Lymph node stromal cell subsets—Emerging specialists for tailored tissue-specific immune responses

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ABSTRACT
The effective priming of adaptive immune responses depends on the precise dispatching of lymphocytes and antigens into and within lymph nodes (LNs), which are strategically dispersed throughout the body. Over the past decade, a growing body of evidence has advanced our understanding of lymph node stromal cells (LNSCs) from viewing them as mere accessory cells to seeing them as critical cellular players for the modulation of adaptive immune responses. In this review, we summarize current advances on the pivotal roles that LNSCs play in orchestrating adaptive immune responses during homeostasis and infection, and highlight the imprinting of location-specific information by micro-environmental cues into LNSCs, thereby tailoring tissue-specific immune responses.

1. Introduction
Lymph nodes (LNs) as part of the secondary lymphoid organs (SLOs) are dispersed throughout the body in close proximity to vascular and lymphatic branching points. Their primary function is to act as hubs of constant immune surveillance in which lymph- and blood-borne constituents are continuously filtered and checked by cells of the adaptive immune system. In humans, LN position and number show slight inter-individual variations, while mice possess 22 LNs that develop exactly at the same anatomical sites (Hoorweg and Cupedo, 2008; Van den Broeck et al., 2006). Each LN is responsible for draining the surrounding tissue at its specific anatomical localization, therefore the influx of antigen-presenting cells migrating from the tissue to the draining LN after antigen uptake represents a unique tissue-derived cell population. In addition, the antigen-pool is often exclusive to the respective draining site, e.g., tissue-specific antigens, food-borne antigens or antigens of microbial origin. Lately, more and more studies have uncovered how much the micro-environment matters for the generation of distinct tissue-resident immune cell subsets. However, transcriptome analyses revealed similarities in circulating lymphocytes isolated from spleen and LNs, indicating that there are many common immunomodulatory functions inherent to SLOs (Miragaia et al., 2019; Zhao et al., 2020). How much impact the micro-environment has on the functional properties of LNs at distinct locations is currently subject of ongoing investigations. In parallel to the exploration of the tissue-specific shaping of immune cell niches, the micro-milieu within the LN itself has garnered interest. Lymph node stromal cells (LNSCs) are responsible for stabilizing and compartmentalizing the LN and have long been viewed as a simple scaffold facilitating immune cell migration and interaction. Recently, the startling heterogeneity of this understudied cell population

Abbreviations:  
Aire, autoimmune regulator; Aldh1a2, aldehyde dehydrogenase 2; BAFF, B cell activating factor; BECs, blood endothelial cells; cellN, celiac lymph node; CRCs, CXCL12-expressing reticular cells; CSF1, colony stimulating factor 1; DC, dendritic cell; FDCs, follicular dendritic cells; FRCs, fibroblastic reticular cells; FSCs, fibroblastic stromal cells; GF, germ-free; HEV, high endothelial venules; IFN, interferon; IL, interleukin; kDa, kilodaltons; kDA, kilodaltons; LECs, lymphatic endothelial cells; IFRCs, interfollicular FRCs; LNs, lymph nodes; LNSCs, lymph node stromal cells; LT, lymphotxin; LTBR, lymphotxin receptor; medRCs, medulary FRCs; MAdCAM1, mucosal adressin cell adhesion molecule 1; MHC, major histocompatibility complex; mLNs, mesenteric LNs; MRCs, marginal reticular cells; MyD88, myeloid differentiation response protein 88; PDPN, podoplanin; pLN, peripheral LN; PP, Peyer’s Patches; PTAs, peripheral tissue-specific restricted antigens; RA, retinoic acid; RADLH, retinal dehydrogenase; RANKL, receptor activator of NF-κB ligand; resDCs, resident DCs; Scs, stromal cells; scRNAseq, single-cell RNA sequencing; SCS, subcapsular sinus; SLOs, secondary lymphoid organs; SPF, specific pathogen-free; TBRC, T-B cell border FRCs; TNF, tumor necrosis factor; TRCs, T cell zone FRCs; Tregs, regulatory T cells.

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has been revealed, followed by the discovery of various immunomodulatory functions carried out by LNSCs. Moreover, LNSCs are also involved in the initiation of LN development during embryogenesis and early postnatal stages, and recent findings on this topic were excellently summarized elsewhere (Krishnamurty and Turley, 2020; Onder and Ludewig, 2018). In the present review, we aim to summarize current advances in our understanding of how LNSC subsets influence immune cells across the LN. LECs guide immune cells arriving with the afferent lymph via CCL21 secretion and maintain the local macrophage niche by providing CSF-1. Moreover, LECs line the lymphatics of the medullary sinuses throughout the medulla (not depicted). 3) MRCs are located below the capsule in close proximity to the B cell follicles. They produce CXCL13 and RANKL and are capable of replenishing FDCs after FDC ablation during infection. 4) In the cortex, CXCL13+ FDCs are residing in the B cell follicles and present antigen to B cells. 5) In the light zone of germinal centers, FDCs foster B cell affinity maturation, while CXCL12-producing CRCs are inducing B cell proliferation. 6) In the paracortex, TRCs guide T cells and DCs via the expression of CCL19 and CCL21. Moreover, they build a conduit system to efficiently channel lymph throughout the paracortex. 7) HEVs are dispersed throughout the LN, facilitate immune cell entry by the expression of adhesion molecules and are composed of an inner layer of BECs, followed by pericytes and lastly a layer of perivascular FRCs. 8) In the medulla, where the lymph is collected before LN egress, medRC are producing APRIL, BAFF and IL-6 to sustain the local plasma cell population. BEC, blood endothelial cell; CRC, CXCL12-expressing reticular cell; CSF-1, colony stimulating factor 1; DC, dendritic cell; FDC, fibroblastic dendritic cell; FRC, fibroblastic reticular cell; HEV, high endothelial venule; LEC, lymphatic endothelial cell; LN, lymph node; medRC, medullary FRC; MRC, marginal reticular cell; SC, stromal cell; SCS, subcapsular sinus; TRC, T cell zone FRC.

2. LN micro-architecture

A general infrastructure is shared among all murine LNs. The LN is mantled by a capsule, under which the subcapsular sinus (SCS) acts as a conduit for the afferent lymph. Beneath the SCS lies the LN cortex, which is interspersed with multiple B cell follicles. A centrally oriented paracortex disemboques into the medulla, from which the effert lymphatics reconnect to circulation. All LNs possess a hilus, from which a main artery enters the medulla to branch into an articulate network, forming high endothelial venules (HEVs), which interpose the LN with a higher vascular density in the LN periphery (Jafarnejad et al., 2019; Kelch et al., 2015) (Fig. 1).

The LNSC network tightly regulates immune cell trafficking in these distinct zones. LNSCs can be broadly separated into three subsets, CD45+ CD31+ PDPN+ fibroblastic reticular cells (FRCs), CD45+ CD31+ PDPN+ lymphatic endothelial cells (LECs) and CD45+ CD31+ PDPN+ blood endothelial cells (BECs). FRCs can be further subdivided into the major subsets of marginal reticular cells (MRCs), follicular dendritic cells (FDCs), T cell zone FRCs (TRCs) and medullary FRCs (medRCs). The capsule contains a layer of CD34+ stromal cells (SCs), whose function has not been well characterized, yet (Raumbuecher et al., 1994; Rodda et al., 2018). Besides the capsule, CD34+ SCs can also be found in the adventitia of larger vessels, named "adventitial SCs" (Sitnik et al., 2016). LECs situated in the SCS facilitate lymphocyte and dendritic cell (DC) migration across the sinus-cortex interface (Jalkanen and Salmi, 2020), permit the passage of <70 kilodaltons (kDa) lymph-borone antigens into the conduit system (Gretz et al., 2000; Rantakari et al., 2015), while molecules <500 kDa can pass through LECs via transcytosis (Kahari et al., 2019). LECs are lining lymphatic vessels and are therefore also abundantly found in the medullary sinuses in the medulla (Jalkanen and Salmi, 2020). MRCs, characterized by the expression of CXCL13, mucosal addressin cell adhesion molecule 1 (MAdCAM1) and receptor activator of NF-κB ligand (RANKL), line the

![Diagram of LN micro-architecture](image-url)
outer edge of the cortical area and are in close proximity to B cell follicles, where they are thought to give rise to FDCs during B cell follicle development (Jarjour et al., 2014) and drive B cell follicle formation during infection (Dubey et al., 2019). CD21/35/CXCL13 FDCs are located inside the B cell follicles, where they present antigens to B cells and support germinal center formation (Pikor et al., 2020; Rodda et al., 2015). In germinal centers, FDCs are positioned in the light zone, while CXCL12-CRCs are present in the dark zone, where they conjoinly foster B cell affinity maturation and proliferation (Banard et al., 2013; Rodda et al., 2015). CCL19/21 TRCs are situated in the interfollicular area of the cortex and throughout the paracortex, where they form a conduit system to facilitate lymph flow and foster T cell and DC interaction (Fletcher et al., 2015). In the medulla, cell density decreases and a meshwork of LECs lining the medullary sinuses collects efferent lymph, which is sampled by macrophages (Jalkanen and Salmi, 2020). Here, CXCL12+ medRCs act as a source of plasma cell survival factors, guiding their migration (Huang et al., 2018). The LNs’ capillary system forms specialized HEVs, consisting of an inner layer of BECs adjacent to pericytes and an outer layer of perivascular FRCs. Leucocyte entry into the LN parenchyma is assisted by CCL21 chemotactic gradients and expression of the adhesion molecules GlyCAM-1, ICAM-1, MadCAM-1 and CD34 on BECs (Girard et al., 2012). The transcriptome of HEVs is distinct from the surrounding capillary endothelium, which underlines their high degree of specialization. Moreover, it was shown that the HEV transcriptome is highly similar across the mesenteric LNs (mLNs), peripheral LNs (pLN) and Peyer’s Patches (PP), indicating that HEV function is not influenced by the anatomic location of the LNs (Lee et al., 2014).

With the advent of single-cell RNA sequencing (scRNAseq) several recent studies have demonstrated that the current subset classification is likely not sufficiently representing LNSC heterogeneity. In LECs isolated from human LNs, six subsets were identified, some of which are located in the SCS and the medullary sinuses and express neutrophil chemotactic attractants (Takeda et al., 2019). The existence of multiple LEC subsets was recently also observed in murine LNs (Xiang et al., 2020). FRCs may represent the most heterogeneous LNSC population. In addition to FDCs, MRCs, TRCs and perivascular cells, five additional subsets were initially described in FRCs isolated from murine pLN (Rodda et al., 2018). Also, as many as 14 subsets could be identified among non-endothelial LNSCs sorted from both pLN and mLNs (Pezoldt et al., 2018). There, besides subsets expressing Ccl19, Inmt, Cxcl19 and Nr4a1, which most likely orchestrate the LN’s reticular network, several CD34+ subsets were also identified (Pezoldt et al., 2018). Since CD34+ cells are frequently referred to as ‘adventitial’ SCSs, the term of fibroblastic stromal cells (FSCs) was introduced to represent all subsets among CD45+CD31+PDLP cells identified by scRNA-seq, including the highly heterogeneous FRC subsets as well as the CD34+ subsets (Pezoldt et al., 2018; Takeuchi et al., 2018). In addition to these studies, further light was recently shed on the heterogeneity of FRCs with the help of Ccl19-EYFP reporter mice. Here, in addition to FDCs, MRCs, TRCs and perivascular cells, two subsets of medRCs, two population of FRCs located at the T-B cell border (named T-B cell border FRCs, TBRCS) and a subset of interfollicular FRCs (IFRCs) were identified (Perez-Shibayama et al., 2020). Moreover, the generation of Cxcl13-Cre/TdTomato reporter mice enabled the dissection of the cellular composition of the B cell follicle reticular cell network. In total, seven subsets were identified, including two populations of FDCs (termed germinal center light zone FDCs and dark zone FDCs), two medRC subsets and a subset of MRCs, TBRCS and IFRCs (Pikor et al., 2020).

3. LNSC function during steady-state conditions

Due to the compartmentalization of LNs, LNSCs carry out several functions spanning from lymph filtering, over immune cell entry and guidance to the secretion of survival factors and a supporting role in peripheral tolerance and the mounting of immune responses, which will be discussed in detail in the following sections.

3.1. LNSCs and the conduit system

The lymphatic vascular network formed by LECs mediates the transport of soluble antigens and antigen-loaded DCs from the tissue to the draining LNs, which enables the transmission of the immunological state of the drained tissue into the LN (Kedl and Tamburini, 2015). This conduit network collects lymph from the tissue and transports the fluid in an unidirectional manner to the SCS (Forster et al., 2012). Upon entry into the SCS, the constituents of the lymph are separated according to their size. Small particles with a low molecular weight, including chemokines, peptides, cytokines and small metabolites, directly enter a conduit system of collagen cores sheathed by FRCs (Grete et al., 2000). The accurate separation is enforced by the tight plasmalemma vesicle associated protein diaphragm, which is directly connected to the FRC collagen conduit at the LN sinus (Rantzakari et al., 2015; Sixt et al., 2005). The content of the conduit network is either sampled by FDCs in the follicles to elicit humoral responses through B cells or by DCs adjacent to FRCs to prime T cell responses (Roozendaal et al., 2009; Sixt et al., 2005). As the FRC network sheathing the collagen conduit system resides in close proximity to the content of the lymph, it might be able to sample constituents of the lymph rapidly and therefore modulate T cell differentiation in concert with DCs. Besides antigen import from the tissue, the conduit system can also be utilized by B cells to export secreted IgM antibodies out of the LN (Thierry et al., 2018).

3.2. LNSCs and lymphocyte trafficking

The initiation of antigen-specific T cell responses requires a tight interaction of naive T cells and antigen-loaded DCs. How can T cells and DCs, arriving at LNs via different gateways, overcome their spatial separation and finally meet each other in the paracortical areas of the LN? Upon their arrival at the LN, tissue-derived CCR7+DCs first have to traverse the cell barrier posed by the SCS floor to reach the LN paracortex, a migratory step driven by CCR7 along the CCL21 gradient (Forster et al., 2008). LECs lining the ceiling of the SCS, but not those lining the floor, produce ACKR4 (also known as CCR1L), which scavenges CCL21 from the SCS lumen, thereby creating a CCL21 gradient across the sinus floor to enable the emigration of CCR7+DCs from the afferent lymph to enter into the LN parenchyma (Ulvmar et al., 2014). Subsequently, CCR7+DCs further migrate into the T cell zone following the chemotactic cues CCL19 and CCL21, which are secreted by FRCs located in the T cell zone (Braun et al., 2011). CLEC-2 expression by DCs also facilitates their crawling along the endothelial cells lining afferent lymphatic vessels and along FRCs lining the reticular network towards the T cell zone through the podoplanin (PDVPN):CLEC2 signaling axis (Acton et al., 2012). Meanwhile, HEVs produce CCL21 and transcytose CCL19 from the parenchyma to facilitate circulating CCR7+T cell recruitment and ingress into the parenchyma (Baekkenvold et al., 2001; Stein et al., 2000). CCL19 and CCL21 produced by FRCs in the T cell zone further induce the migration of CCR7+T cells towards them, where they finally encounter antigen-bearing DCs (Forster et al., 2008). In contrast to T cells, B cell trafficking within the LNs additionally relies on CXCR5-mediated homing towards CXCL13-rich B cell follicles, where they encounter FDCs (Bajenoff et al., 2006; Wang et al., 2011).

3.3. LNSCs and immune homeostasis

At steady state, FRCs in the T cell zone are the major source of the survival factor interleukin (IL)-7, which supports naïve T cell homeostasis, and inhibition of IL7 signaling leads to impaired T cell survival and homing to LNs (Link et al., 2007). FRCs can also support B cell maturation, survival and proliferation in B cell follicles via the production of B cell activating factor (BAFF) (Cremasco et al., 2014). LECs help maintain immune cellularity in the SCS and foster the homeostasis
of the CD169+ SCS macrophage pool via secretion of colony stimulating factor 1 (CSF1) (Mondor et al., 2019). MRCs located in the LN cortex at the outer edges of B cell follicles can also support macrophage and innate lymphoid cell (ILC) homeostasis via expression of RANKL (Camara et al., 2019; Perez-Shibayama et al., 2019).

LNSCs also partake in immune responses via multiple mechanisms. In response to interferon (IFN)γ and tumor necrosis factor (TNF), FRCS and LECs can upregulate the expression of inducible nitric oxide synthase (NOS2) and produce nitric oxide to restrict T cell proliferation in a cell-contact-dependent manner (Łukaczk-Körnek et al., 2011; Siegert et al., 2011). Sustained expression of the major histocompatibility complex (MHC) II in FRCS serves as another mechanism of T cell restraint. Under homeostatic conditions, FRCS express MHCII albeit to a low extent (Dubrot et al., 2014). Upon inflammation, MHCII expression is elevated by IFNγ-mediated upregulation of the class II transactivator (Dubrot et al., 2014). Despite endogenous expression, the majority of MHCII molecules on FRCS are potentially captured from DCs by transfer of MHCII complexes in a cell-contact-dependent manner (Dubrot et al., 2014). The presentation of peptide-MHCII complexes by FRCS to naïve CD4+ T cells induces CD4+ T cell dysfunction (Abe et al., 2014; Dubrot et al., 2014). In contrast to mechanisms of restraint, it has recently been reported that FRCS can also benefit T cell fitness during their activation. Upon receiving signals from activated T cells, FRCS upregulate immune-stimulatory molecules such as IL6 (Brown et al., 2019). FRC-deriving IL-6 promotes IL-2 together with TNF production and leads to chromatin remodeling in newly activated CD8+ T cells, thereby enhancing CD8+ T cell survival, metabolism and their capacity to differentiate into tissue-resident memory populations (Brown et al., 2019). In addition, FRCS in the mLN s form a niche for group 1 ILC homeostasis via IL15 expression (Gil-Cruz et al., 2016).

3.4. LNSCs and immune tolerance

Immune tolerance against self and commensal antigens is pivotal for preventing autoimmunity and overt inflammation, and emerging studies reveal a role of LNSCs in peripheral tolerance. Under steady-state conditions, LNSCs, particularly FRCS and LECs, express a range of peripheral tissue-restricted antigens (PTAs) and directly present to naïve CD8+ T cells in the context of MHCII, finally leading to the deletion of self-reactive T cells (Fletcher et al., 2010; Lee et al., 2007). This process is independent of the autoimmune regulator (Aire), but rather depends on the transcription factor Deaf1, thus it is different from medullary thymic epithelial cell-mediated deletion of self-reactive thymocytes (Cohen et al., 2016; Fletcher et al., 2016; Yip et al., 2009). Different LNSC subsets express distinct groups of PTAs and have their own characteristic antigen display (Cohen et al., 2010; Fletcher et al., 2010). For instance, LECs are the only LNSC population expressing the melanocyte-specific protein tyrosinase, mediating the deletion of tyrosinase-specific T cells (Fletcher et al., 2010). LECs can further restrict self-reactive CD8+ T cells by engaging PD-1 on T cells via PD1 expression (Cohen et al., 2014). Recently, also LNSC-mediated deletion of self-reactive CD4+ T cells by engaging PD-1 has been reported. FRCS and LECs are capable of capturing self-antigen-MHCII complexes from DCs, thereby limiting CD4+ T cell proliferation and survival in an antigen-specific manner (Dubrot et al., 2014). Additionally, LECs can serve as an antigen reservoir and transfer the self-antigen β-galactosidase to DCs, which subsequently present it to naïve T cells and induce CD4+ T cell tolerance (Rouhani et al., 2015).

Furthermore, LNSCs indirectly contribute to immune tolerance by modulating regulatory T cells (Tregs), a subpopulation of T cells that is essential for the maintenance of peripheral tolerance. Self-antigen-MHCII presentation by LNSCs supports the non-proliferative maintenance of antigen-specific Tregs (Baptista et al., 2014), and very recently, it has been reported that the continuous presentation of self-antigens by LNSCs is critical for the generation of antigen-specific Tregs, thereby restricting the formation of T follicular helper cells and germinal center

<table>
<thead>
<tr>
<th>Infection/inflammation</th>
<th>LNSC phenotype</th>
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<tr>
<td><strong>Virus infection</strong></td>
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<tr>
<td>Vaccinia virus</td>
<td>LNSC subsets show distinct kinetics for expansion: BECs and LECs expand rapidly, peaking at day 14 post infection, while the expansion of FRCS is delayed and sustained by non-circulating progenitor cells (Abe et al., 2014). LNSCs upregulate MHCII expression until day 10 post infection (Abe et al., 2014). LN dark zone FDCs elevate the expression of the chemokines Cxcl1 and Cxcl6 and the cytokine Il6, as well as integrin-binding and extracellular matrix remodeling proteins, but down-regulate Ccl12 and Ccl19 transcripts (Piker et al., 2020). LN light zone FDCs down-regulate Ccl13 transcripts (Piker et al., 2020). FRCS prevent exaggerated innate immune responses through MyD88-dependent restriction of IL15 production in mLN s and PPs (Gil-Cruz et al., 2016). LNSCs proliferate rapidly and robustly (Gregory et al., 2017). Expansion of FRC networks is long-lived, but the transcriptional program induced by infection is transient (Gregory et al., 2017). Sustained amplified LNSC networks support subsequent immune responses (Gregory et al., 2017). LCMV infects FRCS, which results in disruption of the FR network and a concomitant loss of immunocompetence (Mueller et al., 2007; Scandella et al., 2009). Infected FRCS upregulate expression of PDI, which may contribute to viral persistence in FRCS during chronic infection (Mueller et al., 2007). During acute, LN-restrict infection with LCMV Armstrong strain, type I IFN-dependent stimulation induces a rapid switch into an antiviral state in LN FRCS, with elevated transcriptional signatures of IFN-α receptor signaling pathway, IFN-stimulated genes, genes related to antigen presentation, and chemokines and cell activation markers. This switch into immunostimulatory state of FRCS is crucial to prevent viral dissemination and to facilitate antiviral immune response (Perez-Shibayama et al., 2019). FRCS and the conduit network of LN s are damaged, correlating with a loss of T and B cells zones and increased fibrosis (Fletcher et al., 2015).</td>
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<tr>
<td>Lymphocytic choriomeningitis virus (LCMV)</td>
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<td>Ebola, Lassa and Marburg virus</td>
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<td>Bacterial infection</td>
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<td>Listeria monocytogenes</td>
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<td>Yersinia pseudotuberculosis</td>
<td>SCs in PPs secrete regulatory cytokine IL-11 to limit intestinal villus invasion by Listeria (Dinson et al., 2018). At day 3 post infection, the number of FRCS in mLN s is significantly reduced (Pezoldt et al., 2018). FRCS display an activated phenotype with increased MHCII expression until 4 weeks post infection, a time point when Yersiniae were cleared from mLN s (Pezoldt et al., 2018). Pathogen-derived LPS induces disruption of CCL21 and CXCL13, resulting in enhanced virulence of the pathogen (St John and Abraham, 2009).</td>
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<td>Salmonella typhimurium</td>
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<td>Parasite infection</td>
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<td>Helminth (200 L3 stage parasites)</td>
<td>Enhanced LT expression in B cells drives FR expansion, which in turn supports B cell follicle expansion and antibody production (Dubey et al., 2016).</td>
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<tr>
<td>Inflammation</td>
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<tr>
<td>LPS</td>
<td>LNSCs upregulate the expression of genes encoding chemokines and molecules involved in the acute-phase response and the antigen-processing and antigen-presentation machinery (Malhotra et al., 2012).</td>
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<tr>
<td>PolyIC</td>
<td>A subset of LNSCs shows unique up-regulation of PTAs and Aire (Fletcher et al., 2010).</td>
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<tr>
<td>Immunization</td>
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B cell responses (Nadaf et al., 2020). In addition, it was demonstrated that LNSCs contribute to the efficient de novo generation of Tregs in gut-draining LNs, namely mLNs and the celiac lymph node (cellLN) (Cording et al., 2014; Pezoldt et al., 2018). FRCs isolated from mLNs express high levels of the retinoic acid (RA)-synthesizing enzyme retinal antigen-presentation machinery ( Gregory et al., 2017 ; Malhotra et al., 2012 ) and different pathogens ( Abe et al., 2014 ; Gregory et al., 2017 ), and SC regression. LNSC subsets display distinct expansion kinetics in response to antigenic Treg-inducing micro-environment ( Cording et al., 2014 ). Besides this, it could be further revealed that mLN SCs can directly modulate incoming LN-resident DCs (resDCs) and instruct them with Treg-inducing properties, thereby contributing to the maintenance of intestinal tolerance (Pezoldt et al., 2018).

4. LNSCs in infection and inflammation

Upon infection, LNs often experience hypertrophy to accommodate the clonal expansion of activated T and B cells. After the resolution of the infection, LNs return to their homeostatic size as lymphocytes egress and contract. These dramatic changes in size are mainly mediated by LNSCs. Under homeostatic conditions, PDPN (also known as gp38) regulates the actomyosin contractility of FRCs, which controls physical tension throughout the FRC reticular network (Astarita et al., 2015). Upon infection, increased amounts of naïve lymphocytes and antigen-loaded migratory DCs flow into the LN parenchyma. The enhanced CLEC2 expression on infiltrating DCs, which is induced by antigen-uptake and expression on infiltrating DCs, promotes terminal cell reactions, somatic hypermutation and IgG production (Wu et al., 2009).

Consequently, LNSCs respond differently in the context of diverse infections or inflammatory settings. In order to escape immune surveillance, some pathogens such as lymphocytic choriomeningitis virus (LCMV), can directly target and infect LNSCs, finally inducing damage to the conduit network and resulting in an altered LN architecture (Table 1).

The effects of the LNSC response to infections extend beyond expanding LNs for lymphocyte accommodation. LNSCs can modulate a magnitude of immune responses towards infections. The selective ablation of FRCs results in failure to mount proper T and B cell responses against influenza virus (Cremasco et al., 2014). Lymphotoxin (LT)/lymphotoxin receptor (LT, R) signaling is pivotal for the maturation of LNSCs. Loss of LT, R on LNSCs results in impaired immunocompetence and increased susceptibility to acute LCMV infections (Chai et al., 2015). A recent study revealed that the lack of LT, R signaling in early life results in a long-lasting altered SC composition in mLNs and consequently impaired virus-specific antibody responses against rotavirus (Li et al., 2019). Besides the supportive role in mounting immune responses, LNSCs can also restrain excessive inflammation during enteropathogenic infections. FRC recognition of pathogen entry via myeloid differentiation response protein 88 (MyD88) signaling downregulates IL15 expression in FRCs, thereby restricting exaggerated ILC activation and subsequent inflammatory cell damage (Gil-Cruz et al., 2016).

5. Impact of the LN micro-environment on the phenotype of SCs

One factor that stands out in the context of LN genesis and the maintenance of SC structural integrity is LT, R signaling. In the mature LN, many SC subsets express LT, R and rely on the expression of LT in lymphocytes and myeloid cells for their survival (Kumar et al., 2015). After inhibition of LT, R in mature LNs, CD35 expression is eliminated and FDC networks collapse (Gommerman et al., 2002). FRCs in general are diminished after CD4+ T cell depletion, emphasizing this T cell population as a critical source of LT (Zeng et al., 2012). Moreover, although TRCs can generate a basic T cell infrastructure, they cannot reach full immunocompetence in the absence of LT, R signaling (Chai et al., 2013). For HEV integrity continued LT, R-signaling is essential (Browning et al., 2005; Liao and Ruddle, 2006; Veerman et al., 2019), and in the absence of CD11c+ DCs lymphocyte homing to the LNs is dramatically reduced and HEVs revert to an immature HEV phenotype with a marked downregulation of HEV-specific markers, which was at least partly due to the absence of DC-mediated LT-signaling (Mousson and Girard, 2011). Very recently, it was shown that a transient attenuating of LT, R signaling in utero leads to a permanent alteration of the mLNs SC composition, suggesting a long-lasting impact of early-life LT, R signaling on LNSC phenotype (Li et al., 2019). Aside from these general mechanisms affecting SC maintenance in all LNs as well as LT, R-specific remodeling processes during infection, LT signaling is decisive during the onset of LN organogenesis. Although LN development is not subject of the present review, the following interesting aspect should be briefly discussed: LT, R signaling is pivotal for the maturation of LT, R mice lack PPs and all LNs (Futterer et al., 1998), while LT, R mice lack PPs and most LNs, but retain mLNs and cervical LNs, indicating location-specific differences in LN development dependent on LT signaling (Alimzhanov et al., 1997; Koni et al., 1997).

Since 22 murine LNs always develop at defined anatomical sites, the mouse represents an ideal model to study the influence of the surrounding micro-environment on a sessile cell population with longevity, such as the LNSCs in individual LNs. Although scRNAseq analysis demonstrated a similar SC subset compositions in mLNs and pLN (Peretz-Shibayama et al., 2020; Pezoldt et al., 2018; Pikor et al., 2020; Rodda et al., 2018), scRNA-seq and RNA-seq analysis also reveal a distinct overall transcriptome of mLN FRCs and pLN FRCs (Malhotra et al., 2012; Pezoldt et al., 2018). Interestingly, it was recently shown that even the FRCs transcriptomes of individual LNs of the mLN chain are discrete

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<th>Infection/inflammation</th>
<th>LNSC phenotype</th>
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<td>DC and monocyte-derived IL17 induce early proliferation of FRCs (Benahmed et al., 2014)</td>
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<tr>
<td>IL17 produced by locally differentiating Th17 cells drives SC activation in inflamed LNs through metabolic reprogramming, which is required to support SC proliferation and survival (Majumder et al., 2019)</td>
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<td>FDCs expand in number via direct differentiation from MRCs into mature FDCs, and express high amounts of CXCL13, BAFF and IL6 (Jaurjou et al., 2014; Wu et al., 2009)</td>
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<td>CXCL13 secretion of FRCs in the follicular zone delineates new transient boundaries of the growing follicles (Mionnet et al., 2013)</td>
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<td>IL6 production of FDCs promotes germinal center reactions, somatic hypermutation and IgG production (Wu et al., 2009)</td>
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Another interesting aspect to study is the proximity of gut-draining LNs to the intestinal microbiota and therefore the microbe micro-environmental factors. These findings suggest that a newly forming LN functionally adapts to the new tissue draining site and can be influenced by microbe micro-environmental factors. FRCs from LNs to the intestinal microbiota and therefore the microbe micro-environmental factors.

Microbiota

RNaseq analysis of FSCs isolated from these transplanted LNs as well as endogenous mLNs from GF and SPF mice enabled the assessment of the contribution of microbiota on LNSC phenotype (Cording et al., 2014; Pezoldt et al., 2018). RNAseq works collapse after interruption of LTβR signaling. 2) Microbiota can illicit transcriptional changes in mLN FRCs, and their presence induces HEV maturation via CD103+RALDH+ DCs that induce PNAβ upregulation and HEV portal enlargement, while vitamin A can boost the Treg induction capacity of celLN SCs. celLN, celiac lymph node; DC, dendritic cell; FDC, fibroblastic dendritic cell; FRC, fibroblastic reticular cell; HEV, high endothelial venules; LN, lymph node; LNSC, lymph node stromal cell; LTβR, lymphotxin-β receptor; mLN, mesenteric lymph node; SC, stromal cell; SLO, secondary lymphoid organ; TRC, T cell zone FRC.

(Emrich et al., 2019). To determine whether LNSCs can stably retain their location-specific properties, LN transplantation experiments can be applied (Mebius et al., 1993). After surgically resecting the endogenous intestinal or skin-draining LN, a transplanted LN can readily engraft at the cleared site (Hammerschmidt et al., 2008). During engraftment, the hematopoietic compartment is replaced by cells from the recipients’ draining tissue and the circulation. However, LN-resident cells, predominantly SCs of donor origin, remain (Hammerschmidt et al., 2008). It was observed that the FSC transcriptome of transplanted mLNs to a skin-draining site did not completely adapt a pLN FSC phenotype several weeks after transplantation (Pezoldt et al., 2018). Akin to this, it has been reported that the transplanted LNs stably retain functional properties, such as MAdCAM-1 expression on HEVs originating from mLNs, whereas skin-draining pLN SCs do not gain MAdCAM1 expression if transplanted to the mesenteries (Ahrendt et al., 2008; Hammerschmidt et al., 2008). Together, these data indicate that once matured, the LN’s FSC compartment can retain part of its characteristic features for a prolonged period of time even upon translocation to a different anatomical site. Interestingly, the conservation of mLN SC function with regard to MAdCAM-1 expression is only observed if an intact mLN is transplanted. When single-cell suspensions of SCs from skin-draining LNs were transplanted into the mesenteries using a collagen sponge, the establishing LN HEVs indeed express MAdCAM1 (Buettner et al., 2015). These findings suggest that a newly forming LN functionally adapts to the new tissue draining site and can be influenced by micro-environmental factors.

Another interesting aspect to study is the proximity of gut-draining LNs to the intestinal microbiota and therefore the microbe’s capacity to shape LNSC subset composition, phenotype and function. FRCs from mLNs, which express high levels of Aldh1a2, act in concert with CD103+DCs to contribute to the elevated RA levels in mLNs (Hammerschmidt et al., 2008; Malhotra et al., 2012; Molenaar et al., 2011). Retinal dehydrogenase (RALDH) enzymatic activity has been shown to be a vital constituent for the neonatal to adult addressin switch in HEVs in mLNs and pLN, which does not occur in germ-free (GF) mice (Zhang et al., 2016). Transplantation of mLNs either from GF or colonized specific pathogen-free (SPF) mice into the skin-draining popliteal fossa of SPF mice enables the assessment of the contribution of microbiota on LNSC phenotype (Cording et al., 2014; Pezoldt et al., 2018). In addition to the ‘naturally existing’ neonatal window, when mLNs are stably imprinted with tolerogenic properties (Pezoldt et al., 2018). In addition to the ‘naturally existing’ neonatal window, when mLNs are stably imprinted with tolerogenic properties (Pezoldt et al., 2018). In addition to the ‘naturally existing’ neonatal window, when mLNs are stably imprinted with tolerogenic properties (Pezoldt et al., 2018).
have demonstrated the unique tolerogenic properties of the celLN (Cording et al., 2014; Hauet-Broere et al., 2003; Nutsch et al., 2016; Pezoldt et al., 2018), and transplanted celLN from mice fed with vitamin A-deficient diet lost their superior tolerogenic properties in the non-tolerogenic skin-draining site, while transplanted celLN from mice fed with standard diet retained their tolerogenic properties (Cording et al., 2014). These data suggest a vitamin A-mediated imprinting of tolerogenic properties into celLN SCs, yet whether this imprinting also takes place during early life is still unclear.

6. LNSCs tailor tissue-specific immune responses

LNSCs are influenced by their micro-environment, but besides being receptive to micro-environmental cues, these cells are also capable of tailoring tissue-specific immune responses (Fig. 2). Adoptively transferred T cell receptor-transgenic naïve T cells could upregulate the expression of gut-homing molecules α4β7 and CCR9 upon antigen-specific priming in mLNs transplanted into the skin-draining poplitea fossa, while pLN transplanted into the gut mesenteries failed to foster the generation of gut-homing T cells (Hammerschmidt et al., 2008; Molenar et al., 2009). Thus, SCs not only maintain expression of location-specific adhesion molecules, but also modulate T cell differentiation in a location-specific manner. Additionally, LNSCs show location-specific tolerogenic properties. As mentioned above, gut-draining LNscs (mLns and celLN) foster high de novo Treg induction (Cording et al., 2014). When gut-draining LNscs and pLNscs were transplanted into the skin-draining poplitea fossa and gut mesenteries, respectively, gut-draining celLN and mLNs engrafted at the non-tolerogenic site still fostered efficient de novo Treg induction, while pLNscs transplanted to the tolerogenic gut mesenteries failed to support high Treg induction (Cording et al., 2014; Pezoldt et al., 2018). Together, these data demonstrate that gut-draining LNscs stably retain their efficient Treg-inducing capacity, a feature maintained by the LNSC compartment. Furthermore, in an intranasal tolerance induction model, the surgical removal of cervical LNscs and their subsequent replacement with pLNscs abrogated intranasal tolerance induction (Wolvers et al., 1999), further demonstrating the importance of stably imprinted functional properties of LNSCs for the modulation of tissue-specific immune responses.

7. Conclusion

Quite recently, LNSCs were primarily appreciated as structural constituents defining and organizing the highly compartmentalized architecture of LNscs. Advances in the past decade have expanded our understanding of the functionality of LNSCs. Besides forming a scaffold, LNSCs produce chemokines to direct the migration of lymphocytes and DCs into and within LNscs. Furthermore, they produce survival factors to promote the survival of incoming immune cells to provide high-quality accommodations. LNSCs can also sense the tension of the environment, and either restrain or promote lymphocyte activity to maintain immune homeostasis. The advances in scRNA-seq have further revealed characteristic gene expression signatures of unique LNSC subsets. Remarkably, LNSCs retain location-specific information and tailor tissue-specific immune responses to a degree that was largely ignored, so far. Upon infection, LNSCs are not static, but are dynamically regulated in response to cellular and molecular cues, thereby coordinating adaptive immunity to control the infection. Additionally, LNSCs can contribute to peripheral tolerance towards self- or microbiota-derived antigens. Recent studies highlighted the concept of the neonatal window of opportunity for the establishment of immune tolerance. Indeed, there is also a neonatal window of opportunity for LNSCs to be stably imprinted with tolerogenic properties by micro-environmental factors. In conclusion, the latest advances and continued efforts towards a precise understanding of the role of LNSCs in coordinating immune responses under steady-state conditions and during infection will provide a novel perspective for the design of vaccines, therapies and immunomodulatory strategies against various immune-mediated diseases.

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