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## Lymph node fibroblastic reticular cells steer immune responses

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### Abstract

Lymph nodes (LNs), where immune responses are initiated, are organized into distinctive compartments by fibroblastic reticular cells (FRCs). FRCs imprint immune responses by supporting LN architecture, recruiting immune cells, coordinating immune cell crosstalk, and presenting antigens. Recent high-resolution transcriptional and histological analyses have enriched our knowledge of LN FRC genetic and spatial heterogeneities. Here, we summarize updated anatomic, phenotypic, and functional identities of FRC subsets, delve into topological and transcriptional remodeling of FRCs in inflammation, and illustrate the crosstalk between FRCs and immune cells. Discussing FRC functions in immunity and tolerance, we highlight state-of-the-art FRC-based therapeutic approaches for maintaining physiological homeostasis, steering protective immunity, inducing transplantation tolerance, and treating diverse immune-related diseases.

### Diversity of FRCs in mice and humans

Nonhematopoietic-derived FRCs in mammalian LNs crucially regulate innate and adaptive immune responses. By producing growth factors, chemokines, or cytokines or presenting antigens, FRCs support LN architecture and vasculature, generating extracellular matrix (ECM) components and choreographing the survival, activation, proliferation, differentiation, and crosstalk of immune cells [1,2]. Recent progress in single-cell genetic analyses and high-resolution imaging and the availability of various reporter mice have revealed the extensive heterogeneity of FRCs. From the outer layer of LNs, marginal reticular cells (MRCs) are located underneath the subcapsular sinus (SCS) and at the surface of the outer follicle (Figure 1, Key figure). The interfollicular FRCs (IFRCs) line the SCS and the lymphatic vessels coursing between follicles. In mouse LNs, follicular dendritic cells (FDCs) form a dense network inside the primary and secondary B cell follicles, and the

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Author contributions

L.L. and J.S.B. designed the structure and perspectives of the review and wrote the manuscript. J.W. screened literature information, drew the schematic cartoons, and revised the manuscript. R.A. and C.M.J. revised the manuscript.

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latter are further categorized into light zone (LZ) and dark zone (DZ) along with their own specific FDCs (LZ FDCs and DZ FDCs) [3]. T–B border reticular cells (TBRCs) are located at the T–B boundaries in mouse LNs [4]. The T cell zone (T-zone) is filled with T-zone reticular cells (TRCs). The deep cortex periphery (DCP) reticular cells (DRCs) fill the DCP [5] and the medullary reticular cells (MedRCs) form a dense network in the mouse LN medulla [2,6]. Murine FRC subsets originate from embryonic fibroblast activation protein- $\alpha$  (FAP)<sup>+</sup> mesenchymal **lymphoid tissue organizer (LTo) cells** (see Glossary) [7,8], although the mechanisms directing the differentiation into different FRC lineages are not well known.

Murine LN FRC subsets are distinctive in their anatomical position, phenotype, and functions (Table 1). For instance, B cell-interacting reticular cells (BRCs) include MRCs, LZ and DZ FDCs, and TBRCs [3]. In a murine model, RANKL<sup>+</sup> MRCs cooperate with lymphatic endothelial cells (LECs) to generate a macrophage supportive niche after inflammation [9]. FDCs differ from other subsets by lacking podoplanin (PDPN) – a mucin-type protein characterizing most other FRC subsets [3]. LZ FDCs are CXCL13<sup>+</sup>CXCL12<sup>-</sup> whereas DZ FDCs are CXCL13<sup>-</sup>CXCL12<sup>+</sup> [3]. BRCs are a prominent source of CXCL13, essential for B cell migration and differentiation and reminiscent of CCL21, which is produced by TRCs, and needed for T cell migration and differentiation [3]. One study recently reported that MRCs and FDCs produce CXCL13 and shape CXCL13 gradients; on binding ECM components, CXCL13 formed immobilized gradients in the B cell follicle that drove B cell trafficking [10]. Of note, there are two forms of CXCL13, immobile and soluble, which are regulated by cathepsin B (Cath-B), generating soluble CXCL13 [10]. Cath-B (*Ctsb*)-deficient (*Ctsb*<sup>-/-</sup>) mice have smaller LNs with variable B cell localization and defective follicle morphology compared with wild-type (WT) controls, suggesting that soluble CXCL13 might play a key role in follicle formation [3,10]. Moreover, TBRCs support **plasmablasts** at the interface between the germinal center (GC) and the T-zone by producing a proliferation-inducing ligand (APRIL), CXCL12, and IL-6 and thus protecting the early source of **affinity matured antibodies** [4,11]. In the T-zone, TRCs generate soluble and immobilized CCL21 that triggers CCR7<sup>+</sup> immune cell chemotaxis [12]. MAdCAM and BP3 (CD157) define MRCs (MAdCAM<sup>+</sup>BP3<sup>+</sup>), MedRCs (MAdCAM<sup>-</sup>BP3<sup>-</sup>), and TRCs (MAdCAM<sup>+</sup>BP3<sup>-</sup>) [11]. Perivascular reticular cells (PRCs) are CD34<sup>+</sup> and surround high endothelial venules (HEVs) and other blood vessels [8,13]. However, the function of LN PRCs is not well delineated. One study showed the triggering of PDGFR $\beta$ <sup>+</sup> perivascular stromal cells by agonistic antilymphotoxin- $\beta$  receptor (LT $\beta$ R) monoclonal antibodies (mAbs) and tracked FRC cluster marker genes, including *Mfge8*, *Cxcl13*, *Vcam1*, *Icam1*, *Madcam1*, *Prp*, *Cr2*, and *Pdgfrb*, which demonstrated that PRCs are likely to harbor adult progenitors of other FRC subsets, although this possibility remains to be rigorously tested [14]. TRCs, producing CCL19, CCL21, CXCL12, and IL-7 [15], include three subclusters: CCL19<sup>low</sup> TRCs are located at the T–B border; CXCL9<sup>+</sup> TRCs populate interfollicular regions; and CCL19<sup>hi</sup> TRCs ensheath **lymphatic conduits** and secrete ER-TR7 (a protein produced by FRCs whose encoding gene and molecular structure are unknown), laminins, nidogen-1 (also known as entactin, a basement membrane glycoprotein alongside collagen IV), fibronectin, and collagen I, IV, and XIV, as well as other stromal components constituting the conduit network [8,16–18]. Recent investigations have defined additional and sometimes rare FRC clusters. For instance, one study defined nine distinct

murine FRC clusters [6] and another report recently defined Gremlin1<sup>+</sup> FRCs at T–B cell boundaries in human and mouse LNs [19]. Identifying the functions of FRCs is necessary to understand the LN microenvironment, and to that end various transgenic reporter mice and pharmacological interventions as well as intensive investigations using inflammatory and tolerant animal disease models have been conducted.

In this review, we summarize the most recent insights into LN FRC heterogeneity (Table 1). We update LN FRC structural and secretome remodeling during inflammation and dissect FRC–T cell interactions during T cell activation as well as FRC–B cell crosstalk during GC responses. Summarizing the roles that FRCs play in homeostasis, immunity, and tolerance, we delve into FRC–based therapeutic strategies. Finally, we emphasize the contribution of novel transgenic mouse models to the assessment of specific functions of FRC subsets as well as the current limitations and prospects that exist for FRC therapeutic strategies in mice and humans. Recent discoveries demonstrate that LN FRCs not only sense immune stimuli but also regulate the immune response. Hence, deep insight into their functions might offer a broader set of mechanisms of action for translational medicine.

## LN remodeling during inflammation

Inflammation stimulates rapid FRC expansion in mouse LNs, resulting in LN swelling, immune cell recruitment, vascular expansion, and alteration of conduit transport function [20,21]. Moreover, inflammation stimulates FRC expansion and upregulates CCL19, CCL21, and IL-7 in mouse LNs, which then recruit T and B cells and dendritic cells (DCs) into mouse LNs [15,22]. The recruited cells in turn can alter FRCs: for example, DCs can regulate FRC microtubule networks and thus FRC adhesion and contractility through PDPN–C-type lectin-like receptor 2 (CLEC-2) in mice [23,24]. Moreover, B cells can promote FDC proliferation and vice versa [25]. For instance, in the LNs of mice infected with the helminth *Heligmosomoides polygyrus*, B cell lymphotoxin (LT) triggers the production of transcription factor B cell-activating factor (BAFF) on FDCs [25]. Subsequently, BAFF works synergistically with IL-4 to increase B cell LT, thus forming a positive feedback loop linking B cell LT, FDC BAFF, and IL-4 promoting FDC expansion, subsequently resulting in LN swelling and increasing the vasculature relative to uninfected mice [25]. Moreover, as identified in murine models, LN vascular endothelial cells proliferate by 2 days post-immunization with Complete Freund's Adjuvant and keyhole limpet hemocyanin (CFA/KLH), increasing LN blood vasculature and altering the conduit system relative to naïve LNs [26]. Of note, the conduit network in the T-zone becomes intermittent during acute LN expansion in mice, suggesting that there is disruption in the flow of low-molecular-weight molecules (<70 kDa) including antigens, cytokines, and DCs [24]. FRCs can reduce matrix production during LN expansion, leading to partially disrupted conduit flow, as evidenced by inflamed LNs in mice that were immunized with Incomplete Freund's Adjuvant and ovalbumin (IFA/OVA) [24]. Furthermore, as shown in mouse LN B-follicles, FDCs support the conduits that serve as channels for the transportation of lymph-borne antigens and CXCL13 [27]. Conduits are the sole egress route for IgM, which must be rapidly exported to the periphery for acute defense against pathogens, as shown in mice [27]. Of note, inflammation induces murine **FRC 'stretching'**, which promotes conduit permeability, thus enhancing IgM distribution [27]. Overall, in

response to inflammatory stimuli, FRCs support LN structural changes through secretome remodeling and by interacting with leukocytes and endothelial cells.

### FRC–T cell crosstalk during T cell activation

TRCs can directly and indirectly regulate T cell positioning, survival, and differentiation. For indirect effects, FRCs can form different niches that facilitate T–DC interactions [1,15,28]. For direct regulation, mouse and human FRCs can limit T effector functions through nitric oxide (NO) or constitutive cyclooxygenase (COX) enzymes [1,8]. As revealed by co-culture of human FRCs with CD4<sup>+</sup> or CD8<sup>+</sup> T cells, FRCs can also mediate **T cell energy** by expressing indoleamine-2,3-dioxygenase, the adenosine 2A receptor, prostaglandin E<sub>2</sub>, and the transforming growth factor beta receptor (TGFβR), which act to constrain the proliferation of both naïve and preactivated T cells, skewing their differentiation away from a central memory T cell phenotype [29]. Besides constraining T cell expansion, one study demonstrated that by secreting IL-6, *in vitro*, co-cultured mouse FRCs enhanced IL-2 and tumor necrosis factor (TNF) production in CD8<sup>+</sup> T cells [30]. Moreover, as evidenced by flow cytometric analysis of adoptive transferred CD8<sup>+</sup> T cells into influenza-virus-infected mice, FRCs augmented CD8<sup>+</sup> T cell differentiation into **tissue-resident memory CD8<sup>+</sup> T cells** [30]. Of note, additional TRC-derived molecules, including IL-7, IL-15, IL-33, delta-like 4 (DLL4), CXCL12, CCL19, CCL21, and CD40, can also support CD4<sup>+</sup> and CD8<sup>+</sup> T cell survival and proliferation as well as migration (summarized in [1]). Activated T cells can in turn regulate the FRC phenotype and function [30]. For instance, co-culture of activated mouse CD8<sup>+</sup> T cells with TRCs can trigger TRCs to produce more immunostimulatory molecules, such as ICOS ligand, CD40, and IL-6 [30]. Moreover, in murine models of experimental autoimmune encephalomyelitis (EAE) – a model for multiple sclerosis – and dextran sulfate sodium (DSS)-induced colitis, T-cell-derived IL-17 can support FRC metabolic fitness and proliferation, thereby contributing to humoral immune responses, as evidenced by antibody production [31]. However, how FRCs balance their immunostimulatory and immunosuppressive influences and functions is not well understood. Further elucidation of the mechanisms underlying FRC–T cell crosstalk can enrich our understanding of FRC-driven immune responses, in turn inspiring new candidate therapeutic approaches to treat viral infections or inflammatory diseases.

### BRC–B cell crosstalk during GC responses

GC responses require the coordinated migration of B cells into and within follicles. As indicated in the mesenteric LNs (mLNs) of mice infected with the intestinal helminth *H. polygyrus bakeri* (*Hpb*), activated B cells accumulate in B cell follicles, which triggers FDC expansion via LTβR signaling and leads to GC formation [32]. CCL19/CCL21 and CXCL13 gradients recruit T and B cells into LNs, as shown in murine models [8]. Driven by CXCL12 from DZ FDCs, activated T and B cells move into the DZ, where **somatic hypermutation (SHM)** and proliferation ensues (Figure 2) [33]. CXCL13 then directs B cells to move to the LZ to receive antigen from FDCs to then undergo **antibody affinity maturation** [34]. B cells with low affinity undergo apoptosis [35]. High-affinity B cells present acquired antigens to **T follicular helper (T<sub>FH</sub>)** cells and receive ‘help’ from those T<sub>FH</sub> cells for **immunoglobulin class switching** [36]. B cells then differentiate into long-lived antibody-

producing plasma cells or memory B cells or undergo apoptosis [36,37]. As demonstrated by labeling LNs from *Ccl19-Cre* × *iDTR* mice and *Pdgfrb-Cre* × *iDTR* mice, in which FRCs are depleted by treatment with diphtheria toxin (DT), FDCs arise from  $\text{Pdgfr}\beta^+$  perivascular precursors, and by producing IL-6 they promote SHM and IgG production; they also form a B-cell-favorable niche by producing BAFF, ICAM-1, VCAM-1, and IL-15 [38,39]. One study revealed that in the 564 Igi murine model of lupus, interferon alpha ( $\text{IFN}\alpha$ ) from FDCs sustained long-term GC responses and antibody production, suggesting that FDCs might represent a candidate therapeutic target in lupus, although this warrants robust testing [40]. Using the novel *Cxcl13-Cre/TdTom* genetic mouse model, one report showed that the topological remodeling of LZ and DZ FDCs, driven by CXCL12-mediated crosstalk with B cells, dictated the GC response. Mechanistically, topological organization of FDCs retained B cells in the follicle and enriched  $\text{T}_{\text{FH}}$  cells in the LZ in a CXCL12-dependent manner, thus steering B cell interactions towards  $\text{T}_{\text{FH}}$  cells [3]. Of note, the molecular identity and spatial positioning of BRCs appear to be predetermined under steady-state conditions in primary B cell follicles and remain fixed during inflammatory responses [3]. The mechanisms determining the stability of BRC molecular identity and spatial positioning are unknown. Overall, poised BRCs can accommodate GC responses by supplying a scaffold for B cell migration, generating CXCL12 and CXCL13 gradients, secreting B survival factors such as BAFF, or presenting antigens or through topology remodeling of FDCs, ultimately generating memory B cells and long-lived plasma cells [41].

## FRC functions during immune responses

### FRCs can regulate immune homeostasis

FRCs can maintain immune homeostasis and protect tissues from autoimmune attack. For instance, as discussed earlier, mouse and human TRCs can restrict T cell activation through various molecules such as NO, COX, indoleamine-2,3-dioxygenase, the adenosine 2A receptor, prostaglandin  $\text{E}_2$ , and the  $\text{TGF}\beta\text{R}$  [1,8,29]. As revealed in murine models, TRCs also restrict T cell responses by presenting peripheral tissue-restricted antigens [15,42]. TRCs induce deletional tolerance of MHC class I-restricted  $\text{CD8}^+$  T cells and trigger hyporesponsiveness of MHC II-restricted  $\text{CD4}^+$  T cells in mice [28]. FRC MHC class II also maintains homeostatic **regulatory T cells (Tregs)** in mouse LNs [42], which are central to immune homeostasis [43]. As unveiled in murine models, IL-33 is primarily secreted by TRCs and MedRCs [44] and sustains Tregs, group 2 innate lymphoid cells (ILC2s), and macrophages [45–47], but suppresses proinflammatory **type 1 T helper (Th1) cells** [48]. Relative to WT littermates,  $\text{Pdgfr}\beta\text{-Cre}^{+/-}$  × Laminin  $\alpha 5^{\text{fl/fl}}$  mice lacking laminin  $\alpha 5$  in LN FRCs harbor increased numbers of Tregs in the T-zone [49] overlapping with an IL-33-rich area [44], suggesting that IL-33 might contribute to a pro-tolerant LN niche by supporting Tregs. Overall, by producing functional molecules, presenting autoantigens, and secreting cytokines, FRCs can support pro-tolerant immune cells and constrain proinflammatory cells, thereby contributing to immune homeostasis and self-tolerance. Of note, as examined in mouse spleens, FRCs that were directly infected with lymphocytic choriomeningitis virus (LCMV) clone 13 upregulated the expression of **programmed death ligand 1 (PD-L1)** [50]. Moreover, blocking PD-L1 via an anti-PD-L1 antibody induced severe immunopathology in lymphoid FRCs, suggesting that the PD-L1 signaling pathway



protected FRCs from being destroyed by LCMV [50]. PD-L1 upregulation, however, also resulted in viral persistence and immunosuppression during chronic infection [50,51], suggesting that FRCs might play a role in balancing immune homeostasis and host defense against chronic infections.

### Targeting FRCs to maintain immune homeostasis

**Crescentic glomerulonephritis** is an inflammatory disease featuring the rapid deterioration of kidney function. In the draining LNs (dLNs) of mice with crescentic glomerulonephritis, activated FRCs contribute to the inflammatory milieu by overproducing ECM components [52]. The conditions of the surgical removal of kidney dLNs, depletion of FRCs in CCL19-Cre  $\times$  iDTR mice treated with DT, or blockade of PDPN using an anti-PDPN antibody could each ameliorate murine kidney injury relative to controls [52]. Moreover, in a murine model of cecal ligation and puncture (CLP) sepsis, intraperitoneal administration of *ex vivo*-expanded FRCs after sepsis onset reduced mortality [53]. Mechanistically, FRC administration prevented sepsis-induced apoptosis of splenic leukocytes and reduced the expression of proinflammatory cytokines including TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IFN $\gamma$ , IL-17, and IL-6 (assessed from peritoneal lavage and blood samples) relative to controls [53]. Also, in murine infection models with *Yersinia pseudotuberculosis*, irreversible disruption of lymphoid tissue homeostasis has been reported to persist for a long time [54]. This is relevant as it can potentially lead to further disease or immune dysfunction and interfere with tissue immunity because of a failure to achieve tissue structure restoration [54]. Such an effect is referred to as '**immunological scarring**' [54], which can be resolved by delivering healthy FRCs to mice [55]. For instance, repetitive renal ischemia-reperfusion injury (IRI) can lead to immunological scarring, as evidenced by fibrotic kidney dLNs and FRC senescence (mechanisms still undefined) [55]. Adoptive transfer of 'normal' FRCs into IRI mice has led to migration of these into abnormal kidney dLNs, thus leading to the restoration of normal LN architecture and amelioration of LN fibrosis, relative to control mice not receiving FRC administration [55]. Overall, these studies suggest that FRCs might represent efficient targets for the prevention of immunopathology in murine lymphoid tissues; they also suggest that protection of FRC integrity might be an efficient strategy to reverse immunological scarring and preserve LN function under certain pathological conditions, which certainly merits further investigation.

### FRCs in immune activation

To trigger effective immune responses, naïve T and B lymphocytes must enter LNs through HEVs to encounter antigens [56]. HEV integrity requires interactions between TRCs and platelets through PDPN/CLEC-2 to prevent hemorrhage into the LNs, as unveiled using Pdpn<sup>fl/fl</sup>  $\times$  Pdgfr $\beta$ -Cre and Clec-2<sup>fl/fl</sup>  $\times$  Pf4-Cre mouse strains lacking FRC PDPN and platelet CLEC-2, respectively [57]. In mice, B cells entering LNs undergo GC responses for antibody production and, as discussed earlier, BRCs are essential for this process [3]. Using *Ccl19-Cre* mice, one study demonstrated that type I IFN $\alpha$  receptor (IFNAR) signaling in CCL19<sup>+</sup> FRCs was required for innate and adaptive immune cell activation [58]. Specifically, prior to LCMV Armstrong (Arm) exposure, IFNAR signaling in CCL19<sup>+</sup> FRCs could poise gene expression towards an antiviral innate state that included the expression of MHC I and II at the transcriptional and protein levels; moreover, upregulation of the *Fit27*

and *Gas6* genes was associated with antiviral responses, *B2m* and *H2d1* were linked to antigen presentation, and *Ly6a*, *Ltc4s*, and *Sparc* were associated with immune cell activation [58]. After infection, IFNAR signaling drove immunostimulatory reprogramming of FRCs, including increased expression of genes involving IFNAR signaling, antigen presentation, and chemokines [58]. Moreover, the expression of the cell 'exhaustion' marker programmed death 1 (PD-1) was significantly higher in LCMV Arm-specific CD8<sup>+</sup> T cells from *Ccl19-Cre* × *Ifnar<sup>fl/fl</sup>* mice compared with *Cre*-negative controls, demonstrating that IFNAR-dependent remodeling of FRCs could protect LCMV-specific CD8<sup>+</sup> T cells from **T cell exhaustion**. [58]. Additionally, IL-33 from FRCs also supported the expansion of antiviral CD8<sup>+</sup> T cells in mice infected with LCMV clone 13 [44]. The disparate reports on IL-33 activities suggest that this cytokine might be supportive for both pro-tolerant Treg and pro-immune T cells and the responses might be context dependent, which thus warrants further investigation to assess these differential outcomes [59]. Thus, the specific role of IL-33 in FRC support appears to be contextual, relying on the coordination of other coexisting cues in the microenvironment to ensure the right immune response at the right time.

### FRCs in cancer immunity

LN metastases have been accepted as key determinants of cancer progression. In the dLNs of mice having received subcutaneous injection of colon adenocarcinoma cells, the LN FRC network contributes to a premetastatic microenvironment, reflected by an increased laminin  $\alpha4:\alpha5$  ratio and suggesting suppression of an antitumor immune response [60]. In mice subcutaneously inoculated with B16.F10 melanoma cells, the FRC network in tumor dLNs can remodel at multiple levels, including the secretion of ECM components, chemokines, and cytokines as well as immune cell recruitment, activation, and differentiation; all of these factors can contribute to the establishment of an immunosuppressive niche to support the tumor [61]. In particular, TRCs in tumor dLNs downregulate CCL21 and IL-7 compared with TRCs from non-dLNs, thus limiting CD4<sup>+</sup> T cell priming [61]. In tumor dLNs from invasive breast cancer patients, discontinuous HEVs with thinning of the endothelium as well as dysregulated CCL21 production from perivascular FRCs have been observed [62]. Overall, in response to tumor-derived factors, LN FRC remodeling seems to occur at the transcriptional, translational, and structural levels, contributing to a pre-premetastatic immunosuppressive niche in LNs. A deep understanding of such a FRC-supported niche, as well as the definition of the tumorigenesis that is associated with various FRC phenotypes, may offer additional strategies to counter a tumor-favorable microenvironment and boost the antitumor properties of specific immune cells.

### FRCs can constrain excessive immune responses

Keeping immune responses at proper levels is important to avoid damage by hyperactive immune cells. As revealed in murine models, after the peak immune response, the expanded FRCs shrink back to homeostatic levels and downregulate CCL21, thereby retarding T cell priming and restricting excessive immune responses [2]. Restraint of antibody-forming cells is an efficient way to control immune responses, hence avoiding antibody-mediated autoimmunity. For instance, CCL2<sup>+</sup> FRCs in the T-zone and medulla of mouse LNs limit antibody-forming cell survival by attracting CCR2<sup>+</sup> monocytes [63]. The monocytes inhibit

plasmablast and plasma cell survival by producing reactive oxygen species [63]. In addition, in murine models, FDCs promote the removal of apoptotic GC B cells by secreting phosphatidylserine-binding protein milk fat globule epidermal growth factor 8 (Mfge8), which facilitates the ingestion of dying B cells [37]. Mfge8<sup>-/-</sup> mice lacking Mfge8 have excessive numbers of B cells and suffer lupus-like autoimmunity relative to WT controls, highlighting the role that FDCs play in restricting excessive immunity [35,64]. Taking these findings together, FRCs are highly involved in the dynamic regulation of the proper abundance of T and B lymphocytes in mouse LNs, which is critical to restrict unwanted immune responses and avoid autoimmunity.

## FRCs in tolerance

### Targeting FRCs for transplantation immunity

In murine allogeneic stem cell transplantation, **graft-versus-host disease (GVHD)** is a major risk factor for prolonged humoral immunodeficiency and vaccine unresponsiveness. It can cause irreversible damage to LN FRC networks and HEVs, leading to immunological scarring [65]. As identified in a murine model, the Fas and Fas ligand (Fas–FasL) cytotoxic pathway is involved in LN GVHD development through FRC damage [65]. Blocking the Fas–FasL pathway via a FasL mutation prevents FRC damage and hence, ameliorates murine GVHD [65]. Furthermore, in an allogeneic murine bone marrow transplantation model, DLL1/4-mediated Notch signals have been revealed as major regulators driving GVHD pathogenesis [66]. FRCs, which are a source for Notch ligands during alloreactive T cell priming, deliver Notch signals to donor CD4<sup>+</sup> T cells early after transplantation [66]. In *Ccl19-Cre*<sup>+</sup> mice, depletion of the *Dll1* and *Dll4* genes in FRCs and CD21/35<sup>hi</sup> FDCs can prevent GVHD [66]. Moreover, following allogeneic skin or heart transplantation in mice, donor mast cells migrate from the allograft to dLNs and stimulate the herpes virus entry mediator (HVEM) on FRCs, leading to FRC senescence with overproduction of collagen I relative to naïve LNs [16]. These changes can result in a proinflammatory niche in dLNs with immunological scarring after organ rejection, as evidenced by the significant fibrosis seen in dLNs [16]. Moreover, adoptive transfer of normal *ex vivo*-expanded FRCs to recipient mice can ameliorate fibrosis and improve allograft survival [16]. These results suggest that FRCs are prominent contributors to the proinflammatory milieu in LNs, at least in mice; this is relevant as LN damage can be controlled by systemic administration of non-activated FRCs in mice [16]. Therefore, protection of FRCs may represent an efficient strategy to maintain the normal microarchitecture in lymphoid organs, which can ideally avoid GVHD and improve transplant outcomes.

### Transplantation tolerance

Studies on transplantation tolerance have focused on: (i) defining the role of LN structures; (ii) dissecting cell and molecular interactions; and (iii) revealing decisive factors in balancing immunity versus tolerance. The migration of immune cells into LNs is usually considered a prerequisite to the achievement of immunity and is also required for tolerance induction, relying on integral LN structures supported by FRCs [67]. For instance, targeting of mouse LN stromal fibers using anti-ER-TR7 has altered the HEV basement membrane structure and CCL21 distribution within the LNs [67]. These changes resulted in impaired



Treg migration in LNs and ultimately caused allograft rejection in a murine cardiac transplantation model [67]. Furthermore, in terms of altering cell interactions, TRCs can regulate CD4<sup>+</sup> T cells through CD40–CD40L interactions [68]. Co-stimulatory blockade with an anti-CD40L mAb plus alloantigen delivery by donor-specific splenocyte transfusion (DST) induced alloantigen-specific tolerance in mice. As a result, treating the recipients with one dose of anti-CD40L mAb (i.v.) dramatically enhanced murine cardiac transplantation tolerance relative to controls [49].

From another angle, FRC-derived laminins are decisive factors that may contribute to the balancing of immunity versus tolerance. Specifically, laminin  $\alpha$ 4 and  $\alpha$ 5 expression and their distribution in the cortical ridge (CR) and basement membrane of HEVs have been correlated with tolerance and immunity, respectively; this was revealed in the dLNs of mice receiving subcutaneous injection of colon adenocarcinoma cells, in the mLNs of mice with colitis (transfer of colitis microbiota model), and in the LNs of mice immunized with DST and tolerized by DST + anti-CD40L mAb [60,69]. Those results demonstrated that mouse LN FRCs reacted to inflammatory or tolerogenic cues by expressing laminins, although the underlying mechanisms driving laminin expression await further study. *In vitro* assays also showed that laminin  $\alpha$ 4 suppressed CD4<sup>+</sup> T cell activation and favored Treg induction at the expense of the suppression of Th1, Th2, and Th17 polarization [60]. By contrast, laminin  $\alpha$ 5 had opposite effects [60]. CD4<sup>+</sup> T cells recognized laminin  $\alpha$ 5 via integrin  $\alpha$ 6 for activation and Treg differentiation. During Th17 differentiation, T cells recognized laminin  $\alpha$ 5 via the receptor  $\alpha$ -dystroglycan (DG) [60]. Depletion of LN FRC laminin  $\alpha$ 5 in Pdgfr $\beta$ -Cre<sup>+/-</sup>  $\times$  Laminin  $\alpha$ 5<sup>fl/fl</sup> mice increased HEV size and number and increased Treg numbers in the T-zone relative to Cre-negative littermates [49]. Moreover, depletion of FRC laminin  $\alpha$ 5 in this study also improved murine cardiac allograft survival [49]. Collectively, these results demonstrated that modification of FRC-derived laminins could have a major effect on T cell differentiation and the balance between immunity versus tolerance, as shown in mice. Overall, LN FRCs can be recognized as key contributors of LN structures required for transplantation tolerance. We posit that the administration of FRCs to recipient mice, or modification of the phenotype and function of FRCs (e.g., modulation of genetic profiles), should be explored as a potential immunomodulation strategy to improve allograft survival outcomes.

### Concluding remarks

Immunotherapy has historically focused on hematopoietic cells, with far less consideration of the engineering of non-hematopoietic cells. FRC subsets constitute special niches supporting the trafficking, activation, differentiation, and crosstalk of immune cells in lymphoid organs. During viral infections, transplantation, autoimmune diseases, and cancers, the FRC topological and secretory responses exert spatial and molecular regulation on immune cells, thereby steering immune responses. Recent evidence suggests that FRCs might be efficient targets for therapeutic immunomodulation, although this will require rigorous investigation.

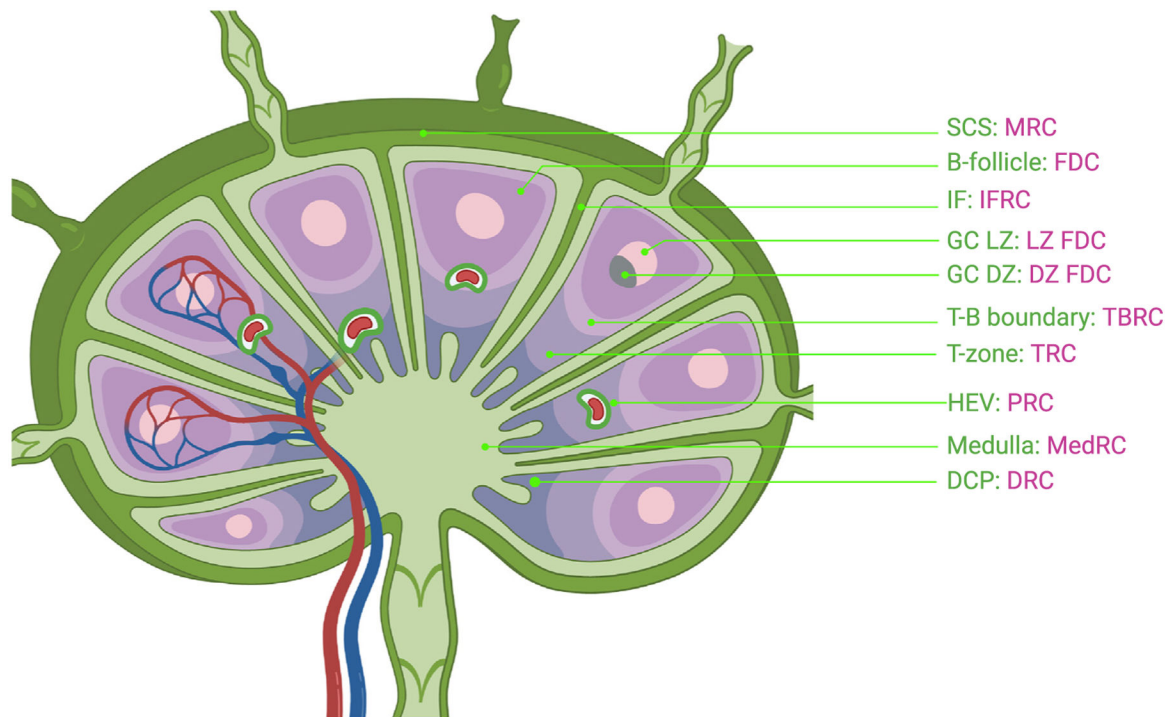
The adoption of several new array and histological technologies has advanced our understanding of many immune events. For instance, single-cell RNA-seq has characterized

further the molecular identities of FRCs in mouse and human LN, thymus, and lungs [70–73]. In addition, histocytometry is a tool that allows the spatial analysis and phenotyping of cell types *in situ* [74]. Also, **cytometry by time of flight (CyTOF)** analyzes cells through multidimensional deep phenotyping [75]. However, many of the investigations analyzing FRCs have been descriptive and thus now require deeper mechanistic insights into the phenotypes and functions of FRC clusters (see Outstanding questions). Recent advances have benefited from several highly advanced transgenic mouse models. For instance, one study developed a mouse model to trace cellular lineage based on the expression of Fap, a marker for FRCs [7]. Specifically, breeding transgenic Fap<sup>TA</sup> mice with Teto-Cre × Rosa26<sup>lox-stop-lox-tdTomato</sup> mice generated FC<sup>Tomato</sup> mice, allowing fate mapping of Fap-expressing cells on induction of tdTomato expression [7]. As a result, the origin of murine FRCs has been traced to embryonic FAP<sup>+</sup> mesenchymal LTo cells [7]. High-resolution imaging and histological analyses with novel transgenic mice will certainly prompt more delineations of property-determining genes in FRCs, which might ultimately contribute to the definition of new FRC subsets or identification of novel functions for known subsets.

FRC-based therapeutic approaches are currently focused on *ex vivo*-expanded FRCs or on the modulation of FRC molecules via genetic or pharmaceutical methods. However, many limitations and uncertainties exist. For example, current advanced mesenchymal stromal cell (MSC) therapies and various clinical factors can also dictate the outcome of FRC administration. These include: (i) cell manufacture (e.g., isolation method, origin tissue); (ii) cell administration (e.g., local or systematic injection); and (iii) recipient conditions (e.g., inflammation, cancer) [76]. The mechanisms underlying *in vivo* blood endothelial transmigration and the homing of fibroblasts to particular tissues are unknown. Thus, FRC administration approaches remain in the preclinical stage, and standardized and syngeneic FRCs are likely to be required. Moreover, *ex vivo*-cultured FRCs lack stimuli from B and T cells and DCs, raising questions about the immunological states of such FRCs. Most human LNs are harvested from patients harboring specific pathologies and, as discussed, FRCs are sensitive to inflammatory stimuli. Thus, human FRCs are not likely to exist in a homeostatic state *ex vivo* and their transfer could potentially exacerbate, rather than ameliorate, disease conditions. A strategy to meet these challenges may be to reverse the non-homeostatic phenotype of FRCs by bioengineering approaches (e.g., pharmaceutical blockade, genetic modification during FRC preparation) [76]. Of note, most therapeutic investigations to date have been based on major population subsets such as TRCs. However, novel advances might be attained when also focusing on smaller FRC subsets (e.g., LZ/DZ FDCs, MRCs, TBRCs, MedRCs). Overall, these promising exploitations might broaden the avenue for FRC-based immunomodulation attempts in pathologies such as cancers, autoimmune diseases, transplantation, and infectious diseases.

## Key figure

### Distinct fibroblastic reticular cell (FRC) subsets distribute and support different regions of mouse lymph nodes



**Figure 1.**

Diverse FRC subsets enrich and support different lymph node (LN) regions in mice. Marginal reticular cells (MRCs) line the floor underneath the subcapsular sinus (SCS) and at the surface of the outer B cell follicle (B-follicle) [3,78]. The interfollicular FRCs (IFRCs) line the SCS and the lymphatic vessels coursing between B-follicles [84]. The follicular dendritic cells (FDCs) form a dense network supporting primary B-follicles and germinal centers (GCs). Light-zone (LZ) and dark-zone (DZ) FDCs support the LZ and DZ, respectively [3]. T–B border reticular cells (TBRCs) are located at the T–B boundaries [4]. The T cell zone (T-zone) reticular cells (TRCs) form the network supporting the T-zone [6]. The deep cortex periphery reticular cells (DRCs) fill the deep cortex periphery (DCP) [5]. The medullary reticular cells (MedRCs) form a dense network in the medulla [2,6]. The perivascular reticular cells (PRCs) surround high endothelial venules (HEVs) and other blood vessels [8,13]. This figure was created using BioRender (<https://biorender.com/>).

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## Glossary

**Affinity matured antibodies**

antibodies produced by B cells after acquiring sufficient affinity for efficient antigen binding

**Antibody affinity maturation**

process whereby antibody affinity increases through SHM

**Crescentic glomerulonephritis**

pathological condition of the kidney; characterized by rapid loss of renal function through cellular inflammation and overgrowth in the glomerulus

***Cxcl13-Cre/TdTom***

mouse strain in which the *Cxcl13* promoter directs *Cre* recombinase and RFP (*TdTomato*) expression, making *Cxcl13*<sup>+</sup> cells display a red fluorescent signal

**Cytometry by time of flight (CyTOF)**

application of mass cytometry used to simultaneously quantify multiple targets on single cells

**FRC ‘stretching’**

morphological remodeling of FRCs in reactive LNs (e.g., promoting the transportation of IgM during immune responses).

**Graft-versus-host disease (GVHD)**

syndrome characterized by inflammation in various organs after allogeneic transplantation, with the transplant bone-marrow-derived donor lymphocytes recognizing and attacking recipient antigens

**Immunoglobulin class switching**

mechanism that changes an immunoglobulin from one isotype to another (e.g., from IgM to IgG).

**Immunological scarring**

irreversible effects on the structure of lymphoid organs caused by inflammation, impairing the ability to respond against new antigenic challenges

**Lymphatic conduits**

type of vasculature channeling small molecules from the LN SCS to the parenchyma.

**Lymphoid tissue organizer (LTo) cells**

precursors of non-hematopoietic stromal cells in secondary lymphoid organs

**Plasmablasts**

immature state of antibody-producing B cells.

**Programmed death ligand 1 (PD-L1)**

protein involved in restricting immune responses by binding to PD-1. This signaling axis inhibits T cell effector function and, for example, prevents T cells from killing PD-L1<sup>+</sup> cells such as cancer cells

#### **Regulatory T cells (Tregs)**

specialized CD4<sup>+</sup>Foxp3<sup>+</sup>CD25<sup>+</sup> T cells; important in regulating immune responses, maintaining tolerance to self-antigens, and preventing autoimmunity

#### **Somatic hypermutation (SHM)**

cellular mechanism that diversifies B cell receptors for recognition of new foreign antigens

#### **T cell anergy**

state where T cells are functionally inactivated in a hyporesponsive state

#### **T cell exhaustion**

state of T cell dysfunction defined by impaired effector function and the expression of inhibitory receptors

#### **T follicular helper (T<sub>FH</sub>)**

B-follicle T cell subset that mediates T cell help for B cells, including B cell survival, selection, and differentiation into either long-lived plasma cells or memory B cells

#### **Tissue-resident memory CD8<sup>+</sup> T cells**

group of memory CD8<sup>+</sup> T cells that persist in peripheral non-lymphoid organs

#### **Type 1 T helper (Th1) cells**

CD4<sup>+</sup> T cell subset producing TNF $\alpha$  and IFN $\gamma$  and stimulating cell-mediated immune responses, typically against intracellular bacteria and protozoa

#### **Type 2 T helper (Th2) cells**

CD4<sup>+</sup> T cell subset producing IL-4, IL-5, IL-9, and IL-13 and stimulating humoral immune responses against extracellular parasites

#### **Type 17 T helper (Th17) cells**

CD4<sup>+</sup> T cell subset producing IL-17; involved in host protection against extracellular bacteria and fungi

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### Highlights

High-resolution genetic and phenotypic studies have recently unveiled discrete fibroblast reticular cell (FRC) subsets in mice and humans; these harbor distinct functions and microanatomic distributions. FRC clusters create a variety of environmental niches in lymph nodes (LNs).

The B cell-interacting reticular cells (BRCs), including marginal reticular cells (MRCs), light-and dark-zone follicular dendritic cells (LZ/DZ FDCs), and T–B border reticular cells (TBRCs), establish a feed-forward system determining germinal center responses in mice. The molecular identity of LZ/DZ FDCs is predetermined in the steady state.

FRCs contribute to the regulation of inflammation-induced LN remodeling in mice and humans. Inflammation stimulates rapid FRC expansion, resulting in LN swelling, immune cell recruitment, vascular expansion, and alteration of conduit transport function.

FRCs contribute to the maintenance of immune homeostasis, thereby protecting tissues from autoimmune attack. The maintenance of FRC integrity can reverse immunological scarring and preserve LN function for immune activation and for anticancer immunity.

FRCs can constrain excessive immune responses. In murine transplantation, administration of FRCs to recipients or modification of FRC gene expression can ameliorate LN pathology and improve allograft survival.

### Outstanding questions

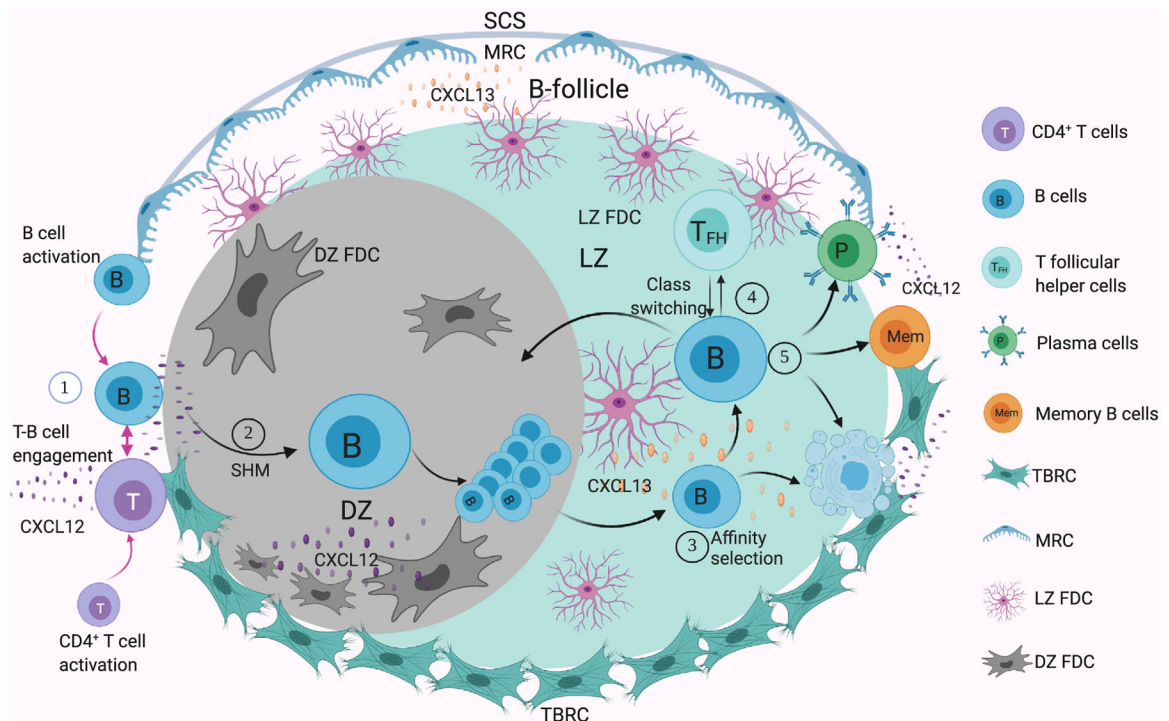
During ongoing immune reactions, is there crosstalk among FRC subsets? Understanding the interactions and transformation among FRC subsets may be helpful to clarify the functions of specific subsets at a higher resolution.

In the context of infectious diseases, three factors affecting immune cell epigenetic programming are: (i) direct infection; (ii) pathogen-associated molecular patterns from microorganisms; and (iii) cytokines [80]. Is FRC-mediated LN structural remodeling another factor regulating immune cell epigenetic programming? LN structural changes affect immune cell recruitment and vascular permeability, thus potentially serving as important epigenetic factors.

FDCs capture HIV-1 virions via CR1/2 and internalize them into non-degradable endosomal compartments, escaping surveillance by CD8<sup>+</sup> T cells. The HIV-1 virion is recycled back to the cell surface to infect surrounding T<sub>FH</sub> cells. What is the molecular machinery for antigen and HIV-1 endocytosis in FDC non-degradable compartments? Understanding this process may enable ongoing attempts to generate protective immunity against HIV-1.

Is it possible to create a favorable LN structure for tolerance before transplantation? Is it possible to create a tumor-reactive artificial LN niche favoring T cell responses, facilitating drug delivery, or promoting vaccination strategies? Modulation of LN niches might be helpful in improving transplantation outcomes, ameliorating certain immune diseases, or facilitating antitumor immunity.

Migrating immune cells encounter diverse signals from various FRC ligands. How are these signals hierarchically organized and how do immune cells integrate such complex signals? How do FRCs balance immune reactions and tolerance? Clarification of the proimmunity and pro-tolerant regulatory effects of FRCs is a prerequisite for putative therapeutic strategies that envision the modulation of gene expression in FRCs.



**Figure 2.**

B cell-interacting reticular cells (BRCs) support germinal center (GC) responses in mouse lymph nodes. During initiation of the GC response, ① CD4<sup>+</sup> T and B cells are activated and engaged outside GCs [35]. ② B cells move into the GC dark zone (DZ) and undergo somatic hypermutation (SHM) and proliferation [3,35]. ③ B cells migrate to the GC light zone (LZ), receive antigen and survival signals from follicular dendritic cells (FDCs), and undergo antibody affinity selection. B cells with low affinity undergo apoptosis [35,37]. ④ High-affinity B cells then present acquired antigens to, and receive help from, T follicular helper cells (T<sub>FH</sub>) for immunoglobulin class switching [35,36]. ⑤ Surviving B cells differentiate into plasma cells or memory B cells [35]. The remaining cells without help from T<sub>FH</sub> cells undergo apoptosis or return to GC DZs to repeat this cycle [35]. Abbreviations: MRCs, marginal reticular cells; TBRCs, T–B boundary FRCs. This figure was created using BioRender (<https://biorender.com/>).



Table 1.

## Murine LN FRC subset phenotypes and functions

FRC subset	Phenotype	Function
MRC	Madcam <sup>+</sup> BP3 <sup>+</sup> RANKL <sup>+</sup> CXCL13 <sup>hi</sup> CD21/CD35 <sup>-</sup> [3,10,77]	<ul style="list-style-type: none"> <li>Support ILC3s by secreting IL-7 [78]</li> <li>Support sinusoidal macrophages via RANKL [9]</li> <li>Attract CXCR5<sup>+</sup> DCs for type 2 immune responses [77]</li> </ul>
LZ FDC	CXCL12 <sup>-</sup> CXCL13 <sup>+</sup> CD21/CD35 <sup>hi</sup> [3]	<ul style="list-style-type: none"> <li>Orchestrate B cell clustering and follicle formation by CXCL13 [3]</li> <li>Present antigen via CD16 and CD32 [35,79]</li> <li>Support B cell survival via BAFF [6,72]</li> </ul>
DZ FDC	CXCL12 <sup>+</sup> CXCL13 <sup>-</sup> CD21/CD35 <sup>low</sup> [3]	<ul style="list-style-type: none"> <li>Drive B cells to GC DZ via CXCL12 [72]</li> <li>Form a meshwork in GC DZ [72]</li> </ul>
TRC	MAdCAM <sup>-</sup> BP3 <sup>+</sup> CCL2 <sup>hi</sup> CCL19 <sup>+</sup> CXCL12 <sup>+</sup> CXCL13 <sup>-</sup> CD21/CD35 <sup>-</sup> [6,11]	<ul style="list-style-type: none"> <li>Drive T and B cell and DC migration, via CCL19, CCL21, CXCL12 [15]</li> <li>Support T cells, lymphoid tissue inducer (LTi) cells, DCs, ILCs by secreting IL-7, IL-15, IL-33 [1,80-82]</li> </ul>
MedIRC	MAdCAM1 <sup>-</sup> BP3 <sup>-</sup> CXCL12 <sup>hi</sup> Leptin receptor <sup>hi</sup> CXCL13 <sup>-</sup> CCL21 <sup>low</sup> [11]	<ul style="list-style-type: none"> <li>Support macrophages and plasma cells via APRIL, IL-6, BAFF [11]</li> <li>Interact with antibody-secreting cells, NK cells, and macrophages [11]</li> </ul>
TBRC	CXCL12 <sup>hi</sup> BP3 <sup>+</sup> CCL19 <sup>+</sup> CCL21 <sup>+</sup> IL-6 <sup>+</sup> [3,8]	<ul style="list-style-type: none"> <li>Form a niche supporting DC and T and B cell interactions</li> <li>Support local plasmablasts via APRIL [4]</li> </ul>
IFRC	Ch25h <sup>+</sup> RANKL <sup>+</sup> MAdCAM1 <sup>-</sup> [3]	<ul style="list-style-type: none"> <li>Form a niche where the conduits extend from, transporting DCs and lymph-borne materials to the inner region of associated HEVs [83]</li> </ul>
DRC	CXCL13 <sup>-</sup> CD35 <sup>-</sup> ER-TR7 <sup>hi</sup> CXCL12 <sup>hi</sup> CCL21 <sup>hi</sup> [5]	<ul style="list-style-type: none"> <li>Form a dense irregular network in the DCP region by highly expressing desmin, ER-TR7, PDGFRβ, CCL19, and CXCL12 [5]</li> </ul>
PRC	CD34 <sup>+</sup> Madcam <sup>+</sup> Integrin α7 <sup>+</sup> [58]	<ul style="list-style-type: none"> <li>Surround HEVs and other blood vessels [8,13]</li> <li>Likely to harbor the adult progenitors of other FRC subsets [13,14]</li> </ul>