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LYMPHATIC VESSELS IN HEALTH AND DISEASE

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Abstract

The lymphatic vasculature plays vital roles in tissue fluid balance, immune defense, metabolism and cancer metastasis. In adults, lymphatic vessel formation and remodeling occurs primarily during inflammation, development of the corpus luteum, wound healing, and tumor growth. Unlike the blood circulation, where unidirectional flow is sustained by the pumping actions of the heart, pumping actions intrinsic to the lymphatic vessels themselves are important drivers of lymphatic flow. This review summarizes critical components that control lymphatic physiology.

In normal tissues, fluid and plasma elements from the systemic blood circulation are exchanged at the capillary level to bring nutrients to cells and to eliminate waste. A critical function of lymphatic vessels is to return excess fluid back to the circulation. Fluid is initially collected by a lymphatic capillary plexus (the initial lymphatics) and transported to collecting lymphatic vessels in the form of lymph (Figure 1). The collecting lymphatics have circumferential smooth muscle coverage and luminal valves that propel and maintain unidirectional flow of lymph fluid. Along the way, the lymph, which is rich in antigens and immune cells, drains through lymph nodes, thereby allowing for efficient organization of immune responses. Antigen is picked up by dendritic cells in peripheral tissues and transported via lymphatics to lymph nodes where lymphocytes survey for specific antigens. Thus, lymphocytes can maintain immunocompetence by circulating from one lymph node to another rather than to each individual tissue of the body. Digested lipids are also absorbed by virtue of mesenteric lymphatic transport (reviewed in [1]).

Disruption of lymphatic drainage leads to disabling and incurable lymphedema. Lymphedema, or lymphatic insufficiency, is characterized by painful regional swelling as fluid accumulates interstitially. Over time, lymphedema results in disfigurement and compromised immune defenses in the affected region. Congenital diseases, parasitic infections, lymphadenectomy and post-surgical radiotherapy in cancer patients are main causes of impaired lymphatic function. Moreover, malignant tumors can directly alter surrounding lymphatic vessels, allowing cancer cells to invade and be transported via the lymphatic drainage to the lymph node.

LYMPHATIC VESSEL DEVELOPMENT

Lymphatic vessels begin to form *in utero* around week 6–7 in humans and embryonic day E9.5-10.5 in mice, after the cardiovascular system is established and functional. Endothelial cells (ECs) derived from the anterior cardinal veins commit to the lymphatic lineage to form lymph sacs. Lymphatic vessels sprout from these lymph sacs in a process known as lymphangiogenesis. These new vessels merge with separate lymphatic capillary networks to

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form a lymphatic capillary plexus (reviewed in [2]). As the lymphatic vascular tree expands, connections to blood vessels are lost, except where lymph returns to the blood in the subclavian veins. Expression of a non-receptor cytoplasmic tyrosine kinase *syk* (spleen tyrosine kinase) and a signal-transducing adaptor protein *slp76* (SH2 domain-containing leukocyte protein of 76 kDa), in ECs as well as in circulating hematopoietic cells, is critical to the separation of blood and lymphatic vessels.[3, 4] Loss of these mediators results in abnormal connections between lymphatic and blood vessels during embryogenesis.[4]

Commitment to the lymphatic lineage begins with the expression of *prox1* (prospero-related homeobox-1) by ECs on the dorsal side of the cardinal vein. Triggered by expression of *sox18* (SRY-related HMG-box 18), *prox1* expression provides the first indication of lymphatic competence and is indispensable for lymphatic vessel development. [5, 6] It is initially expressed in mice at E9-9.5. Together with venous EC fate regulator protein COUP-TFII (chicken ovalbumin upstream promoter transcription factor 2), the Prox1 transcription factor upregulates numerous lymphatic-specific genes while suppressing certain blood vessel markers.[5, 7] *Lyve1* upregulation also provides an early indication of lymphatic competence, though its expression is non-essential for lymphatic development.[8]

Another requirement for a functioning lymphatic network is expression of the forkhead transcription factor *FoxC2*. FoxC2 protein regulates the expression of numerous genes involved in the specification of initial versus collecting lymphatic vessel phenotype. During development, FoxC2 works together with Prox1 and fluid flow to form the lymphatic valves, which are essential for collecting lymphatic vessel function.[9] Deletions or inactivating mutations of *FoxC2* cause an irregular distribution of smooth muscle cells (SMCs) along the collecting vessels and prevent valve formation.[9, 10] Mutations in *FoxC2* are implicated in a congenital form of lymphatic insufficiency known as the lymphedema distichiasis syndrome.[11]

Numerous growth factors cooperate in the development and maintenance of lymphatic vessels. They do so by binding cognate receptors located on the surfaces of lymphatic endothelial cells (LECs). This association activates (or suppresses) enzymatic activity that transduces signals to alter cellular biological processes. Two major ligand-receptor pathways that govern LEC biology are the vascular endothelial growth factor (VEGF)-VEGF Receptor (VEGFR) pathway and the angiopoietin (Ang)-TIE(tyrosine kinase with Ig and EGF homology domain) axis. Lymphangiogenesis and sprouting of the first Prox1-positive LECs during embryogenesis are driven by VEGF-C and VEGF-D-mediated activation of VEGFR-2 and VEGFR-3. These players remain heavily involved in lymphatic maintenance during adulthood.[12, 13] Ang molecules stimulate postnatal vessel growth, remodeling and maturation.[14–16] A homozygous knockout of Ang-2 (*ang-2*$^{-/-}$) produces a strong mesenteric lymphedema phenotype in newborn mice.[14] In addition, the transmembrane ligand ephrinB2 is critical for remodeling of the primary lymphatic capillary plexus into a hierarchical vessel network. *EphrinB2*$^{-/-}$ mice display defective remodeling of the capillary plexus and lymphatic hyperplasia. These vessels also lack intraluminal valve formation.[17]

CD11b+ macrophages play important roles in inflammation- and tumor-induced lymphangiogenesis. Activated CD11b+ cells are capable of forming tube-like structures *in vitro* that display lymphatic markers including LYVE-1, Prox-1 and podoplanin.[18, 19] CD11b+ macrophages also produce VEGF-C and VEGF-D, allowing them to stimulate lymphangiogenesis within the local lymphatic vessel network.[18, 20–22] Expression of *lyve1* and *vegfr3* by CD11b+ cells has been observed in the mouse conjunctiva, with a higher number of VEGFR3-positive cells appearing in inflamed eyes.[19] Furthermore, expression of *vegfr3* by CD11b+ cells is implicated in tumor-mediated chemotaxis of activated

macrophages by VEGF-C-expressing tumors and may contribute to tumor metastasis.[19, 22, 23]

The structural composition of mature lymphatic vessels is important for moving interstitial fluid and lymphocytes through the lymphatic vessel lumen. Discontinuous, button-like junctions connect LECs of the initial lymphatics to one another. These buttons facilitate fluid and lymphocyte entry into the lymphatic system by allowing transport between LECs. They are composed of adherens junction protein VE-Cadherin, tight junction proteins claudin-5 and zonula occludens-1 (ZO-1), tight junction-associated Ig-like transmembrane proteins endothelial cell-selective adhesion molecule (ESAM), junctional adhesion molecule-A (JAM-A), and PECAM-1/CD31.[24] The LECs of the collecting lymphatics, which function to transport the drained fluid and cells over long distances, form continuous zipper-like connections without the button-like openings. Zippers are comprised of the same adhesion molecules as the buttons of the initial lymphatics, indicating that the key difference between buttons and zippers lays in the morphology of the individual LECs and the organization of a common constellation of junctional proteins.[24–26]

FLUID HOMEOSTASIS AND LYMPHATIC PUMPING

Lymphatic networks are found in nearly all vascularized tissues, with the exception of bone marrow and the central nervous system (though some connections between the cerebrospinal fluid and lymphatics exist).[27] Lymph collection occurs in the initial lymphatic vessels (Figure 1), which are structures comprised of a single layer of overlapping endothelial cells, typically without associated SMCs. As the vessels carry lymph back toward the blood circulation, the amount of SMC coverage increases. This increased coverage identifies the pre-collecting and larger collecting lymphatic vessels. SMC coverage is periodically interrupted by intraluminal valves, which are composed primarily of ECs and matrix. [28, 29]

Multiple factors drive lymphatic pumping. Lymphatic transport is driven externally by arterial pulsations, skeletal muscle contractions, and in the mesentery, by smooth muscle contractions. Transport is also autonomously driven by contractions that are unique to the collecting lymphatic vessels (Figure 2, Movie 1). These vessels are composed of individual pumping units known as lymphangions, each of which has an inlet and an outlet valve. These valves, along with the SMCs that line the collecting lymphatics, represent the main mechanisms by which lymphatic contractions maintain unidirectional flow of lymph.[30]

These autonomous contractions are critically modulated by extrinsic and intrinsic forces, which include lymph formation rate, local compression due to muscle movement, local interstitial fluid pressure, lymphatic flow rate and inflammatory mediators. One critical regulator of autonomous lymphatic contraction is nitric oxide (NO). NO is responsible for the reduction in tone in the lymphangion during the relaxation phase of its contraction. NO production inhibits the contraction by depolarizing ATP-sensitive K(+) channels located on the plasma membranes of SMCs.[31] NO-induced relaxation allows for diastolic filling of the lymphangion and thus prepares the vessel for its next contraction. [32, 33]

NO is primarily produced by three isoforms of nitric oxide synthase (NOS), the most relevant of which is endothelial NOS (eNOS). eNOS is expressed by the LECs of the collecting lymphatic vessels and is critical for VEGF-C-mediated lymphangiogenesis.[34, 35] eNOS expression and NO production are higher in the lymphatic bulb surfaces of the valve relative to the tubular portions.[36, 37] It has been predicted that during phasic pumping, the shear forces from fluid flow activate eNOS in the endothelium to produce NO.[28, 33–36, 38–40] While the total NO concentration is critical, temporal and spatial gradients of NO are fundamental to its action on vessels (Figure 2).[41, 42] The short, active half-life of NO *in vivo* produces these spatial and temporal gradients. In order to produce a lymphatic contraction,

NO is released in response to the elevated shear force caused by a contraction from an area near the valve during the systolic phase.[36] This NO release starts the diastolic relaxation necessary for lymphangion filling and the next cycle of contraction. Using mathematical simulations, it has been shown that the ability for phasic lymphatic contractions to be self-sustained is very sensitive to the half-life of the effector molecules. In this context, NO seems ideally suited to this purpose.[43]

The impact of NO on lymphatic pumping has been extensively studied using numerous methods that modulate NO production. Pharmacological blockade of eNOS using inhibitors —N^{w}-Nitro-L-Arginine (L-NAME), N^{G}-monomethyl-L-arginine (L-NMMA), N^{w}-nitro-L-arginine (NOLA) or the non-specific inhibitor methylene blue—in mesenteric and thoracic duct vessels *ex vivo* results in an acute increase in the pumping frequency that is accompanied by a decrease in lymph fluid velocity.[33, 40, 44–46] These findings are supported *in vivo*, where L-NMMA treatment produces an increase in the frequency of contractions of the afferent vessel to the popliteal lymph node.[30] However, the NO effects on pumping are context-dependent and influenced by experimental conditions.[38, 39] For instance, Koller et. al. report that blockade with L-NAME prevents the increase in lymphatic contraction frequency associated with lymph flow in vessels afferent to the iliac lymph node in rats.[39]

Chronic depletion of eNOS using an eNOS knockout mouse (eNOS−/−) results in decreased lymph flow.[34] eNOS−/− also produces a decrease in contraction strength corresponding with an increase in the frequency of contractions in the afferent lymphatic vessels of the popliteal lymph node.[30] The prevailing model suggests that when eNOS is blocked, the lymphatic segment contracts more frequently to maintain lymphatic flow appropriate for the existing level of preload.[30, 33, 34, 40]

There is a somewhat paradoxical increase in lymphatic diameter with eNOS inhibition *in vivo*, indicating that other regulatory molecules are involved with the integrated control of lymphatic pumping.[30, 38, 47, 48] For instance, arachidonate metabolites are well known mediators of spontaneous contractions.[49, 50] The cyclooxygenase inhibitor aspirin and inhibitors of thromboxane synthase suppress phasic contractions *ex vivo*.[48, 49] Acetylcholine induces NO-dependent lymphatic vessel relaxation that is antagonized by atropine but not by cyclooxygenase inhibitors, indicating a separate mechanism for muscarinic influence.[51, 52] 5-hydroxytryptamine (5-HT) modulates the rate of lymphatic pumping by decreasing the frequency of contractions induced by vessel perfusion of isolated guinea pig mesenteric vessels through actions on lymphatic SMCs.[53] Working in the opposite direction, histamine increases the frequency of contractions while decreasing their amplitude.[54] Activated macrophages have also been implicated in the relaxation of lymphatic vessels through generation of NO and prostaglandins.[55] Extracellular glucose and intracellular glucose transporters increase pumping frequency and induce constrictions of the rat thoracic duct.[47] VEGF-C increases lymphatic pumping of rat mesenteric vessels *in vivo* through VEGFR3, which results in release of intracellular Ca^{2+} within LECs and activation of eNOS.[13, 56] Pharmacological inhibition of Rho kinase in SMCs of afferent lymph vessels results in vessel dilation and cessation of pumping *ex vivo*.[57] Pharmacological inhibition of myosin phosphatase produces vessel constriction with reduced lymphatic pumping frequency.[57] The identification of new molecules that impact lymphatic pumping is an active area of research. These molecules may provide new pharmacological targets for treatments of lymphatic insufficiency.

LECs function under low oxygen tension (8–35 mmHg) compared to surrounding tissue and blood vessels. The mean pO_2 levels in lymphatic capillaries, collecting lymphatic vessels and thoracic ducts rise sequentially from ~8 to ~20 to ~35 mmHg, respectively. The reduced oxygen availability in lymphatics promotes an increase in NO bioavailability.[31]

Furthermore, the lower pO_2 levels in lymphatics relative to surrounding tissue indicates that the metabolic activity and oxygen consumption of the collecting lymphatic vessels is high, emphasizing the active nature of many processes of these vessels.[58]

Another isoform of NOS—inducible NOS (iNOS)—is expressed predominantly by surrounding immune cells and is not expressed in LECs under physiological conditions. Unlike *eNOS*$^{-/-}$ mice, *iNOS*$^{-/-}$ mice show no reduction in the strength of lymphatic contractions under normal conditions. However, iNOS does attenuate contractions during inflammation by overwhelming the critical temporal and spatial NO gradients produced by eNOS in LECs. Induction of cutaneous inflammation in mice results in infiltration of iNOS-expressing bone marrow-derived cells, which produce elevated levels of NO. Knockdown of iNOS in these cells allows the NO gradients to be maintained by eNOS function and prevents attenuation of lymphatic contractions, underscoring a mechanism by which immune cells alter lymphatic fluid flow.[30, 40]

LYMPHATIC FUNCTION AND THE IMMUNE RESPONSE

Collected lymph fluid is rich in antigens and humoral factors that are either drained from surrounding tissues or are constitutively present in lymphatic vessels. This antigen and immunocyte-rich fluid is interrogated as it passes through the lymph node (LN) on its way back to the systemic circulation. In this manner, the antigen and antigen presenting cells (APCs) draining from peripheral tissues are efficiently concentrated in the draining LN. During the normal homeostatic state, the cells that enter the LN from the lymphatics are primarily dendritic cells (DCs) and memory T cells.[59, 60] DCs constantly sample self-antigens and migrate to LNs where they maintain an immature status characterized by low-level expression of co-stimulatory molecules. They control self-reactive T cell activity by inducing self-tolerance through anergy and clonal deletion. In this way, LNs function as additional niches alongside the central tolerance mechanisms present in the thymus to generate peripheral tolerance. Non-hematopoietic stromal cells that are present in the LN (e.g. LECs or fibroblastic reticular cells (FRCs)) also promote tolerance by their expression of peripheral tissue antigens.[61–63] Foreign antigens, however, elicit profound immune responses within the LN upon their presentation by activated dendritic cells, among other mechanisms.

The Lymph Node

Functional lymphatic vessels are required for the maintenance of the LN micro-architecture, which supports optimal interactions between antigen presenting cells (APCs) and rare antigen-specific lymphocytes[64–67] (Figure 3). In the lymph node the primary follicles are composed of B cells with follicular DCs located in the cortex of the lymph node. T cells and DCs distribute in the paracortical area. The majority of lymph node macrophages reside in the marginal sinus and medullary cords. LECs surround the LN and concentrate in the medulla and sinus area.

The compartmentalization of cells in LNs is orchestrated by lymphoid chemokines. Chemokines CCL21 and CCL19 recruit and direct the distribution of chemokine receptor CCR7-expressing cells, mostly T cells and DCs, while CXCL13 attracts CXCR5-expressing B cells. A lipid signaling molecule, sphingosine-1-phosphate (S1P), along with its receptor, facilitates the egress of lymphocytes from LNs into efferent lymphatic vessels.[68, 69] Blood vessels enter the lymph node from the hilum, run through the medulla and branch within and distribute throughout the cortex. In the paracortex, blood vessels specialize into high endothelial venules (HEVs), which facilitate lymphocyte homing from systemic circulation to LNs.[70, 71]

The LN conduit system connects the lymphatic sinus with the walls of the blood vessels and enables the incoming factor(s) from the lymph to move rapidly into the paracortical T cell area.[72–74] The conduit system is implicated in providing physical support required for the rapid initiation of an adaptive immune response after immunization. Soon after immunization, a special subset of conduit DCs rapidly take up and process free antigen moving along the conduit. However, large antigens and microbial particles cannot access the LN via this conduit system. These particles enter the LN sinus where they are sampled by macrophages and B cells. [75–77]

Upon activation in the tissue, DCs rapidly migrate to LNs and initiate cell responses within hours of antigen presentation.[78] DC entry into the peripheral lymphatics is CCR7-dependent.[79] The migration of activated DCs towards the T cell zone in the draining LN also relies on CCR7, which is orchestrated by CCL21 and CCL19 expressed by HEVs and FRCs.[80, 81]

Inflammation

A fine-tuned inflammatory response generates an immune reaction to foreign antigens while preventing overt reactions to self-antigens. The LN structure undergoes dramatic changes following antigen activation, including angiogenesis and lymphangiogenesis, decreased expression of genes contributing to peripheral LN addressin (PNAd), and decreased chemokine production of CCL21 and CXCL13.[64, 65, 82–86] These temporary changes in the LN hinder naïve T-cell and DC access and their interactions while enhancing the ability of effector cells to leave the LN and prevent pathological immune-mediated damage in the LN. Viral infection and an immune-stimulant known as polyinosinic:polycytidylic acid increases FRC suppression of the bystander CD8 T cell responses.[86, 87] However, disruption of lymphatic vessel or their autonomous contractions impairs fluid drainage and, thus, the body's ability to activate an immune response to a pathogen, which leads to persistent infection.[64, 88–92] During clinical lymphedema, immune cell accumulation and impaired immune response are also frequently observed.[93]

During acute inflammation, lymphangiogenesis occurs in the area of inflammation and the draining LN. B cells contribute heavily to LN lymphangiogenesis in response to inflammation.[64, 82, 94] In LPS-induced peritoneal inflammation, macrophages promote lymphangiogenesis by expressing multiple lymphangiogenic growth factors such as VEGF and VEGF-C.[18, 95, 96] However, changes to lymphatic drainage during lymphangiogenesis appear to depend on the antigen and the site of immunization. Lymphatic drainage is reduced in response to oxazolone skin painting-triggered inflammation, as well as when lipopolysaccharide is applied in a peritoneal model. However, lymph transport increases when complete Freund's adjuvant is applied in the foot pad of the mouse.[64, 94, 97] Moreover, gene expression patterns in LECs during inflammation are stimulus-dependent.[98]

During chronic inflammation, lymphangiogenesis promotes *de novo* formation of ectopic lymphoid tissue known as tertiary lymphoid organs (TLOs). These form during autoimmunity, microbial infection and chronic allograft rejection. TLOs share considerable morphological, cellular, chemokine and vascular characteristics with LNs. Chronic rejection of transplanted human kidneys is marked by a substantial increase in lymphatic vessel density within and surrounding immune cell infiltrates compared to organs that engraft well.[99] Additionally, the rejection of corneal grafts can be predicted by the presence of lymphangiogenesis.[100] In the synovium of rheumatoid arthritis patients, the lymphangiogenic factor VEGF-C is increased.[101] Lymphangiogenesis has also been noted in other autoimmune pathologies such as inflammatory bowel diseases, Crohn's disease and ulcerative colitis, further highlighting the important role lymphatic vessels play in these immune processes.[102]

LYMPHATIC METASTASIS

Cancer cells invading the tumor margin enter enlarged lymphatics and travel with the lymph flow to the draining LNs (Figure 4).[103] The growth of metastasizing tumors in LNs is a critical event in disease progression that profoundly impacts patient prognosis and treatment decisions.[104, 105] Cancer progression shares many features of wound healing and inflammatory conditions, including angiogenesis,[64, 82, 94] lymphangiogenesis,[106, 107] and immune cell recruitment.[108, 109] As such, tumors have been described as “wounds that do not heal.”[110] These changes provide an expanded lymphatic network that enhances molecular and cellular delivery to the draining LN. Lymph flow from tumors has been reported to be elevated relative to normal tissue (Figure 4). This is due to the increased interstitial fluid pressure within the tumor mass, which is elevated as a result of the hyperpermeable tumor blood vasculature and the lack of lymphatic transport inside of tumors. Increases in interstitial flow have been positively correlated with cancer cell dissemination to the draining lymph node.[111, 112]

Extensive studies utilizing molecular markers such as LYVE-1 and podoplanin have revealed the existence of lymphatic vessels within and around primary tumors. [113–119] These studies have shown that secretion of VEGF-C and VEGF-D by tumor cells correlates with the development of tumor-associated lymphatic networks in numerous animal models. This corresponds with increases in vessel diameter, volumetric flow rate and cancer cell dissemination to LNs.[103, 115, 116, 118, 120–125] Despite several observations of LEC proliferation within tumors, intratumoral lymphatic vessels are physically collapsed and non-functional.[116, 126] These observations collectively suggest that cancer cells disseminate by invading and utilizing lymphatic vessels within their margins.[116, 122] Moreover, functional lymphatics in the tumor periphery have been shown to exhibit abnormal draining patterns, indicative of dysfunctional valves in these vessels.[125]

Many human tumors do not show evidence of lymphangiogenesis within and around the tumor even though they commonly metastasize to LNs. Clinically, the correlation between VEGF-C/D expression in human tumors and metastatic spread is quite strong. It is therefore likely that VEGF-C/D expression by human tumors facilitates metastasis by priming existing lymphatic vessels in the tumor periphery and in the draining LN in addition to the generation of any new lymphatic vessels.[127]

Lymphangiogenesis has been observed within the tumor-draining LN prior to tumor seeding (Figure 4). The hypoxic microenvironment of the tumor causes the draining LN to become chronically inflamed. As a consequence, the draining LN is bathed in fluid from the tumor that consists of inflammatory mediators and tumor-secreted antigens, cytokines and growth factors. In the end, the tumor-draining LN is more immunologically tolerant of the invading cancer cells compared to a LN that does not drain the tumor.[111]

Multiple studies have shown that blocking VEGF-C or VEGF-D signaling reduces metastasis to the LN, thus providing promising targets for therapies in lymphatic metastasis.[103, 121, 122, 128, 129] Unfortunately, targeting VEGF-C/VEGFR-3 signaling in animal models works well to prevent LN metastasis but does not affect tumor growth once the tumor has seeded in the LN. This indicates that effective anti-VEGF-C/D treatments may be limited to the neoadjuvant or preventive settings.[103, 130]

While anti-VEGF-C/VEGFR3 targeted therapies are still in their clinical infancy, anti-angiogenic therapies that target VEGF-A have been pursued in human studies. VEGF-A, which promotes hematogenous metastasis by generating tumor blood vessels, also promotes lymphatic metastasis.[122, 131, 132] While these promising new therapies that target VEGF-A have yielded a weaker impact on metastatic disease in the clinical when compared to results

presented in animal model-based preclinical studies, they have shown tremendous promise in specific disease settings such as metastatic colon cancer.[133] Some preclinical studies have put forth a provocative hypothesis that anti-VEGF therapies administered at high doses might promote metastasis.[132, 133] However, this has not been recapitulated in other preclinical studies or in recent clinical trials.[130, 134]

CONCLUSION

Lymphatic vessels are pivotal to maintaining fluid balance, immune defense and uptake of dietary fats. Moreover, lymphatic vessels facilitate tumor metastasis. It has become increasingly clear that lymphatic vessels differ greatly from blood vessels structurally and in the molecular mechanisms that drive their function. Notably, transport of fluid is mediated by mechanisms intrinsic to the collecting lymphatic vessels themselves rather than by a centralized pump such as the heart. Cell signaling pathways that are relevant to lymphatic specification and function are being uncovered at a rapid rate. Better imaging techniques have allowed for real-time visualization of lymphatic pumping, tumor metastasis, immune function and characterization of various lymphatic phenotypes at cellular and subcellular levels. With the knowledge provided by these tools, key players such as FoxC2 have been linked to lymphatic insufficiency and tissue edema. The role of lymphangiogenesis in the immune rejection of transplanted tissues has been characterized and novel treatments have been developed to address this issue. Moreover, secretion of lymphangiogenic growth factors such as VEGF-C by tumors has been connected to NO production, prostaglandin synthesis and dissemination of cancer cells to lymph nodes, linking it to lymphatic metastasis in animal models. Despite these advancements, numerous molecular pathways important to lymphatic (patho)physiology remain poorly understood. The fact that tumors invade draining lymph nodes has made understanding lymphatic physiology crucial to the development of new cancer treatments. Future investigations into how these numerous molecular pathways communicate with one another to regulate the many facets of lymphatic function will provide invaluable tools for the management of lymphatic-associated diseases.

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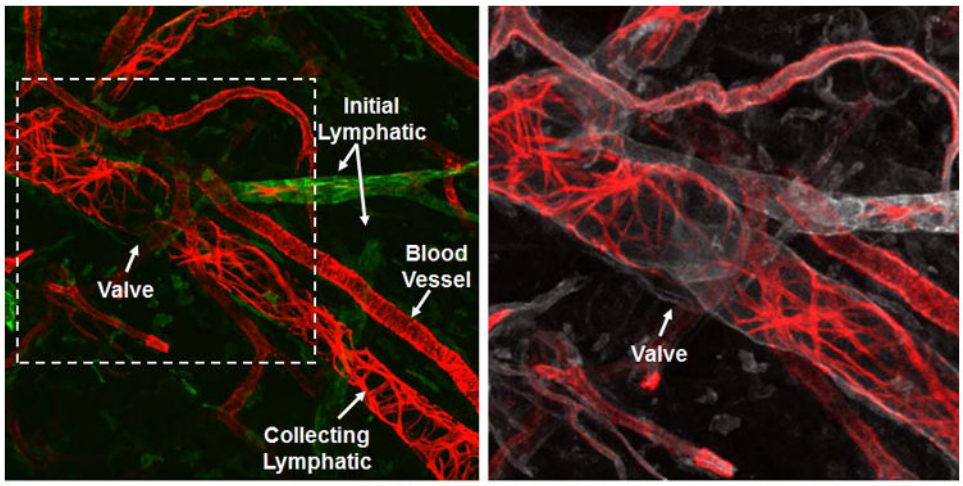

FIGURE 1. Initial and Collecting Lymphatics

The lymphatic vessels of the ear of an athymic nude mouse are shown. LYVE-1 (green) indicates the initial lymphatic vessels. αSMA (red) indicates the SMCs of the collecting lymphatic vessels and blood vessels. The circumferential αSMA staining pattern of the collecting lymphatic vessels is distinct from the more homogenous pattern of the blood vessels. CD31 (white) indicates all endothelial cells in the field and shows an intraluminal valve in the collecting lymphatic vessel.

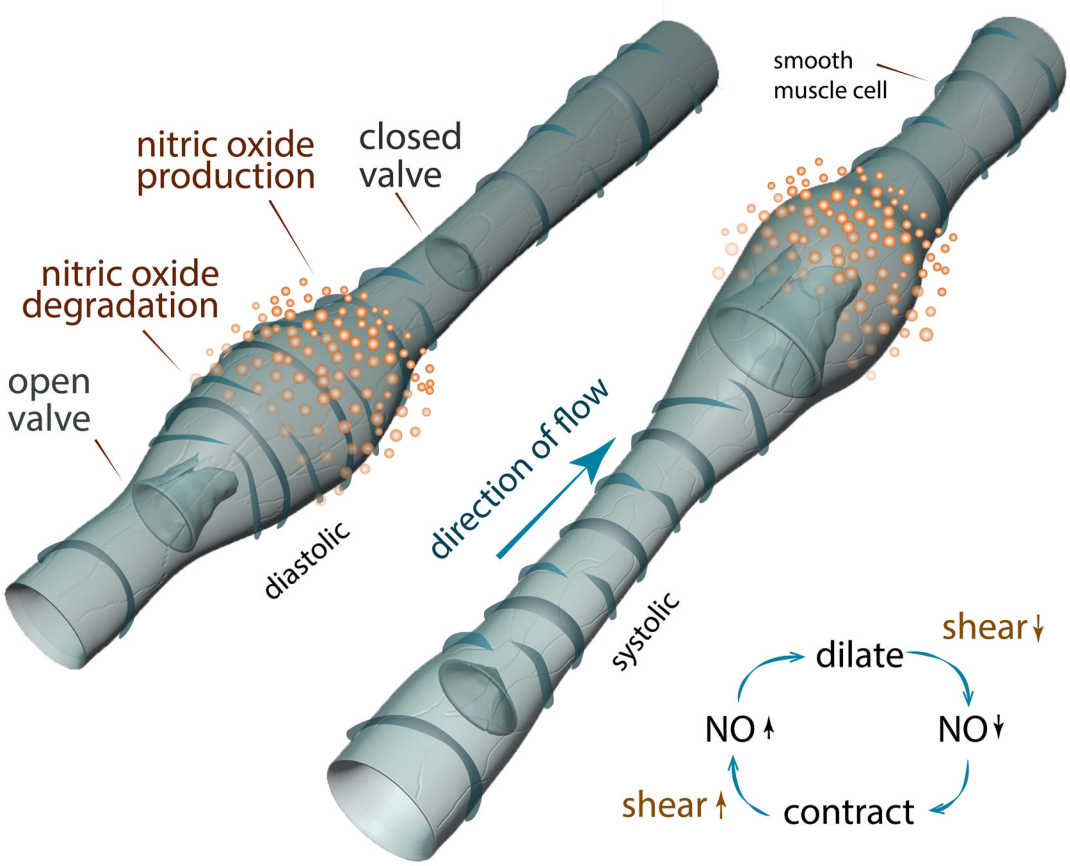

FIGURE 2. Lymphatic Contraction

This illustration of the diastolic and systolic phases of an autonomous lymphatic contraction shows the NO dependency. In the diastolic phase, local NO release allows for the relaxation of the vessel wall and filling to occur. As the NO degrades, the vessel constricts, driving flow into the next lymphangion. It is hypothesized that the increase in flow and shear stress as a result of a contraction, stimulates NO production, allowing the diastolic filling to occur. The spatial and temporal gradients of NO are critical to proper contraction function and are mediated by eNOS in LECs.

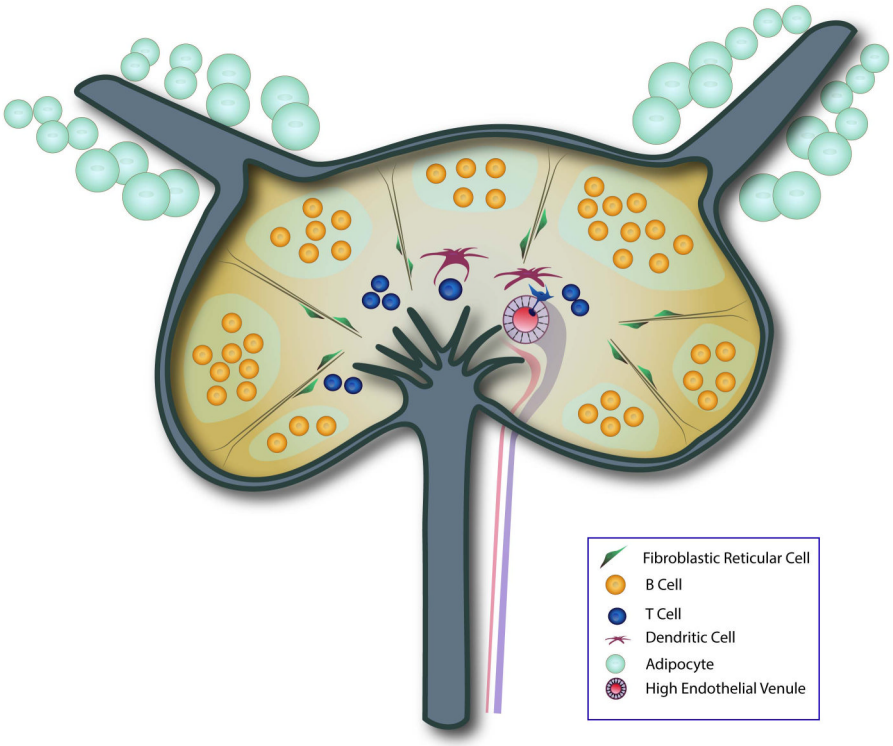

FIGURE 3. Microarchitecture of the Lymph Node

This simplified schematic of the lymph node highlights key structural features critical for the proper activation of an immune response. The adipose-encased afferent collecting lymphatic vessels move antigen-rich lymph into the subcapsular sinus. Fluid and small antigens can then filter into the lymph node cortex, where B cell follicles are found. Reticular fibers, bound by their associated FRCs and specialized DCs, traverse the cortex to rapidly bring antigen to the paracortical and medullary regions where T cells reside. HEVs in the paracortical area bring naïve T cells into the node as well to interact with DCs. In the medulla, there are lymphatic vessels that drain the lymph node and collect fluid into the efferent lymphatic vessel.

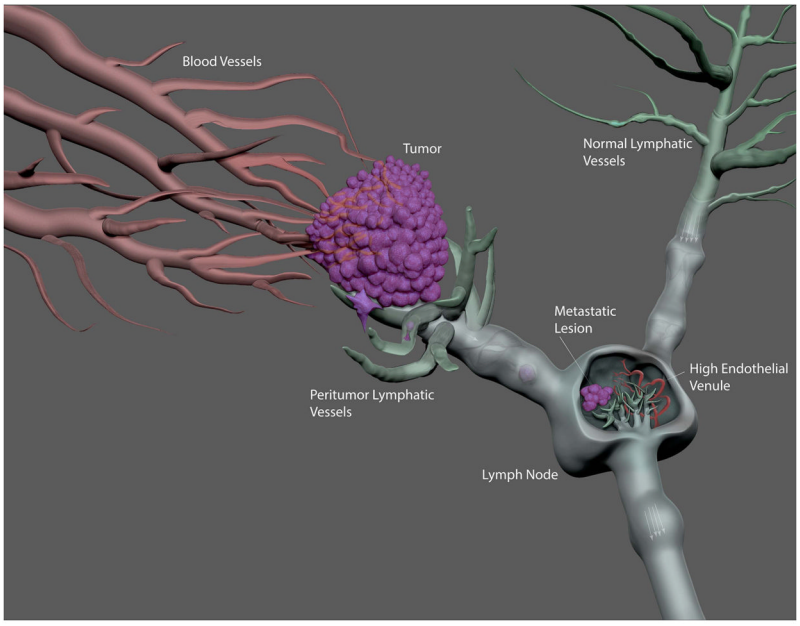

FIGURE 4. Components of Lymphatic Metastasis

In contrast to functional blood vessels that can be found throughout the tumor, functional lymphatic vessels are found in the margin of tumors. These tumor margin lymphatic vessels tend to be enlarged and have greater lymph flow compared to lymphatic vessels draining normal tissues. These functional lymphatics are penetrated by invading cancer cells, which travel to the draining lymph node where they evade the immune system and start to form a secondary metastatic tumor. Understanding the growth of the cancer cells in the lymph node is critical to the development of effective treatment for these metastatic lesions.