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Stromal cell contributions to the homeostasis and functionality of the immune system

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Abstract

A defining characteristic of the immune system is the constant movement of many of its constituent cells through the secondary lymphoid tissues, mainly the spleen and lymph nodes, where crucial interactions that underlie homeostatic regulation, peripheral tolerance, and effective development of adaptive immunity take place. What has only recently been recognized is the role that non-haematopoietic stromal elements have in multiple aspects of immune cell migration, activation and survival. In this Review, we summarize our current understanding of lymphoid compartment stromal cells, examine their possible heterogeneity, discuss how these cells contribute to immune homeostasis and the efficient initiation of adaptive immunity, and highlight how targeting of these elements by some pathogens can influence the host response.

Immune system function involves highly dynamic interactions between diverse cell populations specialized to protect the body against unwanted invaders. Over the past century, many studies have documented the location and organization of cellular immune constituents in primary [G] and secondary lymphoid tissues [G]. Recently, the use of intravital multi-photon laser scanning microscopy [G] has afforded researchers an inside look into the dynamic events that take place in lymphoid and non-lymphoid tissues¹. Very few such studies, however, have examined how these activities are influenced by the surrounding tissue environment, in particular the non-haematopoietic stromal cells and other cells that support the parenchyma of the lymphoid organs and peripheral tissues.

In this Review, we discuss our current understanding of the many roles of the stromal cells in secondary lymphoid organs (SLO), as well as the specialization of various stromal subsets. These cells contribute to immunity through the provision of chemokines and cytokines, by acting as a scaffold for cell trafficking, and through antigen presentation and the expression of adhesion and inhibitory molecules, leading to either tolerance or active immunity. The phenotype and location of lymphoid stromal cells, and the signals they produce, can provide regional control to nurture or direct the most appropriate responses to pathogens. The induction of tertiary lymphoid tissues that contain stromal networks strikingly similar to those in SLOs is a further indication of the importance of these networks for immune responses (BOX 1). We also discuss evidence that several pathogens target lymphoid system stromal cells during infection and consider the implications of such targeting for pathogen persistence and immunosuppression.

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A primer on secondary lymphoid organ organization

The SLOs form a network of structurally and functionally heterogeneous tissues designed as a filtration and surveillance system to capture pathogens and their antigens, presenting the latter in an appropriate manner to cells of the immune system. Of these tissues, the lymph nodes and spleen have been the most extensively studied, although the structures of Peyer's patches [G] and other mucosa-associated lymphoid tissues (MALT) have many important similarities. The complex microarchitecture of SLOs is supported by networks of endothelial and mesenchymal stromal cells².

Structure of the spleen

The spleen is one of the body's main filters for the blood, which also situates the organ in a prime position to capture antigens and allow cells of both the innate and adaptive immune systems to respond rapidly to blood-borne pathogens. The spleen is surrounded by a fibrous capsule from which trabeculae protrude into the tissue, providing support for the organ and its vasculature³. Blood vessels enter the spleen at the hilum and branch into central arterioles, which are surrounded by the organized lymphoid compartments known as white pulp (Fig. 1). Branches of the central arterioles terminate at marginal zones (MZ), which form the interface between the white pulp and the red pulp of the spleen. In mice, the splenic white pulp consists of T cell zones surrounding a central arteriole, as well as associated B cell follicles and the surrounding MZ. In the human spleen the MZ is larger and is surrounded by a perifollicular zone that separates the white pulp from the red pulp⁴. Lymphocytes and other immune cells primarily enter the spleen at the MZ. Many lymphocytes exit the red pulp through the splenic veins⁵, whereas the remainder of the cells make their way into the white pulp at regions where the T cell zones abut the MZ, known as the MZ bridging channels^{6, 7}. It is less clear where lymphocytes exit the white pulp, although they might do so through a network of efferent lymphatic vessels that form around central arterioles in the white pulp and drain via trabeculae and the splenic hilus⁸. Conversely, activated T cells might exit the white pulp through the MZ bridging channels⁹ and enter the blood through the sinuses in the red pulp.

The T cell zones in the spleen contain both CD4⁺ and CD8⁺ T cells and subsets of dendritic cells (DCs). These cells are supported by a network of stromal cells known as fibroblastic reticular cells (FRCs). In the B cell follicles, B cells are supported by a network of follicular dendritic cells (FDCs) and a more diffuse network of other stromal cells towards the periphery of the follicles (see Fig. 1 and Supplementary information S1 (figure)). The stromal cells of the MZ, particularly surrounding the B cell follicles, seem to have several unique features, and as such have been suggested to represent a novel subset of stromal cells known as marginal reticular cells (MRCs)¹⁰. The surrounding MZ contains MZ macrophages (MZMs), MZ metallophilic macrophages (MMMs) that express the receptor CD169, subsets of DCs, and MZ B cells amongst a dense network of stromal cells. Antigens, particles, and pathogens that enter the spleen from the blood are taken up by the MZ phagocytes. MZ B cells and DCs can then migrate into the white pulp, where they deliver or present antigen to B and T cells, respectively^{3, 11}.

Structure of the lymph nodes

Lymph nodes are encapsulated lymphoid organs that receive an extracellular fluid filtrate (lymph) through a series of non-haematogenous vessels (lymphatics) draining the tissues and organs of the body. Immediately beneath the lymph node capsule is the subcapsular sinus (SCS) into which afferent lymphatics empty, delivering molecules, antigens, microorganisms, and cells such as lymphocytes and antigen-presenting cells (APCs) from the tissues (Fig. 2). Macrophages line the SCS and capture antigens and particulates that enter the lymph node through the lymph¹², similar to the macrophages in the splenic MZ. Lymphocytes mainly enter

from the blood through specialized high endothelial venules [G] (HEVs) in the cortex¹³. HEVs are often arranged in regions between the B and T cell zones known as cortical ridges¹⁴. As in the spleen, the T cell zones contain CD4⁺ and CD8⁺ T cells and subsets of DCs, anchored to a network of FRCs and reticular fibres. Likewise, the B cell follicles contain networks of FDCs and a more peripheral network of stromal cells, which border T cell zones and the SCS. Similar to the spleen, the stromal cells found between B cell follicles and the SCS have unique properties that distinguish them as MRCs¹⁰.

Vascular endothelial cells and lymphatic endothelial cells in lymph nodes are other abundant non-haematopoietic cells in SLOs. In lymph nodes, the specialized vascular endothelial cells in HEVs have a distinct morphology¹⁵ and express molecules crucial for lymphocyte entry into the lymph node, such as peripheral node addressin (PNAd; also known as CD62L ligand) and the chemokine CCL21. Lymphatic endothelial cells express the CD44 homologue lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), as well as various adhesion molecules and CCL21, which are involved in cellular entry into the lymphatics¹⁶. Lymphocyte entry into the cortical and medullary lymphatic sinuses also requires sphingosine 1-phosphate receptor type 1 (S1P1) and its ligand S1P¹⁷, which is produced and secreted into the lymph by non-haematopoietic cells, possibly lymphatic endothelial cells¹⁸. Additional study is needed to determine if control of lymphocyte entry into lymphatics via S1P1 occurs solely via T cell intrinsic signalling¹⁹ or if signalling in endothelial cells controls a stromal barrier²⁰, or both.

The organization and phenotype of stromal subsets in SLO

The non-haematopoietic cell types in the spleen and lymph nodes can be divided into at least six subsets on the basis of location, function, and phenotype (Table 1, see Supplementary information S2 (table)). Here we focus on the stromal cell subsets that make up the parenchyma of SLOs, including FRCs, FDCs, MRCs, splenic red pulp fibroblasts and lymph node medullary fibroblasts, rather than the vascular endothelial cells and lymphatic endothelial cells involved in blood and lymph flow through SLOs (reviewed in^{16, 21}). In this section, we describe some of the known phenotypes of the heterogeneous stromal cell populations and their topological organization in SLOs. In subsequent sections, we describe the functions of the lymphoid stromal cells, in particular the FRCs.

Fibroblastic reticular cells

Reticular networks have been observed in tissues including lymph nodes for more than 100 years (cited in²²), although the cellular components of these networks only began to be characterized in the 1960s^{13, 23, 24}. It was initially assumed that the roles of FRCs in lymphoid organs were mainly structural, possibly to assist in the expansion and contraction of the lymph nodes during immune responses and to facilitate antigen or antibody transport. Subsequently, Anderson and Shaw²⁵ proposed that transport of cytokines from the afferent lymph to HEVs involved conduits formed by FRCs. This was then directly demonstrated in the lymph nodes²⁶ and spleen²⁷, heralding a new appreciation of the functions of the lymphoid stroma.

The FRCs in the spleen and lymph nodes T cell zones surround the central arterioles and HEVs, respectively, forming a dense network filled with lymphocytes²⁵. We now know from live imaging studies using two-photon microscopy that lymphocytes in these regions are in constant dynamic motion and that the lymphocytes move along FRC strands that act as guidance paths for cell migration²⁸. FRCs produce and ensheath the collagen-rich reticular fibres, forming an enclosed conduit structure that is separate and distinct from the parenchyma of the lymphoid tissue (Fig. 3). Small molecules, such as chemokines and antigens, can enter the conduit network in lymph nodes from the lymph, and are delivered rapidly to T cell zones and HEVs²⁶. High molecular-weight molecules are unable to enter the conduit lumen and instead are trapped by SCS macrophages and accumulate in the cortical sinuses, or continue around

the SCS and drain through efferent lymphatic vessels. Such functional exclusion of large molecules and particles could restrict the types of signals delivered by the FRC conduits and prevent abuse by pathogens. In the spleen, T cell zone FRCs also collect blood-borne molecules in conduit structures²⁷. Interestingly, molecular exclusion apparently differs in the spleen, since 70 kD dextran enters the splenic conduits²⁷, yet is excluded from lymph node conduits²⁶. This raises the possibility that the system is tuned to allow delivery of distinct signals through the conduit networks in the T cell zones of different SLOs.

The reticular fibres in the T cell zones are rich in collagen and the surrounding FRCs produce tissue transglutaminase and other molecules that are involved in the formation of these fibres (Table 1, see Supplementary information S2 (table)). FRCs, similar to many fibroblasts in different tissues, express an antigen recognized by the antibody ER-TR7²⁹, and FRCs can be differentiated from other (non-endothelial) lymphoid stromal cells on the basis of podoplanin (gp38) expression³⁰. FRCs are also readily identified by their production of the chemokines CCL21 and CCL19³¹, which are crucial for delineating the T cell zones and which have important roles in promoting the migration of lymphocytes and DCs that express CCR7³². FRCs in T cell zones of humans and rodents express extracellular matrix (ECM) components (such as ER-TR7, fibrillin, and the common basement membrane components laminin and fibronectin), and intracellular molecules found in some fibroblasts (such as desmin and alpha-smooth muscle actin). These may have functional roles in FRC network formation, contractile capabilities, and presentation of molecules such as chemokines. FRCs also express integrin subunits and the adhesion ligands intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1). Nevertheless, so far no truly FRC-specific marker has been defined, and little is known about expression of cytokines and membrane receptors such as pattern recognition receptors.

Follicular dendritic cells

Follicular dendritic cells cluster in the centre of B cell follicles in the spleen, lymph nodes, and other lymphoid tissues including the Peyer's patches. They form a dense network within which B cells search for antigen and receive differentiation signals after activation. FDCs express Fc receptors (CD16, CD23 and CD32), complement receptors (CD21 and CD35), and complement components such as C4 (Table 1). This facilitates the capture and presentation of unprocessed antigen, particularly in the form of immune complexes. FDCs also express high levels of VCAM1 and mucosal vascular addressin cell adhesion molecule 1 (MAdCAM1), as well as molecules common to all subsets of stromal cells in the lymphoid tissues, such as desmin and laminin. Importantly, FDCs produce the chemokine CXCL13, which directs B cells expressing the receptor CXCR5 into follicles in the steady state³³. During an immune response, germinal centre reactions drive the formation of long-lived plasma cells and memory B cells whose immunoglobulin genes have undergone somatic hypermutation and class switch recombination. Interactions between B cells, FDCs, and follicular T helper cells [G] occur within the germinal centre in a highly dynamic manner^{34, 35}. In the GC 'light-zone', B cells interact with FDCs expressing CXCL13, whereas interactions with CXCL12-expressing stromal cells occur in the germinal centre 'dark-zone'. It is unclear whether these CXCL12⁺ cells are a subset of FDCs, or represent a distinct subset of stromal cells. Movement of B cells between the two germinal centre compartments seems to occur readily, although stromal chemokine signals might retain B cells in different states of activation in the two compartments at different times³⁶. Hence, the timing of B cell movement between light and dark zones and, thus, B cell activation and differentiation might be controlled by the B cell zone stromal cells.

Marginal reticular cells

Networks of non-haematopoietic cells form a layer underneath the SCS in lymph nodes, in the MZ of the spleen, particularly at the edges of B cell follicles, and also in MALT. These stromal

cells have a phenotype that is distinct from that of stromal cells in the T and B cell zones (FRCs and FDCs, respectively). MRCs express many markers in common with other subsets of stromal cells, such as ER-TR7, desmin, laminin, VCAM1, MAdCAM1 and a molecule recognized by the antibody 1BL-11^{10, 37} (Table 1). However, MRCs seems to be unique in the expression of the tumour necrosis factor family member TRANCE (RANKL)¹⁰. These cells may be integral organizers of SLO structure during organogenesis (Katakai, JI). It is interesting to note that many of the MRCs at the edges of the B cell follicles express CXCL13, similar to the centrally located FDCs^{10, 38}. These stromal cells also form a functional conduit network that can deliver antigens from the lymph node SCS to B cell follicles³⁹. The stromal cells in the splenic MZ, together with the MMMs, might have roles in the delivery of antigen to the B cell follicles, similar to that described in lymph nodes^{39, 40}. Thus, MRCs might be a specialized subset of lymphoid stromal cells with important roles in the capture and delivery of antigens.

Splenic red pulp fibroblasts and lymph node medullary fibroblasts

The cords in the red pulp of the spleen have a crucial role in filtering the blood. They are composed of a highly compact network of fibroblasts and reticular fibres that contributes to immune defence through the production of cytokines, including interleukin-6 (IL-6)⁴¹. Macrophages in the red pulp cords also have numerous anti-microbial functions, in addition to phagocytosing red blood cells and recycling iron, which might be supported by this stromal network. The red pulp stromal cells express several integrin chains (Table 1), which potentially assist in the localization of different cells in the red pulp⁴². For example, short-lived plasma cells, with high levels of CXCR4 and the integrin lymphocyte function-associated antigen 1 (LFA1), and low levels of CXCR5 and CCR7, localize in the splenic red pulp. Red pulp fibroblasts express ICAM1 (which binds LFA1) and the chemokine CXCL12 (which binds to the receptor CXCR4)^{41, 43}. These molecules help direct the movement of plasma cells into the red pulp, where they secrete large amounts of immunoglobulins into the circulation. During acute stress from infections such as malaria, endotoxins, or IL-1, the splenic red pulp fibroblasts activate and fuse, forming so-called barrier cells^{44, 45}. These changes to the fibroblasts in the cords and sinuses of the red pulp might alter or restrict blood flow and filtering during times of stress. Changes in fibroblast function in the marginal sinuses around the white pulp could also have a role in controlling cellular entry or exit at certain times.

A dense network of fibroblasts and reticular fibres form a framework in the medullary cords of lymph nodes, as well as a loose network within medullary sinuses^{46, 47}. Many macrophages and plasma cells, as well as DCs, lymphocytes, and mast cells can be found in the lymph node medulla⁴⁶. Given this unique environment, it is likely that the medullary reticular cells have specialized roles in immunity. Indeed, because expression of CXCL12 is largely restricted to the medulla in lymph nodes⁴³, it is possible that reticular cells in this region may play similar roles to red pulp fibroblasts in directing plasma cell localization, but clearly much more remains to be learned about this stromal population.

Roles of stromal cells in lymphocyte migration in SLOs

A fundamental challenge faced by the immune system is the complexity of antigen-specific recognition and the need for individual cells to locate their cognate antigen within the vast network of lymphoid tissues. Cellular entry into SLOs has been most extensively studied with respect to lymph node HEVs, which involves a series of molecular interactions between leukocytes and the specialized vascular endothelial cells (reviewed in^{48, 49}). Entry into the spleen occurs through the marginal sinuses in the MZ, which express MAdCAM1, although the exact steps involved are unknown⁴⁹. Cells that enter lymph nodes from the tissues do so through the lymphatics, and mainly consist of APCs and activated T cells. Entry into lymphatics from the tissues requires CCR7 expression by the migrating cells^{50, 51}, yet does not appear to require integrins since DCs lacking all integrin heterodimers migrate to lymph nodes, whereas

CCR7^{-/-} DCs do not⁵². By whichever route cells enter the SLOs, immediately after entry they come into direct contact with the stromal microenvironment.

T cell trafficking in SLOs

T cells come into immediate contact with the FRC network after crossing lymph node HEVs or with the MZ and red pulp fibroblasts after entering the spleen. T cell movement within the lymphoid tissues is remarkably dynamic, with cells moving at average speeds of 8-11 $\mu\text{M}/\text{min}$ ^{53, 54}. FRCs in the T cell zone produce significant amounts of CCL21, and to a lesser extent CCL19, which is found on the surface of the network along with ECM components³¹. CCL21 binds to the receptor CCR7 and provides directional clues to T cells, because lack of CCL21 expression in *plt/plt* mice [G] prevents the localization of T cells in the white pulp of the spleen^{7, 55}. Moreover, FRCs and CCL21 seem to delineate the boundary of the T cell zones, and T cells have been observed to turn around once they reach the T cell-B cell border²⁸. This is most probably due to the lack of CCL21 expression in CXCL13-rich B cell follicles and the absence of the receptor for CXCL13, CXCR5, on naïve T cells.

In both spleen and LN, naïve T cells crawl along the surface of the FRC network and change direction apparently randomly at intersections in the FRC network while moving along the pathways corresponding to the processes and cell bodies of these stromal cells^{7, 28} (**Fig. 3**). The motility of T cells in LN is at least partially dependent on CCL21⁵⁶⁻⁵⁸. CCR7 ligands such as CCL21 occupy the receptor CCR7 on T cells in SLOs and might result in continuous signalling to drive T cell motility⁵⁹. The integrins LFA1 and very late antigen 4 (VLA4), however, do not seem to be required for T cell movement in SLOs. These integrins are not activated by the co-expression of CCL21 in the lymphoid environment, which is devoid of the strong shear forces present within blood vessels where these integrins do become activated⁶⁰. Studies suggest that other chemokines probably also have a G α i-dependent role in motility, since blocking chemokine signalling with pertussis toxin induced a greater decrease in T cell movement than removing only the CCR7 signals. However, in the absence of chemokine signals T cells retain some motility, indicating that non-G α i-dependent signals might also contribute^{57, 61}. Stromal cell-derived adhesion molecules, integrins and glycoproteins (such as VCAM-1 and podoplanin) might provide traction for lymphocytes and/or modulate the way in which they migrate through the SLOs. Expression of sialic acid by stromal cells in the spleen is important for the trafficking of lymphocytes into the white pulp⁶². FRCs express MHC class I molecules as well as the inhibitory molecule PD-L1⁶³, and they might deliver signals to T cells moving along their surface, potentially influencing tolerance and immunity by tuning responsiveness or activation state.

Chemokine signals from the lymphoid stroma (such as CCL21) provide strong retention signals to T cells that allow them to stay in SLOs for as long as 12 hours or more while they search for antigen⁶⁴. To continue their body-wide surveillance T cells must exit SLOs, and they do so in an S1P-dependent manner⁶⁵. S1P levels are considerably higher in the blood and lymph than in SLOs due to the action of S1P lyase⁶⁶. T cells modulate expression of S1P1 as they traffic between tissues, expressing low levels when in the blood and lymph, and upregulating expression and response sensitivity in SLOs over time⁶⁷. This cyclical pattern of expression controls the ability of T cells to overcome CCL21 retention signals in SLOs⁶⁸ and enter the lymphatics¹⁷; this same process might also control cell movement out of the spleen¹⁹.

B cell trafficking in SLOs.

Upon entering the spleen or lymph nodes, CXCR5⁺ B cells migrate into B cell follicles in response to CXCL13 produced by FDCs. Upon exiting HEVs, B cells first crawl on the FRC network as they make their way towards B cell follicles²⁸. Once there, B cells migrate at approximately 6 $\mu\text{M}/\text{min}$ ⁵³ in close association with the dense networks of FDCs in the centre

of the follicles (**Fig. 3**). Given that stromal cells, possibly MRC, at the periphery of the B cell follicles in both spleen and lymph nodes also express CXCL13, it is likely that B cells move in a similar manner along these cells. FDCs capture and present antigen and immune complexes, and express several accessory molecules that are important for B cell responses (such as complement and Fc receptors). Whether the signals that B cells receive in different regions of the follicles (from FDCs or MRCs) differ and influence their movement has not yet been explored. Like T cells, naïve B cells require S1P1 to exit the SLOs¹⁹. Plasma cells also rely upon S1P signals to exit the spleen and transit to the bone marrow⁶⁹.

Additional functions of SLO stromal cells

Interactions with antigen presenting cells

The SLOs are ideally suited to capture antigen from around the body and they contain numerous subsets of DCs and macrophages, which are specialized both in function as well as location in the lymphoid organs. A key issue of interest is the relationship between these APC populations and the stromal elements of these SLOs. Resident and migratory subsets of CD11c⁺ DCs are found in lymph nodes, whereas the spleen contains mostly lymphoid organ-resident DCs⁷⁰. DCs accumulate near HEVs in lymph nodes and in the MZ bridging channels in spleen, improving the chances that T and B cells will encounter their cognate antigen upon entering these SLOs^{9, 71, 72}. Resident DCs form a stable network within lymph nodes and this network organization appears to be established by DC adherence to the FRCs within the T zone²⁸.

CD8α⁺ resident DCs in particular are tightly associated with the CCL21-rich FRC network^{28, 73}. The CD8α⁻ subset of resident DCs is found in the splenic MZ, particularly at the bridging channels⁷⁴, but whether the corresponding subset occupies a discrete region in LN T cell zones has not been reported. This raises the intriguing question of whether the stromal cell components of SLO play a primary role in subregion-specific localization of distinct DC subsets, in turn influencing where specific types of immune activation events will occur. Such a possibility is given some support by the finding that the two subsets of migratory DCs in lymph nodes, Langerhans cells and interstitial DCs (dermal DCs from the skin) are asymmetrically distributed in the LN, being found in deep T cell zones and in the region separating the T and B cell zones, respectively⁷⁵. New studies are needed to determine if the FRCs in these regions differ in expression of adhesion or chemotactic molecules that might guide such distinct tissue distributions.

DCs migrating from the tissues must express CCR7 to readily enter lymphatics, and CCL19/21 expression is required for DCs to home to T cell zones in spleen and lymph nodes^{76, 77}. In the spleen, CD169⁺ MZ macrophages require CCL21 to localize to the MZ⁷⁸. Migrating DCs might also use the stromal networks as a substrate for movement, similar to lymphocytes. Local expression of the chemokines CCL19 and CCL21 by FRCs can promote immune responses by co-stimulating T cell activation⁷⁹, increasing T cell interactions with APCs⁸⁰, promoting the maturation of DCs⁸¹, stimulating endocytosis and antigen presentation by DCs⁸², and evoking the extension and probing of dendrites by DCs⁸³. Activated DCs also produce chemokines, such as CCL3 and CCL4, which assist in the recruitment of rare antigen-specific T cells through directed trafficking on FRCs⁸⁴. Therefore, interactions between FRCs and DCs, and the chemokines they produce, assist in the priming of immune responses.

Stromal cells also contribute in other ways to APC functionality. DCs that associate with FRCs in the T cell zones take up antigen transported in the conduits and present it to T cells^{73, 85}. Macrophages can also be found in close association with stromal cells in SLOs. The CD169⁺ macrophages in the SCS of lymph nodes and the MZ in spleen sit on a network of MRCs. These phagocytes capture antigens and they can transfer antigens to resident or arriving

DCs or to B cells^{11, 40, 86}, although at present there is no evidence that stromal cells contribute to the presentation function of these macrophages as FRC conduits do with DCs.

Lymphoid stroma and control of immune responses

Infections by pathogens result in marked changes to the SLOs, many of which are driven by responses of the innate and adaptive immune systems. In lymph nodes, the egress of cells is blocked for several days⁸⁷. Similarly, cell accumulation in the white pulp of the spleen increases soon after infection. This concerted change in the trafficking of cells through SLOs is due to the release of inflammatory mediators, such as type I interferon, which transiently suppresses egress⁸⁸. Inflammation also induces an increase in arterial vessel diameter and increased CCL21 presentation on HEVs, resulting in increased blood flow through the lymph node and cellular input^{89, 90}. Increased expression of vascular endothelial growth factor (VEGF) results in the rapid proliferation of vascular endothelial cells, including cells of the HEVs, and growth of the blood vessels^{91, 92}. In addition, the expansion of lymph node lymphatic sinuses by VEGF-A could assist in the recruitment of cells such as APCs from the tissues⁹³. Though DCs and B cells are critical for these processes^{92, 93}, it was recently shown that CD45⁺CD31⁺gp38⁺ lymph node stromal cells (FRCs) are the main cell type expressing VEGF mRNA in lymph nodes⁹⁴. Remodelling of the SLOs, particularly inflamed lymph nodes, which increase dramatically in size⁹⁵, suggests that expansion of the stromal network might also involve the proliferation of FRCs. This implicates FRCs as key players in the modulation of SLO cellularity and function during immune responses.

Activated T cells decrease their expression of CCR7, and increase expression of other chemokine receptors such as CCR5 and CXCR3, allowing them to respond to chemokines expressed in inflamed tissues. Concomitant with these events, FRCs in responding SLOs downregulate expression of the CCR7 ligands, CCL21 and CCL19, and FDCs reduce expression of the CXCR5 ligand, CXCL13⁹⁶. This marked reduction in chemokine expression by FRCs and FDCs is greatest at the peak of the antigen-driven immune response, and results in altered trafficking by naïve T and B cells, as well as DCs. Because CCL21 and CXCL13 signals guide T and B cells in SLOs and influence their motility, modulation of these signals reflects another level of control over the lymphoid environment by the stromal network. Coordinated regulation of lymphoid chemokine signals might influence the balance of retention and egress signals received by naïve T and B cells, altering their residence time in activated SLOs. Decreased CCL21 expression by FRCs could also promote the egress of effector T cells. Furthermore, DCs responding to CCL21 signals will be impaired in their ability to accumulate in SLOs in which the expression of lymphoid chemokines is decreased. So, the decrease in CCL21 expression in activated SLOs could alter the accumulation of nonresponding T cells and assist in the formation of long-lived memory cells by promoting effector cell access to limited survival signals on lymphoid stroma. Changes in CXCL13 expression by FDCs might influence B cell responses in germinal centres and limit access by non-activated cells. A potential drawback of these SLO changes is a transient reduction in the ability to prime an immune response to a second pathogen while the first response is underway⁹⁶. Such changes could contribute to the immune suppression that is observed during several human infections, including measles and influenza.

The lymphoid stroma may also shape immune responses in other ways. For example, it was recently found that stromal cells from mesenteric lymph nodes, but not peripheral lymph nodes, express the vitamin A metabolite retinoic acid (RA)⁹⁷. T cells primed in this stromal environment had a gut-homing phenotype (expression of $\alpha_4\beta_7$ and CCR9). Lymphoid stromal cells from mesenteric lymph nodes were also considerably better at inducing IgA responses than stromal cells from peripheral lymph nodes⁹⁸. Together, these data indicate that the stroma can direct the quality and type of immune responses in SLOs. Moreover, antigen presentation

by the stroma is capable of contributing to immune responses, for example, increasing the T cell response after viral infection⁹⁹. Conversely, during chronic infections where antigen levels are already very high, this might overstimulate responding lymphocytes, resulting in immune exhaustion¹⁰⁰. In combination with inhibitory ligands (such as PD-L1) expressed by FRCs and other non-haematopoietic cells⁶³, these studies demonstrate that lymphoid stromal cells have a significant impact on the size and quality of immune responses.

Lymphoid homeostasis

In young adults, the populations of mature lymphocytes that circulate through SLOs are maintained at a remarkably constant level by the steady release of mature lymphocytes from the thymus and bone marrow, balanced by death of the cells in the periphery. Mature mouse T cells survive for at least 8 weeks after thymic emigration in the absence of antigen activation, and this life span is decreased in the absence of MHC class II molecules in the case of CD4⁺ T cells or MHC class I molecules for CD8⁺ T cells (reviewed in ¹⁰¹). The other well established factor regulating the life span of naïve T cells is IL-7¹⁰². Lymphocytes undergo a slow rate of turnover in SLOs. Under lymphopaenic conditions, T cells divide multiple times and partially repopulate the SLOs¹⁰¹, a process that requires IL-7¹⁰². This proliferation of T cells occurs in the T cell zones of SLO, and it is absent in lymphotoxin- α deficient mice, which lack lymph nodes, organized T and B cell zones in the spleen, CCL21 and CXCL13, and which have disorganized FRC and FDC networks¹⁰³.

These observations indicate that T cells compete for survival factors in SLOs and that the overall size of the lymphocyte pool might be controlled by competition for such signals. *In vitro* experiments with SLO stromal cells suggest that they can support the survival of resting T cells¹⁰⁴. More recently, it was found that FRCs in the T cell zone might be a primary source of IL-7 and CCL19 in lymph nodes, and that together these factors are important for T cell homeostasis¹⁰⁵. Interestingly, mice expressing IL-7 with a fluorescent reporter indicate that cells near the lymph node SCS might express significant amounts of IL-7¹⁰⁶, raising the possibility that MRC also express IL-7 or have contaminated the putative FRC preparations claimed to produce this trophic cytokine. Physical competition for these apparently limited resources, and a dependence on migration through the SLOs to obtain them, probably represents a tightly regulated pathway to control the T cell population size. B cells also require a survival factor, B cell-activating factor (BAFF), for homeostasis, although these cells do not require IL-7 signals in mice¹⁰⁷. FDCs express BAFF and can control B cell homeostasis through provision of this signal to BAFFR-expressing B cells¹⁰⁸. Lastly, *in vitro* experiments suggest that T cells might be required for the proper formation of FRC networks⁹⁵, which raises the intriguing possibility that cross-talk between immune cells and the SLO stroma is important for the health and/or survival of both cell types.

Peripheral tolerance

Most T cells that express antigen receptors with a strong affinity for self- antigens are deleted in the thymus. However, the deletion mechanism is incomplete, requiring mechanisms of peripheral tolerance to prevent the activation of self-reactive T cells and autoimmunity. Beyond trans-suppression by regulatory T cells, deletion of autoreactive cells in lymph nodes is believed to be important and to involve the cross-presentation of self-antigens to T cells by DCs, which can take up antigen in the tissues or peripheral organs and migrate to draining lymph nodes⁷⁰. DCs may not be the only cell type involved in such intra-nodal tolerance induction, however, as recent reports have found that a subset of stromal cells in the lymph nodes express the autoimmune regulator (*Aire*) gene [G] and a range of tissue-derived self-antigens^{109, 110}. It is not yet known if these cells are a unique stromal subset or whether several different cell types have this property, because Aire-expressing cells in the periphery were found to be both bone-marrow and non-bone-marrow derived in one study using transgenic

Aire-GFP mice¹⁰⁹. CD8⁺ T cells interacting with the self-antigen expressing stromal cells were subsequently deleted^{109, 110}, suggesting that this might be an important mechanism of peripheral tolerance. However, further experiments will be needed to determine whether interactions with DCs are also required to initiate or complete the tolerogenic process, and whether different cell types (DCs or stromal cells) are involved in tolerizing CD4⁺ versus CD8⁺ T cells. Nevertheless, it is quite intriguing to consider the possibility that the very cells that migrating naïve T cells must constantly contact as they move within lymph nodes are capable of tissue-specific antigen presentation and potential tolerization of such T cells.

Pathogen interactions with lymphoid stromal cells

It is becoming increasingly apparent that lymphoid stromal cells, in particular FRCs, are targeted by several intracellular pathogens (Table 2). Lymphocytic choriomeningitis virus strain Clone-13 (LCMV CL-13) directly infects T cell zone FRCs in the spleen and lymph nodes⁶³. Viral infection in the red pulp, MZ, and B cell follicles indicates that CL-13 might also infect red pulp stroma, MRCs, and possibly FDCs. Viral targeting of FRCs results in altered conduit function and contributes to viral persistence in this chronic infection model. Similarly, infection of mice with the WE strain of LCMV results in infection of FRCs¹¹¹. In the LCMV WE model, loss of the gp38⁺ cell network, or possibly reduction in gp38 expression was observed¹¹¹. During CL-13 infection destruction of FRCs was prevented by expression of the inhibitory molecule PD-L1⁶³. Infection of macaque monkeys with simian immunodeficiency virus (SIV) also alters homeostatic chemokine expression progressively throughout the debilitating chronic infection¹¹². Infection of mice with *Leishmania donovani*, a parasite that can infect ER-TR7⁺ cells in lymph nodes¹¹³, also results in loss of gp38⁺ cells in the spleen¹¹⁴, as well as FDCs¹¹⁵.

Targeting of the lymphoid stroma by pathogens can have important consequences for SLO function and pathogen clearance. Highly pathogenic infection of non-human primates by Ebola, Marburg, or Lassa viruses results in infection of FRCs, as well as endothelial cells, and corresponds with considerable apoptosis of cells in the SLOs^{116, 117}. During acute and latent infection of mice with mouse cytomegalovirus, endothelial cells and stromal cells are the main target of infection in the spleen and bone marrow, which could contribute to latency and immune suppression¹¹⁸⁻¹²⁰. Infection of mice with the malaria parasite *Plasmodium yoelii* results in marked changes to the red pulp fibroblasts^{45, 121}. Red pulp stromal cells become highly metabolically active with expanded endoplasmic reticulum, and potentially have multiple roles in protection. The fusing of red pulp fibroblasts and the formation of splenic barrier cells contributes to protection from malaria by restricting parasite access to much of the cords. However, during lethal *P. yoelii* 17XL infection, large numbers of splenic red pulp stromal cells become parasitized and contribute to disease, which might reflect a different tropism, infectivity, or rate of replication by the 17XL strain. Lastly, prion proteins have been found to be associated with stromal elements in the spleen, and in granulomas¹²². Thus, various pathogens and prion proteins appear to use lymphoid stromal cells to spread and persist, which can have important consequences for SLO function and immunity. In addition, there have been rare reports of patients with aggressive tumours resulting from FRC neoplasms in the spleen and lymph nodes¹²³.

Concluding remarks

The presence of non-haematopoietic elements in SLOs has been recognized by anatomists for more than a century. For most of this time, these cells were considered to have mainly a structural role, forming the skeleton of the SLO within which the haematopoietic components carried out their function. This view has given way over time to a more holistic concept, one in which the stromal components are actively engaged in functional interactions with the

lymphoid and myeloid cells, providing survival signals, pathways for migration, channels for delivery of antigens and inflammatory stimuli, and chemokine cues that help establish the B and T cell zones of SLOs. Stromal networks are a dynamic component of SLOs, and consist of a diversity of cell types with distinct but overlapping functions. At least some stromal cells contribute to tolerance induction rather than active immunity. Cells in the stroma are the targets of infectious agents, either as direct sites for replication or as substrates for functional changes that in turn affect adaptive immune responses. In short, the stromal components of the immune system are key players, yet about whose biology we know much less than the haematopoietic cells we typically consider to constitute the building blocks of host defence. If we are to better understand the immune system as a whole, then a deeper understanding of stromal cell biology, from cellular heterogeneity and plasticity to molecular composition to intercellular communication mechanisms and more, is surely a key element in moving towards that goal. Such increased knowledge of the biology and function of stromal cells could provide avenues of therapeutic value for vaccine design or the treatment of autoimmune diseases and infections.

BOX 1

Stromal elements of tertiary lymphoid tissues

Tertiary lymphoid tissues (TLT) can form at sites of inflammation in organs and peripheral tissues. They are induced by acute inflammation, for example in the lungs after respiratory influenza virus infection¹²⁴. Other viral and bacterial infections also induce TLT in mice and humans (reviewed in¹²⁵). Chronic inflammatory states, such as those accompanying autoimmune reactions including arthritis, diabetes, and multiple sclerosis, also induce TLT formation¹²⁵. The signals that drive TLT formation remain contentious^{126, 127}. However, many studies have used transgenic expression of chemokines or cytokines to drive TLT formation¹²⁵, suggesting that the dissection of these pathways could benefit from a greater focus on more relevant infection- or autoimmune-induced inflammation models.

It is of particular interest that TLT develops many of the structural characteristics of secondary lymphoid organs (SLOs). These include peripheral node addressin (PNAd)⁺ high endothelial venules, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1)⁺ lymphatics, organized B and T cell zones, and stromal cells, which express CCL21 and CXCL13. TLT structures can be loosely arranged and have fewer of the elements that make up SLOs, or they can form large highly organized tissues, corresponding with significant local inflammation and considerable expression of lymphotoxins and chemokines. Although relatively little is known about the structure and function of stromal cells in TLT, ER-TR7⁺ fibroblasts are present that express CCL21, as well as CD35⁺ or CD21⁺ stromal cells expressing CXCL13^{124, 128}. It is unclear whether these stromal networks form conduits and provide traction for lymphocyte motility in these tissues, as they do in SLOs. Understanding how the stromal networks in TLT can influence immune cell trafficking, survival, and differentiation into pathogenic effector cells is of considerable significance for determining ways to modulate these structures during inflammatory diseases and devise therapeutic interventions to benefit the patient.

Online summary

- The immune system combats invading pathogens with a broad cellular and molecular arsenal. Integral to this defence are the secondary lymphoid organs (SLOs). It has been increasingly recognized in recent years that the non-haematopoietic stromal cells that make up much of the structure of the SLOs play many important roles in immunity.

- The spleen and lymph nodes have a complex microarchitecture. Lymph nodes have T and B cell zones in the cortex and networks of specialized blood and lymphatic vessels that snake through the organ. The spleen consists of T and B cell zones in the white pulp surrounding central arteries, around which is a marginal zone separating the lymphoid compartments from the red pulp.
- Heterogeneous populations of non-haematopoietic stromal cells are present and occupy distinct topographic locations in SLOs. These include the fibroblastic reticular cells (FRCs), follicular dendritic cells (FDCs), marginal reticular cells (MRCs), red pulp fibroblasts, vascular endothelial cells (including HEV in lymph nodes), and lymphatic endothelial cells.
- SLO stromal cells (in particular FRCs and FDCs) produce chemokines critical for the migration and motility of T and B lymphocytes into and within SLOs (CCL19/21 and CXCL13, respectively).
- Subsets of SLO stromal cells interact with antigen presenting cells, in particular dendritic cells (DCs). Interactions between DCs and stroma, and chemokines produced by both cell types, help induce and shape immune responses.
- Stromal cell subsets in SLOs are also involved in the homeostasis of lymphocytes via the production of CCL19 and interleukin-7 (FRCs) or BAFF (FDCs). Certain SLO stromal cells also contribute to tolerance induction rather than active immunity via expression of Aire.
- Regulation of SLO stromal cell function during inflammation can control immune responses through changes in chemokine production and cellular input or output from the tissues.
- A number of pathogens target SLO stromal cells through direct infection of these cells or indirect changes to stromal cell function. This has important consequences for SLO function and immunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biography

Scott N. Mueller

Scott N. Mueller received his Ph.D. (for research with F. Carbone) from the University of Melbourne, Australia, and undertook postdoctoral training at Emory University, Atlanta, Georgia (with R. Ahmed) and then at the National Institute for Allergy and Infectious Disease at the National Institutes of Health, Bethesda, Maryland (with R. Germain). He is currently an Australian Research Council research fellow in the Department of Microbiology and Immunology at the University of Melbourne, Australia. His research involves analysis of stromal cells and *in vivo* imaging to examine stromal cell networks and host-pathogen interactions.

Ronald N. Germain

Ronald Germain received his M.D. and Ph.D. (for research with B. Benacerraf) in 1976 from Harvard University, Boston, Massachusetts, USA. Since then, he has investigated basic T cell immunobiology, first on the faculty of Harvard Medical School, Boston and, since 1982, in the Laboratory of Immunology at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. Over the years, he and his colleagues have contributed to our understanding of MHC class II structure–function relationships, the cell biology of antigen processing and the molecular basis of T-cell recognition, especially the role of self-recognition and the organization of signaling networks involved in ligand discrimination. More recently, his laboratory has been focused on live imaging of the immune system and the development of new methods for quantitative modeling and simulation of biological processes.

Glossary

Primary lymphoid tissue, The primary lymphoid organs are the thymus and bone marrow, which are involved in the development of mature T and B lymphocytes, respectively.; Secondary lymphoid tissue, The secondary lymphoid organs, including the spleen, lymph nodes and mucosa-associated lymphoid tissues, assist in the induction of immune responses by capturing and presenting antigens and facilitating interactions between cells of the innate and adaptive immune system.; Multi-photon laser scanning microscopy, A fluorescence-imaging technique that takes advantage of the fact that fluorescent molecules can absorb two photons nearly simultaneously during excitation before they emit light. This technique utilizes infrared lasers that reduce heat damage and light scattering and allow imaging deeper into tissues, permitting study cellular interactions in real time.; Peyer's patches, Groups of lymphoid nodules present in the small intestine (usually the ileum). They occur massed together on the intestinal wall, opposite the line of attachment of the mesentery. Peyer's patches consist of a dome area, B cell follicles and interfollicular T cell areas. High endothelial venules are present mainly in the interfollicular areas.; High endothelial venule, A specialized venule that occurs in secondary lymphoid organs, except the spleen. HEVs allow continuous transmigration of lymphocytes as a consequence of the constitutive expression of adhesion molecules and chemokines at their luminal surface.; Follicular T helper cells, A CD4+ T helper cell that is essential in determining B cell antibody class switching, which localizes to B cell follicles during immune responses.; *plt/plt* mice, Paucity of lymph-node T cells (*plt*). A murine mutation that leads to loss of expression of the chemokines CCL21 (*CCL21-ser* gene) and CCL19 in lymphoid organs, resulting in altered lymphoid architecture and migration of CCR7-expressing T cells and mature DCs.; Autoimmune regulator (*Aire*) gene, The autoimmune regulator (*Aire*) gene is a transcription factor expressed mainly in the thymus by medullary thymic epithelial cells that is involved in the transcription of diverse tissue-specific antigens that once processed and presented promote negative selection of self-reactive T cells..

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Figure 1. Organized architecture of the spleen

Schematic representation of the organization of the spleen (left panel). The white pulp consists of T cell zones (also known as the periarteriolar lymphoid sheath (PALS)) containing networks of fibroblastic reticular cells (FRC) surrounding a central arteriole, together with B cell follicles containing a central network of follicular dendritic cells (FDC). Marginal zones (MZ) surrounding the white pulp contain marginal reticular cells (MRC), particularly at the edges of the B cell follicles. Blood and leukocytes entering the spleen pass through branches of the central arteriole, which end in the marginal sinuses and red pulp. In the cords of the red pulp, a dense network of reticular fibroblasts and fibres construct an open blood network, which is marked by its lack of a typical endothelial cell lining. Large numbers of macrophages phagocytose dying or damaged red blood cells in the red pulp (not shown). Immune cells enter the white pulp at regions where the T cell zones abut the MZ, known as the MZ bridging channels. An image of a section of mouse spleen generated using multicolour immunofluorescence microscopy illustrates the organization of the white pulp, red pulp, and MZ (centre panel). The distribution of CD3⁺ T cells (white), B220⁺ B cells (blue), CD169⁺ MZ macrophages (cyan), CD11c⁺ dendritic cells (DCs) (green), and ER-TR7⁺ stromal cells (red) is shown. The distinct organization of stromal cells in different regions of the spleen is shown by single-colour immunofluorescence staining (right panel). Networks of stromal cells and reticular fibres form in the white pulp, including the fibroblastic reticular cells (FRCs) in T cell zones, follicular dendritic cells (FDCs) in B cell follicles (ER-TR7⁻) and marginal reticular cells (MRCs) in the MZ. A dense network of stromal cells and reticular fibres is present in the red pulp. Scale bars represent 130 μ m.

Figure 2. Organized architecture of lymph nodes

Schematic representation of the organization of a lymph node (left panel). Afferent lymphatics enter lymph nodes and deliver lymph to the subcapsular sinus (SCS), which forms a channel around the periphery of the lymph node. Lymphatic sinuses run from the SCS through the cortex to the medulla, and exit the lymph node via efferent lymphatic vessels on the opposite, hilar, side of the organ. B cell follicles containing follicular dendritic cell (FDC) networks are arranged in the lymph node cortex and are separated from the SCS by a layer of marginal reticular cells (MRC). The T cells zones in the paracortex, which contain many fibroblastic reticular cells (FRC), are separated by the cortical ridge, an area rich in T cells, dendritic cells (DCs), blood vessels, and FRC. Blood vessels enter and exit the lymph node on the hilar side, and snake through the lymph node like the branches of a tree. Specialized high endothelial venules (HEVs) in the paracortex and cortical ridge allow entry of leukocytes from the blood. An image of a mouse popliteal lymph node generated using multi-colour immunofluorescence microscopy illustrates the distribution of CD3⁺ T cells (white), B220⁺ B cells (blue), CD11c⁺ DCs (green), LYVE1⁺ (Lymphatic Vessel Endothelial Receptor 1) lymphatics (cyan), PNAd⁺ (peripheral node addressin) HEVs (yellow), and ER-TR7⁺ stromal cells (red) (centre panel). The organization of stromal cells in the lymph node is shown by single-colour immunofluorescence staining for ER-TR7 (right panel). Scale bars represent 200 μ M.

Table 1
Stromal subsets in secondary lymphoid organs

The full version of this Table, including references, is available online. MALT, mucosa-associated lymphoid tissue; TLT, tertiary lymphoid tissue; SCS, subcapsular sinus; MZ, marginal zone; α -SMA, alpha-smooth muscle actin; LT β R, lymphotoxin-beta receptor; TNFR1/2, tumour necrosis factor receptor 1/2; ICAM1, intercellular adhesion molecule 1; VCAM1, vascular cell adhesion molecule 1; MAdCAM1, mucosal vascular addressin cell adhesion molecule 1; VEGF, vascular endothelial growth factor; IL-7, interleukin 7; BAFF, B cell activating factor; TRANCE, tumor necrosis factor-related activation-induced cytokine; PDGFR α/β , platelet-derived growth factor receptor α/β ; IL-6, interleukin 6; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; JAM-A, junctional adhesion molecule-A; S1P, sphingosine 1-phosphate; PNA α , peripheral node addressin; ESAM1, endothelial cell adhesion molecule.

	Tissue & location	Selected markers	Functions
Fibroblastic Reticular cells (FRC)	Lymph nodes, spleen, peyer's patches, MALT, TLT. T cell zones.	ER-TR7, podoplanin (gp38), laminin, desmin, fibrillin, fibronectin, α -SMA, LT β R, TNFR1/2, VCAM1, ICAM1, collagen I, II, IV, integrin α 1, α 4, β 1, MHC-I, VEGF, CXCL16, CCL21, CCL19, IL-7.	Structural support; production of reticular fibers; formation of conduit network; chemokine production & expression; substrate for lymphocyte migration; APC adhesion; T cell homeostasis; antigen presentation.
Follicular Dendritic cells (FDC)	Lymph nodes, spleen, peyer's patches, MALT, TLT. B cell zones.	CD35, CD21, CD16, CD23, CD32, FDC-M2 (complement C4), VCAM1, ICAM1, MAdCAM1, laminin, desmin, CXCL13, CXCL12, BAFF.	Antigen capture; presentation of immune complexes; chemokine production & presentation; B cell homeostasis.
Marginal Reticular cells (MRC)	Lymph nodes, spleen, peyer's patches, MALT. SCS (LN) & MZ (spleen).	ER-TR7, VCAM1, ICAM1, MAdCAM1, TRANCE (RANKL), laminin, desmin, podoplanin (gp38), 1BL-11, CXCL13.	Structural support; chemokine production; conduit function.
Red-pulp fibroblasts	Spleen.	ER-TR7, desmin, laminin, integrin α 3, α 4, α 5, β 1, PDGFR α/β , ICAM1, IL-6, CXCL12.	Construct splenic cords & direct blood flow; assist in removal of dying red blood cells; control of splenic blood flow, macrophage and plasma cell attraction and retention.
LN medullary fibroblasts	Lymph node	ER-TR7, desmin, laminin, collagen III, CXCL12(?).	Macrophage and plasma cell attraction and retention?
Lymphatic endothelial cells (LEC)	Lymph nodes, spleen (?), peyer's patches, MALT, TLT.	CD31, LYVE1, VCAM1, ICAM1/2, ER-TR7, podoplanin (gp38), laminin, VE-cadherin, claudin-5, JAM-A, Prox1, Toll-like receptors, CCL21, S1P.	Transport of lymph, antigens, & cells; Facilitate entry of leukocytes into lymphatics, chemokine production & presentation.
Vascular endothelial cells (VEC)	Lymph nodes, spleen, peyer's patches, MALT, TLT.	PNA α (LN), CD31, CD34, VE-Cadherin, laminin, JAM-A/B/C, ZO-1/2, ESAM1, claudin-5, CCL21 (mice).	Transport of blood; entry of cells including lymphocytes from the blood into tissues.

Table 2
Pathogens that target stromal cells in SLO

CMV, lymphocytic choriomeningitis virus; MCMV, murine cytomegalovirus; SIV, simian immunodeficiency virus.

Pathogen	Role of stroma	Refs
LCMV clone-13 (m) *	Infection of FRC (spleen & lymph nodes)	58
LCMV WE (m)	Infection of FRC & loss of gp38+ cells (spleen)	107
MCMV (m) - acute & latent	Infection of splenic endothelial cells.	114-116
Ebola, Marburg & Lassa	Infection of FRC (spleen & lymph nodes)	112,
(nhp)		113
<i>SIV (nhp)</i>	Decrease in CCL21 & CCL19 expression in LN	108
<i>Leishmania major (m)</i>	Infection of ER-TR7+ fibroblasts (lymph nodes)	109
<i>Leishmania donovani (m)</i>	Reduction in FDC & gp38+ FRC after infection (spleen)	110,
		111
<i>Plasmodium yoelii (m)</i>	Alteration to RP stromal cell function, & parasitization of RP stroma (lethal strain 17XL)	43, 117
Prion (m)	Associated with FDC & stromal fibroblasts in spleen & granulomas	118

* murine (m); human (h); nonhuman primates (nhp)