Review

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Stromal infrastructure of the lymph node and coordination of immunity

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The initiation of adaptive immune responses depends upon the careful maneuvering of lymphocytes and antigen into and within strategically placed lymph nodes (LNs). Non-hematopoietic stromal cells form the cellular infrastructure that directs this process. Once regarded as merely structural features of lymphoid tissues, these cells are now appreciated as essential regulators of immune cell trafficking, fluid flow, and LN homeostasis. Recent advances in the identification and *in vivo* targeting of specific stromal populations have resulted in striking new insights to the function of stromal cells and reveal a level of complexity previously unrealized. We discuss here recent discoveries that highlight the pivotal role that stromal cells play in orchestrating immune cell homeostasis and adaptive immunity.

Stromal contributions to the initiation of adaptive immunity

The enormous repertoire of antigen receptors in the immune system provides a level of versatility to match the vast array of potential antigens one may encounter in a lifetime. However, this versatility comes at the cost of pure numbers for any antigen-specific cell. Each distinct naive T cell is exceedingly rare, and immunity depends upon the encounter of these rare lymphocytes with an antigen-bearing dendritic cell (DC). If left to chance, such an encounter would never occur, but adaptive responses are initiated with remarkable speed and reliability. This is made possible by strategic placement of LNs throughout the body. Non-hematopoietic stromal cells direct the formation of these structures (Box 1), and the mature LN is populated by a variety of endothelial and non-endothelial stromal cell subsets which provide the crucial infrastructure necessary for controlled movement of leukocytes into and within the LN (Table 1). However, in comparison to their hematopoietic counterparts, the role of these cells in supporting immune responses has, until recently, been largely overlooked.

The basic concept of stromal cell mediated recruitment, compartmentalization, and homeostatic maintenance of immune cells has long been appreciated. Pioneering research in the late 1990s established crucial roles for stromal cell produced chemokines CCL19, CCL21, CXCL12, and CXCL13 in the attraction, retention, and organization of circulating lymphocytes within lymphoid tissues [1–4]. The expression of CCL19 and CCL21 in lymphatic vessels has likewise been linked to the migration of antigen-bearing DCs [5]. However, these findings alone do not sufficiently address the exquisite spatial and temporal control of leukocyte movement and antigen transport observed in the LN. Instead, there is a complexity to the ordering of stromal cell architecture, and the shaping of the directional cues they produce, that has remained largely unaddressed. Moreover, active immune responses are accompanied by large-scale changes to LN architecture and alterations to leukocyte migration patterns, suggesting that stromal cells are not static structures, but must be dynamically regulated.

More recent studies have sought to understand the finer intricacies of how the LN microenvironment directs cellular movement and homeostasis. These studies have revealed a striking level of complexity and heterogeneity in stromal cell populations not previously appreciated. In this review we discuss recent advances in our understanding of how the LN stroma coordinates the movement and positioning of immune cells. We highlight studies that redefine the functional identity of previously described stromal cell subsets, and discuss the emergence of newly defined populations. We also discuss the evolving role of the LN stroma in directing active immune responses and the mechanisms that drive these functions.

Lymphocyte recirculation and LN surveillance

Rare antigen-specific lymphocytes continuously survey lymphatic tissues, entering through specialized blood vessels termed high endothelial venules (HEVs, Table 1), exiting through the cortical and medullary sinus to the efferent lymph, and returning to circulation via the thoracic duct. This entire process occurs within a matter of several hours, and thus millions of lymphocytes enter and exit each peripheral LN on a daily basis.

The timely initiation of adaptive responses is predicated on the efficiency of lymphocyte surveillance of lymphatic tissues and recirculation; hence, significant efforts have been made to understand the mechanisms that drive this process. The stepwise interactions between lymphocytes and HEVs necessary for lymphocyte ingress are largely

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Box 1. Lymph node development

The development of LNs begins during embryogenesis and is driven by the interactions of hematopoietic lymphoid tissue inducer (LTi) cells with non-hematopoietic mesenchymal cells. This process is initiated upon expression of CXCL13 by mesenchymal cells [93]. Interestingly, these poorly defined mesenchymal cells have recently identified as adipocyte precursors that are reprogrammed to give rise to LN stromal cells [94]. The stimulus leading to their initial expression of CXCL13 is not yet entirely clear, although this is thought to depend on the production of retinoic acid by nearby nerve fibers [93]. Upregulation of CXCL13 in turn attracts CXCR5-expressing LTi cells. LTi cells express both RANK and RANK-L, and clustering of these cells likely allows homotypic interaction through this signaling axis [95]. Signaling via RANK:RANK-L on LTi cells leads to their upregulation of LTα1β2, which then triggers differentiation of mesenchymal cells to lymphoid tissue organizer (LTo) cells (also known as stromal organizer cells) [96–98]. LTo cells then contribute to further recruitment of LTi cells, thus initiating a positive feedback loop that fuels the continued recruitment and development of LN tissue progenitors. LTo cells additionally begin to attract and retain lymphocytes through the production of CCL19 and CCL21 and the expression of adhesion molecules ICAM-1, VCAM-1, and MAdCAM [99]. LTo cells eventually give rise to the various major LN stromal cell subsets that populate the mature LN, including FDCs, FRCs, and MRCs. A more thorough examination of the mechanisms driving early LN development and the differentiation of various LN stromal cell subsets can be found in several topical reviews [99–101].

mediated by the coordinated action of a distinct set of selectins, integrins, and chemokines [6,7], while opposing molecular cues within lymphatic tissues govern lymphocyte retention and egress [8].

Lymphocyte recruitment to the LN

Despite continuous population turnover, resting LN and splenic cellularity remains strikingly constant under resting conditions, and thus the drivers of lymphocyte ingress and egress must somehow equalize. Exactly how this occurs has largely been unclear. Evidence from Mionnet *et al*. suggests that HEV endothelial cells (ECs) help to maintain normal population homeostasis through the formation of temporary holding areas for incoming lymphocytes [9]. Close inspection of HEV EC morphology revealed that the distinctive cuboidal, or 'high' morphology of HEV ECs, is actually a result of numerous lymphocytes nested within pockets formed on the abluminal side of the cell (Figure 1D). These pockets allow migrating lymphocytes to exit the flow of circulation before being granted access to the LN. The subsequent transition from HEV pockets to LN parenchyma depends upon physical constraints – when space is made available through cell egress in the LN sinus, new lymphocytes are permitted entrance across the HEV basal lamina. Hence, the rate of ingress is matched to the rate of egress, and the proper resting cellularity of lymphoid organs is maintained.

Whether this transition occurs passively, with lymphocytes stochastically moving to fill empty space, or is actively controlled by HEV ECs in response to environmental cues remains unclear. However, there are other notable requirements for the extravasation of circulating lymphocytes across the HEV basal lamina into the lymphoid organ parenchyma. Movement of lymphocytes across

Table 1. Previously described stromal cell subsets of the LN.

Stromal cell type	Molecular Identifiers	Description
Lymphoid Tissue organizer (LTo)	PDPN$^+$, CD31$^-$, MAdCAM$^+$, RANKL$^+$, ER-TR7$^+$	LTo cells differentiate from mesenchymal cells upon interaction with LTαβ-expressing lymphoid tissue inducer cells during lymphoid tissue organogenesis. These cells recruit and retain hematopoietic cells to the LN anlagen and are thought to give rise to several major stromal cell types including FRCs and MRCs.
Follicular dendritic cell (FDC)	PDPN$^+$, CD31$^-$, ER-TR7$^-$, CD35$^+$	FDCs are found within the B cell areas of the LN cortex and play a crucial role in organizing the B cell follicle through expression of CXCL13. FDCs are a major source of B cell survival factors including BAFF and APRIL. FDCs efficiently acquire and retain antigen and are crucial for the formation of germinal centers.
Fibroblastic reticular cell (FRC)	PDPN$^+$, CD31$^-$, ER-TR7$^+$	FRCs densely populate the T cell areas of the LN. These cells produce and then ensheath extracellular matrix, forming a fiberoptic-like reticular structure. A large, interconnected network of these reticular structures permeate the LN parenchyma and facilitate the flow of lymph and the transport of small molecules. FRCs are a heterogeneous group of cells that contribute distinct functions based on their anatomical location within the LN, including support of HEV integrity, recruitment and survival of T cells within the paracortex, and survival of B cells.
Marginal reticular cell (MRC)	PDPN$^+$, CD31$^-$, MAdCAM$^+$, RANKL$^+$, ER-TR7$^+$	MRCs are a newly identified subset of stromal cells localized to the outer edge of LN follicles beneath the subcapsular sinus. MRCs constitutively produce CXCL13 and maintain many of the characteristics of LTo cells, although the precise immunological function of these cells remains unclear.
Integrin α7 pericytes (IAP)	PDPN$^-$, CD31$^-$, ITGA7$^+$	IAPs are a newly identified subset of stromal cells that encircle blood vessels in the LN cortex and medulla. Little is known about the function of these cells, although transcriptional analysis suggests they are highly contractile and exhibit many characteristics similar to FRCs.
Blood endothelial cell (BEC)	PDPN$^-$, CD31$^+$	BECs line the blood vessels within the LN. While a specialized subset of BECs that line the HEV have been extensively studied owing to their role in lymphocyte ingress, little is known about the immunological functions of non-HEV BECs. Both HEV and non-HEV ECs proliferate extensively during immune responses, likely reflecting the need to increase blood flow to the growing LN.
High endothelial venule EC (HEV EC)	PDPN$^-$, CD31$^+$, MECA-79$^+$, PNAd$^+$	A specialized subset of BECs that line the post-capillary venules within the paracortex of the LN. HEV ECs actively regulate the ingress of circulating lymphocytes to the LN parenchyma.
Lymphatic endothelial Cell (LEC)	PDPN$^+$, CD31$^+$, LYVE-1$^+$	LECs line the afferent and efferent lymphatic vessels, the medullary sinuses, and both the ceiling and floor of the LN subcapsular sinus.

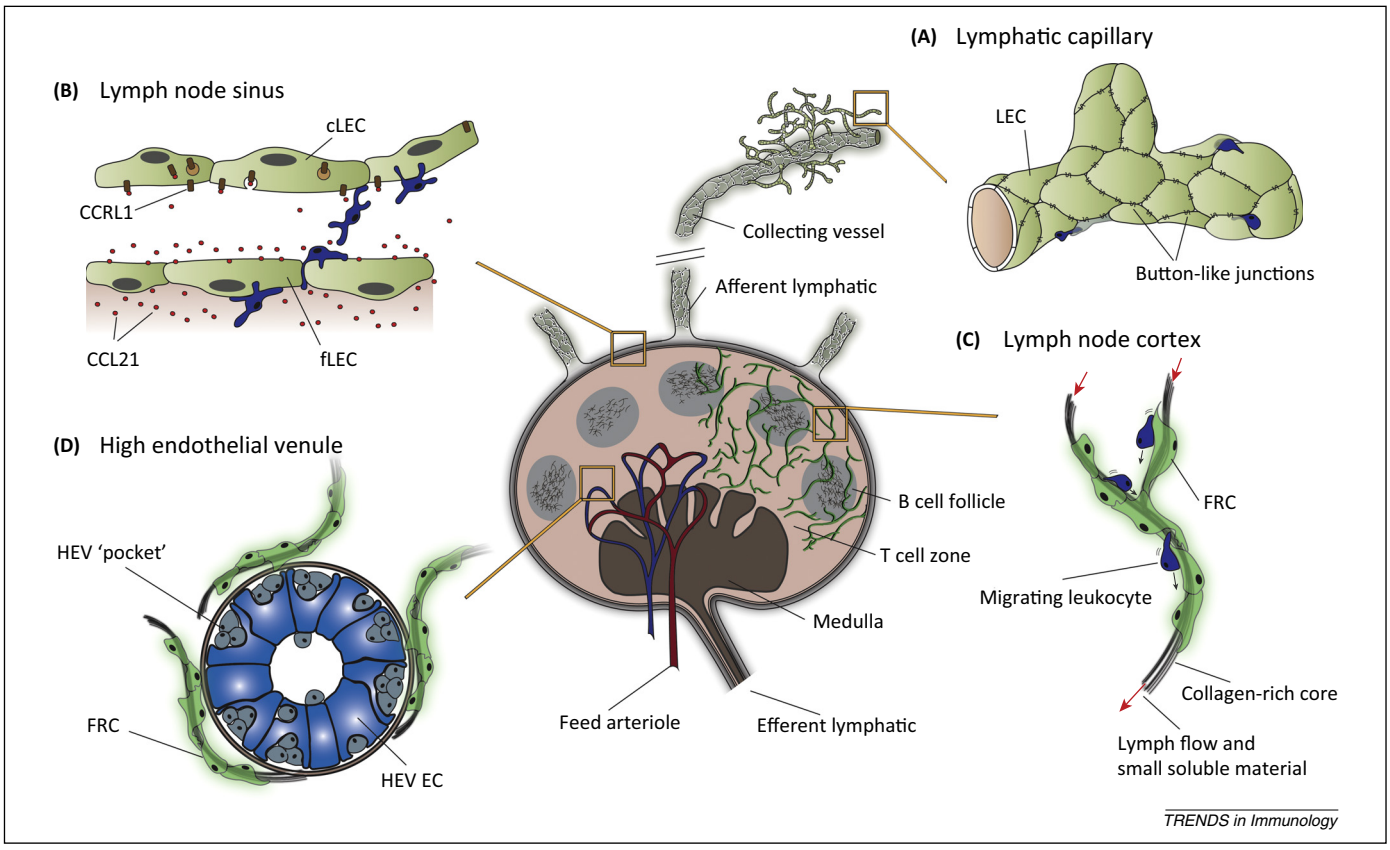

Figure 1. Stromal cells coordinate immune responses by directing immune cells into and within lymph nodes (LNs). **(A)** Antigen-bearing dendritic cells (DCs) and free soluble antigen enter the lymphatics through the initial lymphatic capillaries. These capillaries are formed from specialized lymphatic endothelial cells (LECs) joined with discontinuous, button-like junctions that allow the passage of fluid and cells without disrupting junction integrity. **(B)** As DCs migrate into the LN they must traverse the subcapsular sinus. The atypical chemokine receptor CCRL1, which is expressed exclusively by LECs lining the ceiling of the subcapsular sinus (cLECs), scavenges CCL21 and establishes a gradient that is most concentrated on LECs lining the floor of the subcapsular sinus (fLECs) and the LN cortex. This gradient directs DCs to transition from migrating along cLECS to fLECS, and finally passing into the LN cortex. **(C)** Within the LN, immune cells crawl along the fibroblastic reticular cell (FRC) network which directs the positioning of immune subsets and provides survival signals to both B and T cells. FRCs additionally form a conduit network that facilitates the transport of fluid and fluid-borne signals and antigen through the LN. **(D)** Circulating lymphocytes enter the LN through the high endothelial venule (HEV). Lymphocytes are temporarily retained within pockets formed by specialized blood endothelial cells (BECs) lining the HEV until there is sufficient space to migrate into the LN. HEVs are encircled by FRCs, which direct lymph-borne signals to the HEV as well as help to maintain HEV integrity.

the HEV basal lamina additionally depends on the activity of autotaxin (ATX), an endothelial cell produced enzyme that catalyzes production of the lipid mediator lysophosphatidic acid (LPA) [10,11]. LPA in turn induces morphological changes to HEV ECs that appear necessary for movement of lymphocytes across the HEV. Local inhibition of the ATX/LPA axis results in an excess accumulation of lymphocytes within HEV EC pockets or in the sub-HEV EC space.

Additional immune–stromal interactions may participate in the regulation of HEV function. For instance, DCs are required for the homeostatic maintenance of HEV EC function and lymphocyte homing to LNs in a lymphotoxin-dependent manner [12]. Furthermore, in the absence of continuous lymphotoxin β receptor (LTβr) signaling, blood endothelial cells (BECs, Table 1) in the LN fail to develop properties typical of HEV ECs, including polarized ICAM expression and production of CCL19 and CCL21 [13]. Notably, lymphocytes are no longer found sequestered within HEV pockets, and ingress of circulating lymphocytes largely is impaired.

It should also be emphasized that the HEV functions as a barrier as much as a port of entry. The continuous influx of lymphocytes across the HEV, particularly during initiation of immune responses, likely requires constant rearrangement of the junctions between ECs. Fibroblastic reticular cells (FRCs, Table 1) that encircle the HEV are believed to provide crucial support in this regard through interaction with CLEC-2-expressing platelets [14]. Ligation of CLEC-2 with PDPN, a mucin-type glycoprotein expressed on the surface of FRCs and various other stromal subsets, mediates several immunologically important functions (Box 2). Interaction of platelet-bound CLEC2 with PDPN on FRCs specifically induces the release of S1P by platelets, which in turn elicits an upregulation of VE-cadherin on HEV ECs. In the absence of CLEC-2-PDPN signaling, HEV integrity is compromised and bleeding occurs within the node.

Lymphocyte egress from the LN

Egress of lymphocytes primarily begins at the blunt ended cortical sinuses populating the T zone of the LN, which then flow into the medullary sinus and the efferent lymphatics [15,16]. Transmigration across the sinus endothelium is thought to occur through specific portals, although this remains to be more thoroughly explored. However, the molecular requirements for egress have been thoroughly studied and found to be principally dependent on

Box 2. The CLEC-2:PDPN signaling axis

CLEC-2 (or CLEC1b) is a member of a small subgroup of C-type lectin receptors characterized by signaling through a single ITAM motif and utilization of the adaptor molecule spleen tyrosine kinase (Syk) [102]. Other members of this subgroup, including Dectin-1 and DNGR1, have emerged as key regulators of myeloid cell function [103]. While CLEC-2 expression has likewise been identified in myeloid cell populations [104], its function has been most extensively studied in platelets, wherein CLEC-2 promotes coagulation in response to the snake-venom toxin rhodocytin [102].

More recently, CLEC-2 has been shown to interact with podoplanin (PDPN or gp38), a well conserved mucin-type transmembrane glycoprotein expressed endogenously in various lymphoid and non-lymphoid tissues [105]. Notably, PDPN is highly and constitutively expressed on LECS and FRCs, but is absent from BECs [106]. PDPN contains only a short, nine amino acid cytoplasmic tail, but has nevertheless been shown to functionally interact with the ezrin, radixin, and moesin (ERM) family of proteins and mediate activation of RhoA [107].

As with rhodocytin, engagement of CLEC-2 by PDPN triggers activation of platelets – a process that has been found to be crucially important for the normal development and maintenance of lymphatic and blood vasculature [108]. The loss of either PDPN or CLEC-2 expression results in a blood-in-lymph phenotype. Mechanistically, this has been attributed to loss of platelet-mediated inhibition of LEC proliferation and migration. CLEC-2 signaling triggers the release BMP9 from stored granules in platelets, which in turn blocks lymphatic endothelial tube formation [109]. In addition, intrinsic PDPN signaling in LECs has been shown to directly inhibit tube formation through activation of RhoA [110]. Interestingly, while platelet/megakaryote-specific loss of CLEC-2 results in the development of blood-filled LNs, global deletion of CLEC-2 results in a proliferative defect in LECs that impairs LN development completely, suggesting that other CLEC-2-expressing cell types are involved in this process [111].

The CLEC-2:PDPN signaling axis has emerged as a key facilitator of a variety of immune–stromal interactions. In this review we note several immunologically important outcomes of CLEC-2:PDPN mediated interactions, including maintenance of HEV integrity, migration of DCs, and modulation of FRC contractility during immune responses. Additional known functions of this signaling axis include lung and heart tissue development [112,113], ectopic germinal center formation by PDPN-expressing Th17 cells [114], and tumor progression and metastasis [105].

lymphocyte expression of sphingosine-1-phosphate receptor (S1PR1) and a differential concentration of S1P within lymph and LN tissue [17–19]. S1P is largely absent within the LN parenchyma, while high concentrations within the blood and lymph are established by hematopoietic cells and lymphatic endothelial cells (LECs, Table 1) respectively [20,21]. The S1PR1 receptor is rapidly internalized upon contact with S1P, and thus naive lymphocytes that enter the LN from the blood initially lack the capacity to respond to S1P, preventing immediate egress into the lymph [22]. Thus, while many of the cortical sinuses through which lymphocytes egress are found in close proximity to HEVs, direct migration from HEV into the sinus is not typically observed [15]. Instead, incoming lymphocytes remain within the LN parenchyma until reacquiring expression of S1PR1 and gaining access to the sinus, a process which has been found to occur within 20 minutes to 1 h [15,23]. Interestingly, the average dwell time of most T and B cells has been observed to be much longer, lasting roughly 6–10 h for T cells and 12–24 h for B cells [15,24]. This would suggest that the rate of egress is not limited by acquisition of S1PR1 expression but may instead reflect the competing effects of retention signals. In the absence of CCR7, for instance, egress from the LN occurs more rapidly [21]. Ultimately, future studies will need to more thoroughly dissect the mechanisms governing dwell time and recirculation dynamics of naive lymphocytes.

Recruitment and retention of lymphocytes during immunity

While the lymphocyte population in resting LNs is maintained at a fairly constant level, T and B cell numbers can increase substantially during an active immune response. Within hours of immunogenic challenge, lymphocyte recruitment is enhanced while egress is transiently shut down [25]. This process is largely initiated by innate signals originating from the effected peripheral tissues. Lymph-borne cytokines and chemokines are transported into the LN cortex through a reticular conduit network formed by FRCs that extends from the LN capsule to the HEVs [26–28]. These factors can be transcytosed across the HEV EC and displayed on the lumenal surface of the vessel, enhancing recruitment of naive circulating lymphocytes [26]. Concurrent upregulation of CD69 on lymphocytes in response to inflammatory cues results in decreased responsiveness to S1P and a transient halt to cellular egress from the node [29]. This process effectively primes the LN for the ensuing immune response by increasing the pool of potential antigen-specific naive lymphocytes.

Massive alterations to the stromal network must take place within the first few days of immune activation to facilitate this net cellular influx and support the resulting enlarged population. Early stages of an immune response are associated with a proliferative expansion of the primary feed arteriole, bringing a greater supply of blood circulation to the LN [30]. HEVs also grow in both size and number [31]. Interestingly, although HEVs become more numerous, this occurs in proportion to the overall growth of the LN and thus the density of these vessels remains constant [32].

This initial expansion of the LN vasculature is driven by innate immune factors and may occur in the absence of antigen [30]. LN-resident $CD11c^+$ DCs appear to be crucial for this stage of vascular expansion, but drive the process through mechanisms distinct from the direct triggering of LTβr, which was found to be necessary for homeostatic maintenance of HEV ECs [32]. Instead, DCs are thought to indirectly influence vascular expansion by enhancing the production of VEGF by FRCs [33]. By contrast, subsequent expansion and remodeling of the LN vasculature depends on B and T cells [34]. In the case of LCMV infection, continued LN expansion has been shown to occur independently of VEGF, and instead requires B cell derived LTβ [35]. This proposed biphasic expansion of LN ECs presumably mirrors the transition from innate activation to initiation of adaptive immune responses.

The FRC network likewise undergoes morphological changes and proliferative expansion to accommodate increases in lymphocyte numbers [34,36]. As with expansion of the blood vasculature, FRC growth also appears to take place in two phases. Early expansion of FRCs is dependent on the presence of DCs and trapping of naive lymphocytes [36]. The exact mechanisms by which naive lymphocytes may drive this process are yet unclear.

However, direct triggering of PDPN signaling in FRCs through interaction with DC-expressed CLEC-2 has recently been shown to reduce FRC contractility, allowing these cells to stretch and accommodate increases in LN volume [37,38]. Reduced contractility may additionally trigger proliferative expansion of the FRCs [38]. By contrast, late-phase expansion of the FRC network depends upon interaction of the stromal network with activated lymphocytes through LTαβ and LIGHT [36].

These studies collectively illuminate a generalized expansion of multiple components of the LN stromal cell support network in response to inflammation and infection, and highlight the influential role these cells play in driving the ensuing immune response. In contrast to the events leading up to an immune response, significantly less is known about the resolution of LN swelling and return to homeostasis. Are there mechanisms in place to limit the signals driving stromal cell expansion? Does LN cellularity and architecture revert upon loss of these signals? FRCs are believed to internalize PDPN upon signaling, and this may be one means through which LN swelling is limited or reversed [37,38]. In addition, activated migratory DCs are relatively short-lived, and thus reduced FRC contractility may be tied to the turnover rate of CLEC-2-expressing DCs in the inflamed node [39,40]. Similarly, B and T cell derived signals, which promote stromal cell expansion, may be similarly lost as these cells egress from the LN. However, there may also be alternative signaling pathways that attenuate stromal cell growth or proliferation that arise during the resolution of an immune response. These possibilities will need to be more directly addressed in future studies. It is also unclear how the stromal network copes with conditions of chronic infection or inflammation. Ultimately, a better understanding of these processes may have important clinical relevance.

Antigen transport to LNs

In addition to coordinating lymphocyte recruitment, stromal cells contribute to the initiation of adaptive responses by facilitating the transport of antigen to the LN. Antigen is brought from peripheral tissues to regional LNs through an expansive system of lymph vessels. Collection of lymph begins with blind-ended lymphatic capillaries, which are formed of loosely-connected ECs with discontinuous 'button'-like junctions (Figure 1A) [41]. These specialized junctions allow ECs to form overlapping flaps that ensure unidirectional uptake of fluid from surrounding interstitial space into the vessel lumen. Lymphatic capillaries eventually converge into collecting vessels which, unlike the initial capillaries, contain continuous junctions and are surrounded by smooth-muscle cells [42]. Smooth-muscle cells, along with movement of the surrounding tissue, provide the necessary pumping action to regulate the movement of lymph, and a system of valves separating segments of lymph vessels ensures directional flow to the draining node [43].

DC-mediated antigen transport

Stromal cells function as a crucial highway for tissue-derived migratory DCs. *En route* to the T cell zone of draining LNs, antigen-bearing DCs must crawl along ECs lining afferent lymphatic vessels and FRCs lining the reticular network of the LN. The PDPN:CLEC-2 signaling axis has been identified as a key facilitator of these interactions [44]. Expression of PDPN extends throughout the stromal reticular network of the LN cortex as well as the lymphatic endothelium, and, through its interactions with CLEC-2, functions as a crucial factor driving the migration of DCs from peripheral tissues to the LN. While expression of CLEC-2 on DCs is normally low at resting-state, activation and maturation results in its upregulation. Upon binding PDPN, CLEC-2 signaling induces formation of actin-rich protrusions and facilitates movement of DCs along the stromal cell network. This, in conjunction with chemotactic cues, guides the directional migration of DCs into the lymphatic vasculature as well as their positioning within the LN cortex.

The primary chemotactic cues directing peripheral migratory cells into lymphatics have been well established [45,46]. Both CCR7 ligands CCL19 and CCL21 have been implicated in DC migration to the LN, but contribute to chemotaxis by distinct mechanisms [47]. LECs constitutively produce CCL21, which is then immobilized on extracellular matrix or cell surfaces through interactions with heparin sulfate glycosaminoglycans (GAGs) [48]. Gradients of immobilized CCL21 are formed around initial lymphatic vessels and these gradients direct DCs migration by haptotaxis [49].

Interestingly, numerous inflammatory chemokines such as IL-8, RANTES, and MCP-1 have also been shown to bind GAGs and can presumably be displayed on LECs [50]. However, inflammatory leukocytes do not accumulate around lymphatic vessels or migrate to the draining node. Instead, migration into lymphatic vessels is largely restricted to CCR7-expressing DCs. Recent studies have suggested that the accumulation of inflammatory cytokines on LECs is prevented by the scavenging activity of the atypical chemokine receptor D6, which is specifically expressed on the cell surface of LECs. D6 binds to and internalizes numerous inflammatory chemokines, but does not interact with CCL19 or CCL21 [51,52]. In the absence of D6, inflammatory leukocytes are found to accumulate around lymphatic vessels and within the draining LN. This excess accumulation of inflammatory cells actually results in congestion of lymphatic vessels and impedes DC migration.

By contrast, CCL19 is not immobilized on cell surfaces, but freely diffuses [53]. In peripheral tissue, it has thus been suggested that DCs direct chemotaxis in an autologous manner by secreting CCL19, which then diffuses in the direction of interstitial fluid flow [45]. Migration of DCs within the initial lymphatic capillaries depends on active crawling along the surface endothelium, and directionality of movement within these vessels has been linked to the rate of fluid flow [48].

Once in the larger collecting vessels, DCs are passively swept along by the flow of lymph until reaching the LN subcapsular sinus (SCS) [48]. Upon arriving at the draining LN, migrating DCs must next traverse the SCS – a process which involves a recently described transition from migration along the endothelial cells lining the ceiling of the LN sinus [termed ceiling LECs (cLECs)] to the

floor-lining endothelial cells [floor LECs (fLECs)] [54]. This process is also driven by CCR7-directed migration along a CCL21 gradient. Interestingly, this gradient is established by the expression of the atypical chemokine receptor CCRL1, which functions as a scavenger receptor for CCL19 and CCL21 [54–56]. Expression of CCRL1 is restricted to cLECs, thus preventing surface display of CCR7 ligands on the SCS ceiling and effectively directing migration of DCs through the fLECs and into the LN parenchyma (Figure 1B).

Cell independent antigen transport

While uptake and transport via migratory DCs is typically recognized as the major avenue of antigen delivery from non-lymphoid tissues into LNs, soluble antigen can also freely drain via the afferent lymph. This occurs rapidly, with antigen arriving at the LN within a matter of minutes. Upon arriving at the LN, smaller antigens with a hydrodynamic radius of less than ~4 nm (or MW of less than 70 kDa) rapidly permeate the LN cortex through FRC-formed conduits (Figure 1C), making these antigens readily accessible to resident DCs, cognate B cells, and follicular DCs (FDCs, Table 1) that are in close contact to the FRC network [28,57,58]. By contrast, larger particles, such as viruses, are excluded from the conduit network. Larger material is instead primarily captured by medullary and subcapsular sinus macrophages [59], and can then be transferred to B cells in the LN cortex [60–62]. The vast majority of material present in the afferent lymph is captured and filtered out as it passes through the LN [63]. This process that has been found to not only influence adaptive immune responses, but prevent systemic dissemination of lymph-borne pathogens [61,64,65]. Recent evidence suggests that LECs residing along the subcapsular sinus may also capture and store antigen. Interestingly, this archiving function appears to be restricted to proliferating LECs which emerge under inflammatory conditions [66]. This would suggest that archiving of free antigen by LECs occurs only in conditions of immunogenic challenge.

It should be noted that, while cell independent trafficking of antigen to the LN has been convincingly shown to occur during model immunization, the extent to which this occurs during a natural infection, in which antigen is introduced in more limiting quantities, is less clear. In addition, the immunological consequence of DC-borne antigen transport versus soluble antigen transport to the LN will need to be examined. DCs carrying antigen to the LN may be conditioned by signals derived from the site of infection or the stromal cells upon which they migrate. Cell-free antigen lacks this additional information, and thus may elicit a distinct immunological outcome upon arrival within the LN.

Immune cell positioning and homeostasis in LNs

Lymphoid organs are carefully organized into discrete functional compartments. This compartmentalization is crucial for optimal resource management and efficient generation of adaptive immune responses. Significant progress has been made in the last several years toward deciphering the physical and chemical cues that direct immune cells to their proper destination within the lymphoid tissue parenchyma. Entry and movement of lymphocytes within the densely packed LN depend on close physical interactions with the stromal network [67]. Lymphocytes appear to crawl along the LN stroma, but largely remain within strictly defined geographical regions that are delineated by specific chemokine expression patterns [67]. Entry and retention of lymphocytes in the paracortical T cell zone is dependent on the expression of CCR7 and interaction with its ligands CCL19 and CCL21, while incoming naive B cells additionally depend upon CXCR5-mediated homing toward CXCL13-rich follicles [2,5,68]. The role of stromal cells in orchestrating this process has long been appreciated; however, the specific contributions of FRCs, FDCs, and other stromal subsets, as well as the precise means by which they shape the lymphoid tissue landscape, are only recently coming to light.

Studies in which FRCs were specifically ablated *in vivo* (via administration of diptheria toxin to mice conditionally expressing the diptheria toxin receptor in CCL19-expressing cells) have confirmed the pivotal role these cells serve in both organizing lymphocyte positioning within lymphatic tissues as well as in maintaining cell homeostasis and viability [69,70]. FRC-depleted LNs lose segregation of B and T cell compartments, fail to maintain normal T cell numbers, and are rendered incapable of mounting virus-specific CD4 and CD8 T cell responses. Interestingly, only naive lymphocytes require FRC-derived signals for retention within the LN because depletion of FRCs during an ongoing immune response did not result in a loss of activated lymphocyte numbers or failure to mount antiviral immunity [70]. Failure to support T cell survival is likely due to the loss of FRC-produced IL-7 [71]. This phenomenon is similarly observed following long-term fibrosis of LNs in HIV/SIV-infected subjects in which the FRC network is damaged or inaccessible [72,73]. Unexpectedly, Cremasco *et al.* also found that the loss of FRCs was equally devastating to resident B cell populations, and likewise resulted in impaired germinal center formation and humoral immunity [69]. FRCs localized to the B cell follicle were found to be major producers of the B cell survival factor BAFF, thus indicating that FRCs may not only organize and maintain the paracortical T cell zone, but also help to establish and maintain B cell homeostasis in the follicle. Whether BAFF-expressing FRCs in the B cell follicle represent a distinct population of stromal cells remains to be addressed. Recent work by Mionnet *et al* has suggested that a previously unidentified population of stromal cells, distinct from conventional FRCs (based on transcriptional profile), populate the T cell area of the LN [74]. Moreover, these cells, termed 'versatile stromal cells' (VSCs), could be instructed via interactions with B cells to produce CXCL13. A phenotypically distinct, CXCL12-expressing population of reticular stroma (CRCs) has also been found to populate the T-zone proximal region of the primary B cell follicle as well the germinal center dark zone [75]. The contribution of these newly identified stromal populations to immune cell compartmentalization and homeostasis will ultimately need to be more thoroughly parsed out in future studies. However, these findings nevertheless indicate a greater level of heterogeneity to the LN reticular stroma than previously thought.

Maintenance of B cell homeostasis and the organization of discrete follicles had previously been attributed to the function of FDCs. Indeed FDCs are producers of both B cell chemotactic cues (CXCL13) as well as BAFF, APRIL, and other survival factors, and the loss of these cells results in failure to maintain strict primary follicle organization [76,77]. However, the specific ablation of FDCs alone results in no appreciable decreases in BAFF production in the spleen and LN, and only modest decreases in CXCL13 in the spleen [77]. FDCs were also found to be dispensable for resting B cell homeostasis. Nevertheless, LNs lacking FDCs fail to maintain proper segregation of B and T cell zones and were unable to support germinal center formation upon immune activation. Interestingly, loss of FDCs was found to result in encroachment of CCL21-expressing FRCs into the B cell rich areas, which might suggest that FDCs may additionally contribute to maintenance of strict follicle borders through repression of T cell chemotactic cues or by repelling T zone FRCs.

Marginal reticular cells (MRCs), a recently describe stromal cell subset localized to the subcapsular sinus overlying B cell follicles, have also been implicated in the production of BAFF and CXCL13 [78,79] (Table 1). However, their definitive contributions to LN organization and homeostasis remain to be determined. Failure to maintain B cell homeostasis in the absence of FRCs suggests that MRC-derived BAFF and CXCL13 alone are not sufficient for maintaining B cell follicles. Interestingly, while little is known about the direct functional contributions of MRCs, fate-mapping studies have suggested that this population gives rise to FDCs, and thus may serve as important stromal cell progenitors [80].

Immune cell redistribution upon initiation of adaptive immune responses

The significance of stromal cells in coordinating immune cell positioning and migration within resting lymphoid tissues is fairly well established. However, the contributions of stroma to immune responses are only more recently coming into focus. The failure to mount antiviral T and B cell responses upon acute FRC ablation presents new evidence that the LN stroma is indispensable during immunity [69]. However, it is unclear whether failure to mount effective immune responses following FRC ablation results from impaired cellular positioning or because the collapsed LN architecture can no longer regulate normal cellular ingress or maintain the survival of B and T cell populations.

Using a model of conditional LTBR ablation in CCL19-expressing FRCs, Chai *et al.* describe the formation of an intact, but functionally immature T zone reticular network [81]. Although slightly reduced in size and cellularity, the basic architectural features of the LN remain largely normal, including the formation of distinct B and T cell zones as well as a functional conduit network. However, the loss of LTBR on LN FRCs nevertheless resulted in a loss of immunocompetence and increased susceptibility to viral infection. Failure to establish antiviral immunity in this model was associated with impaired expression of interleukin 7 and homeostatic chemokines CCL19 and CCL21 by FRCs. Whether the loss of FRC-produced chemotactic cues is causal remains unclear, although *plt* mice, which lack both CCL19 and CCL21, exhibit a similar delay in antiviral response.

The importance of stroma-produced homeostatic chemokines during immune activation is an intriguing question because numerous reports suggest that immune cell compartmentalization is transiently disrupted during the initial stages of an immune response to infection [82–88]. This is a general phenomenon found to occur following exposure to several viral, bacterial, and parasitic protozoan infections. Although in some instances this appears to be due to direct targeting and destruction of the FRC network [89], many of these pathogens have been found to elicit a specific transcriptional downregulation of CCL21 and CXCL13 [86]. Disruption of immune cell compartmentalization has also been found to occur in response to administration of lipopolysaccharide (LPS) or in the presence of particular immune adjuvants such as complete Freund's adjuvant (CFA) [86,90].

Whether this transient downregulation occurs by design or represents a commonly exploited means of subverting host adaptive immunity remains unclear. In most instances, adaptive immunity does not appear to be impaired following this transient alteration in LN or splenic architecture. However, in the case of *Salmonella*, LPS-induced disruption of CCL21 and CXCL13 in draining LNs enhances the virulence of this pathogen [88]. Moreover, the loss of these organizational cues during a variety of infections appears to render the host more susceptible to secondary infection [86].

It has been suggested that a temporary downregulation of CCL21 and CXCL13 may benefit the host adaptive immune response by limiting the recruitment of additional naive lymphocytes after initial immune activation, thereby reducing the competition for limited space and resources [86]. An alternative possibility is that CCL21- or CXCL13-mediated retention of lymphocytes within their respective compartments must be relieved to allow favorable intranodal repositioning for antigenic priming. For instance, recent reports suggest that the chemokine receptor CXCR3 mediates T cell localization within the inter-follicular and medullary zones, and enhances interactions with antigen-bearing DCs [91,92]. This occurs through interaction with stromal cell derived CXCL9 and DC-derived CXCL10 [91]. Both CXCL9 and CXCL10 are transiently expressed upon exposure to LPS/poly(I:C) and follow a close reciprocal expression pattern to that of CCL21 and CXCL13. Whether this represents a coordinated response to redirect immune cells to regions rich in antigen-bearing APCs will need to be specifically examined.

Concluding remarks

Stromal cells orchestrate adaptive immune responses by directing the recruitment and positioning of lymphocytes, delivery of antigen, and maintenance of cell populations within secondary lymphatic tissues. These are not static functions, but are dynamically regulated in response to complex cellular or molecular cues.

Recent advances in high-powered imaging techniques and the development of new genetic tools for specific targeting of stromal cell subsets have reshaped the field of stromal cell biology and enabled the study of these cells

at far greater depth than ever before. New studies demonstrate clearly that stromal cells are more heterogeneous and functionally versatile than previously credited. The contributions of newly described endothelial (cLECs and fLECs) and non-endothelial (MRCs, IAPs, VSCs, and CRCs) stromal cell populations to immune cell trafficking and homeostasis are intriguing and warrant future investigation (Table 1). Moreover, our understanding of many previously established stromal populations, including HEV ECs, FDCs, and FRCs, is continuously evolving.

Upon immunogenic challenge, the LN stroma undergoes marked expansion and reorganization. The mechanisms driving this process and the consequences of these alterations are not fully understood, although we have discussed here the involvement of a variety of key signaling pathways known to drive phenotypic and proliferative expansion of LN stroma. A thorough understanding of these processes may have relevant implications for vaccine design. In addition, significantly less is known about what regulates these pathways during immune responses or how LN homeostasis is restored. Addressing these questions may yield findings relevant to the control of long-term disruption of LN homeostasis and fibrosis resulting from conditions of chronic infection or inflammation.

While the topics covered in this review have largely focused on the contribution of LN stroma to immunity, it is important to note that stromal cells are likely to be equally crucial for the immunological functions of other lymphoid organs and peripheral tissues. Many of the same populations of stromal cells may be found in other secondary lymphoid organs, including the spleen and Peyer's patch. Whether these stromal cells are functionally similar to those that populate the LN will need to be directly examined. However, given the environmental and architectural differences of these other lymphoid tissues, there will almost certainly be functional differences.

Ultimately, it has become clear that stromal cells constitute a fundamental, although oft-overlooked, component of the immune system. Recent studies discussed herein have brought better resolution to the complex picture of the LN microenvironment, and have opened the door to a bevy of exciting new avenues to be explored. Ultimately, discovering the precise means by which these cells coordinate the cellular interactions necessary for the initiation of adaptive responses will have important biological and clinical implications.

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References

1 Bleul, C.C. *et al.* (1996) A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J. Exp. Med.* 184, 1101–1109
2 Gunn, M.D. *et al.* (1998) A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 391, 799–803
3 Gunn, M.D. *et al.* (1998) A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 95, 258–263
4 Ngo, V.N. *et al.* (1998) Epstein–Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells. *J. Exp. Med.* 188, 181–191
5 Forster, R. *et al.* (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99, 23–33
6 Springer, T.A. (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301–314
7 von Andrian, U.H. *et al.* (2003) Homing and cellular traffic in lymph nodes. *Nat. Rev. Immunol.* 3, 867–878
8 Cyster, J.G. *et al.* (2012) Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu. Rev. Immunol.* 30, 69–94
9 Mionnet, C. *et al.* (2011) High endothelial venules as traffic control points maintaining lymphocyte population homeostasis in lymph nodes. *Blood* 118, 6115–6122
10 Bai, Z. *et al.* (2013) Constitutive lymphocyte transmigration across the basal lamina of high endothelial venules is regulated by the autotaxin/lysophosphatidic acid axis. *J. Immunol.* 190, 2036–2048
11 Nakasaki, T. *et al.* (2008) Involvement of the lysophosphatidic acid-generating enzyme autotaxin in lymphocyte-endothelial cell interactions. *Am. J. Pathol.* 173, 1566–1576
12 Moussion, C. *et al.* (2011) Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. *Nature* 479, 542–546
13 Onder, L. *et al.* (2013) Endothelial cell-specific lymphotoxin-beta receptor signaling is critical for lymph node and high endothelial venule formation. *J. Exp. Med.* 210, 465–473
14 Herzog, B.H. *et al.* (2013) Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature* 502, 105–109
15 Grigorova, I.L. *et al.* (2010) Lymph node cortical sinus organization and relationship to lymphocyte egress dynamics and antigen exposure. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20447–20452
16 Grigorova, I.L. *et al.* (2009) Cortical sinus probing, S1P1-dependent entry and flow-based capture of egressing T cells. *Nat. Immunol.* 10, 58–65
17 Matloubian, M. *et al.* (2004) Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427, 355–360
18 Pham, T.H. *et al.* (2008) S1P1 receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity* 28, 122–133
19 Schwab, S.R. *et al.* (2005) Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. *Science* 309, 1735–1739
20 Pappu, R. *et al.* (2007) Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* 316, 295–298
21 Pham, T.H. *et al.* (2010) Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J. Exp. Med.* 207, 17–27
22 Lee, M.J. *et al.* (1998) Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science* 279, 1552–1555
23 Lo, C.G. *et al.* (2005) Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. *J. Exp. Med.* 201, 291–301
24 Tomura, M. *et al.* (2008) Monitoring cellular movement in vivo with photoconvertible fluorescence protein 'Kaede' transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10871–10876
25 Hall, J.G. *et al.* (1965) The immediate effect of antigens on the cell output of a lymph node. *Br. J. Exp. Pathol.* 46, 450–454
26 Baekkevold, E.S. *et al.* (2001) The CCR7 ligand elc (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. *J. Exp. Med.* 193, 1105–1112
27 Palframan, R.T. *et al.* (2001) Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. *J. Exp. Med.* 194, 1361–1373
28 Sixt, M. *et al.* (2005) The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity* 22, 19–29

29 Shiow, L.R. *et al.* (2006) CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* 440, 540–544
30 Soderberg, K.A. *et al.* (2005) Innate control of adaptive immunity via remodeling of lymph node feed arteriole. *Proc. Natl. Acad. Sci. U.S.A.* 102, 16315–16320
31 Kumar, V. *et al.* (2012) Optical projection tomography reveals dynamics of HEV growth after immunization with protein plus CFA and features shared with HEVs in acute autoinflammatory lymphadenopathy. *Front. Immunol.* 3, 282
32 Webster, B. *et al.* (2006) Regulation of lymph node vascular growth by dendritic cells. *J. Exp. Med.* 203, 1903–1913
33 Chyou, S. *et al.* (2008) Fibroblast-type reticular stromal cells regulate the lymph node vasculature. *J. Immunol.* 181, 3887–3896
34 Chyou, S. *et al.* (2011) Coordinated regulation of lymph node vascular-stromal growth first by CD11c$^+$ cells and then by T and B cells. *J. Immunol.* 187, 5558–5567
35 Kumar, V. *et al.* (2010) Global lymphoid tissue remodeling during a viral infection is orchestrated by a B cell-lymphotoxin-dependent pathway. *Blood* 115, 4725–4733
36 Yang, C.Y. *et al.* (2014) Trapping of naive lymphocytes triggers rapid growth and remodeling of the fibroblast network in reactive murine lymph nodes. *Proc. Natl. Acad. Sci. U.S.A.* 111, E109–E118
37 Acton, S.E. *et al.* (2014) Dendritic cells control fibroblastic reticular network tension and lymph node expansion. *Nature* 514, 498–502
38 Astarita, J.L. *et al.* (2014) The CLEC-2–podoplanin axis controls the contractility of fibroblastic reticular cells and lymph node microarchitecture. *Nat. Immunol.* Published online October 27, 2014. (http://dx.doi.org/10.1038/ni.3035)
39 Kamath, A.T. *et al.* (2002) Developmental kinetics and lifespan of dendritic cells in mouse lymphoid organs. *Blood* 100, 1734–1741
40 Steinman, R.M. (1991) The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9, 271–296
41 Baluk, P. *et al.* (2007) Functionally specialized junctions between endothelial cells of lymphatic vessels. *J. Exp. Med.* 204, 2349–2362
42 Schmid-Schonbein, G.W. (1990) Microlymphatics and lymph flow. *Physiol. Rev.* 70, 987–1028
43 von der Weid, P.Y. (2001) Review article: lymphatic vessel pumping and inflammation – the role of spontaneous constrictions and underlying electrical pacemaker potentials. *Aliment. Pharmacol. Ther.* 15, 1115–1129
44 Acton, S.E. *et al.* (2012) Podoplanin-rich stromal networks induce dendritic cell motility via activation of the C-type lectin receptor CLEC-2. *Immunity* 37, 276–289
45 Randolph, G.J. *et al.* (2005) Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat. Rev. Immunol.* 5, 617–628
46 Teijeira, A. *et al.* (2014) Taking the lymphatic route: dendritic cell migration to draining lymph nodes. *Semin. Immunopathol.* 36, 261–274
47 Schumann, K. *et al.* (2010) Immobilized chemokine fields and soluble chemokine gradients cooperatively shape migration patterns of dendritic cells. *Immunity* 32, 703–713
48 Tal, O. *et al.* (2011) DC mobilization from the skin requires docking to immobilized CCL21 on lymphatic endothelium and intralymphatic crawling. *J. Exp. Med.* 208, 2141–2153
49 Weber, M. *et al.* (2013) Interstitial dendritic cell guidance by haptotactic chemokine gradients. *Science* 339, 328–332
50 Hoogewerf, A.J. *et al.* (1997) Glycosaminoglycans mediate cell surface oligomerization of chemokines. *Biochemistry* 36, 13570–13578
51 Lee, K.M. *et al.* (2011) D6 facilitates cellular migration and fluid flow to lymph nodes by suppressing lymphatic congestion. *Blood* 118, 6220–6229
52 Lee, K.M. *et al.* (2013) D6: the 'crowd controller' at the immune gateway. *Trends Immunol.* 34, 7–12
53 de Paz, J.L. *et al.* (2007) Profiling heparin–chemokine interactions using synthetic tools. *ACS Chem. Biol.* 2, 735–744
54 Ulvmar, M.H. *et al.* (2014) The atypical chemokine receptor CCRL1 shapes functional CCL21 gradients in lymph nodes. *Nat. Immunol.* 15, 623–630
55 Heinzel, K. *et al.* (2007) A silent chemokine receptor regulates steady-state leukocyte homing in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8421–8426
56 Ulvmar, M.H. *et al.* (2011) Atypical chemokine receptors. *Exp. Cell Res.* 317, 556–568
57 Roozendaal, R. *et al.* (2008) The conduit system of the lymph node. *Int. Immunol.* 20, 1483–1487
58 Roozendaal, R. *et al.* (2009) Conduits mediate transport of low-molecular-weight antigen to lymph node follicles. *Immunity* 30, 264–276
59 Nossal, G.J. *et al.* (1968) Antigens in immunity. XV. Ultrastructural features of antigen capture in primary and secondary lymphoid follicles. *J. Exp. Med.* 127, 277–290
60 Carrasco, Y.R. *et al.* (2007) B cells acquire particulate antigen in a macrophage-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node. *Immunity* 27, 160–171
61 Junt, T. *et al.* (2007) Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature* 450, 110–114
62 Phan, T.G. *et al.* (2007) Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* 8, 992–1000
63 Drinker, C.K. *et al.* (1934) The filtering capacity of lymph nodes. *J. Exp. Med.* 59, 393–405
64 Iannacone, M. *et al.* (2010) Subcapsular sinus macrophages prevent CNS invasion on peripheral infection with a neurotropic virus. *Nature* 465, 1079–1083
65 Gonzalez, S.F. *et al.* (2010) Capture of influenza by medullary dendritic cells via SIGN-R1 is essential for humoral immunity in draining lymph nodes. *Nat. Immunol.* 11, 427–434
66 Tamburini, B.A. *et al.* (2014) Antigen capture and archiving by lymphatic endothelial cells following vaccination or viral infection. *Nat. Commun.* 5, 3989
67 Bajenoff, M. *et al.* (2006) Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* 25, 989–1001
68 Forster, R. *et al.* (1996) A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 87, 1037–1047
69 Cremasco, V. *et al.* (2014) B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. *Nat. Immunol.* 15, 973–981
70 Denton, A.E. *et al.* (2014) Fibroblastic reticular cells of the lymph node are required for retention of resting but not activated CD8$^+$ T cells. *Proc. Natl. Acad. Sci. U.S.A.* 111, 12139–12144
71 Link, A. *et al.* (2007) Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat. Immunol.* 8, 1255–1265
72 Estes, J.D. *et al.* (2008) The role of collagen deposition in depleting CD4$^+$ T cells and limiting reconstitution in HIV-1 and SIV infections through damage to the secondary lymphoid organ niche. *Semin. Immunol.* 20, 181–186
73 Zeng, M. *et al.* (2011) Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. *J. Clin. Invest.* 121, 998–1008
74 Mionnet, C. *et al.* (2013) Identification of a new stromal cell type involved in the regulation of inflamed B cell follicles. *PLoS Biol.* 11, e1001672
75 Bannard, O. *et al.* (2013) Germinal center centroblasts transition to a centrocyte phenotype according to a timed program and depend on the dark zone for effective selection. *Immunity* 39, 912–924
76 Cyster, J.G. *et al.* (2000) Follicular stromal cells and lymphocyte homing to follicles. *Immunol. Rev.* 176, 181–193
77 Wang, X. *et al.* (2011) Follicular dendritic cells help establish follicle identity and promote B cell retention in germinal centers. *J. Exp. Med.* 208, 2497–2510
78 Katakai, T. (2012) Marginal reticular cells: a stromal subset directly descended from the lymphoid tissue organizer. *Front. Immunol.* 3, 200
79 Katakai, T. *et al.* (2008) Organizer-like reticular stromal cell layer common to adult secondary lymphoid organs. *J. Immunol.* 181, 6189–6200
80 Jarjour, M. *et al.* (2014) Fate mapping reveals origin and dynamics of lymph node follicular dendritic cells. *J. Exp. Med.* 211, 1109–1122
81 Chai, Q. *et al.* (2013) Maturation of lymph node fibroblastic reticular cells from myofibroblastic precursors is critical for antiviral immunity. *Immunity* 38, 1013–1024

82 Benedict, C.A. *et al.* (2006) Specific remodeling of splenic architecture by cytomegalovirus. *PLoS Pathog.* 2, e16
83 Cadman, E.T. *et al.* (2008) Alterations of splenic architecture in malaria are induced independently of Toll-like receptors 2, 4, and 9 or MyD88 and may affect antibody affinity. *Infect. Immun.* 76, 3924–3931
84 Glatman Zaretsky, A. *et al.* (2012) Infection with *Toxoplasma gondii* alters lymphotoxin expression associated with changes in splenic architecture. *Infect. Immun.* 80, 3602–3610
85 John, B. *et al.* (2009) Dynamic Imaging of $CD8^+$ T cells and dendritic cells during infection with *Toxoplasma gondii*. *PLoS Pathog.* 5, e1000505
86 Mueller, S.N. *et al.* (2007) Regulation of homeostatic chemokine expression and cell trafficking during immune responses. *Science* 317, 670–674
87 Mueller, S.N. *et al.* (2007) Viral targeting of fibroblastic reticular cells contributes to immunosuppression and persistence during chronic infection. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15430–15435
88 St John, A.L. *et al.* (2009) *Salmonella* disrupts lymph node architecture by TLR4-mediated suppression of homeostatic chemokines. *Nat. Med.* 15, 1259–1265
89 Scandella, E. *et al.* (2008) Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone. *Nat. Immunol.* 9, 667–675
90 Katakai, T. *et al.* (2004) A novel reticular stromal structure in lymph node cortex: an immuno-platform for interactions among dendritic cells, T cells and B cells. *Int. Immunol.* 16, 1133–1142
91 Groom, J.R. *et al.* (2012) CXCR3 chemokine receptor-ligand interactions in the lymph node optimize $CD4^+$ T helper 1 cell differentiation. *Immunity* 37, 1091–1103
92 Woodruff, M.C. *et al.* (2014) Trans-nodal migration of resident dendritic cells into medullary interfollicular regions initiates immunity to influenza vaccine. *J. Exp. Med.* 211, 1611–1621
93 van de Pavert, S.A. *et al.* (2009) Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. *Nat. Immunol.* 10, 1193–1199
94 Benezech, C. *et al.* (2012) Lymphotoxin-beta receptor signaling through NF-kappaB2–RelB pathway reprograms adipocyte precursors as lymph node stromal cells. *Immunity* 37, 721–734
95 Vondenhoff, M.F. *et al.* (2009) LTbetaR signaling induces cytokine expression and up-regulates lymphangiogenic factors in lymph node anlagen. *J. Immunol.* 182, 5439–5445
96 Kim, D. *et al.* (2000) Regulation of peripheral lymph node genesis by the tumor necrosis factor family member TRANCE. *J. Exp. Med.* 192, 1467–1478
97 Benezech, C. *et al.* (2010) Ontogeny of stromal organizer cells during lymph node development. *J. Immunol.* 184, 4521–4530
98 Cupedo, T. *et al.* (2004) Presumptive lymph node organizers are differentially represented in developing mesenteric and peripheral nodes. *J. Immunol.* 173, 2968–2975
99 van de Pavert, S.A. *et al.* (2010) New insights into the development of lymphoid tissues. *Nat. Rev. Immunol.* 10, 664–674
100 Brendolan, A. *et al.* (2012) Mesenchymal cell differentiation during lymph node organogenesis. *Front. Immunol.* 3, 381
101 Koning, J.J. *et al.* (2012) Interdependence of stromal and immune cells for lymph node function. *Trends Immunol.* 33, 264–270
102 Suzuki-Inoue, K. *et al.* (2006) A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2. *Blood* 107, 542–549
103 Sancho, D. *et al.* (2012) Signaling by myeloid C-type lectin receptors in immunity and homeostasis. *Annu. Rev. Immunol.* 30, 491–529
104 Colonna, M. *et al.* (2000) Molecular characterization of two novel C-type lectin-like receptors, one of which is selectively expressed in human dendritic cells. *Eur. J. Immunol.* 30, 697–704
105 Astarita, J.L. *et al.* (2012) Podoplanin: emerging functions in development, the immune system, and cancer. *Front. Immunol.* 3, 283
106 Malhotra, D. *et al.* (2012) Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat. Immunol.* 13, 499–510
107 Martin-Villar, E. *et al.* (2006) Podoplanin binds ERM proteins to activate RhoA and promote epithelial–mesenchymal transition. *J. Cell Sci.* 119, 4541–4553
108 Suzuki-Inoue, K. *et al.* (2010) Essential in vivo roles of the C-type lectin receptor CLEC-2: embryonic/neonatal lethality of CLEC-2-deficient mice by blood/lymphatic misconnections and impaired thrombus formation of CLEC-2-deficient platelets. *J. Biol. Chem.* 285, 24494–24507
109 Osada, M. *et al.* (2012) Platelet activation receptor CLEC-2 regulates blood/lymphatic vessel separation by inhibiting proliferation, migration, and tube formation of lymphatic endothelial cells. *J. Biol. Chem.* 287, 22241–22252
110 Navarro, A. *et al.* (2008) T1alpha/podoplanin is essential for capillary morphogenesis in lymphatic endothelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 295, L543–L551
111 Benezech, C. *et al.* (2014) CLEC-2 is required for development and maintenance of lymph nodes. *Blood* 123, 3200–3207
112 Douglas, Y.L. *et al.* (2009) Pulmonary vein, dorsal atrial wall and atrial septum abnormalities in podoplanin knockout mice with disturbed posterior heart field contribution. *Pediatr. Res.* 65, 27–32
113 Ramirez, M.I. *et al.* (2003) T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. *Dev. Biol.* 256, 61–72
114 Peters, A. *et al.* (2011) Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. *Immunity* 35, 986–996