Lymphatic endothelial cells of the lymph node

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Abstract | The influx and efflux of cells and antigens to and from the draining lymph nodes largely take place through the subcapsular, cortical and medullary sinus systems. Recent analyses in mice and humans have revealed unexpected diversity in the lymphatic endothelial cells, which form the distinct regions of the sinuses. As a semipermeable barrier, the lymphatic endothelial cells regulate the sorting of lymph-borne antigens to the lymph node parenchyma and can themselves serve as antigen-presenting cells. The leukocytes entering the lymph node via the sinus system and the lymphocytes egressing from the parenchyma migrate through the lymphatic endothelial cell layer. The sinus lymphatic endothelial cells also orchestrate the organogenesis of lymph nodes, and they undergo bidirectional signalling with other sinus-resident cells, such as subcapsular sinus macrophages, to generate a unique lymphatic niche. In this Review, we consider the structural and functional basis of how the lymph node sinus system coordinates immune responses under physiological conditions, and in inflammation and cancer.

Fibroblastic reticular cells (FRCs). Specialized reticular fibroblasts located in the T cell areas of lymph nodes and other secondary lymphoid organs that produce collagen-rich reticular fibres and form stromal networks and conduits that are important for the trafficking of immune cells.

High endothelial venules Specialized venules that occur in secondary lymphoid organs, except the spleen. High endothelial venules allow continuous transmigration of lymphocytes as a consequence of the constitutive expression of adhesion molecules and chemokines on their luminal surface.

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Lymph nodes (LNs), and other secondary lymphatic tissues, generate highly specialized microenvironments for mounting effective immune responses[1,2]. In terms of adaptive immunity, the concentration of antigens, antigen-presenting cells and naive lymphocytes in the LNs enforces T cell and B cell activation and their differentiation into effector cells. Although the vast majority of LN cells (approximately 95%) are leukocytes, the different non-leukocytic stromal cell types, including blood vessel endothelial cells (BECs), lymphatic endothelial cells (LECs) and fibroblastic reticular cells (FRCs), are absolutely essential for the function of the organ[3,4]. Many lymphocyte types, such as naive cells and central memory cells, as well as other leukocyte types and their progenitors, enter the LN from the blood via high endothelial venules, which are lined by unique BECs that are specialized for supporting the multistep leukocyte extravasation cascade[5,6]. LNs also have an extensive lymphatic vasculature. Most leukocytes, including effector and memory lymphocytes, activated dendritic cells and monocytes, can enter the afferent lymphatics in peripheral tissues[7–10]. Antigens and other soluble molecules of the interstitial fluid also drain into the afferent lymph. The lymph-borne molecules and cells are then conveyed into the draining LN by unidirectional lymph flow. Within the LN, the afferent lymphatic vessels with circular cross-sectional profiles transform into several branched sinus systems that are lined by a layer of LECs. At the efferent side of the LN, lymphocytes use specialized lymphatic sinuses to enter the efferent lymphatic vasculature, which finally drains these cells back into the blood circulation. In addition to stromal BECs and LECs, multiple different subtypes of non-endothelial stromal cells, including FRCs, are found in LNs[11,12]. These mesenchyme-derived cells produce the collagen-based conduit system, give structural support to the organ and provide survival signals and routes for the directional migration of leukocytes between the different compartments of the LN. As LN blood vessels, FRCs and peripheral lymphatic vessels have been scrutinized in several excellent Reviews[1–6,8,12–15], we focus our discussion on the lymphatic vasculature within the LNs. Therefore, the numerous papers characterizing the immunology of the entire afferent arm of the lymphatic vasculature or the sinus leukocytes (without separating the LN LEC contribution) largely fall outside the scope of this Review.

New technologies have enabled the discovery of unanticipated heterogeneity of sinusoidal LECs. Because these cells form a physical barrier between the lymph-borne material and the LN parenchyma, where the responding naive lymphocytes reside, there has been increasing interest in defining the mechanisms by which antigens and different leukocyte types penetrate through the sinusoidal LECs. Moreover, many previously unappreciated functions, including LN organogenesis, antigen presentation and interactions with other sinus-resident cell types, such as subcapsular sinus macrophages, have also been recently ascribed to LN LECs. In reviewing these new insights into the architecture, phenotype and function of different LN LEC populations, we reinforce their key roles in supporting multiple aspects of immunosurveillance in both health and disease.

Extravasation cascade
The multistep process of leukocyte infiltration through the endothelium. This process proceeds through the stages of leukocyte rolling, adhesion, diapedesis and, finally, migration to the surrounding tissues.

Architecture of the LN sinus systems

The basic architecture of the sinus system is largely conserved between mice, rats and humans, and in other mammals as well[16–18]. The afferent collecting lymphatics penetrate through the collagenous capsule of LNs and open into a narrow subcapsular sinus (FIG. 1). LNs typically have multiple afferent lymphatics opening to the subcapsular sinus at different positions, although small LNs (such as those in mice) can only have a single afferent lymphatic vessel[16,17,19–22]. The subcapsular sinus overlays the whole LN cortex, which includes both B cell follicles and interfollicular T cell areas, and is lined by LECs on both sides. The LECs facing the capsule are called ceiling LECs and those overlaying the lymphocyte-containing LN parenchyma are called floor LECs. The ceiling LECs adhere to the collagenous matrix of the capsule, whereas the floor LECs rest on a meshwork of subsinusoidal conduit bundles and associated stromal cells. In the lumen of the subcapsular sinus, there are numerous traversing trans-sinusoidal tissue cords (also known as pillars or bridges), containing a collagen-rich stroma covered by LECs, and occasional LEC-lined valves. The subcapsular sinus contains both transient migratory leukocytes of the afferent lymph and tissue-resident leukocyte types. Among the resident leukocytes, numerous subcapsular sinus macrophages and

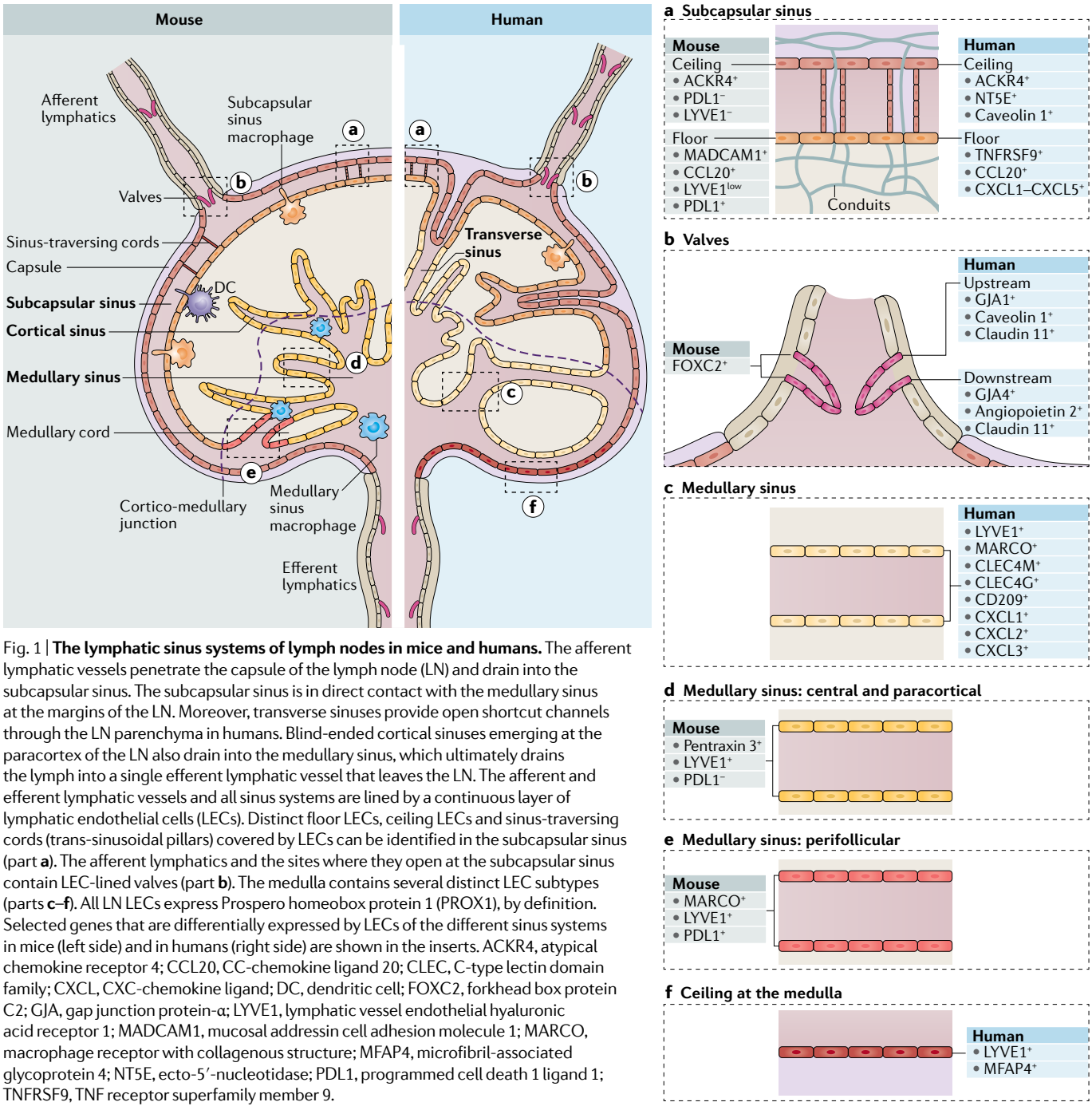

Fig. 1 | **The lymphatic sinus systems of lymph nodes in mice and humans.** The afferent lymphatic vessels penetrate the capsule of the lymph node (LN) and drain into the subcapsular sinus. The subcapsular sinus is in direct contact with the medullary sinus at the margins of the LN. Moreover, transverse sinuses provide open shortcut channels through the LN parenchyma in humans. Blind-ended cortical sinuses emerging at the paracortex of the LN also drain into the medullary sinus, which ultimately drains the lymph into a single efferent lymphatic vessel that leaves the LN. The afferent and efferent lymphatic vessels and all sinus systems are lined by a continuous layer of lymphatic endothelial cells (LECs). Distinct floor LECs, ceiling LECs and sinus-traversing cords (trans-sinusoidal pillars) covered by LECs can be identified in the subcapsular sinus (part **a**). The afferent lymphatics and the sites where they open at the subcapsular sinus contain LEC-lined valves (part **b**). The medulla contains several distinct LEC subtypes (parts **c**–**f**). All LN LECs express Prospero homeobox protein 1 (PROX1), by definition. Selected genes that are differentially expressed by LECs of the different sinus systems in mice (left side) and in humans (right side) are shown in the inserts. ACKR4, atypical chemokine receptor 4; CCL20, CC-chemokine ligand 20; CLEC, C-type lectin domain family; CXCL, CXC-chemokine ligand; DC, dendritic cell; FOXC2, forkhead box protein C2; GJA, gap junction protein-α; LYVE1, lymphatic vessel endothelial hyaluronic acid receptor 1; MADCAM1, mucosal addressin cell adhesion molecule 1; MARCO, macrophage receptor with collagenous structure; MFAP4, microfibril-associated glycoprotein 4; NT5E, ecto-5′-nucleotidase; PDL1, programmed cell death 1 ligand 1; TNFRSF9, TNF receptor superfamily member 9.

B cell follicles
Aggregates of B cells in lymphoid tissues. They contain naive B cells, as well as activated, proliferating and maturing B cells in germinal centres. B cell follicles are contiguous with T cell zones.

sparse sinus-resident dendritic cells are interspersed within the floor LECs[23–26]. Both of these leukocyte types have noticeable cellular protrusions, which constantly survey the lumen of the subcapsular sinus.

At the hilum of the LN, the lymphatic vasculature forms a medullary sinus in all mammalian species studied[16–21] (FIG. 1). The demarcation point between the subcapsular sinus and the medullary sinus at the margin of the LN remains poorly defined[27]. At least in humans, the capsule-facing LECs in the medulla have a unique phenotype[28]. In all mammals, the extensive and wide medullary sinuses converge into the single efferent lymphatic vessel, which emanates from the organ. Notably, the efferent lymphatic vessel eventually becomes an afferent lymphatic vessel for the next, more proximal LN in a chain of draining LNs. The medullary sinus LECs form the sinus-facing surface of the medullary cords, which contain mostly lymphocytes and macrophages. Medullary sinuses are packed with sluggishly flowing lymphocytes egressing from the LN, and they also contain resident intraluminal medullary sinus macrophages[23,29].

In addition to the subcapsular and medullary sinuses, two other sinus systems have been described in LNs[17–21] (FIG. 1). Transverse sinuses (also known as trabecular sinuses or cortical gaps) are LEC-lined deep radial invaginations of subcapsular sinuses, sometimes located in fibrous trabeculae or septae[30]. Although not observed in mice, they are clearly defined in humans, rats and other larger species. Transverse sinuses form open shortcut channels from the subcapsular sinus all the way down to the medullary sinus and may be involved in sub-compartmentalization of the LN. The cortical sinuses (also known as lymphatic sinuses, paracortical sinuses, unit sinuses or peripheral sinuses) — the fourth sinus type — are blind-ended sacs that emerge at the paracortical zone of the LN[20,31]. At the corticomedullary junction, these sinuses drain into the medullary sinuses. Cortical sinuses form a dense labyrinth of lymphatic vessels, which the egressing lymphocytes enter when leaving the LN parenchyma[32–34].

Historically, the sinus anatomy has been mainly deduced from careful analyses of histological sections using light and electron microscopy, possibly in conjunction with lymph-borne marker dyes[16,17]. LEC-selective antibodies and confocal imaging have subsequently been used extensively in the histological analyses. Most recently, technical advances in optical tissue clearing and two-photon microscopy have opened new possibilities for analysing sinus architecture[34,35]. Nevertheless, the variable and highly three-dimensional organization, which differs in different LNs, in different species and kinetically during the ontogeny and immune responses, still poses formidable challenges to the analysis of the sinus system[16,36]. Nevertheless, it is evident that the LEC-lined subcapsular, transverse, cortical and medullary sinuses all represent local, highly differentiated adaptations of the lymphatic vasculature inside the LNs.

Sinusoidal LECs are heterogeneous

All LECs express the homeobox gene *PROX1*, which is the master regulator of their differentiation[37]. In flow-cytometric assays, LN LECs have been classically identified as non-leukocytic $CD45^-CD31^+$podoplanin$^+$ cells. Based on comparative transcriptomic analyses of the pooled total mouse LN LEC population, these cells are unique and differ from peripheral LECs (BOX 1) and from LN FRCs more than from LN BECs[8,38,39]. LN LECs produce several cytokines[38,39]. These include growth factors for naive lymphocytes (IL-7), macrophages (macrophage colony-stimulating factor 1 (CSF1)), FRCs (such as platelet-derived growth factor (PDGF) family members) and BECs (such as vascular endothelial growth factor (VEGF) and angiopoietins). They also synthesize low levels of CC-chemokine ligand 21 (CCL21)[38], a crucial chemoattractant for LN-infiltrating cells, CCL20, several pro-inflammatory chemokines (such as CXC-chemokine ligand 1 (CXCL1), CCL5 and CXCL19) and atypical chemokine receptor 4 (ACKR4;

Box 1 | Peripheral capillary lymphatic endothelial cells

Development
Peripheral capillary lymphatic endothelial cells (LECs) mostly develop from venous endothelial cells by sprouting angiogenesis under the guidance of Prospero homeobox protein 1 (PROX1)[142,150]. Non-venous origins have also been reported[151].

Anatomy
A rich lymphatic capillary network is found in most tissues[152–154]. The network starts as blind-ended sacs that then converge on larger collecting afferent lymphatic vessels, which open into the draining lymph node (LN).

Morphology
Peripheral lymphatic capillaries have punctate, button-like junctions[7], a thin and discontinuous basement membrane and no surrounding perivascular cells. Anchoring filaments bind capillaries to the extracellular matrix[8,10].

Phenotype
Capillary LECs often express lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1), podoplanin and vascular endothelial growth factor receptor 3 (VEGFR3), but they are very heterogeneous and plastic in different organs[8,152,153]. Transcriptomic comparison between capillary LECs (from the diaphragm) and LN LECs revealed clear differences in the expression of several matrix molecules, cell adhesion molecules, chemokines, cytokines and immunostimulatory molecules. For example, capillary LECs expressed less collagenase 3, lymphotoxin-β, macrophage colony-stimulating factor 1 (CSF1), atypical chemokine receptor 4 (ACKR4), antigen peptide transporter 1, β_2-microglobulin, macrophage receptor with collagenous structure (MARCO) and macrophage scavenger receptor 1 (MSR1) than LN LECs, but more cytoskeletal proteins[39].

Functions
Drainage. Accumulation of interstitial fluid leads to the distension of lymphatic capillary anchoring filaments and opening of flap valves[47]. The fluid, soluble molecules and microorganisms enter by passive convection[8,10]. Osmotic tension (via macrophage mediators) induces lymphangiogenesis, which regulates tissue fluid homeostasis and systemic blood pressure[155]. Capillary LECs can also mediate active transcytosis of antigens via clathrin-dependent and caveolin-dependent endocytosis[156].

Leukocyte trafficking. Capillary LECs secrete many chemokines[8,13], including CC-chemokine ligand 21 (CCL21), which is immobilized by heparan sulfates of the extracellular matrix to generate haptotactic gradients for migrating leukocytes on their way to the afferent lymphatics[157].

Lipid metabolism. Capillary LECs have a major role in reverse cholesterol transport (that is, the transfer of high-density lipoprotein-bound cholesterol from the tissues to the blood circulation)[158]. In the intestine, capillary LECs mediate the transport of dietary lipids into the liver[159].

Alterations in diseases. Dysfunction of peripheral capillary lymphatics often leads to lymphoedema[160]. Neolymphangiogenesis occurs in many cancers, and these capillaries are the main route for metastasis formation in many cancers[160,161]. Capillary LECs are modified by microbial infections, and certain microorganisms can use LEC molecules as binding receptors to infect cells (such as binding of Streptococcae to LYVE1 (REF. [162])).

also known as CCRL1), which binds to CCL21. In addition, C-type lectins, scavenging receptors, Fc receptors and Toll-like receptors (TLRs), which are potentially involved in antigen binding and uptake, are selectively enriched in LN LECs[38,39]. The gene expression profiles of LECs in peripheral LNs and mesenteric LNs are highly correlated, and LECs at both locations express comparable levels of Prospero homeobox protein 1 (PROX1), lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1), podoplanin, CD31 and IL-7, for example[38].

Bulk analyses of LN LECs are unable to capture any location-specific differences in distinct parts of the sinus system. Interestingly, many molecules, including podoplanin, VEGF receptor 3 (VEGFR3), PROX1 and CD206, which are used to discriminate LECs from BECs, are expressed at comparable levels in subcapsular, cortical and medullary sinuses[27,40,41], although in one study LYVE1 expression was not found in the subcapsular sinus LECs of human LNs[42]. However, at least in the mouse subcapsular sinus, floor LECs are uniformly podoplanin$^+$LYVE1$^+$, whereas the ceiling LECs largely lack LYVE1 expression[27,43] but show unique expression of ACKR4 (REF.[27]). Recent bulk RNA sequencing efforts with mouse LECs isolated from different LN locations revealed the robustness of the site-specific signatures[43]. For example, nearly 400 genes were upregulated and almost 300 genes were downregulated more than eightfold in LECs from pooled cortical and medullary sinus LECs compared with subcapsular sinus LECs. In unbiased annotation analyses, several differentially expressed genes belonged to categories associated with metabolic functions, stress responses and immune responses. These analyses also identified new location-specific markers, such as Siglec 1 for subcapsular sinus and endomucin for cortical and medullary sinuses, at the protein level in both mice and humans. Moreover, the expression of macrophage scavenger receptor 1 (MSR1) mainly in the floor layer of the subcapsular sinus and in the medullary sinus, but not on cortical sinuses, reinforced the concept of sub-compartmental differences in LN LECs.

Recent single-cell RNA sequencing of CD45$^-$ CD31$^+$podoplanin$^+$ LEC populations from non-cancerous human axillary and head and neck LNs enabled unbiased identification of six different PROX1$^+$ cell clusters[28]. Interestingly, each LN LEC cluster had more than 100 differentially expressed genes. Histological mapping of representative differentially expressed molecules allowed construction of the first comprehensive atlas of the sinusoidal heterogeneity (FIG. 1). These analyses revealed ecto-5′-nucleotidase (NT5E; also known as CD73) and caveolin 1 as selective new markers for ceiling LECs, and tumour necrosis factor receptor superfamily member 9 (TNFRSF9) for floor LECs in the subcapsular sinus. These data also implied that cortical and medullary sinus LECs are transcriptionally indistinguishable. Hitherto unknown LN LEC subtypes were also discovered. Thus, microfibril-associated glycoprotein 4 (MFAP4) was identified as a specific marker for the capsule-facing LECs in the medulla, and claudin 11 for lymphatic valves (which even had distinct LEC phenotypes on the upstream and downstream sides) within the subcapsular sinus.

In mouse peripheral LNs, similar single-cell RNA sequencing analyses identified five main PROX1$^+$ LEC subtypes: valve LECs, ceiling LECs, floor LECs and two types of medullary LECs[44,45] (FIG. 1). Of the medullary LEC subtypes, cells expressing pentraxin 3 were found to cover the central and paracortical areas of the medullary sinus, whereas cells expressing macrophage receptor with collagenous structure (MARCO) overlaid more peripheral, perifollicular areas of the medulla. Additional LEC populations covering the trans-sinusoidal cords, the interfollicular area of the floor and the transition zone between the MARCO$^+$ medullary LECs and the subcapsular sinus floor LECs may exist. The five main LEC subsets in mice correspond to the human LEC subsets, although the existence of distinct pentraxin 3$^+$ and MARCO$^+$ medullary LEC subtypes in human LNs remains to be clarified. For instance, in both species, ceiling LECs are characterized by expression of ACKR4, caveolin 1 and bone morphogenetic protein 4 (BMP4), and floor LECs by expression of CCL20, BMP2 and CD74. Nevertheless, notable species-specific differences in the marker gene expression were also observed[44,45]. These include the floor LEC phenotype mucosal addressin cell adhesion molecule 1 (MADCAM1)$^+$IL-6$^-$ in mice and MADCAM1$^-$IL-6$^+$ in humans and the apparent absence of MARCO expression in most medullary LECs in mice. Moreover, LECs isolated from mouse duodenal LNs have a unique transcriptomic signature compared with ileal LN LECs or pooled caecal and colonic LN LECs, which may reflect differences in the lymph composition[46]. Hence, LN LECs have unique characteristics compared with peripheral LECs or BECs and they display extensive heterogeneity between different sinus systems. Moreover, LN LECs at different anatomical localizations may have distinct molecular adaptations.

Sinusoidal LECs sort macromolecules

Under physiological conditions lymph fluid contains more than 1,000 different proteins and multiple other biomolecules, which represent a combination of the ultra-filtrate of plasma and soluble molecules produced by the parenchymal cells[8,47,48]. The lymph also carries non-soluble material, including particular antigens, bacteria and viruses, to the draining LNs. Most endogenous and exogenous lymph-carried proteins show a marked decrease in concentration between the afferent and efferent lymph, which is indicative of their effective clearance in the draining LN[30,49–51].

In the subcapsular sinus, lymph-borne molecules and particles can undertake five alternative but not mutually exclusive routes. They can be scavenged or phagocytosed by subcapsular sinus macrophages and dendritic cells, whose projections continuously scan the luminal contents above the LEC monolayer[23,24]. In addition, subcapsular sinus macrophages propel immune complexes on their surface into B cell follicles[52]. Sinusoidal LECs directly control the other three macrophage-independent routes, which are conduit-mediated transfer, transcytosis through the sinus floor and direct transfer to the efferent lymphatics[53–56]. Notably, many proteins and oligosaccharides reside in the parenchyma of draining LNs within

seconds of subcutaneous injection, implying very fast sorting decisions in the sinus[53,54,56,57].

Sinusoidal floor LECs form a single, flattened cell layer between the subcapsular sinus lumen and the LN parenchyma, and outside the nucleolar area their cytoplasm can be extremely thin (about 200 nm)[19,21,51,58,59]. Moreover, subcapsular LECs contain endothelial fenestrations and transendothelial channels distended by diaphragms, which offer direct pathways to the subsinusoidal space[19,21,58] (FIG. 2). Diaphragms are cartwheel-like filters, which are composed of plasmalemma vesicle-associated protein (PLVAP) fibrils[60]. The maximal arc length between individual fibres (in BECs) is approximately 7–8 nm, which is similar to the radius of a 70-kDa globular protein[59]. Therefore, sinus diaphragms may physically or physico-chemically contribute to the size-selective transfer of molecules across the LEC floor. They may thereby contribute to the well-established 70-kDa size-exclusion limit for the molecules transported in the conduits, which lie immediately above the ceiling LECs, below the floor LECs and within the LEC-lined trans-sinusoidal cords. In fact, PLVAP-deficient mice lack diaphragms in LN LECs and, concurrently, molecules up to 500 kDa start to enter the conduit system[58], and access of vaccinia virus virions to the conduits is also enhanced[61].

The LEC layer is likely continuous without any open gaps[19], which would allow direct exchange of material between the sinus lumen and LN parenchyma. In addition, numerous functional studies have shown that, upon subcutaneous administration, high molecular-weight molecules (including dextrans and IgM) are largely excluded from the LN parenchyma, whereas many small molecules get instant access through the sinus floor[30,53,56,62,63]. Inter-LEC junctions in LNs contain both components of adherens and tight junctions, and the cells are underlined by a basement membrane[64]. A recent genomic screen revealed that lysophosphatidic acid receptor signalling induces constitutive coupling of β-arrestin to sphingosine-1-phosphate receptor 1 (S1PR1), which helps to maintain the barrier function of LECs[65]. However, the notion of a continuous LEC floor layer has also been challenged, as gaps or pores of 0.1–1.0 μm have been observed by electron microscopy in the sinus floor[22,66–68]. The gaps often contain a transmigrating leukocyte, suggesting that they may be transient structures, and they may also represent transendothelial channels or fenestrae with torn diaphragms. Thus, more studies are needed to firmly establish whether the subcapsular floor LECs form a continuous or non-continuous barrier.

Sinusoidal LECs have numerous surface invaginations, cytoplasmic vesicles, transfer tubules and multivesicular bodies, which are indicative of active endocytotic processes[19–21,63,67]. A few real-time microscopic analyses have noted rapid entry of molecules larger than 70 kDa into the LN parenchyma[53,57]. Subcutaneously administered IgG and IgA antibodies (approximately 150-kDa molecules) also specifically stain their target cells in the parenchyma of the draining LNs in a few seconds in mice[56]. The antibody transfer across the floor LECs was found to be a macrophage-independent, receptor-independent fluid-phase process. The transfer took place through the subcapsular sinus LECs in endocytic vesicles in a dynamin-dependent manner (FIG. 2). The same mechanism also transferred other large proteins and oligosaccharides across the sinus floor, although very large proteins such as pentameric IgM (molecular weight 850 kDa) were excluded[56]. Moreover, sinus LECs can also phagocytose and transfer particulate substances, such as colloidal carbon, to the LN parenchyma[30,63,69]. These data indicate that floor LECs quickly and efficiently transcytose multiple types of endogenous and exogenous cargoes from the subcapsular sinus. This LEC-dependent process allows entrance of antigens into the LN parenchyma well before the influx of antigen-loaded migratory dendritic cells about 10 h later. The transcytosis route through LECs may be exploitable for clinical purposes, as it allows local delivery of intact functional monoclonal antibodies to the draining LNs for modulating immune responses[56].

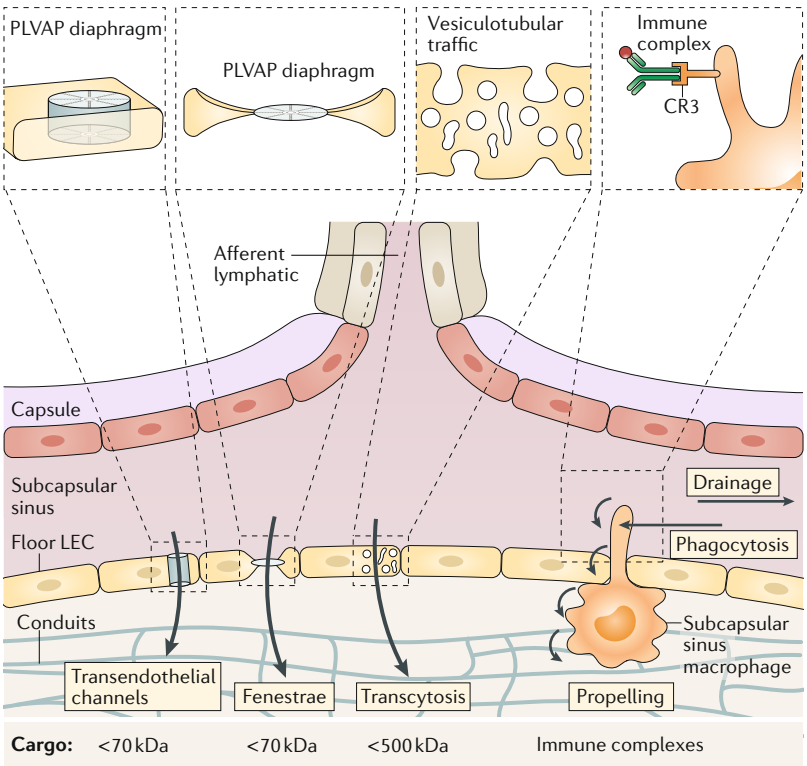

Fig. 2 | **Pathways mediating the transfer of macromolecules across the subcapsular floor.** Floor lymphatic endothelial cells (LECs) express plasmalemma vesicle-associated protein (PLVAP), which forms cartwheel-like diaphragms that guard transendothelial channels (diaphragms at both the luminal and abluminal sides) and fenestrae (a single diaphragm). Molecules less than 70 kDa in size can relatively freely permeate through these filters. Floor LECs are rich in endocytic vesicles and in other cytoplasmic vesiculotubular organelles. Very fast fluid-phase transcytosis through floor LECs takes place constitutively and allows the trans-sinusoidal transfer of molecules of up to 500 kDa. The reticular conduit system below the floor LECs is a fibrillary collagen-based transport channel for parenchymal delivery of molecules less than 70 kDa, but it remains unknown how the cargo gets access to the conduits. In addition, there is a non-LEC-dependent trans-sinusoidal transfer mechanism, in which subcapsular sinus macrophages propel immune complexes to B cell follicles, in the subcapsular sinus of lymph nodes (LNs). The macromolecules can also be scavenged or phagocytosed by sinus macrophages, or can be directly shunted to the next LN. The depicted transfer modes are deduced from mouse studies. CR3, complement receptor 3.

Immunoproteasome
A form of proteasome with unique subunits. Immunoproteasome has distinct cleavage-site preferences, which may improve antigen presentation.

LN LECs present antigen

Apart from controlling antigen distribution within the LN parenchyma, LN LECs can also directly present antigens to immune cells[70]. The high phagocytic and endocytic capacities of LN LECs enable efficient sampling of exogenous antigens, and the cells are rich in molecules involved in the assembly of the inflammation-induced immunoproteasome[39]. Medullary and cortical LECs also transcribe selected peripheral antigens, such as melanocyte-specific tyrosinase, intestinal epithelial protein A33 and pancreatic prohormone (PPY), in an autoimmune regulator (AIRE)-independent manner[71,72].

Both MHC class I and MHC class II molecules are present in human and mouse LN LECs[39,71,73,74]. MHC class II molecules can be transcribed by LN LECs themselves or can be acquired from dendritic cells[39,75]. Molecules involved in antigen processing and loading on MHC class II molecules, such as the transporter associated with antigen processing and the invariant chain, are also found in LN LECs. However, another crucial component of antigen loading, H2-M, is barely seen in LN LECs, at least under non-inflamed conditions. The expression of co-stimulatory molecules (including B7 family members) that is needed for efficient T cell activation is also very low or absent in LN LECs. By contrast, LN LECs synthesize high levels of multiple co-inhibitory molecules, including programmed cell death 1 ligand 1 (PDL1)[76,77]. Low-level expression of MHC class II, the presence of antigen-processing molecules and a virtual lack of co-stimulatory molecules were also detected in human LN LECs without any obvious sinus type-specific differences[28]. The dominance of co-inhibitory signals in LN LECs suggests their involvement in tolerance rather than in driving pro-inflammatory reactions.

The direct role of antigen presentation by LN LECs in mediating peripheral tolerance has been elegantly demonstrated in several genetic animal models. Interaction of both $CD8^+$ and $CD4^+$ T cells with LN LECs presenting endogenous or foreign antigens leads to specific deletion of the reactive lymphocytes[71,78]. LN LECs also express immunosuppressive enzymes, such as indoleamine dioxygenase and inducible nitric oxide synthase, which inhibit dendritic cell maturation and, hence, indirectly contribute to their immunosuppressive functions[74,79]. Nevertheless, LN LECs can also support T cell activation under certain settings by producing IL-7, which transmits proliferative and anti-apoptotic signals to T cells[80], and by antigen archiving (see below). LN LECs thus act both directly as antigen-presenting cells and indirectly to modulate dendritic cell and T cell function and contribute to the maintenance of peripheral tolerance. Therefore, it can be hypothesized that LN LEC dysfunction in the regulation of tolerance may contribute to the pathogenesis of autoimmune diseases.

Sinusoidal LECs control cell traffic

In both rodents and humans, most leukocyte subtypes can enter the draining LN via lymphatics, but only lymphocytes can exit the organ under physiological conditions[81,82].

Entrance to LNs

Dendritic cells. Chemokines and their receptors guide leukocyte transmigration from the blood to lymphoid organs and from the parenchyma to afferent lymphatics[5]. Similarly, lymph-borne migratory dendritic cells arriving at the subcapsular sinus rely on chemokines and chemokine receptors for translocation to the LN parenchyma[13,14] (FIG. 3). Direct intralymphatic injections of dendritic cells into an afferent lymphatic vessel draining to the popliteal LNs have shown that these cells enter the nodes from the subcapsular sinus[83]. The incoming dendritic cells express CCR7, and its ligand CCL21 is produced by many cell types in the LN. Ulvmar et al.[27] found that only the ceiling LECs, and not the floor LECs, express ACKR4, a decoy receptor for the CCR7 ligands CCL19 and CCL21. ACKR4 scavenges chemokines from the sinus lumen and thereby generates chemokine gradients across the sinus floor. Moreover, CCL1 released from floor LECs also facilitates dendritic cell entry to the LN parenchyma by binding to its receptor, CCR8, on dendritic cells[84]. Interestingly, dendritic cells lacking β1, β2, β7 and αV integrins enter the LN as efficiently as wild-type dendritic cells, implying that their counter-receptors, such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1), which are present on the floor of the subcapsular sinus, are not needed for parenchymal penetration[85].

Lymphocytes. When purified naive $CD4^+$ T cells are administered intralymphatically, they preferentially enter the LN through the medullary sinuses (FIG. 3). However, when co-injected with dendritic cells, the T cells start to use the subcapsular sinus LECs for their entrance[83]. The entry routes of other lymphocyte subtypes in isolation and in physiologically representative pools remain to be determined. Trans-sinusoidal migration of naive $CD4^+$ T cells is CCR7-dependent, but the other migratory determinants remain poorly characterized[83]. LEC diaphragms may be involved, as lack of PLVAP (the diaphragm-forming protein) expression enhanced transmigration of subcutaneously administered lymphocytes to the LN parenchyma[58]. Also, MSR1 expressed on subcapsular sinus LECs (and on different macrophage types) supports lymphocyte binding and regulates lymphocyte entrance from the subcapsular sinus to the LN parenchyma[43].

Neutrophils. Both mouse and human LNs contain a subset of neutrophils that are distinct from those present in the blood also under homeostatic conditions[86,87]. The neutrophils most likely enter via high endothelial venules, express MHC class II and mainly locate to the subcapsular sinus lining and interfollicular areas. In fact, LECs lining the subcapsular floor and the medullary sinus express neutrophil chemoattractants, such as CXCL1, CXCL2, CXCL3, CXCL4 and CXCL5, at least in humans[28]. LECs in the human medullary sinus also express CD209, a C-type lectin. In ex vivo binding assays, CD209 supports effective neutrophil adhesion to the medullary sinus by interacting with its ligand Lewis X (also known as CD15) on neutrophils (FIG. 3). This mechanism may keep neutrophils at this crucial location and

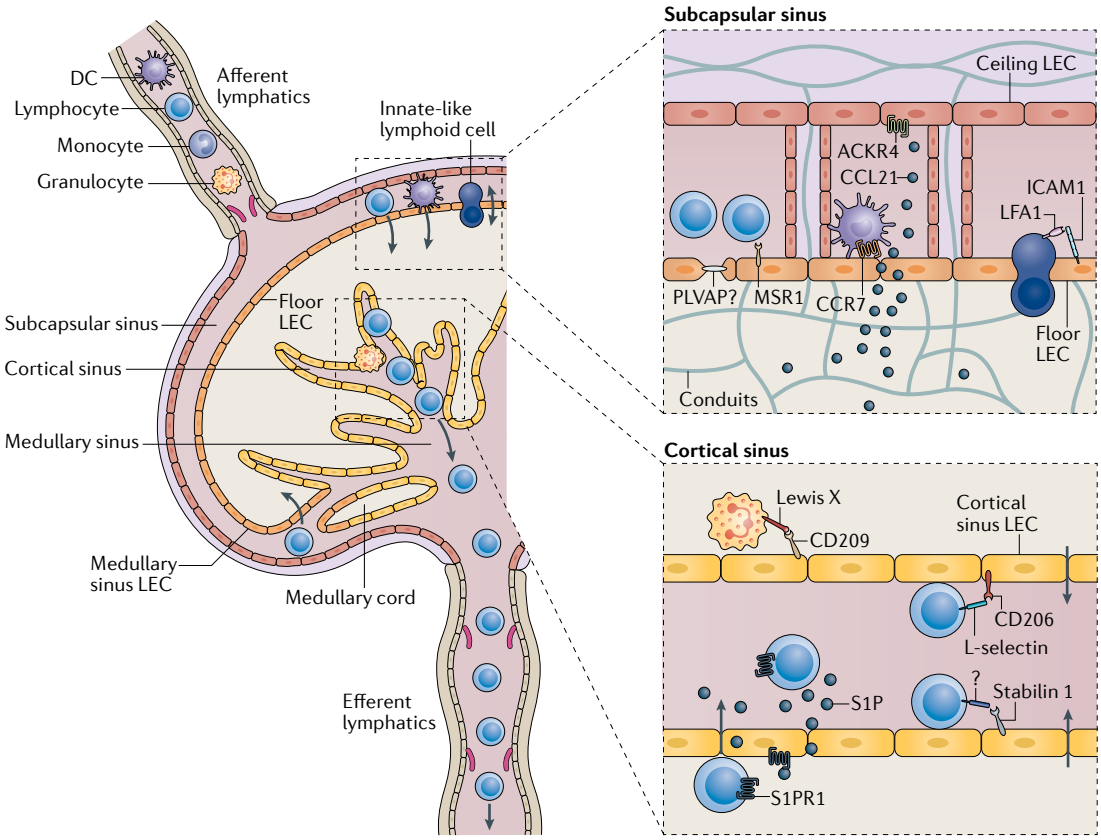

Fig. 3 | **Leukocyte entry and exit through lymphatics in the lymph nodes.** Dendritic cells (DCs) and naive T cells in the presence of DCs enter the draining lymph node (LN) through the floor lymphatic endothelial cells (LECs) in the subcapsular sinus. When the afferent lymph contains only purified naive T cells, the lymphocytes migrate into the LN parenchyma via the medullary sinus LECs. In the subcapsular sinus, innate-like lymphoid cells undergo constant patrolling between the sinus lumen and parenchyma below the floor LECs. Granulocytes can adhere to the cortical and medullary sinuses. Lymphocyte egress from the LN takes place mainly via the cortical sinuses. The best-characterized adhesive and chemotactic signals (verified in vivo in mouse LNs) involved in trans-sinusoidal leukocyte traffic are depicted. ACKR4, atypical chemokine receptor 4; CCL21, CC-chemokine ligand 21; CCR7, CC-chemokine receptor 7; ICAM1, intercellular adhesion molecule 1; LFA1, leukocyte function-associated antigen 1; MSR1, macrophage scavenger receptor 1; PLVAP, plasmalemma vesicle-associated protein; S1P, sphingosine-1-phosphate; S1PR1, sphingosine-1-phosphate receptor 1.

thereby help to limit systemic spread of pathogenic microorganisms. Given that mouse neutrophils use CXCR4 to enter inflamed LNs via lymphatics, and that mouse LECs lack CD209 homologues, the molecular mechanisms for LN entrance are most likely different between mice and humans[28,88].

Other leukocyte types. Classical Ly6C$^+$ tissue monocytes can enter the draining LN via afferent lymphatics without differentiation into macrophages or dendritic cells[89]. The entry is CCR7 independent, but the molecular mechanisms remain to be determined. LNs contain several types of innate-like lymphoid cell below the sinusoidal floor[90,91], but they apparently do not enter via the afferent lymphatics[92]. However, resident CXCR6$^+$ innate-like lymphoid cells, possibly γδ T cells, constantly shuttle across the subcapsular sinus LECs[93] (FIG. 3). Their migration to the sinus lumen is driven by LEC-produced sphingosine-1-phosphate (S1P). In the lumen, they use CCL20 for positioning and CD169 (also known as sialoadhesin) for binding to subcapsular sinus macrophages. Antibody blocking experiments and gene-knockout mice have shown that the migration of innate-like lymphoid cells across the sinus is dependent on interaction between leukocyte function-associated molecule 1 (LFA1) and ICAM1 (REF.[93]). This trans-sinusoidal patrolling behaviour of innate-like lymphocytes likely enhances early antimicrobial responses.

Exit from LNs

Mechanisms controlling LN exit have been studied primarily with lymphocytes as they are practically the only leukocyte type that leaves the LN under normal conditions[29]. Lymphocyte egress is regulated by the balance of tissue-retaining signals (such as CCR7) and emigration-promoting signals. S1P, a lipid chemoattractant, was the first molecule shown to control lymphocyte exit from the LN. Mice treated with the functional antagonist of S1PR1 (FTY720) manifested with empty lymphoid sinuses and retention of lymphocytes on the abluminal side of lymphatic sinuses[94]. Two-photon imaging subsequently verified that FTY720 inhibits T cell migration across sinusoidal LECs[95].

S1PR1 expression on LN LECs, where it modulates junctional stability[96], may have direct effects on this cell type during lymphocyte egress. However, regulated S1PR1 expression on lymphocytes is also instrumental for the exit process[29].

High expression of enzymes involved in S1P production (sphingosine kinases) and the absence of S1P-degrading enzymes (sphingosine lyase) in sinusoidal LECs are important for securing a high S1P concentration in the lymph[97,98]. Moreover, the release of intracellularly produced S1P is a controlled process in LN LECs[99]. Mice lacking spinster 2, a major S1P transporter, in all cells or in LYVE1$^+$ LECs manifest a low S1P concentration, especially in the lymph, and impaired lymphocyte egress from LNs[99–101]. S1P production by LN LECs may be fine-tuned by environmental signals: for example, IL-4 triggers sphingosine kinase expression in LN LECs[102] and hypercholesterolemia induces hyperplasia of cortical and medullary sinuses and a decrease in lymph S1P concentration[103]. Notably, whereas lymphocyte S1PR1 expression and lymphocyte numbers in the lymph oscillate according to the diurnal rhythm, S1P levels in LN LECs and lymph remain constant[104]. Recently, S1P produced by LECs was also found to promote survival of naive T cells[101]. α9β1 integrin signalling in cortical and medullary LECs may also contribute to lymphocyte exit from LNs[105].

The cortical sinuses are the major S1PR1-dependent exit sites for T cells (FIG. 3). Jason Cyster's group made a three-dimensional reconstruction of inguinal LNs using intravital two-photon microscopy and showed that lymphocyte flow begins within blunt-ended sinuses and that some lymphocytes enter these sinuses within minutes of entering LNs via high endothelial venules[34]. Interestingly, several lymphocytes went through a few rounds of migration between the parenchyma and sinuses before leaving the LNs[34]. B cells also exit the LNs through the cortical sinus LECs in an S1PR1-dependent manner[32], and natural killer cells have been reported to use both S1PR1-dependent and S1PR5-dependent mechanisms for their exit[106]. During the secondary immune response, but not in the primary response, T follicular helper cells migrate out of the follicle to the subcapsular sinus via undefined mechanisms[107]. It should also be noted that occasional lymphocytes egress from the parenchyma to the subcapsular sinus and some directly to the medullary sinus[32,33,95]. Real-time imaging has suggested that lymphocytes enter the cortical sinuses through preferred permissive sites of the LEC layer, but the molecular mechanisms governing this behaviour remain to be studied. Also, the adhesion molecules involved in lymphocyte egress remain mostly uncharacterized, although it is known that plasma cells egress from medullary cords using β2 integrins, which may interact with ICAM1 expressed on medullary LECs[108]. With regard to T cells, binding of LFA1 to ICAM1 on LN LECs helps the return of the cells back to the parenchyma and thereby slows down the exit process, giving more time for antigen binding[109]. Moreover, ex vivo antibody blocking assays with human LNs suggest that CD206 and stabilin 1 (also known as CLEVER1) contribute to lymphocyte binding to the medullary and cortical sinuses[110,111]. Hence, the localized production of S1P by LN LECs appears to be the major driving force that overrules the retention signals in the LN, and drives lymphocyte egress through cortical LECs in a process that is dependent on poorly understood adhesive mechanisms.

Sinusoidal LECs and LN organogenesis

LECs remodel heavily during LN organogenesis[112–114] (FIG. 4a). Whole-mount imaging reveals that a disc-like structure is formed from the collecting lymphatic LECs beneath the LN anlage after embryonic days 15.5–16.5 (E15.5–E16.5) in mice[112]. The area is subsequently engulfed by a cup-like LEC sheet by E20.5. Beginning from E16.5, discernible outer and inner sheets of LECs as well as trans-sinusoidal cords are found. The outer layer expresses forkhead box protein C2 (FOXC2) and ACKR4, whereas the innermost LEC layer expresses LYVE1, α2B integrin and MADCAM1 instead. At E18.5, the outer LEC layer expresses PDGFβ and becomes closely associated with smooth muscle cells. In humans, the distinct sinus LEC systems are identifiable during the first trimester[115,116].These data suggest that the ceiling and floor LEC identities of adult LNs are established already during embryonic development, and the development of the capsule is regulated by LEC-derived growth factor signals.

Recently, two alternative scenarios for the initiation of LN formation have been put forward (FIG. 4a). The development of LNs is dependent on bidirectional interactions between lymphoid tissue-organizer (LTo) cells and lymphoid tissue-inducer (LTi) cells[117]. Mesenchymal stromal cells (precursors of FRCs) have been thought to represent the major LTo cell type, which produce CXCL13 to attract the first embryonic haematopoietic LTi cells into the LN anlage[117]. In an elegant series of experiments with cell type-specific knockout mice, LN LECs were identified as the first functional LTo cell type in developing LNs[113]. The major LTi cell-attracting chemokine CCL21 was only found in LN LECs, and not in LN BECs, and LTi cells were identified inside lymphatic vessels at E14–E17. The interaction between LN LECs and LTi cells was largely dependent on receptor activator of nuclear factor-κB (RANK; also known as TNFRSF11A). When LTi cell exit from the LN anlage was prevented by FTY720 treatment, ectopic LN formation was induced, indicating that accumulation of LTi cells in LN lymphatics is an important driver for LN organogenesis[113].

In another recent study, LTi cells were found to enter the LN anlage first through blood vessels, and only later via LN LECs[112] (FIG. 4a). This view is supported by the observations that LTi cells are found in the embryonic LN anlage in the absence of lymphatic vessels[118,119]. Thus, although further analyses of LN development are warranted to conclusively pinpoint the kinetics and steps of LTi cell migration to the LN anlage, these recent studies both indicate that LN LECs are pivotal for LN organogenesis, as collectors of extravasated LTi cells into the LN anlage. Thereby, these data profoundly change the prevailing dogma of LN development and show that LN LECs are more primary LTo cells than FRCs and other mesenchymal cell types.

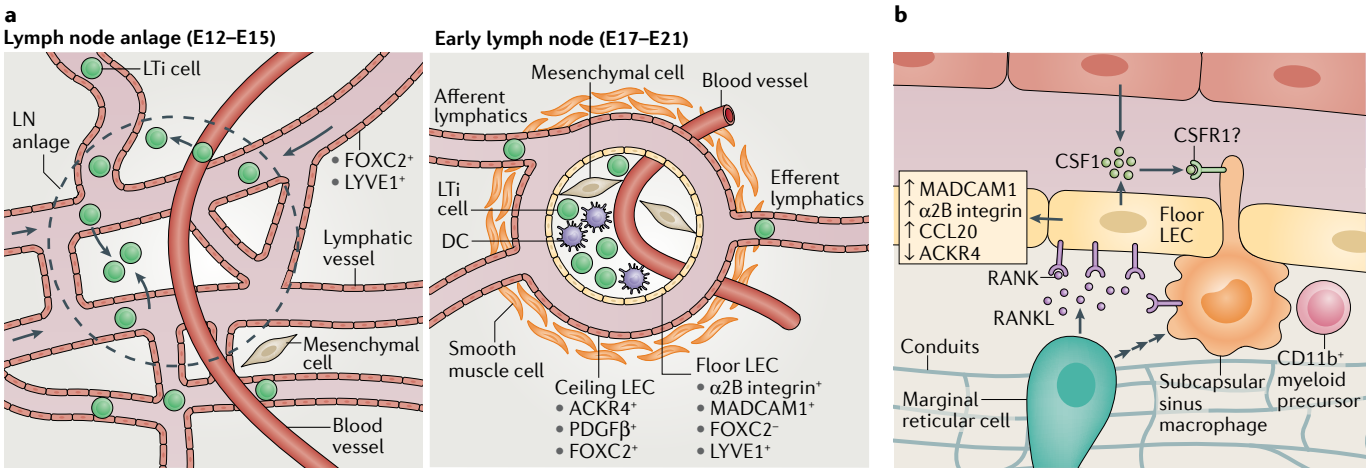

Fig. 4 | **Lymphatic endothelial cells in organizing the lymph node niche. a** | Developmental roles of lymphatic endothelial cells (LECs) in lymph node (LN) organogenesis during embryonic development. Starting from embryonic day 12 (E12) in mice, LECs of the collecting lymphatics in the future LN area (known as the LN anlage) start to form a cup-like projection around the anlage. The LECs may serve as the first lymphoid tissue-organizer (LTo) cell type, which attracts the first haematopoietic lymphoid tissue-inducer (LTi) cells to the anlage. Alternatively, the first LTi cells can enter the anlage through blood vessels, and only later are carried to the anlage by LEC-lined lymphatics. At E17, the LECs have completely engulfed the LN anlage. The floor and ceiling LEC layers become detectable and start to express the characteristic markers. LN LECs are also needed for the recruitment of smooth muscle cells, which then produce the capsule of the LN. **b** | LECs generate a unique niche for subcapsular sinus macrophages. Floor and ceiling LECs produce macrophage colony-stimulating factor 1 (CSF1), which is needed for the survival of subcapsular sinus macrophages. Marginal reticular cells, which are another stromal cell type in LNs and are located below the floor LECs, synthesize receptor activator of nuclear factor-κB ligand (RANKL), which binds to its receptor RANK expressed by floor LECs. This RANKL–RANK signalling supports the differentiation of floor LECs and contributes to their capacity to maintain subcapsular sinus macrophages. RANKL may also directly bind to subcapsular sinus macrophages and is involved in recruiting myeloid precursors ($CD11b^+$ cells) to the subfloor space. The pathways have been identified in mouse LNs. ACKR4, atypical chemokine receptor 4; CSFR1, macrophage colony-stimulating factor receptor 1; CCL20, CC-chemokine ligand 20; DC, dendritic cell; FOXC2, forkhead box C2; LYVE1, lymphatic vessel endothelial hyaluronic acid receptor 1; MADCAM1, mucosal addressin cell adhesion molecule 1; PDGFβ, platelet-derived growth factor-β.

The sinusoidal niche and macrophages

New studies have revealed that LN LECs orchestrate the development of sinusoidal macrophages[120,121] (FIG. 4b). Ceiling and floor LECs were found to be the principal source of CSF1, a major growth factor for macrophages, in LNs[120]. When CSF1 expression by $PROX1^+$ LECs was specifically deleted, the numbers of subcapsular and medullary sinus macrophages were strongly reduced during initial embryonic seeding as well as during recovery after an experimental deletion in adults, whereas other LN macrophage types were unaffected[120]. Another study identified RANK-mediated signalling in LN LECs as a major pathway controlling subcapsular sinus macrophage development and differentiation during embryogenesis and the first days of postnatal development[121]. In adults, the normal recovery of sinusoidal macrophages after infection-induced loss was also largely dependent on RANK synthesis by LECs, although this signalling pathway was not required during steady-state conditions. Interestingly, RANK-deficient LN LECs as well as LN LECs in mice lacking RANKL production by mesenchymal cells showed reduced expression of the floor LEC marker CCL20 and enhanced expression of the ceiling LEC marker ACKR4. The deficient LEC RANK–RANKL signalling pathway was accompanied by lower numbers of $CD11b^+$ cells in the sinusoidal areas[121]. These data suggest that LN LECs assist in the chemotactic recruitment and/or retention of myeloid cells in the sinusoids. They also indicate that LN LECs provide a unique microenvironmental niche inside LNs for the development and differentiation of sinusoidal macrophages.

Sinusoidal LECs in disease

Infection and inflammation

Lymphatic sinuses undergo marked expansion in inflammation. One important factor that drives the lymphangiogenic changes is the production of VEGFA by peripheral cells, FRCs, B cells and macrophages[122–125]. Lymphangiogenesis is accompanied by a transient increase in lymph flow, dendritic cell and lymphocyte migration to the LN parenchyma and activation of immune responses. The egress of lymphocytes from LNs is shut down during the first days of an inflammatory reaction. This is mainly due to interferon-induced CD69-dependent internalization of S1PR1, although CD69-independent mechanisms also contribute in certain infections[126,127]. Collectively, these alterations lead to a 10-fold to 20-fold enlargement of the inflamed LN. Whereas medullary and cortical sinus LECs continue to proliferate after the first week of immunization, the subcapsular sinus starts to involute[128]. Although the basis for sinus-specific differences remains unknown, T cells can exert negative effects on lymphangiogenesis in LNs, which may be partly mediated by interferon-γ (IFNγ)[129]. Moreover, type 1 interferons induce PDL1 overexpression by LN LECs, which coincides with their reduced proliferation[130].

Lymph-borne microorganisms bathe the sinusoids of draining LNs and LN LECs harbour multiple scavenging and phagocytic receptors. During viral infections or vaccinations, proliferating LN LECs capture viral antigens and store them for long periods (antigen archiving)[131,132]. The antigen reservoirs in LN LECs may

then slowly release antigens for transfer to the LN parenchyma, where the induction of protective responses takes place. In several infection models, subcutaneously administered microorganisms and infection-triggered neutrophils have been found to be in contact with sinusoidal LECs in the draining LNs[88,133–135]. Moreover, virus-induced inflammation often causes lymphatic neoangiogenesis in LNs, and certain viruses can directly infect peripheral LECs[136–138]. However, in most viral infections, it remains poorly understood whether LN LECs themselves become infected. Nevertheless, it is known that *Mycobacterium tuberculosis* is transported via the lymphatics and that a mannosylated lipoglycan of the bacterium interacts with CD206 and CD44, which are both expressed by LN LECs. The bacterium is internalized by LECs and can replicate within the LEC cytoplasm. Resting LECs form a permissive site for bacterial replication, whereas IFNγ-dependent induction of nitric oxide in LN LECs restricts bacterial proliferation in activated LN LECs[139]. It remains unknown whether LN LECs share with subcapsular sinus macrophages the capacity to limit the spread of microorganisms.

Cancer

Many malignancies, such as breast cancer and head and neck cancer, first spread to sentinel LNs and then metastasize to distant organs. Cancer cells modify the lymphatics and draining LNs in many ways to generate pre-tumoural niches for dissemination[140,141]. Cancer-induced lymphangiogenesis is believed to be the most important mechanism to create new lymphatics in sentinel LNs for facilitating metastatic spread[140,142]. However, Jeong et al. report preclinical and clinical evidence that the density of $CD31^+$ vessels (that is, both lymphatic and blood vessels) does not increase in metastatic LNs in several tumour models in mice and in patients suffering from colon cancer or head and neck cancer[143]. It thus remains to be studied whether co-opting of existing vessels[144], incorporation of myeloid cell types into lymphatics[145] or exosome-mediated LEC modulation[146,147] provide alternative mechanisms for LN lymph vessel-dependent seeding of metastatic cells.

Interestingly, when the first steps of cancer cell entry to the subcapsular sinus are studied in isolation (without microenvironmental modulation of LNs by the slowly growing primary tumour), LN LECs seem to limit tumour spread to more proximal LNs. Thus, after intralymphatic injections, mouse mammary tumour cells make contacts with the subcapsular sinus LECs, but do not migrate to the medullary sinus[148]. When residing in an avascular subcapsular sinus, tumour cells upregulated invasive programmes and penetrated through the sinus floor within 1 day. In the subcapsular sinus, both physical constraints and chemotactic cues may control tumour cell localization. The width of the subcapsular sinus in mice is less than the diameter of a cancer cell and the frequent trans-sinusoidal cords may further impede tumour cell movement. Moreover, CCL1 is expressed by subcapsular sinus LECs both under normal conditions and in mice and humans with cancer, and malignant cells use CCR8 to bind to CCL1. Blocking of CCR8 or CCL1 prevents the entry of tumour cells from the collecting lymphatics to the subcapsular sinus and their lateral spread within the sinus[149]. Thus, tumour-induced LN LEC remodelling and the role of LN LECs in tumour progression need to be understood in much more detail before their role in promoting or inhibiting tumour spread can be conclusively determined.

Conclusions

Lymphatic vessels in the LNs display several unique adaptations that make them very different from peripheral lymphatics. The subcapsular sinus is the first sinus system exposed to the incoming lymph-borne material, and it shows many structural and functional adaptations, which support key functions in mediating antigen sorting, immune cell trafficking and promotion of peripheral tolerance. Although the highly branched cortical and medullary sinus systems likely contribute to these same processes, they also secure ordered exit of cells and lymph fluid from the LN. The true complexity of the LN sinus systems is only now beginning to be unravelled as single-cell gene expression data are combined with live imaging, three-dimensional positional analyses and conditional knockout models. These analyses have already revealed hitherto unknown contributions of sinusoidal LECs to LN organogenesis and to the generation of intra-organ niches through intimate crosstalk with other stromal and leukocytic components of the LN.

Several burning questions remain to be addressed in the immunology of LN LECs. These cells include at least six different subpopulations. Unfortunately, none of the traditionally used LEC markers (apart from PROX1) captures them all. Moreover, none of the widely used LEC markers is truly specific for LECs, not to mention LN LECs only. Therefore, re-examination of the published literature and more detailed analyses of the different LEC sub-compartments are warranted. Similarly, different mechanistic studies have to be interpreted with care, as there are, to our knowledge, no strictly LN LEC-specific conditional mouse models, and in any antibody/inhibitor experiments the effects on peripheral LECs and systemic effects are also very difficult to control for. Moreover, much of the existing knowledge stems from analyses of easily accessible LNs (inguinal or popliteal LNs), and it remains to be seen how conserved the LN LEC structure and function are among 22 peripheral LNs and multiple non-capsulated Peyer's patches in mice. Moreover, there are also ample examples of species-specific differences in the phenotype and function of LN LECs. For potential medical interventions, it will therefore be important to address the sinusoidal LEC biology in the approximately 400 LNs found in humans. Finally, LN LEC immunology has so far almost exclusively been studied under normal conditions or using a few inflammation models induced with classical adjuvants such as complete Freund's adjuvant or lipopolysaccharide. Therefore, a panoply of new LN LEC responses can be expected to be discovered when these cells are scrutinized under different phases of various inflammatory, infectious and cancerous conditions.

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