# HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes

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Abstract | In search of foreign antigens, lymphocytes recirculate from the blood, through lymph nodes, into lymphatics and back to the blood. Dendritic cells also migrate to lymph nodes for optimal interaction with lymphocytes. This continuous trafficking of immune cells into and out of lymph nodes is essential for immune surveillance of foreign invaders. In this article, we review our current understanding of the functions of high endothelial venules (HEVs), stroma and lymphatics in the entry, positioning and exit of immune cells in lymph nodes during homeostasis, and we highlight the unexpected role of dendritic cells in the control of lymphocyte homing through HEVs.

Lymph
Interstitial protein-poor aqueous fluid in the extravascular space that is channelled in lymphatic vessels and returned to the circulation via the thoracic duct.

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The trafficking of immune cells in lymph nodes has a crucial role in immunity. The hundreds of lymph nodes (~450) that are dispersed in the human body are essential for the encounter of circulating naive lymphocytes with antigens and antigen-presenting cells, such as dendritic cells (DCs), which drain from peripheral tissues through interstitial fluids (lymph). The recirculation of lymphocytes through lymph nodes thus allows the extremely rare populations of naive lymphocytes specific for a given antigen to survey the lymph for the presence of their target antigen in any part of the body. This provides an effective immune surveillance for foreign invaders (such as viruses, bacteria or helminths) and for alterations in the body's own cells (such as abnormal self antigens in cancer).

Sir James Gowans was the first to demonstrate that lymphocytes continuously recirculate from the blood to lymphoid organs and back to the blood as often as one or two times per day during homeostasis[1]. In two landmark studies published in 1964, Gowans and colleagues showed that radioactively labelled lymphocytes that were transfused into the blood of rats migrated rapidly into lymph nodes by crossing specialized blood vessels termed high endothelial venules (HEVs)[2,3]. These experiments conclusively demonstrated that HEVs are the site of a large-scale migration of lymphocytes from the blood into lymph nodes. Fifty years later, we now have a good understanding of the molecular mechanisms regulating lymphocyte recirculation and lymphocyte migration ('homing') to lymph nodes[4,5]. Naive B and T cells extravasate through HEVs via a multistep adhesion cascade[4–8]. After crossing HEVs, T cells migrate to the T cell areas in the lymph node paracortex, whereas B cells enter the B cell follicles in the cortex (FIG. 1a). The intranodal migration and positioning of B and T cells (as well as DCs) are regulated by the chemokine ligands CC-chemokine ligand 21 (CCL21), CCL19 and CXC-chemokine ligand 13 (CXCL13) that are produced by fibroblastic reticular cells (FRCs) in the T cell areas and by follicular dendritic cells (FDCs) in the B cell follicles[9–12]. If lymphocytes do not recognize their specific antigen in the 'visited' lymph node, they leave the lymph node through the efferent lymphatic vessel using the sphingosine-1-phosphate (S1P)–S1P receptor type 1 (S1PR1; also known as S1P1) signalling pathway and return to the circulation through the thoracic duct[10,13]. The time that naive B cells spend 'exploring' a given mouse lymph node (~24 hours) is significantly longer than the time spent by naive T cells (~8–12 hours)[14].

In this article, we review the mechanisms that control lymphocyte recirculation during homeostasis. Rather than focusing on a single step, we discuss the different steps that regulate the trafficking of immune cells in lymph nodes: step one, entry via HEVs, as well as via afferent lymphatics; step two, intranodal migration and positioning; and step three, egress via efferent lymphatics. We emphasize several important recent advances regarding the role of DCs in the control of lymphocyte entry through HEVs[15,16], the entry of naive T cells and DCs via afferent lymphatics[17] and the importance of downregulation of S1PR1 (the receptor required for egress) for the entry of lymphocytes into lymph nodes through HEVs[18]. The entry of DCs into terminal lymphatics, the lymph node stromal cell networks and the S1P–S1PR1-mediated egress of lymphocytes into efferent lymphatics are described only briefly. For additional information, readers are referred to several 'state-of-the-art' reviews on these topics that have been published recently[11,13,19,20].

High endothelial venules (HEVs). Specialized venules (small veins that join capillaries to larger veins) that are lined by plump endothelial cells. HEVs occur in secondary lymphoid organs, except the spleen, and are the main sites of lymphocyte entry from the blood.

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.

Follicular dendritic cells (FDCs). Specialized reticular fibroblasts located in B cell follicles of lymph nodes and other secondary lymphoid organs that present intact antigens to B cells.

## Blood and lymphatic vessels in lymph nodes

An important structural characteristic of lymph nodes is the existence of specialized vascular and lymphatic systems. These include the HEVs (FIG. 1b) in the paracortex, and the lymphatic vessels and sinuses (FIG. 1c) in the paracortex and medulla.

*HEVs.* HEVs are anatomically distinct post-capillary venules found in lymph nodes and other secondary lymphoid organs (with the exception of the spleen) that have fascinated many investigators over the past 100 years[5–7]. The peculiar structure of HEVs in lymph nodes was first observed by Thome in 1898, who described these vessels

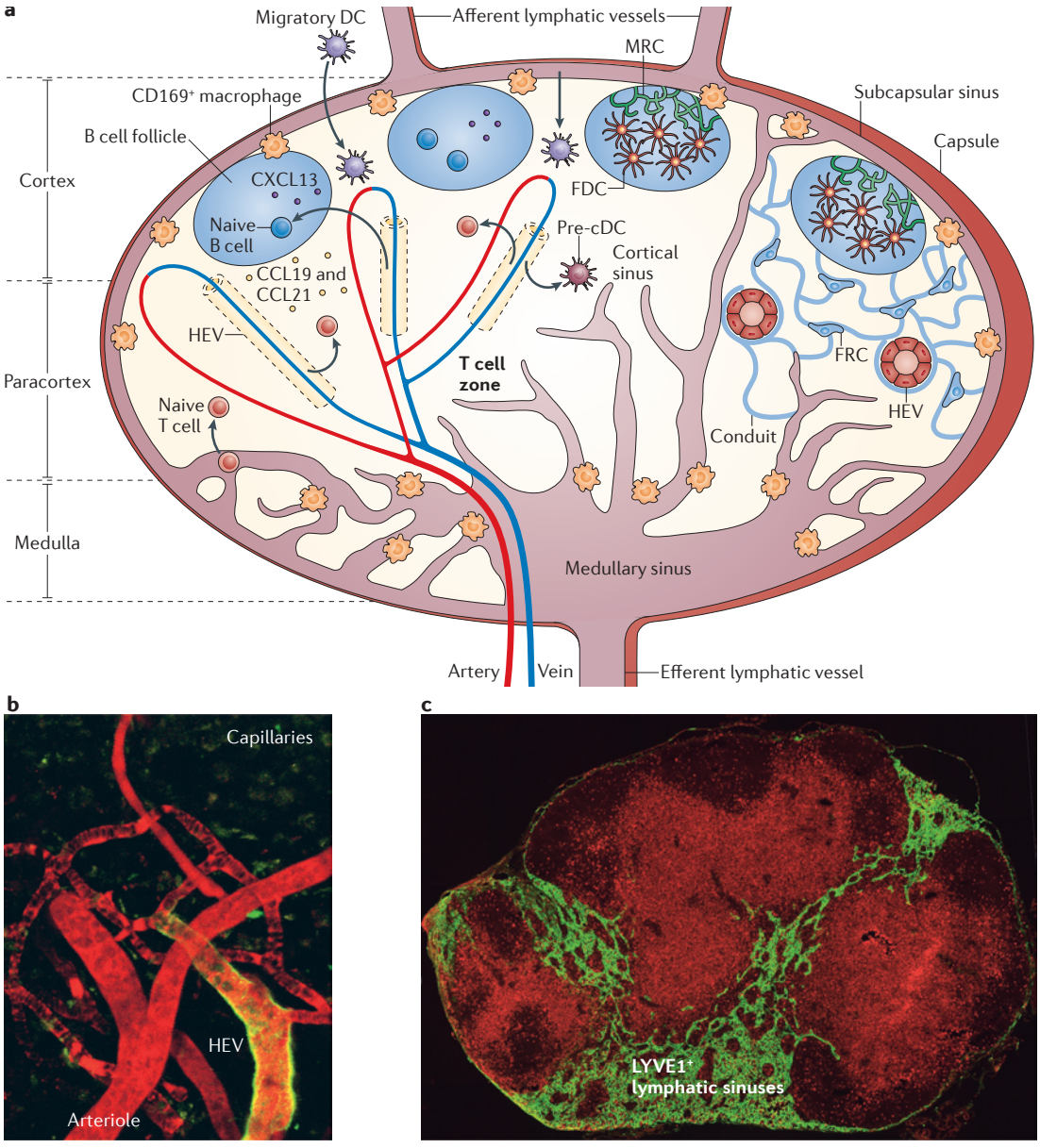



Figure 1 | **Organization of the lymph node and entry of immune cells.** **a** | Lymph nodes are encapsulated bean-shaped lymphoid organs that are subdivided into three main regions: the cortex, the paracortex and the medulla. Naive lymphocytes enter the lymph node via high endothelial venules (HEVs) or afferent lymphatic vessels, and exit through cortical sinuses, medullary sinuses and the efferent lymphatic vessel in the medulla. The entry of dendritic cells (DCs) occurs through afferent lymphatics and the subcapsular sinus (for migratory DCs) or HEVs (for precursor conventional DCs (pre-cDCs)). The cortex contains densely packed B cells and follicular dendritic cells (FDCs) arranged into discrete B cell follicles. FDCs cluster in the centre of the follicles and form a dense network in which B cells search for antigens[11]. By contrast, T cells accumulate in the T cell zones of the paracortex. The fibroblastic reticular cells (FRCs) in the paracortical T cell areas form reticular fibres and stromal networks that function as guidance paths for lymphocytes and DCs[11,12]. **b** | The two-photon microscopy image shows the lymph node microcirculation. Blood vessels are labelled in red with rhodamine-conjugated dextran and a post-capillary HEV is stained in green with MECA-79. **c** | The epifluorescence composite image of a cryosection of an axillary lymph node shows the LYVE1 (lymphatic vessel endothelial hyaluronic acid receptor 1)-expressing lymphatic sinus system (green). $CD3^+$ T cells are labelled in red. MRC, marginal reticular cell.

as being composed of plump endothelial cells that bulge into the vascular lumen[21]. It is this plump appearance that has given rise to the name of high endothelial venules. Although von Schumacher reported the presence of numerous lymphocytes within HEV walls as early as 1899 (REF. 22), the direction and physiological significance of lymphocyte migration through HEVs were not fully appreciated until the classical experiments of Gowans in the 1960s[2]. More recently, the precise localization of HEVs within the peripheral lymph node microcirculation was carefully analysed by von Andrian using intravital microscopy[23]. The study by von Andrian identified a distinct hierarchy of branches in the lymph node venular tree (venular branching orders I to V, from the large collecting venule to the small post-capillary venules) and showed that HEVs are found at the level of venular branching orders III to V (FIG. 2a), which support the bulk of lymphocyte trafficking to lymph nodes. During homeostasis, HEVs are found only in lymphoid organs, but they can develop in non-lymphoid tissues during chronic inflammatory diseases[6,24] and cancer[25], and they are associated with high levels of lymphocyte infiltration into these tissues (BOX 1).

***Lymphatics.*** Afferent lymphatic vessels deliver lymph — which contains tissue-derived antigens and immune cells (including DCs) — to the subcapsular sinus (SCS), a space below the collagen-rich fibrous capsule that covers the lymph node[5,20]. The floor of the SCS is covered by lymphatic endothelial cells expressing lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1), and these cells are interspersed with $CD169^+$ sinus-lining macrophages. After flowing into the SCS, lymph percolates through the widely branched medullary sinuses and blind-ended cortical sinuses, before leaving the lymph node via the efferent lymphatic vessel[20]. Cortical sinuses — which begin in areas adjacent to HEVs and proximal to B cell follicles in the paracortex (FIG. 1a) — were recently found to function as sites for B and T cell egress[26–29]. Medullary sinuses are lined by $LYVE1^+$ endothelium and contain numerous $CD169^+$ medullary macrophages. SCS macrophages capture large molecules, particles and microorganisms that enter the lymph nodes through the lymph, and display antigens to B cells for delivery into follicles[30–32]. Although large lymph-borne molecules do not have direct access to the lymphocyte compartment, small molecules (<70 kDa), such as chemokines and antigens, can reach the T cell areas and B cell follicles directly from the SCS via lymph node conduits[33–35]. T cell zone conduits, which are formed by FRCs that are wrapped around collagen-rich reticular fibres, can rapidly drain lymph into perivascular channels around HEVs[33,34].

## Entry of immune cells through HEVs

The interaction of lymphocytes with the endothelium of HEVs is initiated by the lymphocyte homing receptor L-selectin (also known as CD62L), which mediates the tethering and rolling of lymphocytes along HEV walls[5,8] (FIG. 2b). L-selectin recognizes a family of mucin-like glycoproteins, which are heavily sulphated, fucosylated and sialylated when expressed by the HEV endothelial cells of peripheral lymph nodes[8]. These HEV sialomucins (also known as peripheral node addressins) include CD34, glycosylation-dependent cell adhesion molecule 1 (GLYCAM1; only in mice), podocalyxin, endomucin and nepmucin (also known as CLM9)[8,36]. The crucial carbohydrate determinant for L-selectin recognition is 6-sulpho sialyl Lewis X (sialic acidα2-3Galβ1-4(Fucα1-3(sulpho-6))GlcNAcβ1-R), which is abundantly produced in HEVs and present on both *N*-glycans and extended core 1 and core 2 *O*-glycans that decorate HEV sialomucins[8,37–43].

***Phenotypical characteristics of HEVs.*** Strikingly, many of the HEV-specific antibodies described to date recognize the 6-sulpho sialyl Lewis X epitope[40–43]. The function-blocking antibody MECA-79 (REF. 44) — which has been widely used over the past 20 years to characterize HEVs in both mouse and human tissues[6,8,25] — binds to 6-sulpho sialyl Lewis X on core 1 *O*-glycans[40], a unique feature of HEV sialomucins. Interestingly, the recently described antibodies CL40 (REF. 42) and S2 (REF. 43) recognize 6-sulpho sialyl Lewis X on both *O*-glycans and *N*-glycans from human and mouse HEV sialomucins and exhibit a greater potency than MECA-79 in the inhibition of HEV-mediated lymphocyte homing to mouse lymph nodes[42,43].

Another important phenotypical characteristic of HEV endothelial cells is the expression of specific genes that are not expressed by endothelial cells from other blood vessels[15]. Again, most of these HEV-specific genes appear to be implicated in the synthesis of the 6-sulpho sialyl Lewis X-decorated HEV sialomucins. These include the genes encoding GLYCAM1 and the HEV-specific enzymes *N*-acetylglucosamine 6-*O*-sulphotransferase 2 (GlcNAc6ST2; encoded by *Chst4*) and fucosyltransferase 7 (FUT7; also known as FucTVII)[8,37,38,45]. Although not strictly HEV-specific, other enzymes are also abundantly expressed in HEVs, including GlcNAc6ST1 and *O*-glycan core 1 extension and core 2 branching enzymes[37–39]. Studies in knockout mice have revealed that FUT7 is crucial for the fucosylation of HEV sialomucins[45], that the sulphotransferases GlcNAc6ST1 and GlcNAc6ST2 cooperatively control the synthesis of 6-sulpho sialyl Lewis X in HEVs[37,38], and that core 1 and core 2 branching enzymes are essential for the expression of 6-sulpho sialyl Lewis X on *O*-glycans[39]. Recently, mice deficient in the sialyltransferases ST3GalIV and ST3GalVI were also found to exhibit an impaired synthesis of L-selectin ligands in HEVs[46]. It is the coordinated expression of all these enzymes that gives HEV endothelial cells their unique capacity to capture large numbers of circulating lymphocytes.

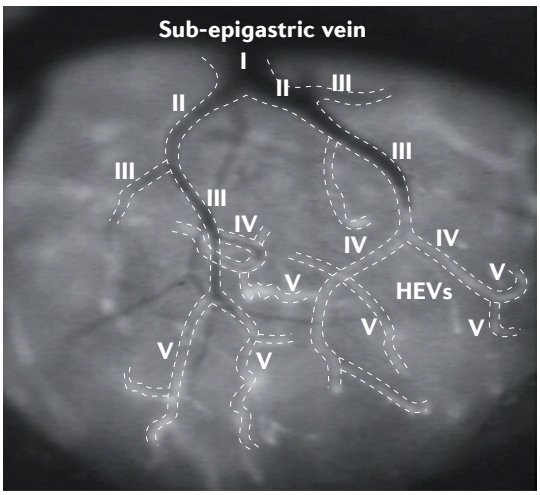
The development of specific protocols for the isolation of HEV endothelial cells from lymphoid organs[47] has opened the way to the identification of genes involved in HEV sulphation pathways[48] and the discovery of new genes that are abundantly expressed in HEVs. These abundantly expressed genes encode the mucin-like protein nepmucin[36], the nuclear cytokine interleukin-33 (IL-33; originally termed NF-HEV)[49] and

Cortical sinuses
Blind-ended lymphatic vessels located in the T cell areas of lymph nodes that mediate the exit of B and T cells from the lymph nodes.

the secreted proteins hevin (also known as SPARCL1)[47] and autotaxin (also known as ENPP2)[50,51]. Although these genes are not HEV-specific, some of them could have important roles in lymphocyte migration through HEVs. For instance, autotaxin — an ectoenzyme that produces lysophosphatidic acid — has been proposed to facilitate lymphocyte entry into lymph nodes[50,51].

***HEV-mediated entry of B and T cells.*** Naive B and T cells rolling along HEV walls enter lymph nodes through a multistep adhesion and migration cascade[4–8] (FIG. 2b). The lymphocytes first undergo chemokine-induced activation of their integrins, which mediate lymphocyte arrest (sticking) on the HEV endothelium. This step has been extensively reviewed in previous articles[5,7,9] and is described only briefly here. The homeostatically expressed chemokines CCL21, CXCL12 and CXCL13 are the crucial factors for lymphocyte extravasation through lymph node HEVs. Naive T cells express CC-chemokine receptor 7 (CCR7), the receptor for CCL21, and CXC-chemokine receptor 4 (CXCR4), the receptor for CXCL12, whereas naive B cells (but not naive T cells) express CXCR5, the receptor for CXCL13, in addition to CCR7 and CXCR4 (REFS 5,7,9).

CCL21 is abundantly expressed by HEV endothelial cells (in mice but not in humans), whereas CXCL12 and CXCL13 are produced by lymph node stromal cells (FRCs and FDCs) and transcytosed to the luminal



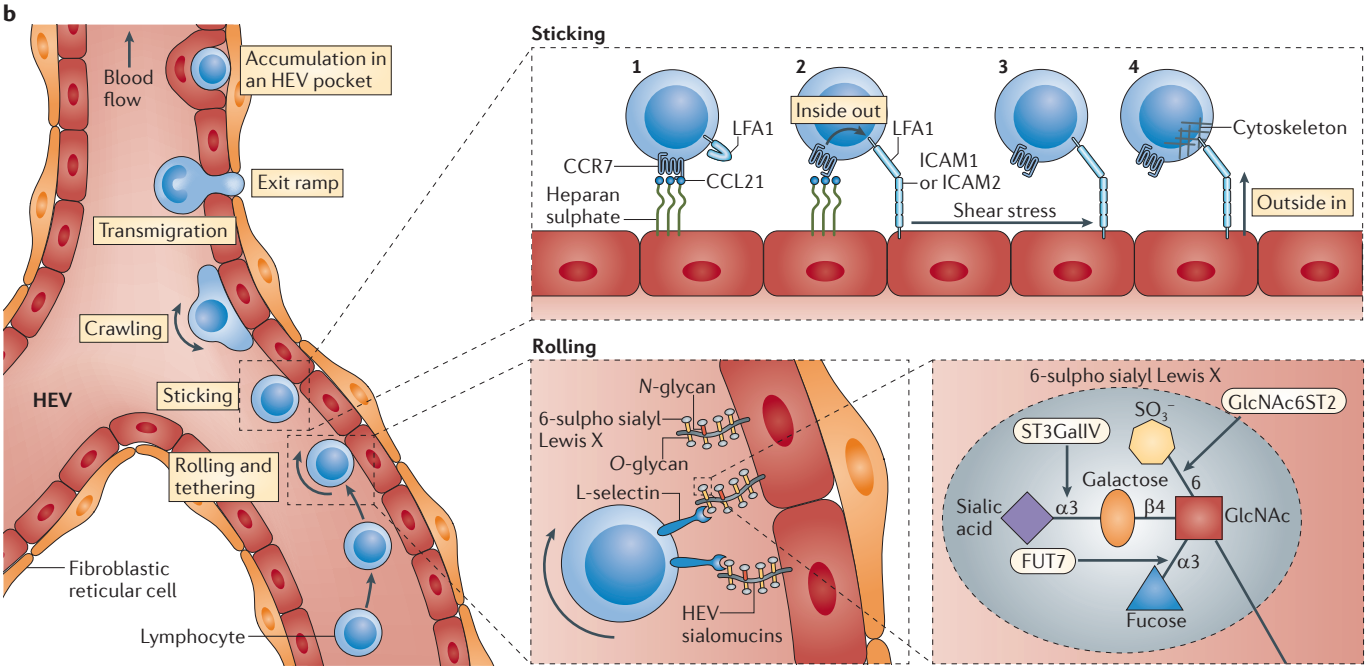




Figure 2 | **Entry of lymphocytes through HEVs — the multistep adhesion cascade.** **a** | The image shows the inguinal lymph node venular tree, visualized by intravital microscopy. The high endothelial venule (HEV) network is revealed following intravenous injection of the fluorescently labelled HEV-specific antibody MECA-79. High-order venules (orders III to V) correspond to HEVs[23]. Image is modified, with permission, from REF. 15 © (2011) Macmillan Publishers Ltd. All rights reserved. **b** | Naive B and T cells migrate through lymph node HEVs via a multistep adhesion cascade (which involves rolling, sticking, crawling and transmigration)[4–8]. Lymphocytes circulating in the blood tether and roll on HEV walls through the binding of L-selectin to 6-sulpho sialyl Lewis X motifs decorating *O*-glycans and *N*-glycans from HEV sialomucins, a family of sulphated, fucosylated and sialylated glycoproteins[8]. Several HEV-specific enzymes (including *N*-acetylglucosamine 6-*O*-sulphotransferase 2 (GlcNAc6ST2) and fucosyltransferase 7 (FUT7)) are involved in the synthesis of 6-sulpho sialyl Lewis X on HEV sialomucins[8,37,38,45]. Subsequently, rolling lymphocytes are activated by chemokines that are either produced by HEVs (such as CC-chemokine ligand 21 (CCL21)) or transcytosed through HEVs[5,7] and that are immobilized on the luminal surface by heparan sulphate[52]. Signalling through the G protein-coupled receptor CC-chemokine receptor 7 (CCR7), together with the shear force of blood flow, induces conformational changes in the lymphocyte integrin lymphocyte function-associated antigen 1 (LFA1)[53], which mediates firm binding (sticking) to intercellular adhesion molecule 1 (ICAM1) and ICAM2 on the endothelium. Next, lymphocytes crawl on the HEV surface for several minutes[54–57], before rapidly transmigrating across the HEV endothelium via 'exit ramps' formed by fibroblastic reticular cells[12]. Some lymphocytes accumulate transiently in 'HEV pockets'[58].

Two-photon intravital microscopy
A fluorescence imaging technique that combines laser-scanning confocal microscopy with long-wavelength multiphoton fluorescence excitation to capture high-resolution three-dimensional images of fluorescent cells or tissues in living animals.

surface of the HEV endothelial cells[5,7]. Endothelial heparan sulphate has recently been shown to be essential for the presentation of CCL21 and for lymphocyte homing through HEVs[52]. The integrin lymphocyte function-associated antigen 1 (LFA1; also known as αLβ2 integrin), which binds to intercellular adhesion molecule 1 (ICAM1) and ICAM2 on endothelial cells, is the major integrin for B and T cell arrest in the HEVs of peripheral lymph nodes. By contrast, integrin α4β7, which binds to mucosal addressin cell adhesion molecule 1 (MADCAM1), is also important for lymphocyte arrest in mesenteric lymph nodes[5,7,53].

Intravital microscopy has been invaluable for the characterization of the lymphocyte–HEV adhesion cascade. In particular, epifluorescence intravital microscopy has been used for the analysis of the initial rolling and sticking interactions[5,23], and two-photon intravital microscopy has been used for the study of the later steps of lymphocyte migration through HEVs[12,54–57]. Indeed, two-photon intravital microscopy analyses have recently revealed that, after firm arrest, a substantial fraction of B cells and T cells crawl along the luminal surface of the HEV endothelium in search of an appropriate site for transendothelial migration[54–57]. Naive T cells crawl at an average migration velocity of ~10 μm per minute, irrespective of the direction of blood flow, and after reaching an exit site they rapidly (within ~1.5 min) transmigrate across the HEV endothelium into the extravascular space[55–57]. Many T cells were found to follow each other through discrete exit sites designated 'exit ramps'[12]. Naive B cells crawl and transmigrate less rapidly than T cells[57], and it is not yet known whether they use the same HEV exit ramps to enter lymph nodes. Two-photon intravital microscopy analyses have also revealed that many lymphocytes accumulate transiently below HEV endothelial cells in 'pockets', which are highly dynamic structures that are continuously altered in size and location by lymphocyte migration and that may contribute to the 'highness' of HEVs[58].

In addition to naive T cells, other T cell populations enter lymph nodes through HEVs during homeostasis. For instance, central memory T cells and forkhead box P3 (FOXP3)-expressing regulatory T cells circulating in the blood have been shown to extravasate through HEVs under steady-state conditions[5,9,59]. These T cells express L-selectin and CCR7 and migrate to the paracortical T cell areas of the lymph nodes after transendothelial migration[5,9,59].

***HEV-mediated entry of other immune cell types.*** HEVs also mediate the entry of other important immune cell types into lymph nodes during homeostasis, including plasmacytoid DCs (pDCs)[60] and the precursors of conventional DCs (pre-cDCs)[61]. In addition to having reduced B and T cell counts[62], CCR7-deficient mice have reduced pDC numbers in lymph nodes owing to impaired homing of *Ccr7*$^{-/-}$ pDCs[60]. Pre-cDCs express L-selectin, and an L-selectin-specific blocking antibody was found to prevent the accumulation of pre-cDCs in lymph nodes[61]. However, the chemokine(s) and chemokine receptor(s) that are important for the migration of pre-cDCs through HEVs have not yet been characterized. Finally, in addition to pDCs and pre-cDCs, low numbers of natural killer (NK) cells have been shown to recirculate through lymph nodes during homeostasis. NK cell migration to resting lymph nodes was inhibited in mice deficient for L-selectin or L-selectin ligands[63].

Together, these observations indicate that although the main function of HEVs is to recruit large numbers of naive B and T cells into lymph nodes, other cell types can also enter lymph nodes through HEVs. HEVs are therefore crucial for the trafficking of immune cells in lymph nodes during homeostasis, and a better understanding of the mechanisms regulating their unique phenotypical characteristics is essential.

## DCs regulate entry through HEVs

Studies performed in rodents 20 years ago revealed that when peripheral lymph nodes are deprived of afferent lymph, HEV-specific genes (such as *Glycam1* and *Fut7*) and markers (such as the addressin recognized by MECA-79) are downregulated and HEVs lose their ability to support lymphocyte traffic[64,65]. Further analyses using HEV endothelial cells freshly isolated from lymphoid organs confirmed that these cells exhibit a remarkable plasticity in adults and rapidly lose their specialized characteristics when isolated from their natural tissue microenvironment[66]. Together, these studies suggested that tissue- and lymph-derived cells and/or factors are important for the maintenance of HEVs *in vivo*. Recently, we discovered that CD11c$^+$ DCs contribute to the regulation of the HEV phenotype and of the HEV-mediated homing of lymphocytes to lymph nodes during homeostasis[15,16] (FIG. 3).

### Box 1 | **HEVs in chronic inflammatory diseases and cancer**

Blood vessels that are structurally, phenotypically and functionally similar to high endothelial venules (HEVs) from lymph nodes appear in the chronically inflamed tissue in many inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel diseases, psoriasis, dermatitis, bronchial asthma, autoimmune thyroiditis, *Helicobacter pylori* gastritis and atherosclerosis, as well as during acute heart allograft rejection and chronic vascular rejection of kidney and heart grafts[6,24]. Such vessels have been proposed to participate in a positive feedback mechanism for increasing lymphocyte entry into the diseased tissue, thus contributing to the amplification and maintenance of chronic inflammation. Interfering with the development and/or maintenance of HEV blood vessels in chronically inflamed tissues is therefore likely to provide therapeutic benefits in many distinct chronic inflammatory diseases in humans.

Recently, it was shown that vessels with HEV characteristics are also frequently found in the stroma of human solid tumours (such as melanomas and breast, colon, lung and ovarian carcinomas). Moreover, a high density of these tumour HEVs is associated with high levels of infiltration of B and T cells (including CD8$^+$ cytotoxic T cells), as well as with a favourable clinical outcome in breast cancer[25]. Blood vessels and tumour angiogenesis are generally associated with poor prognosis, and this was the first time that a specific type of blood vessel was associated with good prognosis. Therefore, it could be important to be able to induce the HEV endothelial cell differentiation programme in tumour blood vessels. This could increase the density of tumour HEVs without increasing tumour angiogenesis and could suppress tumour growth through the enhanced recruitment of cytotoxic and memory lymphocytes that can limit tumour cell metastasis[25]. A better understanding of the mechanisms involved in the maintenance of HEVs[15] may thus allow for the development of novel therapeutic approaches for both chronic inflammatory diseases and cancer.

***DCs control lymphocyte homing to lymph nodes via HEVs.*** Intravital microscopy analyses of DC-depleted mice revealed alterations in lymphocyte–HEV interactions, with increased rolling velocities and decreased adhesion of lymphocytes inside lymph node HEVs[15]. These alterations in lymphocyte rolling and sticking were associated with an inhibition of HEV-mediated lymphocyte homing to lymph nodes and an important reduction in lymph node cellularity[15]. Together, these observations indicated that CD11c$^+$ DCs control the function of HEVs in lymphocyte recruitment.

Independent genetic evidence that CD11c$^+$ DCs are essential for lymphocyte homing to lymph nodes during homeostasis was obtained using CCR7-deficient mice[16]. These mice have reduced lymph node cellularity owing to defective homing of B and T cells[62]. Surprisingly, restoration of CCR7 expression on T cells was not sufficient to restore the homeostasis of these cells in peripheral lymph nodes[16]. By contrast, when CCR7 expression was restored on both T cells and CD11c$^+$ DCs, the lymph nodes recovered normal numbers of T cells and B cells[16]. Together, these data indicated that the expression of CCR7 by CD11c$^+$ DCs is essential for lymphocyte homing to lymph nodes under steady-state conditions.

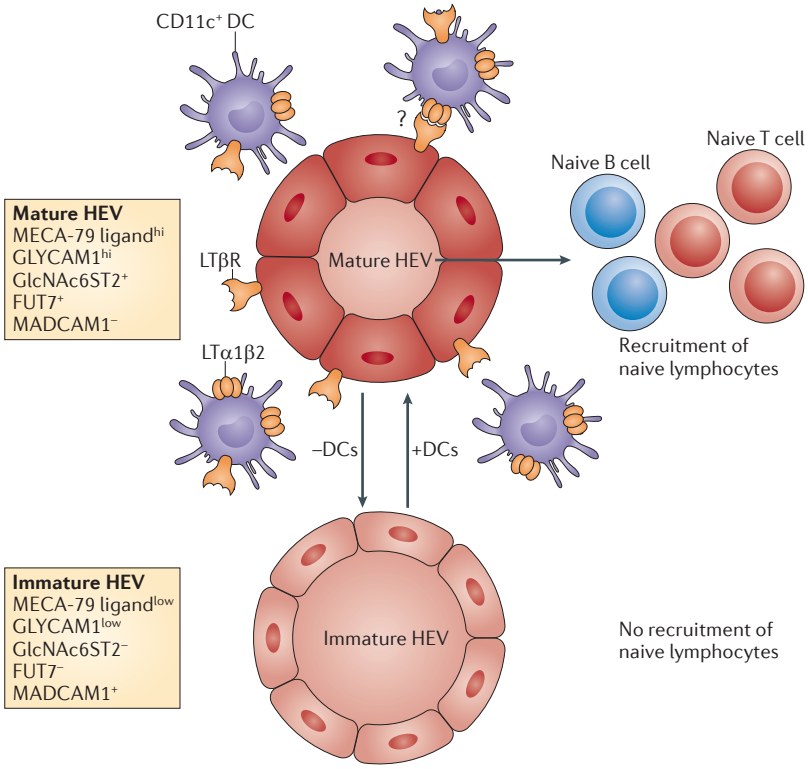



Figure 3 | **DCs regulate HEV phenotype and function.** Dendritic cells (DCs) are strategically positioned close to the walls of high endothelial venules (HEVs) *in vivo*[80,81] and are required for the maintenance of HEV characteristics[15]. In the absence of CD11c$^+$ DCs, the mature adult HEV phenotype in peripheral lymph nodes (MECA-79 ligand$^{hi}$ MADCAM1$^-$) reverts to an immature neonatal phenotype (MECA-79 ligand$^{low}$ MADCAM1$^+$) and the homing of naive lymphocytes to lymph nodes is inhibited[15]. CD11c$^+$ DCs produce lymphotoxin ligands (LTα1β2) for the lymphotoxin-β receptor (LTβR), and DC-derived lymphotoxin is important for HEV-mediated lymphocyte homing[15]. CC-chemokine receptor 7 (CCR7) expression by CD11c$^+$ DCs is also crucial for lymphocyte homing to lymph nodes during homeostasis[16]. Other signalling pathways are likely to be involved in the crosstalk between DCs and HEVs, but these pathways remain to be defined. Similarly, it remains to be determined whether other cell types, in addition to DCs, have a role in the maintenance of the mature HEV phenotype. FUT7, fucosyltransferase 7; GlcNAc6ST2, *N*-acetylglucosamine 6-*O*-sulphotransferase 2; GLYCAM1, glycosylation-dependent cell adhesion molecule 1; MADCAM1, mucosal addressin cell adhesion molecule 1.

***DCs are essential for the maintenance of the HEV phenotype.*** A striking downregulation of HEV-specific genes (*Glycam1, Fut7* and *Chst4*) and an HEV-specific marker (the addressin recognized by MECA-79) was observed in lymph nodes after 8 days of DC depletion[15]. By contrast, there was no effect on the expression of endothelial cell markers, such as CD31 and vascular endothelial cadherin. The possibility that DCs may regulate the HEV phenotype directly was investigated using a co-culture model. Strikingly, when HEV endothelial cells expressing the MECA-79 ligand were co-cultured with CD11c$^+$ DCs isolated from wild-type mice, HEV-specific (*Glycam1*) gene expression was maintained *ex vivo* at levels similar to those found *in vivo* in HEVs from peripheral lymph nodes[15]. Previous studies suggested that continuous stimulation of lymphotoxin-β receptor (LTβR) on HEV endothelial cells by LTα1β2-expressing cells may be required for the induction and maintenance of HEVs in lymphoid tissues[24,67–69]. Interestingly, interruption of LTβR signalling in the cultures containing HEVs and DCs totally abrogated the effect of DCs on HEV-specific gene expression[15]. CD11c$^+$ DCs isolated from peripheral lymph nodes expressed transcripts encoding the LTβR ligands, LTα, LTβ and LIGHT, and DC-derived lymphotoxin was shown to be important for HEV-mediated lymphocyte homing to lymph nodes *in vivo*[15]. Together, these findings indicated that CD11c$^+$ DCs and DC-derived lymphotoxin are involved in the regulation of HEV phenotype and function.

Studies in CCR7-deficient mice with restored expression of CCR7 on T cells revealed that DCs may also contribute to HEV growth[16]. Lymph nodes from these mice had reduced numbers of HEVs, but these numbers could be increased by injection of semi-mature wild-type DCs. Under steady-state conditions, CD11c$^+$ DCs from lymph nodes were shown to express vascular endothelial growth factor (VEGF)[16], which can induce the proliferation of HEV endothelial cells[70]. During homeostasis, CD11c$^+$ DCs may therefore regulate both the phenotype and growth of HEVs.

## Entry via afferent lymphatics

Some immune cells enter lymph nodes via afferent lymphatics rather than through HEVs. The entry and migratory routes of DCs in lymphatic vessels have been well described[19,20,71], but recent evidence indicates that naive T cells, in addition to effector and/or memory T cells, can also enter lymph nodes through afferent lymphatics[17,20] (FIG. 4).

***Migration of DCs and T cells in lymphatics.*** Skin-resident DCs have been shown to enter lymphatics by squeezing through the endothelium of terminal lymphatics in the skin[19,20,71,72]. Under both steady-state and

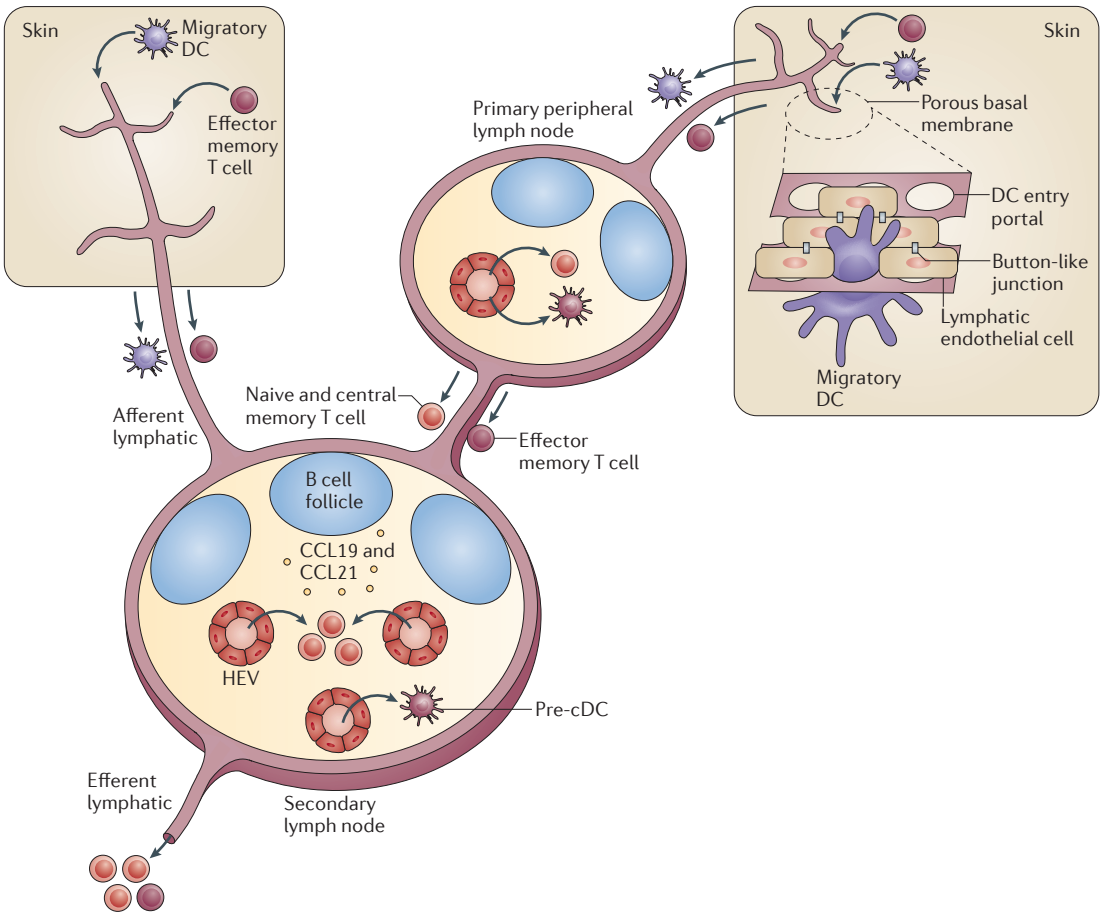



Figure 4 | **Trafficking of DCs and T cells through a chain of lymph nodes.** In the skin, lymphatic vessels originate as wide, blind-ended capillaries that collect interstitial fluid and that possess a thin wall with a discontinuous collagen IV-containing basement membrane[71,72]. These terminal lymphatics are lined by oak leaf-shaped lymphatic endothelial cells that are adjoined to each other by discrete cell junctions (termed buttons), leaving loose flaps in between[100]. Dendritic cells (DCs) have been shown to enter terminal lymphatics by squeezing through and transiently dilating the pores in the basement membrane to subsequently displace the endothelial flap valves towards the lumen of the lymphatic vessel[72]. Similarly to DCs, effector memory T cells also enter terminal lymphatics, circulate in lymph, and migrate to skin-draining lymph nodes through afferent lymphatics[9,20]. However, lymph nodes are often organized in chains, and naive T cells leaving a peripheral primary lymph node (for instance a popliteal lymph node) via efferent lymph, after their entry through high endothelial venules (HEVs), can also enter downstream secondary lymph nodes through afferent lymphatics[17,20]. Naive T cells may thus migrate to secondary lymph nodes via two different routes: HEVs (the 'blood' route) and afferent lymphatics (the 'lymph' route). CCL, CC-chemokine ligand; pre-cDC, precursor conventional DC.

inflammatory conditions, the entry of DCs into terminal lymphatics depends on CCR7 (REFS 62,73). As terminal lymphatics express the CCR7 ligand CCL21, it has been suggested that DCs sense local CCL21 gradients that allow their directional migration towards, and eventually into, lymphatic vessels[74]. Following lymphatic entry, DCs crawl on endothelial cells in the direction of lymph flow, but they get passively transported with the lymph once they reach collecting lymphatics[74].

Analysing T cells collected from afferent lymph vessels draining towards popliteal lymph nodes in sheep, Mackay and co-workers found that these cells had a memory phenotype, whereas most of the T cells leaving these lymph nodes via efferent lymphatics were of naive phenotype[75]. These observations, together with the finding that naive T cells home to lymph nodes via HEVs, led to a model that effector and/or memory T cells, but not naive T cells, home to lymph nodes via afferent lymphatics. However, this model did not take into account the fact that lymph nodes are frequently arranged in chains and that cells leaving a distal lymph node through efferent lymphatics will arrive via afferent lymph in more proximally positioned lymph nodes (FIG. 4). Indeed, in mice it was recently shown that, following microinjection of naive T cells into the afferent lymphatic vessel draining towards a popliteal lymph node, the cells not only home to the T cell zone of the popliteal lymph node, but also to the T cell areas of lymph nodes that are located further downstream, such as the medial iliac lymph node[17]. Evidence that naive T cells can migrate from one lymph node to another via lymphatics has also been provided by Tomura *et al.*[14].

Therefore, although most naive T cells enter lymph nodes via HEVs, some naive T cells leaving a primary lymph node through efferent lymphatics may circulate in lymph, together with effector memory T cells, and enter secondary lymph nodes via afferent lymphatics.

***Delivery of DCs and T cells into lymph nodes via afferent lymph.*** Intralymphatic cell delivery techniques in combination with two-photon intravital microscopy have allowed a detailed analysis of how DCs and T cells arriving via the afferent lymph actually enter lymph nodes[17]. As previously suggested[19,76], both bone marrow- and skin-derived DCs were shown to directly enter the lymph node parenchyma through the floor of the afferent side of the SCS, primarily via the interfollicular regions. DC migration was characterized by a pronounced CCR7-mediated directional movement towards the T cell-rich paracortex, which was completely populated by immigrating DCs within 24 hrs of cell transfer[17]. In addition to CCR7, CCR8 and its ligand CCL1 have been suggested to contribute to the homing of DCs into the lymph node parenchyma[77], whereas integrins seem to be dispensable for this process[78]. Indeed, DCs genetically deficient in β1, β2 and β7 integrins migrated to lymph nodes and accumulated in the T cell areas in a manner indistinguishable from that of wild-type DCs[78].

The transmigration of DCs through the floor of the SCS induces local alterations in lymphatic endothelial cells and $CD169^+$ sinus-lining macrophages. Surprisingly, these changes also allow the subsequent homing of naive T cells, which instantly start to scan the 'gate-opening' DCs for presented cognate antigen[17]. However, when injected alone, intralymphatically delivered T cells could not enter the lymph node parenchyma via the floor of the SCS and were passively transported to the outer areas of the medullary sinuses. From there, T cells actively left the sinuses to enter the medullary cords that are located within the sinus system. Although T cell entry into the medullary cords is independent of CCR7, the subsequent translocation of the cells into the deep lymph node paracortex relies on the expression of this chemokine receptor, which probably senses haptotactic gradients of CCR7 ligands deposited on stromal cells and potentially also on DCs[16]. Consequently, lymph-derived CCR7-deficient T cells are present in high numbers in the medullary cords but are largely excluded from the T cell zone[17]. This observation, in addition to the defect in the homing of CCR7-deficient T cells via HEVs, helps to explain why the lymph node paracortex in CCR7-deficient mice is basically devoid of T cells[62].

Together, these studies have revealed that afferent lymph-derived DCs and T cells use different routes to enter lymph nodes. DCs transmigrate through the floor of the SCS, whereas naive T cells enter through medullary sinuses.

Haptotactic gradients
Gradients of surface-bound ligands that promote directional, receptor-dependent migration of cells towards areas of higher concentrations.

## Intranodal migration and positioning

After entering the lymph node through HEVs or lymphatics, lymphocytes and DCs traffic to their respective subcompartments: the paracortical T cell areas for T cells and DCs, and the follicles for B cells (FIG. 5). Stromal cell networks and stromal cell-derived lymphoid chemokines (namely CCL21, CCL19 and CXCL13) have key roles in guiding immune cells to these lymph node subcompartments[10–12,79].

***T cells.*** After leaving HEVs, T cells enter perivascular channels on the abluminal side of HEVs[12]. HEVs are surrounded by a sheath of FRCs, and two-photon intravital microscopy analyses have shown that lymphocytes are transiently trapped in the perivascular space[56]. Lymphocyte retention or perivascular trapping around HEVs may increase the likelihood of T cell–DC encounters, as DCs have been shown to accumulate around HEVs[80,81]. After this transient retention around HEVs, T cells rapidly migrate along the FRC network[12]. T cell zone FRCs (also known as TRCs[82]) express α-smooth muscle actin (αSMA), ER-TR7 antigen, podoplanin, the T cell homeostatic cytokine IL-7 and the chemokines CCL21 and CCL19, which regulate the intranodal motility and migration velocity of T cells[11,82–84]. Two-photon intravital microscopy studies have revealed that naive T cells crawl along the surface of the FRC network in an apparently random pattern of motion[12,85,86] at an average two-dimensional velocity of ~10–12 μm per min[85,86] (three-dimensional velocity ~15 μm per min[83]). CCL21 is abundantly expressed by T cell zone FRCs[82], and CCR7-deficient T cells exhibit a reduced motility in lymph nodes[83,84]. CCR7 and FRC-derived CCL21 and CCL19 are also crucial for the retention and accumulation of T cells in the paracortical T cell areas of lymph nodes, as CCR7-deficient T cells have been shown to exit lymph nodes more rapidly than wild-type T cells[28]. CCR7 is thus a master regulator of T cell trafficking in lymph nodes, as it promotes T cell entry through HEVs, as well as the motility, compartmentalization and retention of T cells within lymph nodes.

***B cells.*** Rather than rapidly accessing the follicles, B cells remain confined close to HEVs for 3–4 hours after lymph node entry[57] and survey locally concentrated DCs[87]. Interestingly, it has been demonstrated that B cells can be activated by DC-associated antigens soon after their exit from HEVs and before their migration into the B cell follicles[87]. The signals that retain B cells in the vicinity of HEVs have not yet been characterized, but CCR7-deficient B cells have a motility pattern around HEVs similar to that of wild-type B cells, suggesting that CCR7 ligands are not involved[57].

After leaving the HEV area, B cells crawl on the FRC network in the T cell areas before entering the B cell follicles[12]. The homing of naive B cells to the follicles during homeostasis depends on the expression of CXCR5 by B cells and CXCL13 by FDCs[10,88]. FDCs are a specialized subset of lymph node stromal cells that are clustered in the centres of the follicles, and they capture antigens and present them to B cells in an unprocessed form (particularly in the form of immune complexes)[10,11]. Follicular B cells are highly motile and crawl on the FDC network in a 'random walk' at a speed of ~6 μm per min[10,11], surveying FDCs,

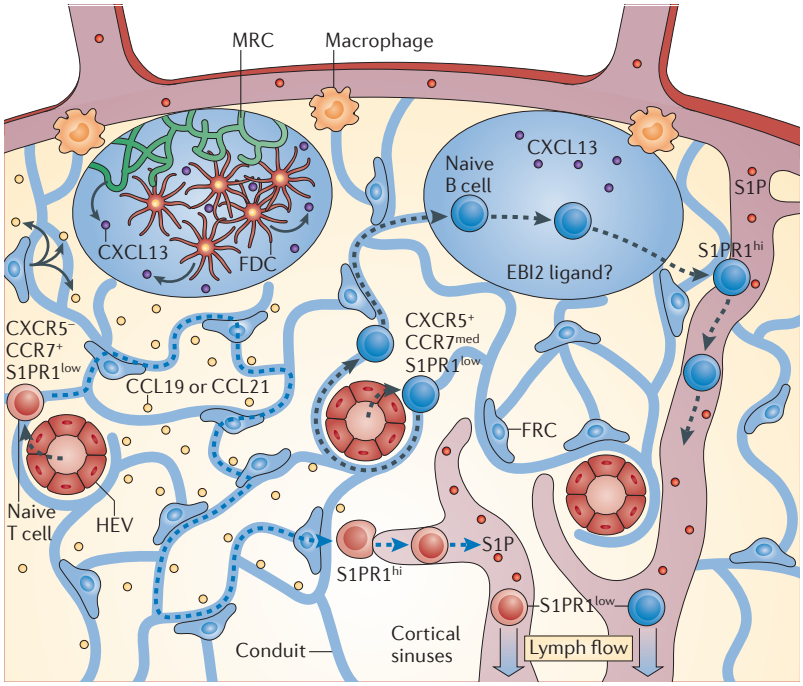



Figure 5 | **The journey of lymphocytes in lymph nodes and their exit through cortical sinuses.** The possible paths taken by naive lymphocytes from the high endothelial venules (HEVs) to the cortical lymphatic sinuses are shown. Two-photon intravital microscopy analyses have revealed that naive B cells cross HEVs more slowly than do T cells and that many B cells persist within the perivascular channels for more than 1 hour[57]. However, soon after their entry into lymph nodes, both B and T cells can encounter dendritic cells (DCs) that accumulate around HEVs[80,81,87]. T cells then randomly crawl along fibroblastic reticular cell (FRC) networks, which function as local roads for T cell trafficking within the lymph node paracortex[12]. Sphingosine-1-phosphate (S1P)–S1P receptor type 1 (S1PR1) signalling overrides CC-chemokine receptor 7 (CCR7)-mediated retention in the T cell areas[28] and allows T cells to cross the lymphatic endothelium and enter the lymph node sinus system[26,29]. B cells also crawl on FRC networks[12] to reach the B cell follicles, using CXC-chemokine ligand 13 (CXCL13)–CXC-chemokine receptor 5 (CXCR5) signalling. During their migration towards the centre of the follicle, B cells upregulate S1PR1 expression[57]. After some time, they desensitize their chemokine receptors (that is, CXCR5 and CCR7) and sense the S1P-mediated egress signal from lymphatics at the edge of the follicles[27,57]. Similarly to T cells[26,29], B cells become rounded after entering the cortical sinuses and flowing in the lymph[27,57]. CCL, CC-chemokine ligand; FDC, follicular dendritic cell; MRC, marginal reticular cell.

sinus-lining macrophages and conduits for antigens[35]. The differential positioning of B cells in the outer and centre follicles has recently been found to be driven by the G protein-coupled receptor EBI2 (also known as GPR183), which directs B cells to perifollicular and interfollicular areas[89,90]. EBI2-mediated B cell positioning has been shown to be important for the early stages of antibody responses[89,90].

B cells may also migrate along stromal networks and conduits formed by marginal reticular cells (MRCs)[35,91], which constitute another subset of lymph node stromal cells. MRCs are found at the edge of the follicles under the SCS, and they express CXCL13, ER-TR7 antigen, podoplanin and RANK ligand[91]. During their journey in the lymph node, naive B cells may thus move along three different stromal cell networks: FRCs in the paracortex; FDCs in the follicles; and MRCs under the SCS[11].

***DCs.*** As described above, CCR7 and its ligand CCL21 provide essential migratory cues to guide lymph-derived DCs through FRC stromal networks into the lymph node paracortex, a process that seems to be independent of integrins[78]. Whereas DCs that have recently left the lymph show highly motile and directional migration, lymph node-resident DCs are largely sessile but continuously protrude and retract their dendrites, a process known as probing (reviewed in REF. 19). Resident DCs form a stable organized network in the T cell areas, contacting FRCs and lymph node conduits that transport antigens from lymph[12,34]. Over time, lymph-derived migratory DCs and pre-cDCs that have entered lymph nodes through HEVs[61] join the network of resident DCs, but the mechanisms and/or factors involved are currently unknown. As CCR7 is indispensable for directed DC migration, it seems likely that internalization and/or desensitization of this chemokine receptor allows DCs to stably integrate into the network of resident DCs.

## Egress through efferent lymphatics

After exploring a given lymph node for several hours, naive lymphocytes that do not encounter their target antigen leave the lymph node through efferent lymphatics (FIG. 5). Important advances have been made in the past few years regarding the mechanisms that regulate the egress of lymphocytes from lymph nodes and their continuous shuttling between lymph, blood and lymphoid tissue[13].

***S1P and S1PR1 control lymphocyte exit.*** S1P and its G protein-coupled receptor S1PR1 have been shown to be required for the egress of both B and T cells[10,13]. S1P was first implicated in the exit of lymphocytes from lymph nodes during homeostasis, when it was discovered that the immunosuppressive drug FTY720 (also known as fingolimod), a potent agonist of S1P receptors, induces the sequestration of lymphocytes in lymph nodes by inhibiting their egress into lymph[92]. Further analysis revealed that FTY720 acts by down-regulating S1PR1 expression on lymphocytes and that S1PR1 expression by B and T cells is required for their egress from lymph nodes[93]. S1P is generated *in vivo* by sphingosine kinases, and genetic inactivation of these kinases in lymphatic endothelial cells has been shown to markedly reduce S1P levels in lymph and to impair lymphocyte egress from lymph nodes[94]. S1P gradients are important for egress, as inhibition of S1P lyase (which degrades S1P and maintains low levels in lymph nodes) has been shown to result in lymphocyte sequestration in lymph nodes[95].

Recent two-photon intravital microscopy analyses indicate that B and T cells exit lymph nodes through cortical sinuses in a multistep process[26,27]. Randomly migrating T cells were shown to contact LYVE1$^+$ cortical sinuses in the interfollicular areas, with some cells probing the sinus surface for more than 10 minutes[26]. About one-third of wild-type but not S1PR1-deficient T cells then crossed the lymphatic endothelium and entered the sinuses at multiple locations[26]. Similarly, B cells were found to egress from the lymph node by

Pertussis toxin
A toxin that blocks Gαi-coupled receptor signalling (including chemokine receptor signalling) by catalysing ADP ribosylation of the Gαi subunit.

migrating from the B cell follicle into adjacent cortical sinuses, and experiments with FTY720 showed that B cells fail to enter into the sinus lumen when S1PR1 is downregulated[27].

S1PR1 signalling appears to act principally by overcoming retention mediated by Gαi-coupled receptors (that is, CCR7 and CXCR5)[27,28,94]. Indeed, experiments using S1PR1-deficient T cells[28] and mice in which the lymphatic endothelium is S1P-deficient[94] have conclusively demonstrated that the treatment of T cells with pertussis toxin (which inactivates Gαi) restores their egress through cortical sinuses in the absence of S1P–S1PR1 signalling.

After S1PR1-dependent entry into cortical sinus central branches, B and T cells become rounded and flow unidirectionally into medullary sinuses and subcapsular regions near the efferent lymphatic vessel[26,27,29,57]. Although cortical sinuses appear to be the major sites of lymphocyte egress from lymph nodes, medullary sinuses and the SCS may also serve as exit sites[26–28].

***Cyclical modulation of S1PR1 regulates trafficking.*** High levels of S1P, such as those found in the blood and lymph, have been shown to induce the rapid downregulation of S1PR1 expression on lymphocytes through receptor internalization[57,96]. A direct analysis of S1PR1 expression on recirculating lymphocytes revealed that S1PR1 is downregulated in the blood, upregulated in the lymph node parenchyma (which contains low levels of extracellular S1P) and downregulated again in the lymph[56,93]. It was thus proposed that cyclical ligand-induced modulation of S1PR1 expression is required for lymphocyte recirculation. After lymph node entry, B cells progressively reacquire S1PR1 as they move away from HEVs[57]. This may explain why B cells located near HEVs do not immediately exit lymph nodes, despite the fact that HEVs are in close proximity to cortical sinuses (FIG. 5). Some lymphocytes, however, can rapidly migrate into cortical sinuses soon (~30 minutes) after HEV entry[29]. The juxtaposition of entry and exit sites for lymphocytes may have an important role in the regulation of entry and exit fluxes.

Recently, G protein-coupled receptor kinase 2 (GRK2) has been shown to contribute to the downregulation of S1PR1 on blood lymphocytes[18]. GRK2-deficient B and T cells in the blood had high levels of S1PR1 on their cell surface (similar to the levels observed on lymphocytes present in lymph nodes of wild-type mice) and had a defect in homing to lymph nodes. No defect in the lymph node entry of GRK2-deficient lymphocytes was observed in mice lacking blood S1P, indicating that GRK2-mediated downregulation of S1PR1 on blood lymphocytes is required to overcome their attraction to S1P in the blood[18]. Intravital microscopy analyses revealed a substantial reduction in the number of GRK2-deficient T cells undergoing the rolling to sticking transition inside lymph node HEVs (as compared with the number of wild-type T cells that undergo this transition), indicating that high levels of S1P–S1PR1 signalling inhibit CCR7-mediated T cell arrest in HEVs[18]. Together, these experiments supported the earlier observation that the homing of lymphocytes to lymph nodes via HEVs is increased in FTY720-treated mice[97], and they convincingly demonstrated that GRK2-mediated downregulation of S1PR1 is essential for the HEV-mediated entry of lymphocytes into lymph nodes[18].

Interestingly, recent data indicate that the egress rates of lymphocytes from lymph nodes critically influence entry through HEVs[58]. The sequestration of lymphocytes in lymph nodes, through specific inactivation of S1PR1, was shown to inhibit the influx of blood-circulating lymphocytes and to induce their accumulation in 'HEV pockets'[58]. HEVs were thus proposed to function as traffic control checkpoints for the maintenance of lymph node cellularity in the steady state[58].

Together, these studies have greatly increased our understanding of the mechanisms that regulate the egress of lymphocytes from lymph nodes. It is now well established that S1P–S1PR1 signalling allows lymphocytes to exit through cortical sinuses, by overcoming retention signals from Gαi-coupled receptors, and that cyclical regulation of S1PR1 expression at the cell surface is crucial for the recirculation of naive lymphocytes through lymph nodes.

## Concluding remarks

The capacity of lymph nodes to recruit naive lymphocytes and to facilitate their encounter with antigens and antigen-presenting cells (such as DCs) is crucial for immune surveillance during homeostasis. In this article, we have reviewed the important advances that have been made during the past decade in our understanding of the mechanisms that regulate the entry of lymphocytes and DCs into lymph nodes through HEVs and lymphatics, intranodal trafficking along stromal cell networks, and exit through lymphatic sinuses. Despite this considerable progress, many fundamental questions remain to be answered.

### Box 2 | **Trafficking of immune cells in inflamed lymph nodes**

Many changes occur in lymph nodes during inflammation. Soon after the initial inflammatory insult, lymph nodes undergo substantial remodelling, which includes increases in size and cellularity, and the expansion of their primary feed arterioles and high endothelial venule (HEV) network[5,24,70,79]. The flow of afferent lymph also increases, and large numbers of mature CC-chemokine receptor 7 (CCR7)-expressing dendritic cells (DCs) are transported to the inflamed lymph node[5,79]. Pro-inflammatory cytokines upregulate the expression of cell adhesion molecules (such as P-selectin and E-selectin) on HEVs, whereas lymph-borne pro-inflammatory chemokines, such as CC-chemokine ligand 2 (CCL2), are transported through lymph node conduits to the HEV lumen (and CXC-chemokine ligand 9 (CXCL9) is also deposited in the lumen)[5,7]. As a consequence, naive B and T cells, activated (effector) T cells, natural killer cells and monocytes migrate through HEVs into inflamed lymph nodes, and thus the input of immune cells into a lymph node draining an inflamed tissue is strongly increased. Concurrently, lymphocyte exit from the inflamed lymph node is blocked[13,98]. This egress shutdown is due to the downregulation of sphingosine-1-phosphate receptor type 1 (S1PR1) expression on lymphocytes[98], and potentially also to S1PR1-induced alterations on lymphatic endothelium[99]. Together, the increase in cell entry and the blockade of cell egress contribute to the rapid accumulation of recirculating cells in the inflamed lymph node. This ensures that a large repertoire of antigen-specific lymphocytes will have a chance to encounter their cognate antigen. In addition, lymph-borne antigens draining from the inflamed tissues are rapidly transported through lymph node conduits[34] to the T cell areas, where they can be presented by resident DCs to B cells and T cells entering the inflamed lymph node through HEVs[80,81]. Finally, the intranodal migration of lymphocytes is profoundly modified in antigen-stimulated lymph nodes[11,79,85]. For instance, the upregulation of CCR7 and EBI2 on activated B cells regulates their movement from the follicle centre along the B–T boundary zone to interfollicular and outer follicular regions[89,90]. This transient repositioning of B cells is crucial for T cell-dependent B cell responses.

For instance, what are the transcription factors and signalling pathways that regulate the expression of HEV-specific genes? Although signalling through LTβR appears to provide a crucial signal for this regulation, other pathways are likely to be involved. Can DCs provide these additional signals? Another important question is the identity of the CD11c$^+$ DC population that is important for the maintenance of HEVs. CCR7$^+$CD11c$^+$ DCs that migrate from lymph into lymph nodes under steady-state conditions may be involved, but a role for blood-derived pre-cDCs in combination with signals such as soluble antigens or pathogen-associated molecular patterns from afferent lymph cannot be excluded.

Another unresolved issue is the mode of lymphocyte migration across HEVs. The relative importance of lymphocyte migration between adjacent endothelial cells and transcellular migration within endothelial cells will need to be carefully studied using advanced two-photon intravital microscopy imaging technologies. Similarly, the precise details of lymphocyte transmigration through lymphatic endothelium during lymph node homing and egress remain to be characterized. The other pathways and molecular factors that may regulate egress, in addition to S1P–S1PR1 signalling, will also need to be defined.

In addition to these issues, there are several other unanswered questions regarding B cell migration. Which signals retain B cells close to HEVs after entry? Can naive B cells enter lymph nodes through afferent lymphatics, in a similar manner to T cells? Which lymph node cells express the ligand for the B cell positioning receptor EBI2? Does EBI2 also have a role on T cells or other immune cells in the lymph node?

In this Review, we concentrated on the mechanisms that regulate immune cell trafficking in lymph nodes during homeostasis. It is important to highlight that many changes occur after antigen stimulation (BOX 2). Similarly, the trafficking of lymphocytes and DCs in other secondary lymphoid organs (such as Peyer's patches and the spleen) and non-lymphoid tissues is regulated by mechanisms distinct from those used in resting lymph nodes. For instance, HEV blood vessels have a different phenotype in Peyer's patches[7], and CD11c$^+$ DCs are not required for their maintenance[15]. However, blood vessels with phenotypical characteristics of lymph node HEVs develop in chronically inflamed tissues and tumour tissues (BOX 1), and a better understanding of the role of HEVs (and lymphatics) in the trafficking of immune cells in lymph nodes may thus have important implications for human chronic inflammatory diseases and cancer.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## Competing interests statement
The authors declare no competing financial interests.

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