tumor. However, these effects are not well characterized. It is possible that chronic inflammatory mediators induced by the tumors, tumor secreted factors or tumor antigen from apoptotic tumor cells can induce peripheral tolerance. In addition, lymphatic endothelial cells can present tumor antigen and may induce immune tolerance.¹⁰⁷ All of these factors need to be considered in the development of immune therapy for metastatic tumors.

Interstitial fluid pressure in cancer is frequently increased as a result of the hyperpermeable tumor blood vasculature and the lack of lymphatic function from inside tumors.¹⁰⁸ Interstitial hypertension in tumors increases interstitial flow leaving the tumor, which has been positively correlated with cancer cell dissemination to the draining lymph node.^{107,109} Cancer cells invading the tumor margin enter enlarged lymphatic vessels and travel with the lymph flow through the collecting lymphatic vessels to the draining LN.¹¹⁰ However, it is likely that the function of collecting lymphatic vessels draining the tumor is impaired, similar to its inhibition during inflammatory conditions. How this impairment inhibits the arrival of antigen in the lymph node and the subsequent immune response is unknown.

Lymphatics in Infectious Diseases

Disruption of lymphatic function leads to lymphedema, the consequences of which are disfigurement and compromised immune defenses in the affected region. Worldwide, infectious diseases are the major causes of acquired lymphedema. However, it is not clear how infectious microorganisms affect lymphatic function. Upon infection, antigen from the bacteria or viral proteins can be picked up by DCs to be transported to the lymph node. Viruses and bacteria are also small enough to pass through the endothelium of lymphatic capillaries and travel through collecting lymphatic vessels to enter the node. Here, they are sampled by sinus macrophages and B cells.^{52–54} Additionally, sinus macrophages prevent pathogens from exiting to the efferent lymph without being interrogated—depleting sinus macrophages leads to systemic infections.⁵⁵ Worth noting, most experimental models of infectious disease use needle injections, which mimic vaccination. However, many infectious microorganisms, such as influenza virus or Herpes simplex virus-1 (HSV), infect mucosal tissues and need to disrupt the epithelial layer before entering interstitial spaces. Thus, the infection route may determine the efficiency of immune responses initiated in the draining lymph node. Needle-injected HSV-1 will become a lymph-borne antigen and is subsequently presented by resident DCs in the lymph node. In contrast, HSV that infects the vaginal mucosa is presented by tissue migrating DCs.¹¹¹ Both migrating and LN resident DCs are required to process the antigen and activate efficient CD4 T cell priming, but their functions are different.⁵⁷

Lymph node resident DCs activate T cells and retain the antigen-specific T cell in the lymph node. Tissue-migrating DCs, which arrive after the initial infection, induce antigen-specific T cell proliferation.⁵⁷ How lymphatic function is affected by infection, and conversely, how alterations in lymphatic function affect immune responses are not clear. However, disruption of lymphatic vessels inhibits the drainage function, leads to lymphedema and reduces antigen transport to the lymph node.⁸⁷ The stagnant fluid in lymphedematous tissue can become a further nidus for infection, thus exacerbating the already immuno-compromised state of the affected tissue. In addition, larger microorganisms, such as mosquito-transmitted filarial parasites, may be trapped and proliferate in lymphatic vessels, blocking lymph flow.¹¹² These examples suggest that lymphatic transport and function of the lymphatic system are tied to immune response, but to date this area of research has been underexplored.

Conclusions

The lymphatic system maintains host peripheral tolerance during normal conditions, and quickly initiates protective immunity against foreign antigens upon stimulation. The lymphatic capillaries, collecting lymphatic vessels, and lymph nodes coordinate efficient antigen delivery, antigen presentation, and cell–cell interaction. Many pathological conditions cause lymphangiogenesis, presumably providing an expanded lymphatic network that allows greater access for antigen and fluid to enter lymphatic vessels. Lymphatic function is also altered in various disease states. However, the role of lymphangiogenesis and changes in lymphatic function in immune regulation is not clear. For example, malignant tumors can cause local lymphangiogenesis via increasing production of VEGF/C/D, allowing cancer cells to invade and be transported via the lymphatic drainage to the lymph node. Metastatic tumors can also induce lymph node lymphangiogenesis. On one hand, tumor lymphangiogenesis increases lymph flow, suggesting it may increase tumor antigen delivery to the tumor draining lymph node. On the other hand, tumor lymphangiogenesis carries with it a poor prognosis,^{105,106} which may be partially due to tumor-induced immune tolerance as well as an increased ability of the cancer cells to travel to the LN. Similarly contradictory predictions of lymphangiogenesis between enhancing immunity and immune tolerance are also seen in transplant rejection. Blocking lymphangiogenesis reduces the rate of acute transplant rejection, but one-year follow-up data indicate that continued function of the transplanted organ is correlated with lymphangiogenesis.^{113–115} In addition, a viral infection generates anti-viral immunity but also causes suppression of the bystander CD8 T cell responses through inducing tolerogenic gene expression in lymph node stromal cells.^{82,116} How the lymphatic system is regulated in a diverse set of diseases and how its function exerts its influence on ultimate immune function needs further study. Understanding the connection between lymphatic function and immune regulation will lead to new therapeutic opportunities in cancer, infection, and autoimmune diseases.

Acknowledgments

We thank Drs. Mark Badeaux, Cristina Kesler and Lance Munn for their critical input on this manuscript.

Author Disclosure Statement

The authors have no financial conflicts of interest.

The authors are supported by NIH R00CA137167, NIH DP2OD008780, NIH R21AI097745 and NCI Federal Share/Proton Beam Income (TPP), Charles King Trust Fellowship and NIH K99HL111343 (SL).

References

1. Drayton DL, et al., Lymphoid organ development: From ontogeny to neogenesis. *Nat Immunol* 2006;7:344–353.
2. Baluk P, et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. *J Exp Med* 2007;204:2349–2362.
3. Oliver G, Alitalo K. The lymphatic vasculature: Recent progress and paradigms. *Annu Rev Cell Dev Biol* 2005;21:457–483.
4. Witte MH, et al., Lymphangiogenesis and lymphangiodysplasia: From molecular to clinical lymphology. *Microsc Res Tech* 2001;55:122–145.
5. Pflücke H, Sixt M. Preformed portals facilitate dendritic cell entry into afferent lymphatic vessels. *J Exp Med* 2009;206:2925–2935.
6. Randolph GJ, V. Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat Rev Immunol* 2005;5:617–628.
7. Schumann K, et al. Immobilized chemokine fields and soluble chemokine gradients cooperatively shape migration patterns of dendritic cells. *Immunity* 2010;32:703–713.
8. Miteva D, et al. Transmural flow modulates cell and fluid transport functions of lymphatic endothelium. *Circ Res* 2010;106:920–931.
9. Weber M, et al. Interstitial dendritic cell guidance by haptotactic chemokine gradients. *Science* 2013;339:328–332.
10. Nitschke M, et al., Differential requirement for ROCK in dendritic cell migration within lymphatic capillaries in steady-state and inflammation. *Blood* 2012;120:2249–2258.
11. Skalak TC, Schmid-Schonbein GW, Zweifach BW. New morphological evidence for a mechanism of lymph formation in skeletal muscle. *Microvasc Res* 1984;28:95–112.
12. Schmid-Schonbein GW., Microlymphatics and lymph flow. *Physiol Rev* 1990;70:987–1028.
13. Breslin JW, et al. Vascular endothelial growth factor-C stimulates the lymphatic pump by a VEGF receptor-3-dependent mechanism. *Am J Physiol Heart Circ Physiol* 2007;293:H709–718.
14. Eisenhoffer J, Yuan ZY, Johnston MG. Evidence that the L-arginine pathway plays a role in the regulation of pumping activity in bovine mesenteric lymphatic vessels. *Microvasc Res* 1995;50:249–259.
15. Gasheva OY, Zawieja DC, Gashev AA. Contraction-initiated NO-dependent lymphatic relaxation: A self-regulatory mechanism in rat thoracic duct. *J Physiol*, 2006; 575:821–832.
16. Hagendoorn J, et al. Endothelial nitric oxide synthase regulates microlymphatic flow via collecting lymphatics. *Circ Res* 2004;95:204–209.
17. Lahdenranta J, et al. Endothelial nitric oxide synthase mediates lymphangiogenesis and lymphatic metastasis. *Cancer Res* 2009;69:2801–2808.
18. Bohlen HG, et al. Phasic contractions of rat mesenteric lymphatics increase basal and phasic nitric oxide generation in vivo. *Am J Physiol Heart Circ Physiol* 2009;297:H1319–1328.

19. Bohlen HG, Gasheva OY, Zawieja DC. Nitric oxide formation by lymphatic bulb and valves is a major regulatory component of lymphatic pumping. *Am J Physiol Heart Circ Physiol* 2011;301:H1897–1906.
20. Fukumura D, Kashiwagi S, Jain RK. The role of nitric oxide in tumour progression. *Nat Rev Cancer* 2006;6:521–534.
21. Kashiwagi S, et al. Perivascular nitric oxide gradients normalize tumor vasculature. *Nat Med* 2008;14:255–257.
22. Shirasawa Y, Ikomi F, and Ohhashi T. Physiological roles of endogenous nitric oxide in lymphatic pump activity of rat mesentery in vivo. *Am J Physiol Gastrointest Liver Physiol* 2000;278:G551–556.
23. Liao S, et al. Impaired lymphatic contraction associated with immunosuppression. *Proc Natl Acad Sci USA* 2011;108:18784–18789.
24. Li X, et al. Glucose and glucose transporters regulate lymphatic pump activity through activation of the mitochondrial ATP-sensitive K⁺ channel. *J Physiol Sci* 2008;58:249–261.
25. Johnston MG, Feuer C. Suppression of lymphatic vessel contractility with inhibitors of arachidonic acid metabolism. *J Pharmacol Exp Ther* 1983;226:603–607.
26. Tal O, et al. DC mobilization from the skin requires docking to immobilized CCL21 on lymphatic endothelium and intralymphatic crawling. *J Exp Med* 2011;208:2141–2153.
27. von Andrian UH, M'Rini C. In situ analysis of lymphocyte migration to lymph nodes. *Cell Adhes Commun* 1998;6:85–96.
28. Anderson AO, Anderson ND. Studies on the structure and permeability of the microvasculature in normal rat lymph nodes. *Am J Pathol* 1975;80:387–418.
29. Hemmerich S, Butcher EC, Rosen SD. Sulfation-dependent recognition of HEV-ligands by L-selectin and MECA-79, an adhesion-blocking mAb. *J Exp Med* 1994;180:2219–2226.
30. Hemmerich S, et al. Sulfation of L-selectin ligands by an HEV-restricted sulfotransferase regulates lymphocyte homing to lymph nodes. *Immunity* 2001;15:237–247.
31. Homeister JW, et al. The alpha(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity* 2001;15:115–126.
32. Hiraoka N, et al. Core 2 branching beta1,6-N-acetylglucosaminyltransferase and high endothelial venule-restricted sulfotransferase collaboratively control lymphocyte homing. *J Biol Chem* 2004;279:3058–3067.
33. Hiraoka N, et al. A novel, high endothelial venule-specific sulfotransferase expresses 6-sulfo sialyl Lewis(x), an L-selectin ligand displayed by CD34. *Immunity* 1999;11:79–89.
34. Bajenoff M, Granjeaud S, Guerder S. The strategy of T cell antigen-presenting cell encounter in antigen-draining lymph nodes revealed by imaging of initial T cell activation. *J Exp Med* 2003;198:715–724.
35. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 2004;427:154–159.
36. Cyster JG. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu Rev Immunol* 2005;23:127–159.
37. Lo CG, et al. Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. *J Exp Med* 2005;201:291–301.
38. Liao S, Ruddle NH. Synchrony of high endothelial venules and lymphatic vessels revealed by immunization. *J Immunol* 2006;177:3369–3379.
39. Mebius RE, et al. Expression of GlyCAM-1, an endothelial ligand for L-selectin, is affected by afferent lymphatic flow. *J Immunol* 1993;151:6769–6776.
40. Mebius RE, et al. The influence of afferent lymphatic vessel interruption on vascular addressin expression. *J Cell Biol* 1991;115:85–95.
41. Hendriks HR, Duijvestijn AM, Kraal G. Rapid decrease in lymphocyte adherence to high endothelial venules in lymph nodes deprived of afferent lymphatic vessels. *Eur J Immunol* 1987;17:1691–1695.
42. Swarte VV, et al. Regulation of fucosyltransferase-VII expression in peripheral lymph node high endothelial venules. *Eur J Immunol* 1998;28:3040–3047.
43. Drayson MT, Ford WL. Afferent lymph and lymph borne cells: Their influence on lymph node function. *Immunobiology* 1984;168:362–379.
44. Hendriks HR, Eestermans IL. Disappearance and reappearance of high endothelial venules and immigrating lymphocytes in lymph nodes deprived of afferent lymphatic vessels: A possible regulatory role of macrophages in lymphocyte migration. *Eur J Immunol* 1983;13:663–669.
45. Gretz JE, et al. Sophisticated strategies for information encounter in the lymph node: The reticular network as a conduit of soluble information and a highway for cell traffic. *J Immunol* 1996;157:495–499.
46. Gretz JE, Anderson AO, Shaw S, Cords, channels, corridors and conduits: Critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol Rev* 1997;156:11–24.
47. Gretz JE, et al. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *J Exp Med* 2000;192:1425–1440.
48. Larsen CG, et al. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* 1989;243:1464–1466.
49. Anderson AO, Anderson ND. Lymphocyte emigration from high endothelial venules in rat lymph nodes. *Immunology* 1976;31:731–748.
50. Mebius RE, et al. The functional activity of high endothelial venules: A role for the subcapsular sinus macrophages in the lymph node. *Immunobiology* 1991;182:277–291.
51. Moussion C, Girard JP. Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. *Nature* 2011;479:542–546.
52. Iannaccone M, et al. Subcapsular sinus macrophages prevent CNS invasion on peripheral infection with a neurotropic virus. *Nature* 2010;465:1079–1083.
53. Roozendaal R, et al. Conduits mediate transport of low-molecular-weight antigen to lymph node follicles. *Immunity* 2009;30:264–276.
54. Phan TG, et al. Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. *Nat Immunol* 2009;10:786–793.
55. Mandl JN, et al. Quantification of lymph node transit times reveals differences in antigen surveillance strategies of naive CD4⁺ and CD8⁺ T cells. *Proc Natl Acad Sci USA* 2012;109:18036–18041.
56. Braun A, et al. Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration. *Nat Immunol* 2011;12:879–887.

57. Allenspach EJ, et al. Migratory and lymphoid-resident dendritic cells cooperate to efficiently prime naive CD4 T cells. *Immunity* 2008;29:795–806.
58. Bouneaud C, Kourilsky P, Bousso P. Impact of negative selection on the T cell repertoire reactive to a self-peptide: A large fraction of T cell clones escapes clonal deletion. *Immunity* 2000;13:829–840.
59. Wilson NS, et al. Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood* 2003;102:2187–2194.
60. Steinman RM, et al. Dendritic cell function in vivo during the steady state: A role in peripheral tolerance. *Ann NY Acad Sci* 2003;987:15–25.
61. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. *Nat Rev Immunol* 2003;3:867–878.
62. Hemmi H, et al. Skin antigens in the steady state are trafficked to regional lymph nodes by transforming growth factor-beta1-dependent cells. *Int Immunol* 2001;13:695–704.
63. Scheinecker C, et al. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J Exp Med* 2002;196:1079–1090.
64. Huang FP, et al. A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 2000;191:435–444.
65. Wilson NS, Villadangos JA. Lymphoid organ dendritic cells: Beyond the Langerhans cells paradigm. *Immunol Cell Biol* 2004;82:91–98.
66. Wilson NS, El-Sukkari D, Villadangos JA. Dendritic cells constitutively present self antigens in their immature state in vivo and regulate antigen presentation by controlling the rates of MHC class II synthesis and endocytosis. *Blood* 2004;103:2187–2195.
67. Cavanagh LL, Von Andrian UH. Travellers in many guises: The origins and destinations of dendritic cells. *Immunol Cell Biol* 2002;80:448–462.
68. Stoitzner P, et al. Migratory Langerhans cells in mouse lymph nodes in steady state and inflammation. *J Invest Dermatol* 2005;125:116–125.
69. Lee JW, et al. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. *Nat Immunol* 2007;8:181–190.
70. Nichols LA, et al. Deletional self-tolerance to a melanocyte/melanoma antigen derived from tyrosinase is mediated by a radio-resistant cell in peripheral and mesenteric lymph nodes. *J Immunol* 2007;179:993–1003.
71. Cohen JN, et al. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. *J Exp Med* 2010;207:681–688.
72. Angeli V, et al. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. *Immunity* 2006;24:203–215.
73. Kim KE, et al. Role of CD11b+ macrophages in intraperitoneal lipopolysaccharide-induced aberrant lymphangiogenesis and lymphatic function in the diaphragm. *Am J Pathol* 2009;175:1733–1745.
74. Jeon BH, et al. Profound but dysfunctional lymphangiogenesis via vascular endothelial growth factor ligands from CD11b+ macrophages in advanced ovarian cancer. *Cancer Res* 2008;68:1100–1109.
75. Kataru RP, et al. Critical role of CD11b+ macrophages and VEGF in inflammatory lymphangiogenesis, antigen clearance, and inflammation resolution. *Blood* 2009;113:5650–5659.
76. Maruyama K, et al. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *J Clin Invest* 2005;115:2363–2372.
77. Halin C, et al. VEGF-A produced by chronically inflamed tissue induces lymphangiogenesis in draining lymph nodes. *Blood* 2007;110:3158–3167.
78. Kataru RP, et al. T lymphocytes negatively regulate lymph node lymphatic vessel formation. *Immunity* 2011;34:96–107.
79. Wang H. Activated macrophage-mediated endogenous prostaglandin and nitric oxide-dependent relaxation of lymphatic smooth muscles. *Jpn J Physiol* 1997;47:93–100.
80. Hoke D, et al. Selective modulation of the expression of L-selectin ligands by an immune response. *Curr Biol* 1995;5:670–678.
81. Mebius RE, et al. The function of high endothelial venules in mouse lymph nodes stimulated by oxazolone. *Immunology* 1990;71:423–427.
82. Mueller SN, et al. Regulation of homeostatic chemokine expression and cell trafficking during immune responses. *Science* 2007;317:670–674.
83. Mallon EC, Ryan TJ. Lymphedema and wound healing. *Clin Dermatol* 1994;12:89–93.
84. Witte CL, Witte MH. Disorders of lymph flow. *Acad Radiol* 1995;2:324–334.
85. Golshan M, Smith B. Prevention and management of arm lymphedema in the patient with breast cancer. *J Support Oncol* 2006;4:381–386.
86. Morrell RM, et al. Breast cancer-related lymphedema. *Mayo Clin Proc* 2005;80:1480–1484.
87. Szuba A, Rockson SG. Lymphedema: Anatomy, physiology and pathogenesis. *Vasc Med* 1997;2:321–326.
88. Alitalo K, Tammela T, and Petrova TV. Lymphangiogenesis in development and human disease. *Nature* 2005;438:946–953.
89. Kerjaschki D, et al. Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. *Nat Med* 2006;12:230–234.
90. Dietrich T, et al. Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation. *J Immunol* 2010;184:535–539.
91. Paavonen K, et al. Vascular endothelial growth factors C and D and their VEGFR-2 and 3 receptors in blood and lymphatic vessels in healthy and arthritic synovium. *J Rheumatol* 2002;29:39–45.
92. von der Weid PY, Rainey KJ. Review article: Lymphatic system and associated adipose tissue in the development of inflammatory bowel disease. *Aliment Pharmacol Ther* 2010;32:697–711.
93. Mumprecht V, et al. In vivo imaging of inflammation- and tumor-induced lymph node lymphangiogenesis by immuno-positron emission tomography. *Cancer Res* 2010;70:8842–8851.
94. Qian CN, et al. Preparing the "soil": The primary tumor induces vasculature reorganization in the sentinel lymph node before the arrival of metastatic cancer cells. *Cancer Res* 2006;66:10365–10376.
95. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009;9:162–174.
96. Murdoch C, et al. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer* 2008;8:618–631.
97. Padera TP, et al. Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science* 2002;296:1883–1886.

98. Mumprecht V, Detmar M. Lymphangiogenesis and cancer metastasis. *J Cell Mol Med* 2009;13:1405–1416.
99. Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003;9:685–693.
100. Kesler CT, et al. Lymphatic vessels in health and disease. *Wiley Interdiscip Rev Syst Biol Med* 2013;5:111–124.
101. Tammela T, Alitalo K. Lymphangiogenesis: Molecular mechanisms and future promise. *Cell* 2010;140:460–476.
102. Sauter B, et al. Consequences of cell death: Exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med* 2000;191:423–434.
103. Albert ML, et al. Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. *J Exp Med* 1998;188:1359–1368.
104. Asano K, et al. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. *Immunity* 2011;34:85–95.
105. Veronesi U, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med* 2003;349:546–553.
106. Morton DL, et al. Sentinel-node biopsy or nodal observation in melanoma. *N Engl J Med* 2006;355:1307–1317.
107. Swartz MA, Lund AW. Lymphatic and interstitial flow in the tumour microenvironment: Linking mechanobiology with immunity. *Nat Rev Cancer* 2012;12:210–219.
108. Padera TP, et al. Pathology: Cancer cells compress intratumour vessels. *Nature*, 2004;427:695.
109. Jain RK, Tong RT, and Munn LL. Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: Insights from a mathematical model. *Cancer Res* 2007;67:2729–2735.
110. Hoshida T, et al. Imaging steps of lymphatic metastasis reveals that vascular endothelial growth factor-C increases metastasis by increasing delivery of cancer cells to lymph nodes: Therapeutic implications. *Cancer Res* 2006;66:8065–8075.
111. Lee HK, et al. Differential roles of migratory and resident DCs in T cell priming after mucosal or skin HSV-1 infection. *J Exp Med* 2009;206:359–370.
112. Babu S, Nutman TB. Immunopathogenesis of lymphatic filarial disease. *Semin Immunopathol* 2012;34:847–861.
113. Stuh T, et al. Lymphatic neoangiogenesis in human renal allografts: Results from sequential protocol biopsies. *Am J Transplant* 2007;7:377–384.
114. Yin N, et al. Targeting lymphangiogenesis after islet transplantation prolongs islet allograft survival. *Transplantation* 2011;92:25–30.
115. Ling S, et al. Crucial role of corneal lymphangiogenesis for allograft rejection in alkali-burned cornea bed. *Clin Experiment Ophthalmol* 2009;37:874–883.
116. Fletcher AL, et al. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. *J Exp Med* 2010;207:689–697.

Address correspondence to:
Timothy P. Padera, PhD
Massachusetts General Hospital
Radiation Oncology-Cox 7
100 Blossom Street
Boston, MA 02114

E-mail: tpadera@steele.mgh.harvard.edu