REVIEW SUMMARY

LYMPHATIC BIOLOGY

Biological functions of lymphatic vessels

Tatiana V. Petrova* and Gou Young Koh*

BACKGROUND: Blood and lymphatic vessel networks form two arms of the vertebrate cardiovascular system that play complementary roles in body homeostasis maintenance and multiple diseases. Lymphatic vessels are lined with lymphatic endothelial cells (LECs), which represent a distinct endothelial cell lineage characterized by a specific transcriptional and metabolic program. The general functions of lymphatic vessels in fluid transport and immunosurveillance are well recognized, as is their specialization into capillaries, serving as an entrance point of interstitial components and immune cells and collecting vessels that deliver lymph to lymph nodes (LNs) and blood circulation. It is becoming increasingly clear that adult lymphatic vessels, exposed to different organ-specific environments, acquire distinct characteristics and in turn execute multiple tissue-specific functions.

ADVANCES: This Review provides an overview of the recent advances in our understanding of new functions of adult mammalian lymphatic vessels, such as immunomodulation, contribution to neurodegenerative and neuroinflammatory diseases, and response to anticancer therapies. LN LECs have been shown to archive antigens and directly regulate immune cell properties, including immune cell survival and positioning within the LN. Rediscovery of meningeal lymphatic vessels has uprooted the dogma of brain immune privilege, and these vessels now emerge as key regulators of neuroinflammation and neurodegeneration. Intestinal lacteals display distinct cellular characteristics that make them especially suitable for dietary fat uptake and designate them as promising targets for the treatment of obesity. Tumor lymphatics have long been recognized as conduits for metastatic cell dissemination; however, recent data show that lymphatic vessels have multiple additional functions, such as forming metastatic cancer cell niches but also controlling productive response to antitumor immune therapies. Last, discovery of vascular beds with hybrid blood and lymphatic characteristics, such as the Schlemm's canal in the eye and the kidney ascending vasa recta, underscores the degree and potential of endothelial cell plasticity.

OUTLOOK: Molecular characteristics of organ-specific vascular beds and understanding their organotypic functions are among the current fundamental questions of vascular biology. Emerging evidence points to the major contribution of lymphatic vessels, a vascular system generally associated only with tissue-drainage functions. High-resolution analyses of endothelial heterogeneity and organotypic lymphatic vessel architecture, in addition to deciphering the molecular codes that LECs use for communication with other cell types, are necessary to fully understand the role of lymphatics in organ physiology and pathology. Integration of such knowledge with research from other fields, such as immunology and bioengineering, will uncover new possibilities for promoting tissue regeneration and developing new therapies for cancer, obesity, neuroinflammation, and neurodegeneration. ■

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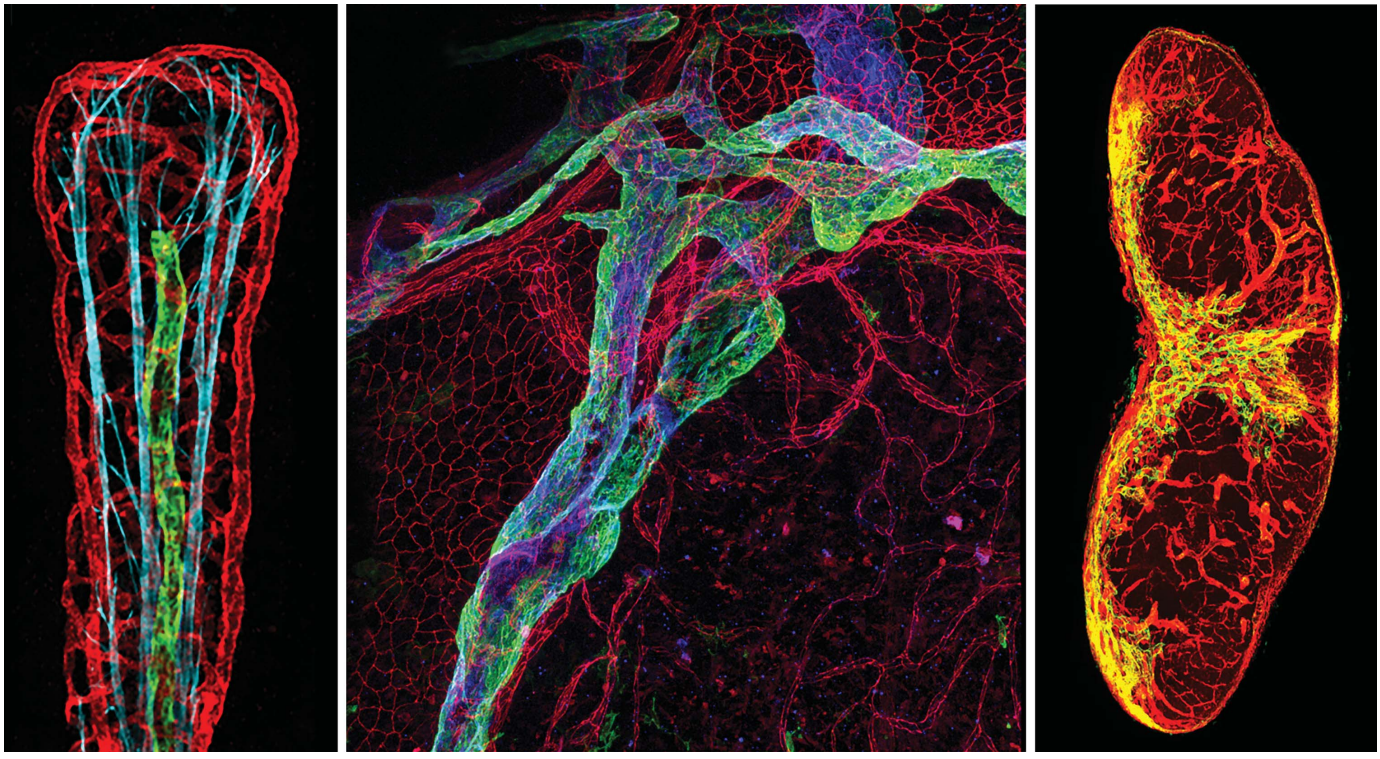

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Organ-specific lymphatic vessels in small intestine, meninges, and LN. (Left) Small intestine. Shown are LYVE-1$^+$ (green) lacteal, CD31$^+$(red) capillary plexus, and α-smooth actin$^+$ (blue) longitudinal smooth muscle cells. (Middle) Meninges. Shown are LYVE-1$^+$ (green) and VEGFR3$^+$ (blue) lymphatic vessels and CD31$^+$ (red) blood vessels. (Right) LN. Shown are LYVE-1$^+$ (green) lymphatic vessels and CD31$^+$(red) blood vessels, including high endothelial venules.

REVIEW

LYMPHATIC BIOLOGY

Biological functions of lymphatic vessels

Tatiana V. Petrova[1]* and Gou Young Koh[2,3]*

The general functions of lymphatic vessels in fluid transport and immunosurveillance are well recognized. However, accumulating evidence indicates that lymphatic vessels play active and versatile roles in a tissue- and organ-specific manner during homeostasis and in multiple disease processes. This Review discusses recent advances to understand previously unidentified functions of adult mammalian lymphatic vessels, including immunosurveillance and immunomodulation upon pathogen invasion, transport of dietary fat, drainage of cerebrospinal fluid and aqueous humor, possible contributions toward neurodegenerative and neuroinflammatory diseases, and response to anticancer therapies.

The lymphatic vascular network is a low-pressure, unidirectional flow system that is present in vertebrates in virtually every organ of the body. In physiological conditions, its main functions are the removal of interstitial fluid (ISF) formed by blood capillary filtrates and tissue immunosurveillance. Lymphatic vessels are lined with lymphatic endothelial cells (LECs), a distinct endothelial cell lineage characterized by specific transcriptional and metabolic programs (*1*–*4*). All LECs express the homeobox transcription factor prospero-related homeobox 1 (PROX1) and receptor tyrosine kinase vascular endothelial growth factor (VEGF) receptor–3 (VEGFR-3). In addition, the majority of LECs express the membrane glycoprotein podoplanin. VEGFR-3 and its secreted ligand, VEGF-C, are the main drivers of developmental and pathological lymphangiogenesis (*2*). The sources of VEGF-C vary depending on tissue and developmental stage and include vascular and visceral smooth muscle cells, fibroblasts, blood endothelial cells, macrophages, and tumor cells (*2*, *5*–*7*). Proteolytic processing of VEGF-C, which generates a ligand capable of activating VEGFR-3, is a key step in the lymphangiogenic process. This processing requires the extracellular protein CCBE1 (collagen and calcium-binding EGF domain-containing protein 1) and the metalloprotease Adamts3 (ADAM metallopeptidase with thrombospondin type 1 motif 3) during development (*8*), but other proteases can participate in adult and diseased tissues (*9*).

Blind-ended, highly branched lymphatic capillaries take up ISF and serve as entrance points for immune cells (Fig. 1A). Lymph is then transported by collecting vessels to the lymph nodes (LNs) and returned to blood circulation through the connection of major lymphatic ducts with veins. Capillary lymphatic vessels have a thin basement membrane and no supporting mural cells. Their oak leaf–shaped endothelial cells are connected by discontinuous junctions (termed "buttons"), which increases permeability and facilitates the uptake of interstitial components and the transmigration of immune cells. Endothelial cells in collecting vessels are elongated and connected by continuous cell–cell junctions (termed "zippers") (*10*). Contractile lymphatic smooth muscle cells (LSMCs) and a prominent basement membrane surround these collecting vessels, so that this vessel type has decreased permeability. LSMC contractions, compression of surrounding skeletal muscle, and arterial pulsations drive lymph propulsion. Intraluminal lymphatic valves in collecting vessels and lymphovenous valves at lymphaticovenous junctions prevent retrograde lymph and blood reflux. Macrophages and dendritic cells (DCs), which frequently occur in association with the lymphatic vasculature, provide factors that regulate lymphatic endothelial proliferation and permeability (*11*–*14*). During inflammation, T helper 2 (T_H2) T cell–derived cytokines inhibit lymphangiogenesis, whereas regulatory T cells (T_{reg} cells) promote lymphatic vascular repair (Fig. 1B) (*15*, *16*).

During mammalian embryonic development, transdifferentiation of venous precursors produces a major population of LECs that give rise to cardiopulmonary, hepatic, meningeal, and dermal lymphatic vessels (*1*, *17*). Nonvenous progenitors substantially supplement dermal lymphatic vessels (*18*), whereas hemogenic endothelium contributes to intestinal lymphatic vessels (*19*). The transcription factor PROX1 pays a major role in establishing and maintaining the lymphatic endothelial transcription program (*1*, *20*). The initiation of lymphatic vascular development corresponds to the need for fluid drainage. In this process, mechanical tissue stretch sensed by integrin β1 in LECs cooperates with biochemical VEGF-C/VEGFR-3 signaling to promote LEC proliferation (*21*, *22*). Specialization of the collecting lymphatic vessels occurs late during gestation and is initiated in response to increased lymph flow. It comprises capillary pruning, intraluminal valve formation, and LSMC recruitment (*23*, *24*). Molecularly, collecting vessel LECs have reduced or absent expression of molecules involved in lymphangiogenesis and immune cell trafficking, such as VEGFR-3, chemokine (C-C motif) ligand 21 (CCL21), and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1). However, they produce the mechanosensitive transcription factors forkhead box C2 (FOXC2) and GATA-binding protein 2 (GATA2), which shows especially prominent expression in lymphatic valves (Fig. 1C) (*23*, *25*–*27*). Similar to blood vessels, platelet-derived growth factor–B (PDGF-B)/PDGF receptor β (PDGFRβ) signaling is necessary for LSMC recruitment (*28*).

A considerable body of knowledge has accumulated about the general molecular mechanisms that regulate lymphatic development and function (*2*, *29*, *30*). It has become increasingly clear that adult lymphatic vessels, which are exposed to different organ-specific environments, acquire distinct characteristics and in turn execute multiple tissue-specific functions (*31*–*33*). These newly understood biological functions of mammalian lymphatics are the subject of this Review.

The role of LN lymphatic vessels in coordinating immune responses

Peripheral lymphatic vessels transport antigens and immune cells to draining LNs, fostering immune response or tolerance (Fig. 2). LNs are strategically positioned throughout the body, having formed during embryogenesis in predetermined locations. Although lymphatic vessels are closely associated with the developing LN anlagen, they are dispensable for initial extravasation of lymphoid tissue inducer cells from blood vessels (*34*–*36*). However, lymphatic vessels play a crucial role in LN expansion by transporting additional lymphoid tissue inducer cells from the periphery to the site of LN development (*36*, *37*). Interstitial flow generated by perinodal lymphatics potentiates stromal production of the chemokine CXC ligand 13 (CXCL13), which is important for retention of lymphoid tissue inducer cells (*36*). Accordingly, either loss of lymphatic vessel transport function through inactivation of the transcription factor FOXC2 or blockade of embryonic lymphangiogenesis impairs embryonic LN development (*35*, *36*). LN development is interrupted in mice with lymphatic endothelial–specific inactivation of lymphotoxin-β receptor (LTβR) and especially with the loss of receptor activator of nuclear factor κB (NF-κB) (RANK) (also known as TNFRSF11a) (*37*, *38*), although

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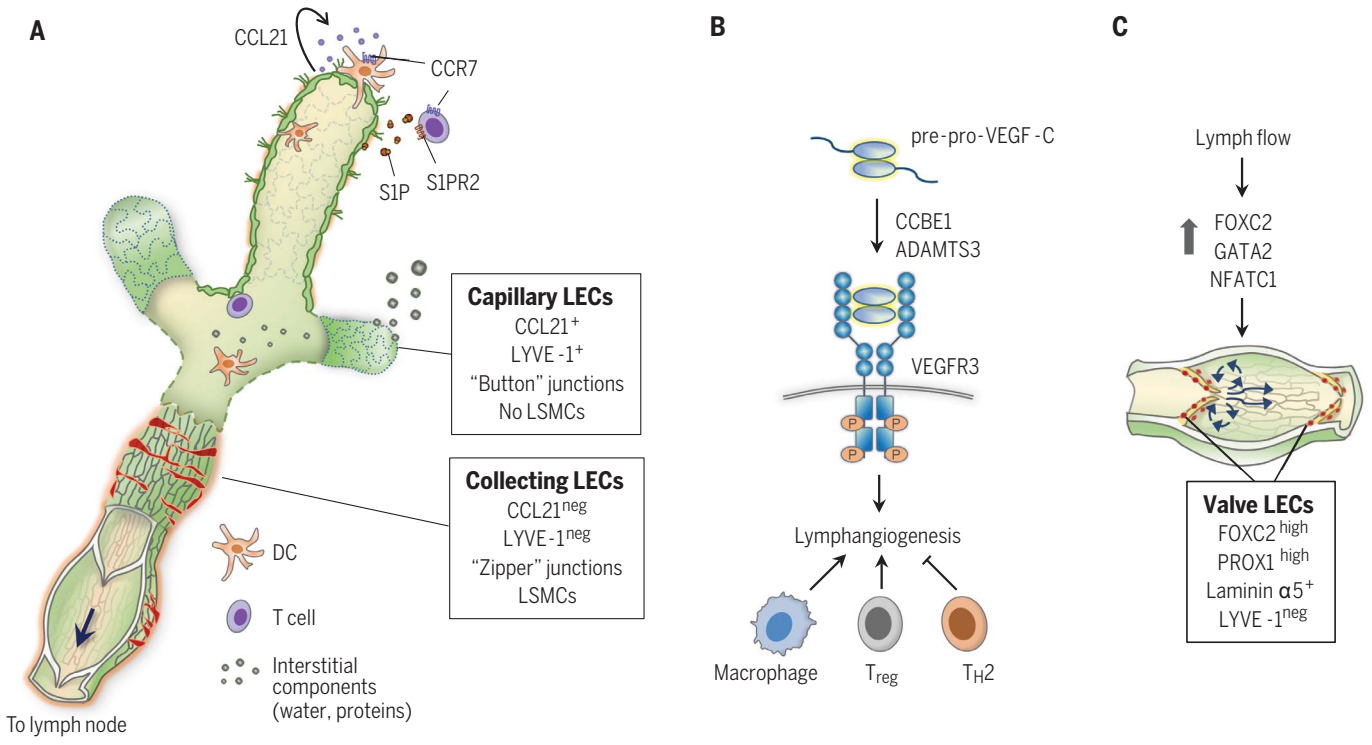

Fig. 1. General features of lymphatic vessels. (**A**) Types of LECs. Capillary LECs are connected by discontinuous junctions and are in direct contact with the tissue microenvironment, which facilitates ISF uptake and migration of immune cells. Collecting-vessel LECs are connected with continuous junctions and surrounded by basement membrane and LSMCs. Valve LECs are attached to specialized extracellular matrix, and the valve region lacks LSMC coverage. Selected features of each population are shown in boxes; more detailed molecular description is available in (*30*). (**B**) CCBE1- and ADAMTS3-dependent processing of VEGF-C produces bioactive VEGF-C, which induces lymphangiogenic responses in VEGFR-3–expressing LECs. During inflammation, T_H2 cytokines reduce lymphangiogenesis, whereas macrophages and T_{reg} cells promote lymphangiogenesis and assist in lymphatic vascular repair. (**C**) Lymph flow maintains the expression of mechanosensitive transcription factors FOXC2, GATA2, and NFATC1 (nuclear factor of activated T cells 1) and collecting lymphatic vessel function. Selected markers of valve LECs are shown; a more detailed description is available in (*154*).

the underlying mechanisms remain to be clarified.

LNs are highly compartmentalized, with discrete niches for T and B cells. LECs from different LN regions are molecularly distinct and perform multiple specialized functions (*39*–*42*). DC migration into peripheral lymphatics relies on LEC-derived CCL21 gradients, whereas CCL21 is produced mostly by fibroblastic reticular cells within the LN (*41*). LECs lining the lymph sinus "ceiling," the external lymphatic vascular layer of the LN, express high levels of CCRL1, a decoy receptor for CCL21. This arrangement creates a CCL21 gradient for facilitating migration of DCs into the LN medulla (*43*). Plasmalemma vesicle–associated protein (PLVAP), normally restricted to fenestrated blood endothelial cells, is also expressed by subcapsular sinus (SCS) LECs. In these cells, PLVAP forms molecular "sieves" or diaphragms covering transendothelial channels, which prevents the passage of larger solutes into follicular reticular cell conduits (*44*). SCS LECs also express the lymphocyte adhesion molecule MADCAM1 (mucosal vascular addressin cell adhesion molecule 1) and integrin α2b [integrin αIIb (ITGA2B) or CD41] (*38*). The role of ITGA2B in LN LECs is not understood, but its expression is further induced during inflammation or tumorigenesis (*38*, *45*).

Medullary LECs express self-antigens and the T cell inhibitory programmed-death ligand 1 (PD-L1) (*46*) and participate in alloreactive $CD8^+$ T cell deletion. Interferon signaling induces PD-L1 expression in LECs (*47*, *48*). The mechanisms for LN LEC self-antigen expression are not well known aside from its independence from the transcription factor autoimmune regulator (AIRE), which drives promiscuous expression of self-antigens in medullary thymic epithelial cells (*46*). LN LECs also induce $CD4^+$ T cell anergy through cross-dressing, although somewhat contrasting mechanisms have been proposed that involve either antigen transfer from LECs to DCs (*49*) or acquisition of preloaded peptide–major histocompatibility complex II (MHC-II) complexes from DCs by LECs (*50*).

LECs are highly endocytotic (*51*). SCS LECs exploit this ability to achieve rapid transcytotic delivery of lymph-borne immunoglobulin G (IgG) into the LN parenchyma (*52*). Medullary LECs use it for soluble antigen acquisition and subsequent presentation to $CD8^+$ T cells to mediate deletional tolerance (*53*). Proliferating LN LECs also can sequester antigens and store them long term ("archiving") during viral infection. These antigens are subsequently released from dying LECs in the contracting lymphatic vasculature during resolution of inflammation, and DCs use them for cross-presentation and generation of $CD8^+$ T cell memory responses (*54*, *55*).

Immune cell compartmentalization is crucial for LN function in host defense. Lymphatic vessels are extensively involved in the regulation of immune cell trafficking and positioning within the LNs. One of the best-studied mechanisms concerns the bioactive lipid sphingosine 1 phosphate (S1P), which acts as a ligand for the family of S1P G protein–coupling receptors. S1P is produced intracellularly, and spinster homolog 2 (SPNS2)–mediated transport of S1P from LECs generates S1P gradients within the LNs, which are sensed by various immune cell populations (*56*). These gradients are necessary for T and B cell egress into efferent lymph and thus are important for lymphocyte recirculation (*57*). Loss of LEC-dependent S1P gradients also leads to LN accumulation of natural killer (NK) cells

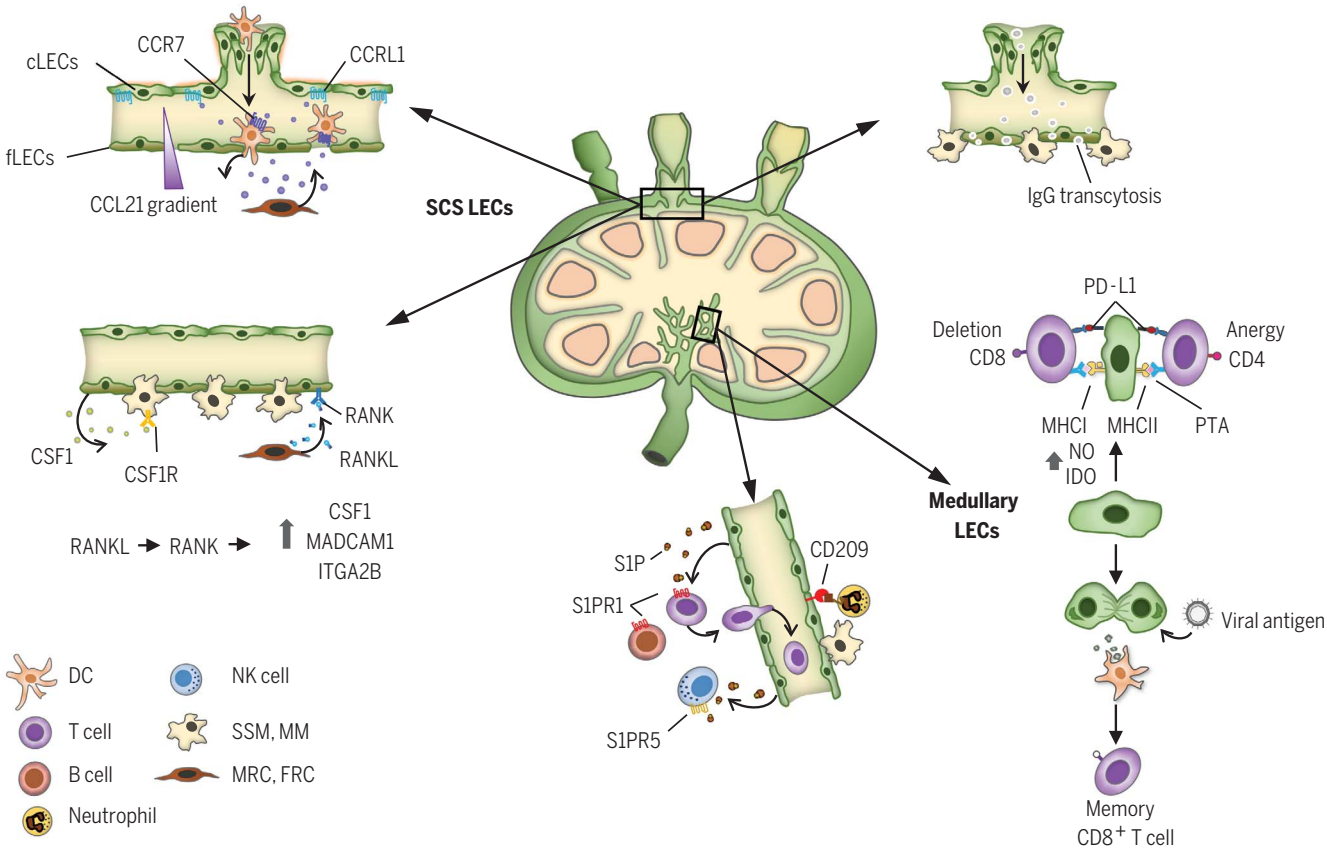

Fig. 2. Compartmentalized functions of LN LECs. Decoy CCL21 receptor CCRL1 produced by "roof" SCS LECs creates a CCL21 gradient and regulates intranodal migration of DCs. LN LECs express high levels of CSF1, which maintains SCS and medullary macrophages. The expression of CSF1, MADCAM1, and ITGA2B in LECs is maintained by RANK expressed on LECs, whereas RANKL is produced by marginal reticular cells. SCS LECs use transcytosis for delivery of lymph-borne IgG. Medullary LN LECs produce peripheral tissue antigens and PD-L1 and induce deletion of alloreactive $CD8^+$ T cells as well as $CD4^+$ T cell anergy. Medullary LN LECs in humans express CD209 to retain neutrophils, which may be important in clearing lymph-borne pathogens. Proliferating LECs take up antigen, which they release during LN contraction to induce DC-mediated T cell memory responses. S1P, produced by medullary LECs, is necessary for recirculation of B and T lymphocytes, for medullar localization of NK cells, and for T cell survival. SSM, subcapsular sinus macrophage; MM, medullary macrophage; MRC, marginal reticular cell; FRC, follicular reticular cell; PTA, peripheral tissue antigen; NO, nitric oxide; IDO, indoleamine 2,3-dioxygenase.

and their mislocalization from the medulla to the T cell zone, where they respond less efficiently to challenge with *Salmonella* infection (*58*).

In addition to providing guidance cues for lymphocytes, S1P supports the survival of naïve T cells by promoting their mitochondrial fitness and migratory capacity (*59*). S1P also regulates peripheral lymphatic vessel junctional organization (*57*). In the skin, capillary LEC-derived S1P selectively enhances migration of $CD4^+$ T cells into initial lymph vessels by promoting both LEC permeability and T cell motility and adhesion to endothelial cells (*60*). Another bioactive lipid, lysophosphatidic acid (LPA), promotes efficient lymphocyte trafficking by inducing porous junctional organization of sinus-lining LECs through cross-talk between the LPA receptor LPAR1 and S1PR1 signaling (*61*).

$CD169^+$ macrophages line the SCS and medullary sinuses. These cells are strategically positioned to rapidly capture lymph-borne pathogens and initiate a specific immune response by relaying antigens to B cells and producing cytokines for DC, neutrophil, and NK cell recruitment. LN LECs are central in the establishment of a niche for SCS and medullary macrophage homing and maintenance (*38*, *62*). LN LECs serve as a major source of macrophage prosurvival factor colony-stimulating factor–1 (CSF1). Lymphatic endothelial–specific inactivation of CSF1 results in loss of SCS and medullary macrophages without affecting other LN macrophage populations (*62*). The RANK receptor controls CSF1 induction of LECs, with nearby $CCL19^+$ marginal reticular cells providing its ligand, RANKL. SCS LEC MADCAM1 and ITGA2B expression also depends on RANK, indicating a general role for RANK–RANKL signaling in shaping the LEC LN-specific program (*38*). A single-cell survey of human LN LECs has identified six distinct subpopulations. SCS LECs produce multiple neutrophil chemoattractants, whereas medullary sinus LECs selectively express C-type lectin CD209, which is important for neutrophil retention. Accumulation of neutrophils by medullary LECs in the immediate vicinity of macrophages may represent a mechanism for lymph-borne pathogen clearance and prevention of their spread (*42*).

Overall, studies of LNs highlight several important features of LECs, which will likely be important for understanding the roles of lymphatic vessels in other organs. These roles may include high endocytic and transcytotic capacity, production of bioactive lipids and growth factors with immunomodulatory and niche-sustaining functions, and an important contribution of lymphatic vessels in establishing gradients of biologically active molecules that govern tissue compartmentalization.

Intestinal lymphatic vessels: New players in gut homeostasis and immunity

Intestinal lymphatic vessels mediate distinct functions in fat absorption, maintenance of

gut immunity, and promotion of intestinal homeostasis (*32*). Each of the millions of small intestinal villi covering the gut mucosa is endowed with a lacteal, a blunt-ended lymphatic capillary. Lacteals are connected to submucosal lymphatic vessels and together constitute the intestinal lymphatic vasculature network, which is the largest lymphatic bed in the human body. Although most other adult lymphatic vessel beds are relatively quiescent, lacteal LECs slowly but continuously proliferate and frequently harbor filopodia (*63*), which suggests a distinct and ongoing lymphangiogenic process. This renewal mechanism may aid lacteals in coping with the constant mechanical and chemical cellular stress caused by intestinal peristalsis and exposure to dietary and microbial products.

In mice, mesenteric LECs appear around embryonic day 12.5 (E12.5) and grow toward the intestinal submucosa at E14.5, when the intestinal villi emerge (*19*, *25*, *64*, *65*). Mesenteric LECs then grow into lacteals at E17.5 and participate in postnatal dietary lipid absorption (*64*). The development of intestinal lymphatic vessels relies on activation of VEGFR-3 by VEGF-C (*64*). A key downstream effector of VEGFR-3 signaling in mesenteric LECs is phosphatidylinositol 3-kinase (PI3K); deletion of the p110α catalytic subunit of this kinase results in intestinal lymphatic-specific developmental defects (*19*).

The structure and function of intestinal lymphatic vessels must be tightly coordinated to maintain intestinal homeostasis. The results of intestinal LEC ablation in mice highlight the importance of the intestinal lymphatic vasculature in whole-body homeostasis. Loss of lacteals in mice harboring the diphtheria toxin receptor under control of the LYVE-1 promoter leads to rapid disruption of the blood-gut barrier and to sepsis and lethality (*66*). Despite the crucial functions of intestinal lymphatic vessels, the specific molecular regulators of their maintenance, renewal, and functions are only beginning to be identified. Although many adult lymphatic vascular beds are quiescent and VEGF-C–independent, intestinal lacteals (*5*, *63*) and meningeal lymphatic vessels (mLVs) (*6*) are exceptions. Intestinal lymphatic vessel maintenance requires continuous VEGFR-3 signaling (Fig. 3A), and genetic deletion of *Vegfc* or *Vegfr3* or a VEGFR-3 signaling blockade shortens lacteal length, reduces the number of lacteal filopodia, and impairs dietary lipid absorption (*5*, *63*). Conversely, by depleting negative regulators of the pathway, VEGF-C–VEGFR-3 signaling hyperactivation also leads to intestinal lymphatic dysfunction. This finding suggests the importance of tight regulation of VEGFR-3 signaling levels for proper intestinal lymphatic development and lacteal maintenance (*67*–*69*). Patients with Hennekam syndrome have a genetic loss of function of *CCBE1*, which activates production of bioactive VEGF-C. With its loss, these patients develop intestinal lymphangiectasia that is characterized by dilated and malfunctioning intestinal capillaries (*70*), further underscoring the role of VEGF-C–VEGFR-3 signaling in intestinal homeostasis. Intestinal villus SMCs are a source of VEGF-C (*5*), but intestinal villus macrophages also produce VEGF-C upon stimulation by gut microbes, implicating the gut microbiota as additional regulators of lacteal integrity (*7*). Further studies involving deletion of *Vegfc* in specific cell types are required to reveal source(s) of VEGF-C in intestinal villi and the regulatory mechanisms of its secretion.

Other molecular regulators of lacteals include Notch ligand δ–like protein 4 (DLL4). Lacteal DLL4 is induced in response to active VEGFR2 or VEGFR3 signaling and regulates LEC migration and rearrangement. DLL4-deficient LECs have reduced directional migration in response to VEGF-C. Furthermore, although wild-type lacteals display a mix of mature button-like and remodeling zipper-like cell-cell junctions, LEC-selective loss of DLL4 decreases the proportion of functional button junctions, leading to lacteal attrition and defective fat absorption (*63*). Calcitonin receptor–like receptor (CLR), which is abundantly expressed in LECs and activated by peptide hormone adrenomedullin, is also required for lacteal maintenance. Selective inactivation of CLR in lymphatic vessels results in intestinal inflammation with systemic lymphatic insufficiency. CLR deletion downregulates the expression of DLL4, indicating that the coordination between CLR and Notch-DLL4 signaling is important for the maintenance of lacteals (*71*). In humans, mutations in *CALCRL*, which encodes CLR, cause autosomal recessive nonimmune hydrops fetalis with lymphatic dysplasia (*72*).

Cross-talk with blood vessels specifically modulates the junctional organization and transport capacity of intestinal lacteals (*73*). In physiologic conditions, blood endothelial–specific VEGF-A receptors VEGFR-1 and neuropilin 1 (NRP1) restrict bioavailability of VEGF-A in the intestine. However, when these are lost, VEGF-A activity increases and enhances VEGFR-2 signaling in nearby LECs and transforms the button-type junctions into zipper-type (*73*). Functionally, such junctional reorganization reduces lymphatic dietary fat uptake and increases resistance to high-fat diet–induced obesity. A high level of VEGF-A has opposite effects on villus blood capillary plexus and lacteals, inducing leakage of blood vessels while closing lacteal junctions. This effect is in part attributable to a mechanism involving inhibition of Rho-associated protein kinases (*73*).

The high rate of adult lacteal LEC remodeling is underscored by the phenotype of mice with lymphatic endothelial–specific inactivation of vascular endothelial cadherin (VE-cadherin) (*74*). Although VE-cadherin is a component of all endothelial adherens junctions, its loss in lymphatic vasculature results in an intestinal-specific phenotype with striking fragmentation and distension of lacteals, deterioration of the mesenteric lymphatic valves, and lymphatic vessel constriction (*74*). Lacteals are surrounded by the villus stroma residing under the rapidly renewing intestinal epithelial cells, and additional studies are needed to elucidate interactions between a lacteal and the surrounding stromal cells for their maintenance and function.

Dietary lipids and fat-soluble vitamins are absorbed by enterocytes and incorporated into large triglyceride-enriched lipoprotein particles, called chylomicrons, which then enter the lymphatics through lacteals. They drain into intestinal submucosal lymphatics and then to mesenteric collecting vessels and LNs. Intestinal lymph is finally delivered into the systemic circulation through the thoracic duct and metabolized by the liver (*32*). Longitudinal villus SMCs surrounding the lacteals facilitate efficient drainage of absorbed lipids by actively contracting and squeezing lacteals under the control of the autonomic nervous system (*75*). Villus SMCs provide factors for lacteal maintenance and serve to compartmentalize the interactions of a portion of immune cells in the laminal propria (*5*, *76*). Lacteals are in close contact with the enteric neurons, with some nerve endings found in the lacteal lumen (*65*, *77*). These lacteal neurons are excited by lymph in vitro, but the precise role of a possible neurolymphocrine interaction remains to be elucidated (*77*). Lacteal LEC themselves express β-adrenergic receptors and can be targets of neurotransmitters or drugs that modulate β-adrenergic receptors (*78*).

Intestinal lymphatic vessels are important components of the gut immunosurveillance system, which promotes mucosal immunity and tolerance. Similar to other tissues, subpopulations of $CCR7^+$ DCs carry antigens from the intestine to the mesenteric LN in response to LEC-derived CCL21, which is essential for the establishment of oral tolerance (*79*). Together with the hematopoietic system, the intestinal epithelium has the highest renewal rate in the body. Accordingly, intestinal DCs also deliver apoptotic intestinal epithelial cells to the mesenteric LN, where they serve as critical signals for the induction of T_{reg} cells (*80*).

During helminth infection, intestinal lamina propria–resident type 2 innate lymphoid cells (ILC2s) up-regulate S1PR expression and egress through intestinal lacteals to reach the blood circulation. Gut-derived activated ILC2s accumulate in the lung, which is another site of

helminth infection, and contribute to protective immunity and tissue repair (*81*). S1P signaling is also used by pathogenic T_H17 cells to egress from the intestine, aggravating kidney autoimmune disease (*82*). A retinoid-related orphan receptor–γ^+ (RORγ^+) CCR7$^+$ subset of ILC3s also traffics from the intestine to mesenteric LNs (*83*), although the functional importance of this trafficking remains to be established. Different parts of the intestine drain to distinct mesenteric LNs, and such LNs are immunologically specific to the functional gut segment that they drain. The proximal small intestine–draining mesenteric LN preferentially gives rise to tolerogenic responses, and the distal mesenteric LN gives rise to proinflammatory T cell responses (*84*). These results highlight the further need to characterize the lymphatic vasculature and lymph composition along the gastrointestinal tract.

CNS-associated lymphatic vessels: A missing link in CSF clearance and CNS immunity

Brain parenchyma is devoid of lymphatic vessels, and clearance of cellular debris and waste products in the central nervous system (CNS) is in part attributable to the so-called glymphatic pathway (*85*). The glymphatic pathway was proposed as a mechanism for small-molecule exchange between the cerebrospinal fluid (CSF) and ISF (*86*, *87*). This pathway promotes CSF flow along the Virchow-Robin spaces, existing between the arterial basement membrane and the glia limitans, which is composed of astrocytic end feet (*86*, *87*). CSF runs from the subarachnoid spaces into the deep brain through the periarterial spaces and then exits along perivenous spaces back into the subarachnoid space. Another path has been described, a perivascular route using the extracellular spaces along the walls of capillaries and arteries that exits the brain in the direction opposite to the arterial blood flow (*88*). Clearance through the glymphatic and perivascular pathways ultimately requires fluid removal from the cranium. Although several exit routes have been proposed, such as arachnoid granulations or perineural pathway, the relative importance of each of these drainage routes remains debated.

The rediscovery of mLVs is one of the most exciting findings in medicine in recent decades because mLVs are potential drainage circuits for CSF and ISF macromolecular clearance as well as routes for immune cell egress from the CSF into the peripheral lymphatic system (*89*, *90*). The participation of mLVs in CSF and brain ISF clearance has been shown in rodents (*89*–*92*), humans, and nonhuman primates (*93*). The mLVs express the classic markers of LECs, such as PROX1, VEGFR3, LYVE-1, podoplanin, and CCL21 (*89*, *90*). These vessels ensure the clearance of CSF macromolecules, antigens, and immune cells to the peripheral lymphatic system through the draining of deep cervical LNs, in a manner similar to that of peripheral lymphatic vessels (*6*, *89*, *92*, *94*). Lymphocytes and CD11c$^+$CCR7$^+$ DCs traffic within mLVs and drain to deep cervical LNs. After mLV ligation, they fail to do so and accumulate instead within the meninges (*89*, *90*). Of interest, although the lymphatic vessel development in most organs occurs in utero, formation of mLVs starts postnatally in response to VEGF-C produced by blood vascular SMCs (*6*). Until the first 2 postnatal weeks in mice, mLVs extend from the basal part of the skull around the middle meningeal artery and sigmoid sinus alongside dural blood vessels toward the dorsal part of the skull. During the 3rd postnatal week, navigating along the transverse sinuses,

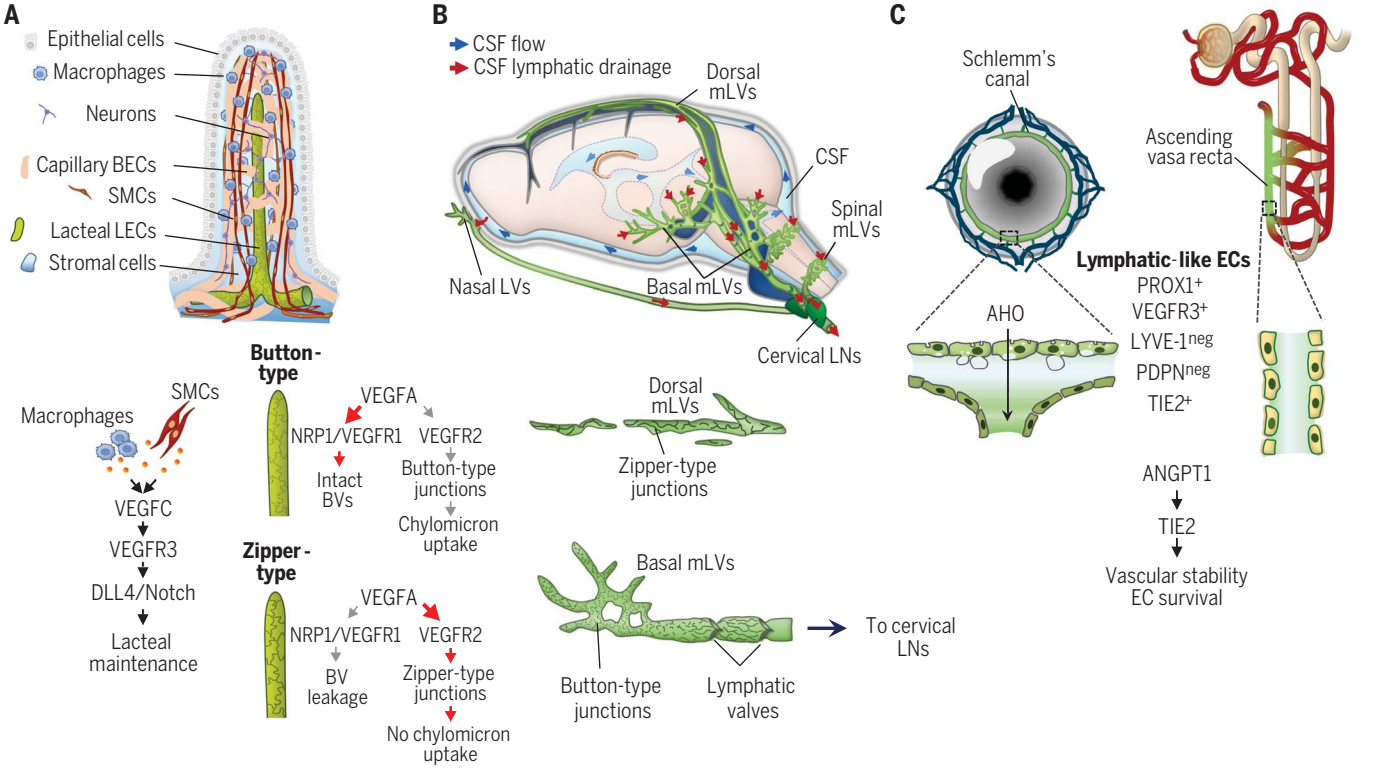

Fig. 3. Organ-specific lymphatic vasculatures. (**A**) Organization of intestinal villi. Each small intestinal villus contains a blind-ended lacteal. VEGF-C, supplied by villous SMCs and macrophages, maintains renewal of lacteals by means of VEGFR3-DLL4 signaling cascade. NRP1 and VEGFR1 on blood capillaries controls bioavailability of VEGF-A and junctional organization of lacteal LECs. BEC, blood endothelial cell; BV, blood vessel. (**B**) CNS-associated lymphatic vessels and routes of lymphatic drainage. Dorsal and basal lymphatic vessels display distinct junctional organization. (**C**) Lymphatic mimicry as vascular adaptation to organ-specific functions: lymphatic-like vessels. SC in the eye drains aqueous humor to reduce intraocular pressure. Kidney AVR drains medullary ISF and preserves medullary osmotic gradient. Lymphatic-like vessels express some (PROX1 and VEGFR3) but not all lymphatic markers. Angiopoietin-Tie2 signaling is necessary for SC and AVR development and maintenance. PDPN, podoplanin; ANGPT1, angiopoietin 1.

mLVs reach the superior sagittal sinus and extend along it toward the olfactory bulb (*6*, *95*). Similar to peripheral lymphatics, blockade of the interaction between VEGF-C and VEGFR3 impairs mLV development. Conversely, excess VEGF-C induces meningeal lymphangiogenesis, suggesting the plasticity and regenerative potential of mLVs (*6*).

In addition to capillary versus collecting lymphatic vessel morphological phenotypes, lymphatic vessels in many tissues display an intermediate precollector phenotype with mixed button- and zipper-like junctional patterns and lymphatic valves but no SMC coverage. Characterization of dorsal skull mLVs suggests that they are phenotypically similar to peripheral lymphatic capillaries because they lack lymphatic valves and have a noncontinuous basement membrane (*89*, *90*). Dorsal mLVs transport macromolecules and cells along the superior sagittal and transverse sinuses (*89*, *90*). However, using controlled low-rate and -volume stereotactic CSF tracer injection, Ma *et al.* did not find substantial CSF reuptake and drainage by dorsal mLVs. These researchers instead suggested that CSF drains along cranial nerves as they exit the skull (*91*). Basal mLVs are located at the skull base and thus are difficult to access and image. Their importance has been highlighted through characterization of their specialized morphologic features and transport capacity, which are distinct from those of dorsal mLVs (*96*). Similar to peripheral lymphatic capillaries, basal mLVs are blunt-ended, with a predominantly button-like junctional pattern (Fig. 3B). Of importance, basal mLVs also have lymphatic valves but lack SMCs and thus display a precollector phenotype, allowing both fluid uptake and maintenance of unidirectional lymph flow (*6*, *89*, *96*). When a fluorescent or contrast tracer is infused into the brain parenchyma or lateral ventricle, tracers are not observed in dorsal mLVs, with the transport preferentially occurring through the basal mLVs into the deep cervical LNs (*89*, *96*). Anatomically, basal mLVs are located close to the subarachnoid space and thus may represent hotspots responsible for CSF and brain ISF drainage into the lymphatic system. The pathways of CSF flow and clearance from the CNS are a rapidly developing area of research. Studies are needed that directly compare the extent of CSF drainage in specific anatomical regions by using standardized methods to allow for a consensus and fully reconcile mixed findings on the roles of dorsal and basal mLVs, the cribriform plexus, and the perineural pathway (*89*–*92*, *96*).

The excitement generated by mLV research results from their emerging function in clearance of brain toxic by-products and thus potential contribution to the pathogenesis of age-related neurodegenerative diseases, especially Alzheimer's disease. Aging impairs mLV structure and function (*6*, *91*, *92*, *96*), and photoablation of dorsal mLVs in young mice results in impaired CSF perfusion and in learning and memory deficits (*92*). In mouse models of Alzheimer's disease, photoablation of mLVs increases brain amyloid-β burden and promotes deposition of meningeal amyloid-β (*92*), indicating that mLVs participate in β-amyloid clearance. Another neuropathological hallmark of Alzheimer's disease is impaired clearance of intracellular aggregates of τ protein, and ablation of mLVs in K14-VEGFR3–Ig transgenic mice exacerbates this impairment (*97*). These data suggest that dysfunction of mLVs potentially contributes to the onset and progression of Alzheimer's disease by disturbing clearance of pathological proteins such as amyloid-β and τ protein and that targeting mLVs may be of therapeutic value.

In contrast to their predicted beneficial role in Alzheimer's disease, mLVs potentially promote disease pathology in autoimmune neuroinflammatory conditions, such as multiple sclerosis. In addition, they may facilitate CNS infection or injury by serving as a route for CNS-derived immune cell and antigen delivery to cervical LNs. Blockade of mLVs reduces disease severity and alleviates the inflammatory response in an animal model of multiple sclerosis, likely by interfering with trafficking and activation of $CCR7^+$ T cells in the draining LNs (*94*). Of interest, responses to inflammation in CNS-associated lymphatic vessels are heterogeneous. Brain inflammation induces VEGF-C–VEGFR-3–dependent expansion of lymphatic vessels associated with cribriform plate, whereas mLVs do not undergo robust lymphangiogenesis (*98*). However, inhibition of VEGFR-3 signaling, even in adult animals, leads to regression of mLVs but leaves cribriform plate lymphatics unaffected (*6*, *94*, *98*).

A highly organized lymphatic network also surrounds the vertebral column, where it navigates in the epidural space and dura mater around the spinal cord (*6*, *99*). Near-infrared and magnetic resonance imaging has demonstrated a directional flow of CSF through the central canal from the ventricle to the caudal end of the spine, with outflow of CSF through spinal lymphatic vessels, leading to the sacral and iliac LNs. The amount of CSF outflow into sacral and iliac LNs is much less than into deep cervical LNs, indicating that mLVs drain a major portion of CSF (*100*). Similar to cranial LVs, spinal lymphatic vessels are VEGF-C–dependent and remodel extensively after spinal cord injury, indicating their potential importance for maintaining and repairing spinal tissues and in CNS immune responses (*6*, *99*).

Further mechanistic and preclinical studies should improve our understanding and allow for manipulation of mLVs for the treatment of brain diseases. Analyses are needed of their relative contribution to immune cell trafficking and to evaluate mLV organization in organisms with more convoluted brains, higher cortical neuronal density, and complex cognitive behaviors. To guide these analyses, the molecular composition of different subsets of CNS-associated lymphatic vessels should be compared with that of other organ-specific lymphatic vessels. The link between mLV dysfunction and impaired waste clearance from the brain in all of these aspects may have far-reaching implications for the diagnosis and treatment of several brain-related pathologies. CSF levels of amyloid-β are decreased in Alzheimer's disease, presumably because they aggregate into fibrils and plaques in the brain; thus, less peptide can diffuse into the CSF. By contrast, τ protein concentrations in the CSF are increased in patients with Alzheimer's disease (*101*). Therefore, analysis and comparison of lymph components between CNS and the periphery will be important for further understanding of the mechanisms underlying CNS waste drainage through the mLVs. An additional important question is how mLVs communicate with the brain, and recent studies have provided initial insights into the direct skull–meningeal vascular connections (*102*, *103*), although the vessel identity has not been well established.

Lymphatic mimicry as a mechanism of vascular adaptation to organ-specific functions

Schlemm's canal (SC) is an endothelium-lined channel that encircles the cornea. It provides an exclusive vascular route for outflow of the aqueous humor, which is continuously produced by the ciliary body and refreshes the anterior chamber of the eye (*33*). SC has morphological, molecular, and functional similarities with lymphatic vessels (Fig. 3C). The aqueous humor is dynamically drained transcellularly by generating giant vacuoles through SC endothelial cells. SC dysfunction or regression during chronic inflammation or aging reduces aqueous humor drainage and elevates intraocular pressure, ultimately leading to glaucoma. In this respect, glaucoma caused by impaired SC could also be regarded as "eye lymphedema" (*33*). Unusually, SC represent an intermediate vessel type between lymphatic and blood vessels (*104*–*106*). SC endothelial cells express the blood endothelial cell markers Tie2 and endomucin and LEC markers PROX1, VEGFR3, and integrin α9 but not LYVE-1 or podoplanin (*104*–*106*). The SC develops by sprouting from the choroidal vein, and endothelial cells of SC are reprogrammed to acquire lymphatic features through PROX1 up-regulation after birth. Mechanical cues from aqueous humor outflow, VEGF-C–VEGFR-3 signaling, and angiopoietin–Tie2 signaling are crucial for the formation and differentiation of SC as well as for maintaining

adult SC integrity (*104*–*108*). *TIE2* and *ANGPT1* loss-of-function mutations have been identified in patients with primary congenital glaucoma (*107*, *109*). These advances uncover previously unidentified molecular pathways in understanding the pathogenesis of congenital glaucoma and adult primary open-angle glaucoma. They also offer new therapeutic avenues: Administration of Tie2-activating antibody alleviates the high intraocular pressure and subsequent retinal neural damage in *Angpt1*- and *Angpt2*-deficient mice by improving SC integrity (*108*).

Another example of vasculature with a hybrid lymphatic and blood vessel phenotype has been described in the kidney ascending vasa recta (AVR), the vascular network that drains ISF in the kidney medulla (*110*). AVR expresses the LEC markers PROX1 and VEGFR-3, but not LYVE-1 and podoplanin, and the blood endothelial cell markers endomucin, CD34, CD31, and PLVAP but not VEGFR-2 (Fig. 3C). PROX1 and VEGF-R3 expression and abundant fenestration in the AVR may be responsible for the relatively high hydraulic conductivity of the AVR and its role in the reuptake of large volumes of ISF, a typical feature of lymphatics. Similar to SC, angiopoietin-TIE2 signaling is required for the prenatal development of AVR (*110*). Of interest, neither SC nor AVR expresses LYVE-1 and podoplanin, which are produced by all peripheral LVs. For podoplanin, its high platelet aggregation capacity would be clearly detrimental for SC and AVR function, but the reason for low LYVE-1 expression is unknown. SC and AVR are interesting examples in which the need for specialized blood vessel function, such as drainage of ISF, leads to emergence of a lymphatic-like phenotype.

A distinct type of lymphatic mimicry by blood vessels has been uncovered in placental spiral arteries (SAs). During pregnancy, SAs undergo rapid remodeling to meet the metabolic needs of a growing fetus. SAs express the lymphatic markers PROX1, LYVE-1, and VEGFR-3, and blockade of VEGFR-3 signaling prevents SA remodeling, leading to fetal growth restriction (*111*). Unlike the SC and AVR, the SAs are not fenestrated or involved in fluid filtering, suggesting that in this case, lymphatic mimicry reflects opportunistic use of VEGF-C, abundantly produced by uterine NK cells, for SA expansion. Collectively, the existence of vascular beds with blood vascular–lymphatic hybrid characteristics underscores the importance of endothelial cell plasticity for vessel adaptation to organ function and calls for further investigation of the underlying molecular mechanisms.

Lymphatic vessels in disease: Toward a broader view of tumor lymphatic vessel functions

Tumor lymphatic vessels execute complex and at times seemingly contradictory functions during cancer progression (Fig. 4). Metastatic cells readily invade permeable peritumoral lymphatic vessels. High levels of lymphangiogenic growth factor VEGF-C, increased tumor lymphatic vascular density, and LN metastasis are all linked to poorer clinical outcomes in multiple cancer types, with especially clear associations observed in melanoma, breast, prostate, and head and neck cancers (*112*, *113*). Inflamed lymphatic endothelium also fosters an immunosuppressive tumor microenvironment by inhibiting DC maturation and limiting cytotoxic lymphocyte function through interferon-γ–dependent production of inhibitory ligand PD-L1, inducible nitric oxide synthase, indoleamine 2,3-dioxygenase (IDO), and transforming growth factor–β (TGFβ) [reviewed in (*114*)]. On the other hand, tumor antigen transport by migratory DCs to draining LN is important for the generation of antitumor immunity (*115*, *116*). Nevertheless,

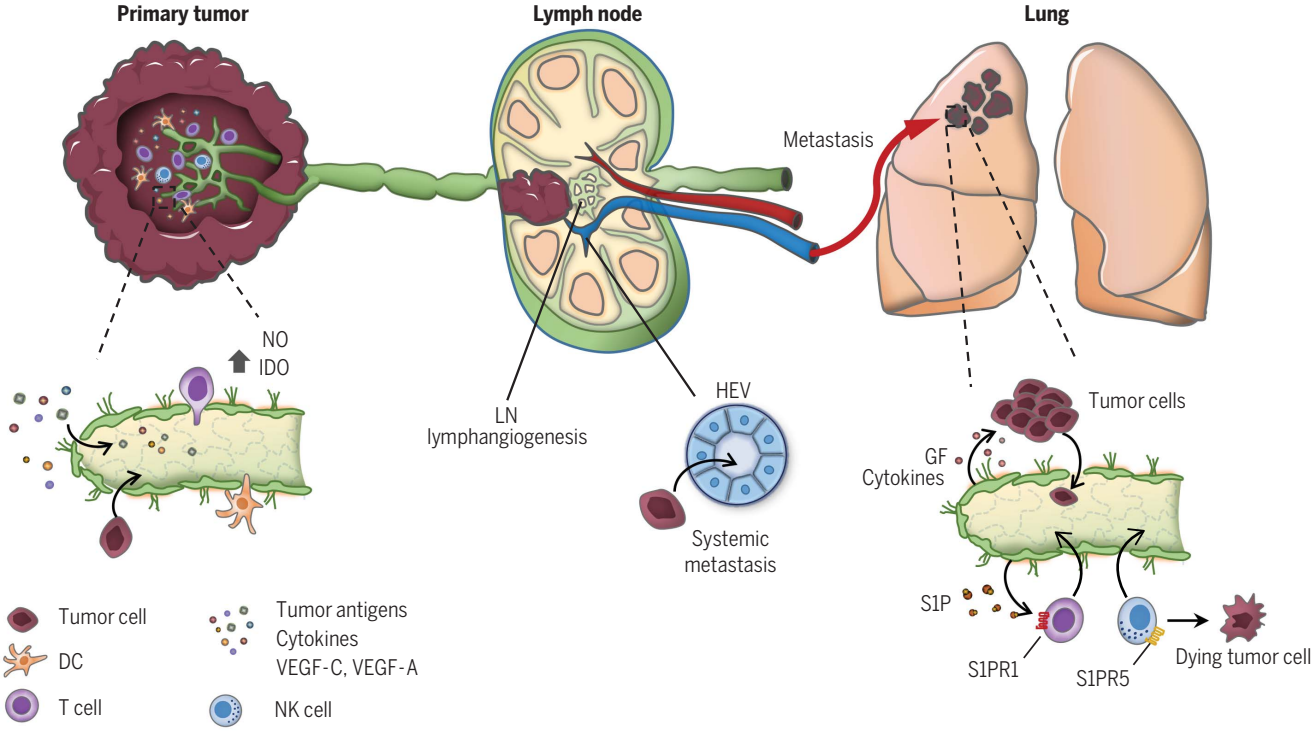

Fig. 4. Functions of tumor-associated lymphatic vessels. Primary tumor lymphatic vessels serve as escape route for metastatic cancer cells to regional LNs. Paracrine signals from tumor LECs such as PD-L1, IDO, and NO create an immunosuppressive microenvironment. Transport of immune cell and tumor antigens to draining LN is necessary for productive antitumor immunity in response to therapeutic vaccination and checkpoint blockade but also induces LN lymphangiogenesis through delivery of lymphangiogenic factors. Lymph-borne metastatic cancer cells migrate on fibroblastic reticular network and invade HEVs to reach the systemic circulation. Metastasis-associated lymphatic vessels serve as a niche for cancer cells by providing secreted factors, such as CXCL12 chemokine. S1P produced by metastasis-associated LECs in the lung promotes egress of NK and T cells, which enhances metastatic outgrowth. GF, growth factor.

antigen presentation and production of effector T cells have also been documented within the primary tumors and in tumor-associated tertiary lymphoid structures (*117*, *118*). Melanoma tumors implanted in K14-VEGFR3-Fc transgenic mice, which lack dermal lymphatic vasculature, fail to develop an inflammatory tumor microenvironment and T cell infiltration (*119*). Furthermore, experimental induction of lymphangiogenesis promotes accumulation of intratumoral $CD8^+$ T cells and improves responses to immune therapies in melanoma models (*120*). High lymphatic vascular density correlates with increased infiltration of intratumoral $CD8^+$ T cells in human colorectal cancer and melanoma (*121*, *122*). Patients with melanoma and high circulating levels of VEGF-C have stronger immune responses to therapeutic vaccination with Melan-A peptide and better clinical responses to anti–CTLA-4 or anti–programmed cell death 1 (PD-1) immunotherapies (*120*). Thus, although the lymphatic vasculature generally facilitates metastasis and even displays "exhausted" and immunosuppressive features in advanced tumors, it is nevertheless essential for generation of antitumor immunity and efficient responses to immune therapies.

Whether lymphatic vessels promote or inhibit tumor progression depends on the propensity of metastatic cells to migrate further than tumor-draining LNs. Using cancer cell infusion into LN or LN photoconversion approaches, Brown *et al.* and Pereira *et al.* found that a significant proportion of lung metastases are formed by LN-derived cancer cells (*123*, *124*). In addition, they found that cancer cells used the LN fibroblastic reticular cell network for their migration toward high endothelial venules (HEVs) to enter blood circulation. These results demonstrate that LN metastases are not a "dead end" of the metastatic process but rather contribute substantially to systemic cancer spread. Outstanding remaining questions are (i) whether this process occurs in human cancers and (ii) its clinical relevance. Genetic analyses of clonal evolution between the primary tumor, LN, and distant metastases in human colorectal cancer indicate a complex picture with common clonal origin found in a subset of cases (*125*). Yet, resection of all regional LNs does not improve the overall survival rate in sentinel-node–positive melanoma patients (*126*). Further work is therefore necessary to establish the molecular determinants that drive invasive behavior of cancer cells within the LNs, the impact of tumor cell conditioning by the LN environment, and how cancer therapies affect these parameters. In melanoma models, LN metastatic cell metabolism differs from that of primary tumors. Such cells up-regulate the transcriptional coactivator yes-associated protein (YAP), which drives fatty acid oxidation in response to increased LN levels of YAP-activating bile acids (*127*).

Metastatic cancer cells arrive in an inhospitable environment and must adapt to survive and expand. Primary tumors systemically condition distant organ environments, which include organ-specific lymphatic vessels, to generate a supportive premetastatic niche. Lymphangiogenesis has been documented not only in primary tumors but also in sentinel LNs, even in the absence of cancer cells, and distant metastases, where it has been associated with poor prognosis (*122*, *128*, *129*). Tumor-derived endothelial growth factors, primarily VEGF-C and VEGF-A, play a key role in LN lymphangiogenesis [reviewed in (*128*)]. Release of the heparin-binding factor midkine by melanoma cells induces systemic premetastatic lymphangiogenesis through activation of mammalian target of rapamycin (mTOR) signaling and further up-regulation of VEGFR-3 in LECs (*130*). An analysis of melanoma-derived lymph demonstrated that it is highly enriched in bile acids (*127*) or particular cancer biomarkers, reflecting early versus advanced metastatic spread (*131*). Results such as these open up a fascinating opportunity to understand the impact of tumor lymph components on draining LN function.

Premetastatic and metastasis-associated lymphatic vessels are functionally important. Expansion of the lung lymphatic vasculature upon transgenic VEGF-C overexpression potentiates outgrowth of arriving cancer cells and facilitates their subsequent dissemination to other organs (*129*). Further work is necessary to establish the role of this process in human disease, and metastasis-to-metastasis spread has already been documented in prostate cancer (*132*). Under inflammatory conditions, LECs abundantly produce exosomes that accumulate in perilymphatic stroma and promote directional migration of DCs (*133*). Metastasizing cancer cells could potentially use a similar mechanism. Metastasis-associated lymphatic vessels promote cancer cell growth and invasion through paracrine production of the chemokine CXCL12, as reviewed in detail elsewhere (*134*). In addition, perilymphatic tumor-associated macrophages facilitate intralymphatic invasion in breast cancer models (*13*, *14*). Metastasis-associated LECs also modulate the local immune environment. LEC-specific inactivation of the S1P transporter SPNS2 curbs metastatic melanoma cell outgrowth in lungs because of increased retention of NK and effector T cells (*135*). Surprisingly, the effects of midkine and SPNS2 are apparent at the sites of metastases but not in primary tumors (*130*, *135*), indicating an important but poorly understood contribution of organ-specific microenvironments.

Tumor-associated lymphatic vessels can play both beneficial and detrimental roles in cancer progression. Accumulating evidence indicates that common cancer therapies induce lymphangiogenic factors and tumor lymphangiogenesis (*136*, *137*), contributing to metastasis. By contrast, functional tumor lymphatic vessels improve delivery of chemotherapy to tumors by reducing ISF pressure (*138*) and potentiate responses to therapeutic vaccination and immune checkpoint inhibitors (*120*). Further work is necessary to fully understand and exploit the role of lymphatic vessels in specific cancer types and the potential of cotargeting lymphatic vessels in combination with other treatments. Initial research has focused on the inhibition of tumor lymphangiogenesis to prevent metastasis. However, similar to other stroma-targeting therapies (*139*, *140*), reprogramming rather than ablation of tumor-associated lymphatic vessels appears to be a more realistic strategy.

Concluding remarks

The field of lymphatic vascular biology is rapidly expanding because of an increased awareness of the role of lymphatic vessels in regulating multiple body functions and diseases, coupled with studies of the underlying molecular mechanisms. Below is a nonexhaustive discussion of what we consider to be crucial future directions in lymphatic vascular biology.

Morphogenetic functions of lymphatic vessels

Functional compartmentalization is a key feature of every organ, maintained by tissue-specific stem cells. The role of lymphatic vessels in the compartmentalization of immune cells in LNs has been clearly demonstrated, but the insights into other organs and the role of lymphatic vessels in stem cell niches are only starting to emerge. For example, lymphatic vessels serve as a main source of mitogens for spermatogenic stem cells in the testis (*141*). Intriguingly, the hair follicle regeneration cycle appears to be coordinated with skin lymphatic capillary remodeling and function (*142*, *143*). Distinct developmental origins potentially contribute to organ-specific features of lymphatic vessels (*17*–*19*). It will be equally important to understand how the specific environment of each organ affects LEC molecular composition and heterogeneity and ability to secrete and regulate the concentration of organotypic growth factors and other bioactive substances. Some potential environmental factors with these effects include extracellular matrix composition and tissue stiffness, availability of nutrients, and the presence of microbial products. A transport function of lymphatic vessels in maintaining morphogenetic gradients has been proposed (*144*) but is still rarely considered. Understanding the morphogenetic functions of the lymphatic vasculature requires refined animal models for the detection and real-time analyses of gradients of bioactive molecules, as illustrated in recent studies of S1P signaling (*58*, *61*), and for genetic targeting of organ-specific vessels (*145*).

Systemic functions of lymphatic vasculatures

The vascular system acts as an "organizer" by ensuring communication among different parts of an organism (*146*). Studies of immune cell trafficking highlight roles for the lymphatic vasculature in this organ-to-organ communication. As discussed above, ILC2s egress from the gut through lymphatics to promote defense against the helminths in the lungs, and encephalitogenic T cells first travel into the lungs and intestine before exiting by means of lymphatics and reaching the brain (*147*, *148*). Brain neuroinflammation is suppressed by gut-derived plasma cells (*149*), whereas pathogenic intestinal T_H17 cells contribute to kidney autoimmune diseases (*82*). Further work is necessary to understand which signals that lymphatic vessels relay during organ-to-organ communication and how such signals coordinate body homeostasis or disease responses. With such knowledge in hand, a transition from single-gene biology to a systemic and quantitative understanding of the organism as a whole may be possible. The development of preventive measures or therapeutic interventions could then arise from this systemic knowledge of vascular communication mechanisms.

Exploiting lymphatic vasculature to improve disease diagnostics and treatments

Lymphatic vessels are emerging as an indispensable component of effective antitumor immune responses. Much of our current knowledge about the functions of tumor lymphatic vasculature derives from the subcutaneous melanoma and breast cancer models engineered to overexpress VEGF-C and foreign antigens. Yet, solid cancers of any given organ arise because of distinct molecular alterations and are molecularly heterogeneous. This heterogeneity goes hand in hand with differential responses of cancer subtypes to therapies and vastly different patient prognoses. The impact of organ-specific vascular specialization and the immune environment on cancer-associated lymphatic phenotypes and functions also needs to be considered. The next challenge will therefore be to assess the role of lymphatic vessels in models that recapitulate specific cancer subtypes or the metastatic site microenvironment to uncover clinically relevant cancer vulnerabilities. Additional approaches for initiating or reinvigorating antitumor immune responses could include modification of the sentinel LN microenvironment through direct tumor-draining LN delivery and vaccination strategies (*150*), design of implantable tumor-reactive artificial lymphoid structures (*151*), or therapeutic promotion of tumor tertiary lymphoid structures (*152*, *153*).

Lymph carries organ-derived metabolic products, growth factors, cytokines, and immune cells. It represents an undiluted signature of the tissue-specific microenvironment (*131*) and as such is an invaluable source of information about organ status and disease progression. Development of methods for lymph-based phenotyping may offer new approaches for diagnostics in, for example, neurodegenerative and neuroinflammatory conditions or for stratification of patients for treatment, as in the context of cancer immunotherapy.

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Roles of organ-specific lymphatic vessels

Lymphatic vessels are spread throughout the human body and have critical functions in mammalian physiology. Petrova *et al.* review emerging roles of the lymphatic vasculature in organ function and pathology and provide perspectives beyond the traditional view of lymphatic vessels in the maintenance of fluid homeostasis. The authors highlight new insights into lymphatic vessel function and lymphatic endothelial cell biology as it relates to intestinal lacteals, lymph nodes, central nervous system meninges, and cancer. Recent steps toward therapeutic opportunities that could alter lymphatic function or growth are also discussed.

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