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Lymph node fibroblastic reticular cells in health and disease

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Abstract

Over the past decade, a series of discoveries relating to fibroblastic reticular cells (FRCs) — immunologically specialized myofibroblasts found in lymphoid tissue — has promoted these cells from benign bystanders to major players in the immune response. In this Review, we focus on recent advances regarding the immunobiology of lymph node-derived FRCs, presenting an updated view of crucial checkpoints during their development and their dynamic control of lymph node expansion and contraction during infection. We highlight the robust effects of FRCs on systemic T and B cell responses and present an emerging view of FRCs as drivers of pathology following acute and chronic viral infections. Lastly, we review emerging therapeutic advances harnessing the immunoregulatory properties of FRCs.

Lymph nodes are immunological meeting places, where T cells, B cells, dendritic cells (DCs), plasma cells and macrophages congregate inside an encapsulated mesenchymal sponge, created by a network of fibroblastic reticular cells (FRCs) and infiltrating lymphatics. The structure of the lymph node is crucial to its function, funnelling antigens and antigen-presenting cells towards rare antigen-specific lymphocytes to maximise their chance of finding each other. Put simply, when antigens meet T or B cells bearing receptors with sufficient affinity and in the appropriate molecular context, an adaptive immune response begins. Here, we discuss the implications of the role of FRCs in facilitating this process.

FRCs are immunologically specialised myofibroblasts **[G]** of mesenchymal origin1–5. They can be differentiated from other lymph node-resident cells by their expression of podoplanin (PDPN) and platelet-derived growth factor receptor-α (PDGFRA), and their lack of expression of CD45 and CD31. They express molecules common to many myofibroblasts, including desmin, vimentin, CD90, CD73, CD103, α-smooth muscle actin (αSMA) and the ERTR7 antigen12. Compared with dermal fibroblasts, FRCs also express a more

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A.L.F. is an inventor on a patent related to the therapeutic use of FRCs.

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immunologically focused gene signature, significantly enriched in genes from antigen presentation and cytokine response pathways[2]. FRCs are found in lymph nodes, spleen, thymus and other lymphoid tissues, but lymph node-derived FRCs are the best studied, and are the focus of this Review.

FRCs comprise 20–50% of the non-haematopoietic compartment in lymph nodes[6]. They form stellate cell–cell contacts to create a three-dimensional open network on which leukocytes migrate[4,7]. FRCs also produce and ensheath a highly-ordered, interconnected web of extracellular matrix (ECM) components, creating the conduit network, which rapidly transports soluble antigens and signalling molecules deep into the lymph node parenchyma[5]. This physical support function of FRCs in facilitating lymph node responses is reviewed in detail elsewhere[8]. Importantly, FRCs provide strength and flexibility to the lymph node, and impose compartmentalization of B and T cells, directing leukocyte traffic using chemokine secretion[1,3,4]. Naïve T cells and DCs are in constant contact with FRCs, migrating along the network while scanning each other for antigen-specific affinity[4]. This intimate contact puts FRCs at the front line of the immune response, where they fundamentally regulate adaptive immunity[2].

Recent advances in FRC biology have shown that the immunological impact of these cells extends beyond the lymph node. Here, we show that normal functioning of the FRC network is essential to immunological health. We describe the crucial molecular cues for FRC development and function, and discuss their role in the creation of the lymph node microenvironment, through interactions with T cells, B cells, DCs and high endothelial venules (HEVs). We discuss the systemic impact of these interactions, by examining newly reported models in which FRCs are deleted, and explore the concept of FRC dysfunction as a driving force for immunodeficiency. Finally, we present novel technological advances that seek to mimic or harness the functions of FRCs therapeutically.

A dual progenitor model of FRC development

Within lymph nodes, FRCs develop from a specialised stromal progenitor, termed lymphoid-tissue organiser (LTo) cells **[G]**. However, LTos are themselves a differentiated intermediate, and evidence was lacking for the identity of the earliest lymph node stromal progenitors. Here we review evidence for a model whereby dual progenitors contribute to the development of LTos. Newly reported developmental steps that differentiate LTos into FRCs are also discussed.

Subsets of FRCs

At least 5 subsets of FRCs have been described in lymph nodes, defined by their location and expression of functional markers. These are outlined in Table 1. As the delineation of FRC subsets is still in its infancy, many studies have referred collectively to these subsets as FRCs, and except where specifically identified in the primary source, we do the same here. T cell zone reticular cells are the best described FRC subset[1,7], followed by the marginal reticular cell subset, which can differentiate into follicular dendritic cells **[G]**[9]. Other subsets are newly described and remain to be fully defined. Precise functions of FRC subsets are discussed below.

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Molecular cues driving lymph node stromal development

The molecular cues and precise cell types that drive the development of lymph node mesenchymal stroma are still incompletely defined (Figure 1). A current model suggests that lymph node imprinting of mesenchymal precursors from which FRCs derive occurs when neuronal fibres release retinoic acid10. In response, these local undifferentiated mesenchymal precursors release C-X-C motif chemokine 13 (CXCL13) to initiate the lymph node anlagen10.

Newer evidence reveals a second stream of mesenchymal precursors that migrate in from adipose tissue adjacent to lymph node sites. During embryogenesis and postnatally, pre-adipocytes can be recruited from these fat pads into the lymph node in a Lymphotoxin β receptor (LTβR)-dependent manner11, with a partial requirement for CXCR412. Upon arrival in the lymph node, these cells differentiated into CXCL13+ early LTos. In adult mice, fat pad-derived stromal progenitors gave rise to approximately 60% of FRCs12. The evidence suggests that both locally derived and distal adipose-derived prescursors are likely to contribute to lymph node formation10,11,13.

CXCL13 attracts **haematopoietic** drivers of lymph node development known as lymphoid tissue-inducer (LTi) cells **[G]** (group 3 innate lymphoid cells; ILC3s), which express C-X-C chemokine receptor type 5 (CXCR5)14. The attraction of ILC3s is a crucial checkpoint in lymph node development15. However, *Cxcl13*$^{-/-}$ mice do not have a complete block in lymph node development; mesenteric and cervical lymph nodes develop, whereas cutaneous lymph nodes are lacking16. Other signals must drive lymph node development or compensate for the loss of CXCL13 in mesenteric and cervical locations.

After anlage specification, receptor activator of nuclear factor k B ligand (RANK-L) signals to RANK expressed by ILC3s. The origin of RANK-L is hypothesised to be ILC3s themselves, through trans-signalling as they cluster together13,17. The RANK signal induces LTis to upregulate expression of lymphotoxin α1β2 (LTα1β2)17.

For both types of stromal precursor, LTβR signalling is a crucial checkpoint that drives differentiation into LTo cells. LTo cells express LTβR, PDPN and CXCL13, and undergo LTβR-dependent upregulation of mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), CCL19, CCL21, intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)18. This expression profile attracts a new wave of ILC3s, thereby increasing the provision of lymphotoxin (LT) ligands and ILC3-attracting chemokines, which enables the hematopoietic compartment and mesenchyme to develop in parallel.

Lymph node stromal development is also dependent on poorly defined interactions between PDPN and its ligand C-type lectin receptor 2 (CLEC-2; gene name *Clec1b*), as *Pdpn*$^{-/-}$ and *Clec1b*$^{-/-}$ mice have lymph node anlagen but do not develop lymph nodes19,20. Mice with platelet-restricted deletion of CLEC-2 possess lymph nodes19, so the crucial source of CLEC-2 for lymph node development is still unconfirmed.

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Maturation and maintenance of FRCs

LTβR signalling is also required at later stages for the final maturation of FRCs. Immediate precursors of FRCs were found in mice where LTβR signals were conditionally blocked in CCL19$^+$ cells, in other words in and downstream of differentiated LTo cells21.These lymph nodes developed a mesenchymal network and a conduit system, but had overall decreased lymph node-wide expression of CCL19, CCL21 and IL-7, which are crucial molecules for the cross-talk with lymphocytes and DCs21. Distinct B and T cell zones were maintained in these lymph nodes, however21, so it is possible that low levels of chemokines are sufficient for lymphocyte organisation.

Studies in rhesus macaques and sooty mangabeys showed that depletion of CD4$^+$ T cells , but not CD8$^+$ T cells, ablated the FRC network, reduced the distinction between B and T cell zones, and decreased IL-7 staining22. Naïve CD4$^+$ T cells were the major source of LTα1β2 in these lymph nodes22, although the molecular mechanism of FRC loss remains to be determined. Interestingly, murine FRCs and marginal reticular cells do not seem to require T and B cells for development at normal frequencies23,24, although the expression of MAdCAM1 protein by marginal reticular cells is significantly reduced in lymphocyte-deficient *Rag1*$^{-/-}$ mice6.

Mature FRCs, particularly marginal reticular cells, show similarities to LTo cells. Both marginal reticular cells and LTo cells express LTβR, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), CCL19 and CCL2123. It is unclear whether the similarity extends to CXCL13 expression; FRCs depleted of MAdCAM1$^+$ cells had similar levels of CXCL13 transcript as sorted MAdCAM1$^+$ marginal reticular cells2, and although by histology CXCL13 seems to be largely restricted to subcapsular marginal reticular cells23, other studies have shown that intrafollicular FRCs (MAdCAM negative) strongly upregulate CXCL13 during infection25. Marginal reticular cells, but not T cell zone FRCs, express the LTo cell adhesion molecule MAdCAM123. Marginal reticular cells (Table 1) are thought to be the postnatal equivalent of LTo cells23,26, but this has not been formally demonstrated. However, Marginal reticular cells have been shown to differentiate into specialized stromal cells found in the B cell zone known as follicular dendritic cells (FDCs)9.

Known crucial checkpoints in FRC development are outlined in Figure 1. While aspects of FRC development remain to be fully defined, their function has been the subject of close scrutiny. Newly developed transgenic mice that allow FRC tracking and conditional deletion have advanced the field significantly in recent years. Here we discuss recent advances in our understanding of the control FRCs exert over immune responses.

Structural organization of lymph nodes by FRCs

Throughout postnatal life, FRCs keep lymph nodes in a response-ready state, through continual interactions with constituent leukocyte populations and other parenchymal cells (Figure 2). These interactions, here discussed, ensure lymphocytes are poised to respond to antigen and inflammation by generating an adaptive immune response.

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FRCs attract and maintain T cells

It is well-described that paracortical T cell zone reticular cells secrete CCL19 and CCL21 to keep $CCR7^{+}IL7R^{+}$ naïve T cells and DCs moving throughout the FRC network searching for antigen-specific interactions, while staying largely confined to the paracortex. FRCs also secrete IL-7, which promotes their survival1,4. Accordingly, deletion of FRCs depletes naïve T cells from lymph nodes and impairs their recruitment to these structures27.

FRCs support B cell survival

New lines of rapidly converging evidence also support a role for FRCs in the maintenance of B cells. It was initially reported that cultured FRCs promote the survival of malignant B cells, which otherwise die rapidly in culture28. FRCs transcribe the B cell growth factor *Baff* and B cell chemoattractant *Cxcl13*2,25, and an intriguing study using fate-mapped mice showed that an intra-follicular subset of $CD21^{-}$ FRCs becomes enveloped by expanding B cell follicles during infection, and that these FRCs rapidly upregulate CXCL13 protein expression during infection through cross-talk with B cells25. Most recently, *in vivo* FRC-depletion experiments27,29, which have been mechanistically dissected using *in vitro* co-cultures29, showed that FRCs promote B cell survival and control the boundaries of primary B cell follicles, through the provision of BAFF29. Importantly, deletion of FRCs in these models did not destroy the lymph node or deplete FDCs27,29 or activated T cells27.

FRCs maintain DCs and promote their migration

The effect of FRC depletion on DCs has also been studied27. Both conventional DCs ($CD8a^{+}$ and $CD8a^{-}$) and migratory DCs were significantly depleted when FRCs were absent from lymph nodes27. DC movement is an active process, requiring amoeboid movement and a scaffold on which to crawl30. Mechanistically, recent advances have shown that PDPN is a key molecule in many aspects of FRC function, including DC motility31. PDPN interacts with the CLEC2, which is upregulated by antigen-bearing DCs, and this interaction regulates the formation of membrane protrusions and motility in DCs31.

FRCs control HEV permeability

FRCs are known to control lymph node endothelial cell proliferation through vascular endothelial growth factor (VEGF) secretion, in a LTβR-dependent manner32. Recently, it has been shown that PDPN expression by FRCs is also required for the regulation of HEV permeability. HEVs carry out two seemingly contradictory roles. They must permit significant lymphocyte trafficking in and out of lymph nodes while maintaining a barrier function to prevent blood components leaking into lymphoid organs33. New data show that platelets, which express CLEC2, permeate venules and contact perivascular $PDPN^{+}$ FRCs surrounding the HEV. The interaction of CLEC2 with PDPN causes the release of stored sphingosine-1-phosphate (S1P) from platelets, which signals through the SIP1R on HEVs to drive upregulation of VE-cadherin expression, thereby reinforcing the cell–cell junctions that are integral to HEV barrier function33. This continuous interaction between platelets and FRCs prevents cellular and acellular components of blood from indiscriminately leaking into lymph nodes.

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The role of FRCs in immune privilege of lymph nodes

Despite being sites where immune responses are robustly initiated, here we discuss evidence showing that lymph nodes are also sites of immune privilege, where effector T cell responses are censored34–45. FRCs contribute to T cell tolerance in three ways.

FRCs mediate deletional tolerance

Early studies noted that T cells with affinity for peptide-MHC complexes presented by lymph node stromal cells became activated, but were swiftly lost from the peripheral T cell pool34–37. When techniques were developed that enabled lymph node stromal cell subsets to be sorted to high purity6, it was shown that FRCs express tissue-specific self-antigens from a range peripheral tissues38,39, and can directly present these to $CD8^+$ T cells for tolerance induction38. FRCs do not express Aire38,39, but do express an Aire-like molecule called DEAF1, which regulates expression of at least some peripheral tissue antigens relevant to the development of autoimmunity37. Accordingly, the ectopic expression of peripheral tissue-restricted antigens by FRCs prevents autoimmunity in animal models by directly deleting autoreactive T cell clones34,36. Strikingly, in a model of chronic versus acute viral infection, direct infection of FRCs (implying the presentation of viral peptides by MHC class I molecules on FRCs) was associated with inability to clear the virus and the acquisition of an exhausted phenotype in responding CD8+ T cells40.

FRCs suppress effector T cell proliferation

As a second method of tolerance induction, FRCs permit T cell activation within lymph nodes, but suppress effector T cell responses41–43. The mechanism is surprisingly generic. *In vitro* evidence suggests that any T cell, regardless of specificity, that begins producing interferon-γ (IFNγ) while crawling on an FRC is likely to have its proliferative capacity dampened through absorption of FRC-generated nitric oxide (NO)41–43. Given that T cell movement correlates highly (93%) with the presence of an adjacent FRC fibre4, this is likely to be a fundamental mechanism preventing T cells from acquiring effector functions within lymph nodes. Also FRCs can acquire intact peptide–MHC class II complexes from DCs, which decreases the proliferation and survival of antigen-specific CD4+ T cells by decreasing antigen concentration44. It is important to note that this suppressive function does not seem to impair systemic immune responses; in fact loss of FRCs is associated with significantly impaired systemic T cell-mediated immunity27,29,46. These observations will be discussed in detail later in this Review.

FRCs support regulatory T cells

FRCs may also constrain effector T cells through effects on regulatory T (T_{Reg}) cells. Several studies have shown that FRCs endogenously express MHC class II molecules2,44,45. Furthermore, FRCs have the capacity to process and present antigen through the expression of molecules from the MHC class II antigen processing and presentation pathway: the invariant chain CD74, lysosome-associated membrane protein 1 (CD107a, LAMP-1), which marks endosomes where peptides are processed and loaded onto MHC class II, and the molecular chaperone H2-M, which catalyzes peptide loading onto MHC class II45. In a lymph node stromal cell transplant model, homeostatic proliferation of

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$CD4^+FOXP3^+$ T_{Reg} cells required MHC class II expression on non-hematopoietic stromal cells. Using K14-OVA mice as a model to monitor antigen-reactive T cells, it was shown that stromal cell expression of OVA increased the proportion of OVA-specific T_{Reg} cells even in distal lymph nodes45. However, within the stromal cell population in lymph nodes, both FRCs and endothelial cells expressed OVA45, so it is not yet certain that FRCs mediate this effect on T_{Reg} cells. In a separate study47, cutaneous, mesenteric and liver-draining celiac lymph node stromal cell transplants were used to show that mesenteric and celiac lymph node stromal cells, but not cutaneous lymph node stromal cells, promote T_{Reg} cell induction, in a manner dependent on DCs. Again, the results are suggestive of an FRC-dependent effect, although the mechanism is yet to be established.

Why are FRCs immunosuppressive?

A current theory is that the antigen-presentation and T cell deletion functions of FRCs evolved to prevent autoimmunity, while T cell-suppressive mechanisms may have evolved to prevent damage to the infrastructure of the lymph node during an acutely inflammatory immune response, supporting T cell egress and acquisition of effector functions external to the lymph node, rather than within it8,48.The lymph node infrastructure, amongst lymphocyte activation, inflammatory mediators, antigen presentation, and arrival of free and opsonised antigen via the lymph, is at risk of damage from "friendly fire", and T cell suppression is likely to ensure the healthy regulation of primed T cells in an inflammatory, antigen-rich environment. As discussed later in this Review, the maintenance of a suppressive environment within lymph nodes evidently does not prevent T cells activated in the presence of FRCs from initiating a robust systemic immune response21,27,29.

FRCs respond dynamically to infection

As we have described, in the absence of infection, FRCs help to keep lymph nodes in a state of self-tolerant readiness. Once an adaptive immune response is initiated, however, FRCs undergo dynamic changes.

FRCs mediate lymph node flexibility

A hallmark of the initiation of an adaptive immune response is the rapid expansion of lymph nodes to accommodate the clonal expansion of activated T and B cells, followed by a return to their homeostatic size as lymphocyte populations egress and contract. New studies place FRCs at the centre of lymph node hypertrophy and resolution49,50 (outlined in Figure 3).

Under homeostatic conditions, PDPN regulates the actomyosin contractility of FRCs, providing physical tension throughout the FRC network49,50. This is achieved by steady-state signalling through ezrin, radixin and moesin (ERM) family proteins **[G]** that link the cell membrane to the underlying actin network49,50. In PDPN-overexpressing NIH/3T3 fibroblasts, phosphorylated ezrin accumulated at the cell cortex and co-localised with PDPN49. The same cells transfected with a non-phosphorylatable ezrin mutant were non-contractile. Contractile force is driven by RhoA signalling, which through Rho-kinase (ROCK), regulates myosin II-mediated actin contractility49. In response to inflammation, the increased availability of CLEC2 on resident and infiltrating activated DCs bound PDPN

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and inhibited PDPN-mediated contractility by clustering and partitioning PDPN molecules within lipid rafts49,51, which uncoupled PDPN from ERM proteins and RhoA activity. The FRCs were then able to elongate, releasing the tension of the FRC network and resulting in a network permissive to expansion and stretching49. This regulation of lymph node tension was also described in another study showing that directly inhibiting PDPN using targeted antibodies results in loss of tension throughout the FRC network50. As a result, as lymphocytes infiltrate and proliferate, lymph nodes are given the flexibility to expand rapidly and accommodate the developing immune response, without compromising the structural integrity of the stromal network49,50. It is interesting to speculate that the contractile capacity of pericytic FRCs may provide physical tensile support to HEVs, in addition to the platelet interactions that have been described33.

FRCs proliferate during infection

The FRC network is capable of significant proliferation in response to infection or lymph node inflammation49,50,52. It is therefore likely that cell proliferation contributes to the ability of FRCs to accommodate lymph node expansion, although the time taken to proliferate seems to depend on the stimulus used, ranging from 24h (LPS50) or 40h (ovalbumin in Monatanide mineral oil adjuvant52, to 12 days (ovalbumin in complete Freund's adjuvant49) or, in an analysis of a lone timepoint, 19 days (*Leishmania major*52). The signals controlling FRC proliferation are still unclear. One study reported that access to T cells and DCs is important53, whereas another study proposed that the effects of DCs are indirect, and that the trapping of naïve T cells is an early trigger for growth and remodelling of the FRC network52. The same study reported that provision of LTβR ligands augmented a later stage of FRC proliferation, through a poorly defined mechanism independent of LTi cells, T cells and B cells52. RANK-L may be an additional stimulus, as lymph nodes from transgenic mice that overproduced this protein were hyperproliferative, with increased numbers of $RANK^{+}$ FRCs and higher production of FRC-linked molecules CXCL13, CCL19, MadCAM-1 and VCAM-154. In wildtype mice, activated T cells are a prominent source of RANK-L54.

As FRCs experience marked changes in mechanical strain during the initial phases of lymph node expansion resulting from B and T cell proliferation, mechanical signals might be a trigger for FRC clonal expansion *in situ*49,50,52. Accordingly, PDPN inhibition induces both FRC elongation and proliferation49,50, which would support a model in which FRC stretching gives rise to reactive proliferation. In at least one model, FRC stretching occured prior to proliferation49. Although the evidence is as yet circumstantial, there is precedent for this type of mechanism operating in lung parenchymal cells55.

The identification of progenitor or proliferative intermediate (transit-amplifying) cell types that might contribute to FRC proliferation is still in its infancy. Two studies report that adipocyte progenitor cells can contribute to the mesenchymal network in adult mice11,12. Their influx to lymph nodes increased in inflammatory conditions12 and the cells differentiated into T cell zone FRCs and marginal reticular cells in reaggregate grafts11. Equally, FRCs or their progenitors may proliferate in situ.

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Loss of FRCs impairs systemic immune responses

The effects of the FRC response to infection extend beyond the lymph node. A systemic requirement for FRCs in the generation of effector B and T cells, and the normal initiation of both humoral and cell-mediated immunity has recently been discovered. A model of systemic LCMV infection (acute WE strain, controlled by $CD8^+$ T cells) was used to test the requirement for mature, immunocompetent FRCs in *Ccl19*-Cre × $Ltbr^{fl/fl}$ mice **[G]**21, which develop a mesenchymal network expressing abnormally low levels of PDPN, CCL19, CCL21 and IL-7. The lymph nodes of these mice have a 60-70% depletion of T cells, B cells, DCs and macrophages. Control mice cleared the virus by day 10 post-infection; $Ltbr^{fl/fl}$ mice could not clear infection and lacked virus-responsive T cells, similar to *plt/plt* mice **[G]** which lack lymph nodes entirely21. Also, localised mouse hepatitis virus infection, which requires a cervical lymph node-generated adaptive immune response for resolution, featuring pDCs and $CD4^+$ and $CD8^+$ T cells, was unresolved 10 days after infection, in contrast to controls21.

Similar results have been obtained from two studies using mice that enable the temporal control of FRC deletion. One study27 selectively depleted FRCs using diptheria toxin (DTx) receptor (DTR) expression driven by fibroblast activation protein-α. Mice receiving DTx were unable to mount an effective response against influenza virus. This systemic effect resulted from a loss of naïve B and T cells and DCs from lymph nodes, because the depletion of FRCs during active influenza virus infection had no effect on the magnitude of the immune response, or on the presence or function of activated B and T cells in lung-draining lymph nodes27. Another study29 immunized *Ccl19*-Cre × iDTR mice **[G]** with a non-replicative influenza A virus expressing the OT-II epitope. OT-II $CD4^+$ T cells did not divide and did not have an activated phenotype in mice where FRCs had been deleted prior to immunization29.

Most recently, the impaired initiation of IgG and IgA humoral immune responses to subcutaneous or oral antigens was reported in a natural graft-versus-host disease model of FRC elimination46.

FRC-associated pathology

As discussed, FRCs fulfil many important functions to regulate immunity, through their effects on the survival, migration and function of T cells, B cells and DCs, as well as regulating lymph node microenvironmental structure and lymph node size. As many of these functions are unique to FRCs, damage to these cells has detrimental outcomes for immunity

FRCs are direct targets of virus infection

Several clinically important human pathogens directly infect FRCs, with systemic consequences. Ebola, Lassa and Marburg viruses, which cause high-mortality hemorrhagic fevers, all induce damage to FRCs and to the conduit network of lymph nodes, correlated with the loss of T and B cell zones and increased fibrosis56–58. Studies in rhesus macaques have shown that FRCs are directly infected by, and are a very early target of, *Zaire ebolavirus*, and that lymph nodes are a site of significant viral replication57,58. Accordingly,

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a prominent hypothesis is that FRC infection contributes to the pathogenesis of Ebola by assisting in the production and dissemination of *Z. ebolavirus* virions within lymphoid tissues58. The complete destruction of lymphoid tissues was among the extensive pathologies observed to be caused by Z. *ebolavirus* in rhesus macaques57.

FRCs are also efficiently infected by lymphocytic choriomeningitis virus (LCMV)40,59,60, for which the availability of mouse models has enabled some mechanistic dissection. The ability of the immune system to clear LCMV correlated negatively with the propensity of the virus to infect FRCs. Infected FRCs upregulated programmed death ligand-1 (PD-L1), a protein that is associated with T cell exhaustion40. PD-L1 expression by infected non-hematopoietic stromal cells did not affect anti-viral $CD8^+$ T cell priming, but it slowed both viral clearance and the onset of fatal immunopathology61. This also fits with the NO-mediated role of FRCs in suppressing effector T cell proliferation41–43, which may be an additional mechanism by which LCMV infection of FRCs slows viral clearance. However, this suppression of the immune response is not sufficient to protect FRCs from T cell-mediated attack. Both studies showed that infected FRCs were nonetheless efficiently destroyed by virus-specific cytotoxic T cells59,60, which crippled the immune response to new antigens60. This finding also correlates with reports of Ebolavirus-infected lymph nodes, which exhibit destruction of lymph node architecture and the conduit network57.

FRC-mediated lymph node fibrosis causes immunodeficiency

FRCs assist the lymph node to recover from acute infection by upregulating IL-7 expression, but chronic inflammation damages FRCs and, in part through these effects, impairs systemic immunity. During prolonged inflammation, T_{Reg} cells upregulate TGF-β1, which suppresses prolonged immune activation within lymph nodes in a beneficial manner, but is also a significant cause of FRC pathology62–64.

In chronic HIV-1 and SIV infection models, using rhesus macaques, a T_{Reg} cell response produces high levels of TGFβ1. This stimulates the TGFβ1RII signalling pathway in FRCs, driving increased collagen synthesis. Secreted collagen accumulates around FRCs to the point that naïve lymphocytes die as they lose complete contact with FRCs and are unable to access their IL-7 output62–65 (Figure 4). High levels of $CD4^+$ T cell apoptosis in HIV^+ patients were previously assumed to occur as a result of direct infection and prolonged inflammation, but lymph node dysfunction driven by FRCs is now believed to be a major contributor to the T cell loss22,62–64.

Loss of $CD4^+$ T cells (and hence LT production) deprives FRCs of the LTβR signals that are required for their maintenance. Administration of a $CD4^+$ T cell-depleting antibody to rhesus macaques reduces the provision of LTβR ligands and depletes FRCs, which in turn induces depletion of naïve $CD8^+$ T cells, creating a negative feedback loop and broad immunosuppression22,63. Importantly, this finding is mirrored in HIV^+ and chemotherapy-treated patients, for whom the loss of $CD4^+$ T cells is also associated with FRC and FDC depletion22. Prolonged T cell immunodeficiency is a major clinical issue post-chemotherapy or irradiation, and persists in 15-40% of treated HIV^+ patients, despite undetectable viral load66,67.

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FRCs are targets of allogenic attack during GVHD

Recently, it was reported that FRCs are an early target of graft-versus-host disease (GVHD)-mediated attack in major and minor MHC-mismatched animal models46. Mice developed fibrotic lymph nodes and suffered an irreversible (>100 days) loss of the lymph node microenvironment, including loss of HEVs, T and B cellularity, and systemic humoral immunity46.

Inherent in these observations is the idea that damage to the FRC network may hamper their ability to promote immune recovery by providing new niches for naïve T and B cells. As such, attempting to reverse lymph node fibrosis is an active area of research.

Therapeutic targeting of FRCs

Given the varied fundamental effects of FRCs on systemic immunity, there are several proposed therapeutic interventions seeking to target, exploit, or replicate the immunoregulatory functions of FRCs.

FRCs drive lymph node recovery

After damage to the lymph node microenvironment, through acute infection, hypoxia or immunodepletion, FRCs are key orchestrators of lymph node reestablishment and remodelling60,68, making them an attractive therapeutic target. FRCs respond to either viral damage or hypoxia by upregulating IL-7 expression to promote the survival of ILC3s68, which in turn drive LTβR-dependent rebuilding of the lymph node, similarly to lymph node development during embryogenesis60,68. How FRCs sense this damage is unknown. Lymph node repair did not occur when IL-7+ stromal cells were depleted68. This finding was supported by research into HIV patients, which found that loss of IL7+ FRCs, which occurred as a result of fibrosis, was associated with prolonged immunodeficiency despite undetectable viral load62,63. As such, the use of drugs to reduce stromal fibrosis has been proposed and pre-clinically tested (discussed below). Irreversible loss of FRCs due to graft-versus-host inflammation also resulted in prolonged humoral immunodeficiency, which persisted despite successful hematopoietic reconstitution46. The ability to restore or prevent damage to FRCs could therefore yield important clinical benefits.

Reduction of lymph node fibrosis

Anti-fibrotic drugs have been proposed to reduce lymph node fibrosis caused by FRCs in response to HIV-1 infection63. Pirfenidone(5-methyl-1-phenylpyridin-2-one) targets the TGF-β1 pathway, with the aim of reducing extracellular matrix production by FRCs. Administration *in vitro* reduced phosphorylation of SMAD2/3 (downstream of TGFβR) and the production of type 1 collagen by FRCs, in a dose-dependent manner64. *In vivo* administration of anti-retroviral therapy (ART) with pirfenidone to SIV-infected rhesus macaques prevented the loss of desmin^{+} FRCs, while reducing collagen I and fibronectin deposition in T cell zones of lymph nodes and increasing systemic CD4^{+} T cell numbers compared with ART alone64. Pirfenidone did not block TGFb1 production, but did inhibit SMAD2/3 phosphorylation64. These exciting data support further clinical testing for the use of anti-fibrotic drugs in HIV-1 infection, to hasten T cell recovery after ART commences by

decreasing FRC-mediated lymph node fibrosis and thereby slow or reduce the progression to AIDS.

Recombinant IL-7 therapy to boost T cell numbers

Administration of recombinant human IL-7 is also being trialled to expand and support naïve and effector-memory T cells in immunodepleted or immunodeficient patients[69]. Beyond direct effects on T cells, it is possible that IL-7 therapy may assist in rebuilding atrophic lymph nodes by supporting ILC3s, which in turn may support the development of LTo cells and restoration of the lymph node architecture. Phase I and II trials are in varying stages of completion (www.clinicaltrials.gov identifiers NCT00105417, NCT02231853, NCT00477321, NCT00684008, NCT01881867, and others).

FRCs as an anti-inflammatory cell therapy

FRCs have also been administered as a cell therapy to animal models of sepsis and acute endotoxemia. They have been shown to significantly reduce mortality when administered as a single dose 4-16 hours after the septic insult[70]. FRCs in this model decreased the level of proinflammatory cytokines in serum and at the site of initial infection, and decreased bacterial counts in the blood, in a mechanism dependent on FRC-mediated synthesis of the anti-bacterial and immunomodulatory molecule nitric oxide[70]. FRCs did not engraft long-term. Stromal cell-based therapies have gained significant clinical attention in recent years, for their ability to contribute to tissue healing and decrease inflammation (reviewed elsewhere[71]). A distinct advantage over traditional single-molecule pharmaceuticals is that these cells can respond dynamically to inflammatory stimuli, targeting multiple molecules or pathways[70,71]. It will be interesting to see whether human FRCs show similar efficacy in relevant models, and whether FRCs may have therapeutic efficacy in other inflammatory diseases.

Conclusion and future directions

In recent years, FRCs have been found to affect all aspects of adaptive immunity. Their role as a physical support for leukocytes is well described, but major findings continue to emerge. FRCs attract, retain and spatially organise naïve lymphocytes, secreting chemokines and cytokines that support the growth, function and survival of other cell populations within the lymph node. FRCs also function as specialised pericytes, controlling HEV permeability and lymphocyte trafficking. New findings show that FRC cytoskeletal tension controls lymph node size, enabling rapid organ expansion during inflammation. FRCs also remodel damaged lymphoid organs following the resolution of the infection, a process that is incomplete during chronic infection, resulting in immunodeficiency. Many open questions remain (Box 1). Further study into FRCs, with their far-reaching immunological effects, may yield therapeutic solutions to the problems of chronic inflammation and lymphocyte activation.

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Biographies

Anne Fletcher received her PhD from Monash University (Australia) and her postdoctoral training at the Dana-Farber Cancer Institute and Harvard Medical School in Boston (USA), in collaboration with Monash University. Anne was recently appointed as a Birmingham Fellow at the University of Birmingham (UK). Research from her group focuses on the immunobiology of lymph node stromal cells, including mechanisms of tolerance induction and cross-talk with leukocytes.

Sophie Acton received her Ph.D. from the University College London, and spent her postdoctoral training between the Dana-Farber Cancer Institute (Boston, USA) and the Cancer Research UK London Institute (UK). Sophie Acton's research focuses on the interactions between leukocytes and stromal cells, with particular focus on cell motility, cytoskeletal regulation and cell–cell communication. She has most recently worked on reciprocal signaling between dendritic cells and fibroblastic stromal cells, controlling lymph node dynamics during acute inflammation.

Glossary

Myofibroblasts

cells which share characteristics with fibroblasts and smooth muscle cells, and are often associated with response to inflammation.

Follicular dendritic cells

non-hematopoietic stromal cells found in B cell follicles, with an important role in antigen presentation to B cells.

Lymphoid tissue-inducer (LTi) cells

CD3-CD4+CD45+ innate lymphocytes that engage in crucial, carefully regulated molecular cross-talk with non-hematopoietic lymphoid tissue organiser cells to induce lymph node formation. Their development is dependent on the transcription factor RORγt.

Lymphoid-tissue organiser (LTo) cells

CXCL13+LTbR+ PDPN+CD45- mesenchymal stromal cells that engage in molecular cross-talk with LTi cells to induce lymph node formation.

ERM family proteins

ezrin, radixin and moesin proteins. ERM proteins are involved in cell adhesion, contraction, and cortical morphogenesis, by linking the plasma membrane to the actin cytoskeleton.

***Ccl19*-Cre × Ltbr$^{fl/fl}$ mice**

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BAC-transgenic mouse model using CCL19 expression to target Cre-recombinase to FRCs and related cells. When crossed to the Ltbr$^{fl/fl}$ mouse, cre-recombinase deletes the LTbR gene in any CCL19+ cells, at the moment LTbR becomes upregulated.

***plt/plt* mice**

paucity of lymph node T cells (plt) is a genetic mutation resulting in loss-of-function of CCL19 and CCL21. These mice have T cell and DC migration defects.

***Ccl19*-Cre × iDTR mice**

BAC-transgenic mouse model using CCL19 expression to target Cre-recombinase to FRCs and related cells. When crossed to the iDTR mouse, administration of diphtheria toxin depletes CCL19+ cells, including FRCs.

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Box 1

The immunobiology of FRCs: poorly understood aspects

- FRCs are heterogeneous, comprising at least 5 subsets. T cell zone reticular cells are well-described; marginal reticular cells moderately so. Other subsets are newly described and not well studied.
- Strong similarities have been noted between FRCs and FRC-like cells in tumours, tertiary lymphoid organs and gut-associated lymphoid tissue such as crypts. The ontogenic and functional relationships between these cells are not well explored.
- Precise mechanisms of antigen acquisition and presentation by FRCs to T cells are not well studied. It is known that FRCs can acquire peptide–MHC complexes intact from DCs44 and that the machinery required for antigen processing and presentation via the MHC class II pathway is present in FRCs45, but can they present antigen via the endogenous pathway? Can FRCs cross-present?
- The molecular response of T cells to FRC-mediated deletion or suppression is not well studied. Understanding this response may provide new opportunities to overcome stromal-mediated suppression in situations of chronic immune activation and in tumours.
- More than 80% of Cre-expressing cells in the spleens of *Ccl19*-Cre mice are poorly defined PDPN$^-$ mesenchymal cells21. *Ccl19*-Cre × iDTR mice show systemic immunological deficiencies29. The contribution of these cells to splenic microenvironmental homeostasis and immunity will be interesting to address.

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Online summary

- FRCs are heterogeneous stromal cells. Subsets of FRCs include T cell zone FRCs that produce IL-7 to support naïve T cells, B cell zone FRCs that express BAFF to support naïve B cells, pericyte FRCs that support HEV barrier function, T cell zone FRCs that inducibly express CXCL13 during inflammation to interact with B cells, and marginal reticular cells that can differentiate into B follicular dendritic cells.
- Crucial checkpoints in mesenchymal stromal cell development are retinoic acid signalling to mesenchymal progenitors, which creates the lymph node anlagen, followed by the attraction of lymphotoxin ligand-bearing ILC3s (usually CXCL13 mediated). LTβR signalling to mesenchymal precursors results in the development of $CCL19^{+}CCL21^{+}CXCL13^{+}RANKL^{+}LT\beta R^{+}MAdCAM^{+}$ lymphoid tissue organizer (LTo) cells. Although it is still unclear precisely how LTo cells relate to mature FRCs, an immature FRC subset has been identified that requires LTβR signalling for the acquisition of an immunologically mature phenotype.
- FRCs give lymph nodes the flexibility to stretch and contract, to accommodate clonal expansion of activated T and B cells. PDPN maintains tension in the FRC network during homeostatic conditions, and this function is inhibited during an immune response when an influx of CLEC2-bearing dendritic cells inhibits PDPN-mediated FRC contractility.
- During a chronic infection such as with HIV-1, regulatory T cells upregulate TGFβ1 production, which signals to FRCs to markedly increase their extracellular matrix production. Naïve T cells can no longer physically contact FRCs and lose access to IL-7, resulting in widespread T cell death and prolonged immunodeficiency.
- Therapeutic advances seeking to mimic or target FRC function include anti-fibrotic drugs to reverse lymph node fibrosis, the administration of recombinant IL-7 to support T cell recovery post-immunodepletion, and their use as a putative anti-inflammatory cell therapy.

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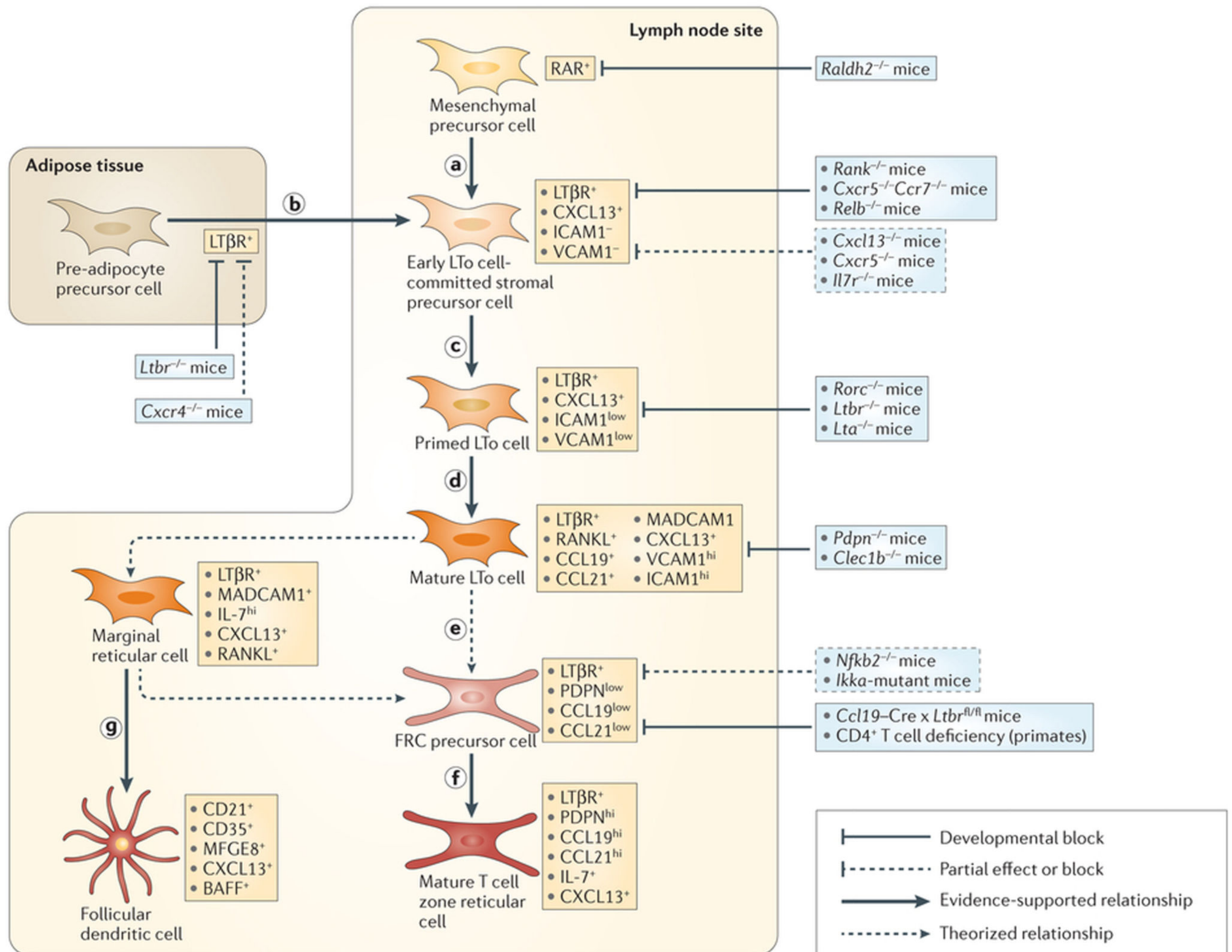

Figure 1. Molecular checkpoints in fibroblastic reticular cell (FRC) development.

FRC development is incompletely defined, but a series of crucial checkpoints have been identified.

a | Lymph node anlagen specification. Retinoic acid, likely provided by adjacent neurons, is required for lymph node anlage specification of retinoic acid receptor + mesenchymal precursors at the site, which develop into CXCL13+ early LTo-committed stromal precursor cells10.

b | Pre-adipocyte precursors. LTβR signalling is a molecular switch that drives lymph node stromal cell development at the expense of adipocyte development11. The migration of pre-adipocytes to the lymph node site is augmented by CXCR412. On arrival, these cells upregulate CXCL13 to become early LTo-committed stromal precursor cells.

c | Early LTos differentiate into primed LTos when then begin to express low levels of ICAM-1 and VCAM-1. Signals involved in this differentiation step are unknown, but precursors do not require ILCs or LTβR signalling to proceed to this stage18.

d | The development of ICAM-1hi VCAM-1hi LTo cells requires cross talk with ILC3s via RANK and lymphotoxin signals. Rorc-/- mice, which lack ILC3s, do not develop lymph

nodes15. There is a partial block in lymph node development in mice lacking CXCL13, CXCR5 and IL-7R, and a complete block in CXCR5-/- CCR7-/- mice14,16,72 11,69,70. RANK signalling to ILC3s is a crucial checkpoint13, inducing the upregulation of LTα1β2. LTβR signalling induces early lymphoid tissue organiser cells to differentiate to a mature form, and is a crucial checkpoint for lymph node development18. Mice with blocks in this signalling pathway develop anlagen and early LTo cells but not lymph nodes13,15,18.

e| It is hypothesised that early FRC precursor cells develop directly from differentiated LTos, but this has not been formally demonstrated. Interactions between PDPN and CLEC2 are a crucial checkpoint for FRC development19,20, as Pdpn-/- and Clec1b-/- develop anlage but no lymph nodes. It is unclear exactly where this block lies, but it is prior to the development of FRC precursors. The source of CLEC2 is undefined.

f | The development of mature FRCs from immature FRC precursors requires lymphotoxin signalling. Lymph nodes lacking the NF-κB2 signalling pathway (*Relb*$^{-/-}$, *Nfkb2*$^{-/-}$ and *Ikka*$^{-/-}$ lymph nodes) are present, but hypoplastic and B cell deficient73,74, which indicates that the canonical NF-κB signalling pathway may partly compensate for loss of LTβR. The loss of LTβR from LTo cells at the point of CCL19 upregulation allows for the development of lymph nodes and FRC precursors, but blocks late-stage FRC development, thus identifying immediate FRC precurors21. In humans and some primates22, but not mice23, $CD4^+$ T cells are a crucial source of LT ligands.

Abbreviations: CLEC2: c-type lectin receptor 2. FRC: fibroblastic reticular cell. ILC3s: group 3 innate lymphoid cells. LN: lymph node. LTβR: lymphotoxin beta receptor. LTos: lymphoid tissue organisers. PDPN: podoplanin.

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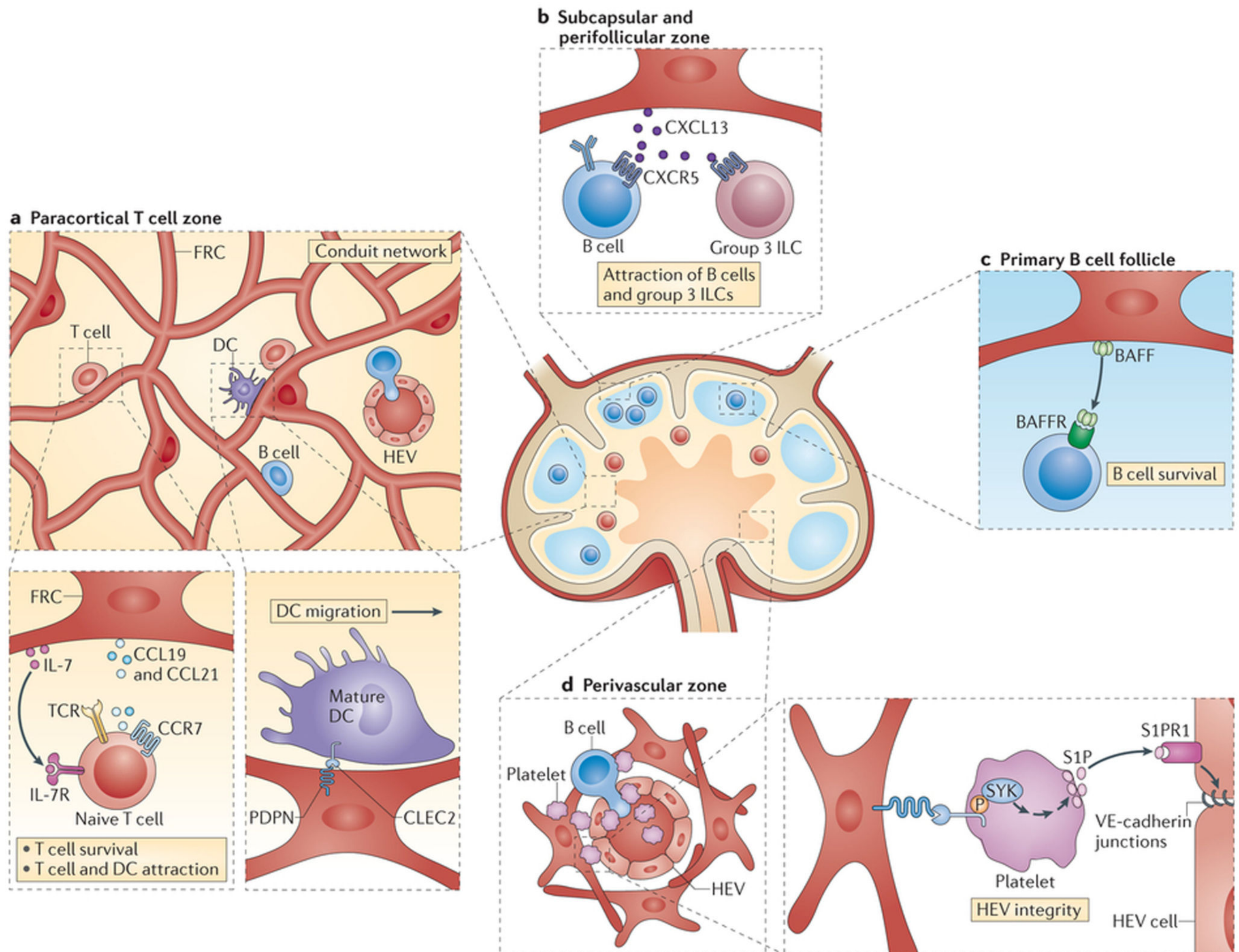

Figure 2. Fibroblastic reticular cells (FRCs) organize the lymph node microarchitecture.

FRCs exist in various lymph node microdomains, where they regulate different leukocyte types and aspects of their function.

a | Paracortical T cell zone. T cell zone FRCs from the lymph node paracortex attract naïve T cells and DCs by their expression of the CCR7 ligands CCL19 and CCL214. T cell zone FRCs express PDPN, which activates CLEC2 on mature antigen-loaded DCs; CLEC2 signalling drives DC migration31 on scaffolding created by FRCs3,4. Paracortical FRCs also create the conduit network, which conveys small molecules from afferent lymphatics towards the paracortex and HEVs5, and secrete the T cell pro-survival factor IL-71.

b | Marginal reticular cells and a subset of T cell zone FRCs express (or inducibly express) CXCL13 to attract CXCR5^{+} B cells and ILC3s23,25,26.

c | FRCs in the B cell follicle produce BAFF to help naïve B cells survive29

d | FRCs maintain tight cell-cell junctions of HEVs33. When leukocytes move into lymph nodes via HEVs, PDPN expressed by FRCs ligates CLEC2 expressed by the small number of accompanying platelets. This signal mediates S1P1 release from the platelet surface, which signals to HEVs to upregulate their VE Cadherin junctions33.

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Abbreviations: BAFF: B cell activating factor. CLEC2: c-type lectin receptor 2. DCs: dendritic cells. FRC: fibroblastic reticular cell. HEV: high endothelial venule. ILC3s: group 3 innate lymphoid cells. LN: lymph node. LTβR: lymphotoxin beta receptor. LTos: lymphoid tissue organisers. MRCs: maginal reticular cells. PDPN: podoplanin. S1P: sphingosine-1-phosphate.

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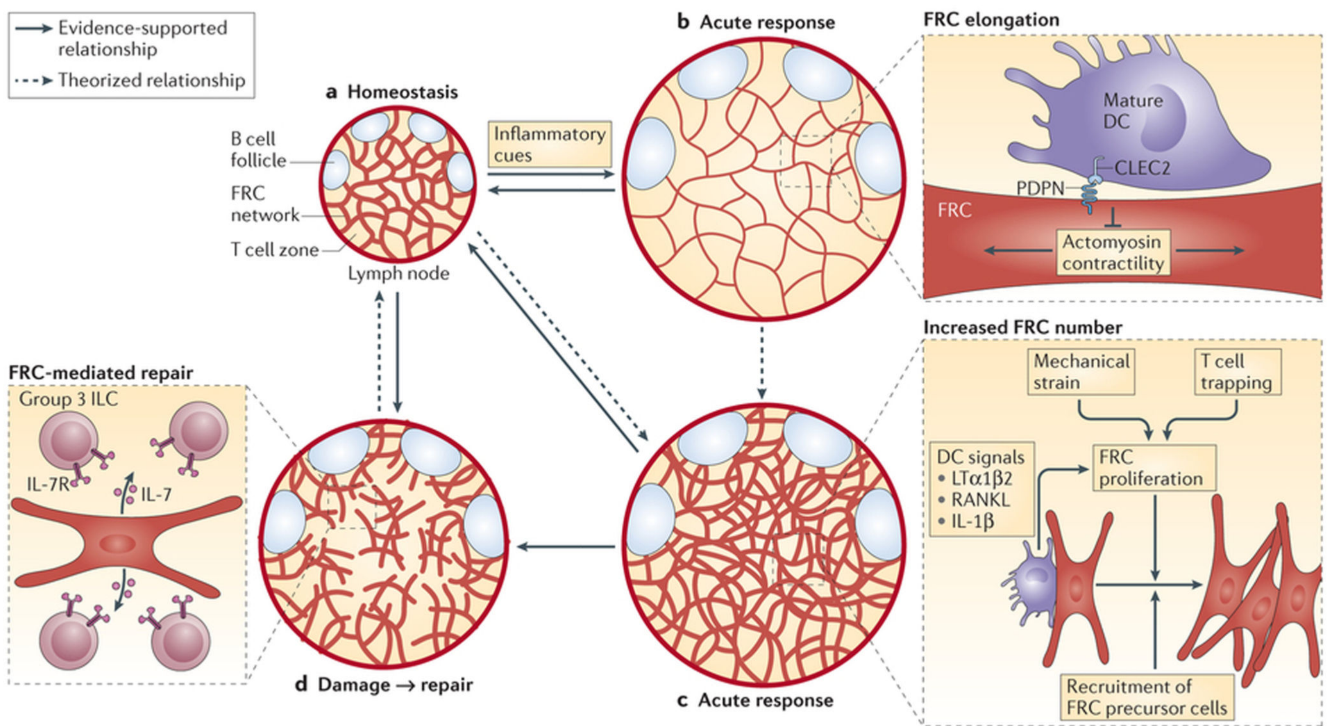

Figure 3. Dynamic response of FRCs to infection.

a | During homeostasis, PDPN+ FRCs form an interconnected tensile network that maintains lymph node size.

b | In an acute response, in response to inflammatory cues, CLEC2 expression is upregulated by both lymph node-resident and infiltrating migratory dendritic cells31,75. CLEC2 inhibits PDPN-driven actomyosin contractility of FRCs resulting in FRC elongation and reduced lymph node tension, which enables rapid organ expansion in the absence of stromal proliferation49,50.

c | Acute responses also trigger FRC proliferation and/or the recruitment of FRC precursors to restore FRC:T cell ratios in the face of T cell proliferation49,50,52,53.

d | Following acute damage to the FRC network, FRCs upregulate IL-7 production to recruit ILC3s, which help to regenerate and rebuild the lymph node to repair damage60,68. Amid chronic infection such as HIV, resolution of damage may take years, or may not occur63,64. Abbreviations: CLEC2: c-type lectin receptor 2. FRC: fibroblastic reticular cell. ILC3s: group 3 innate lymphoid cells. LN: lymph node. PDPN: podoplanin.

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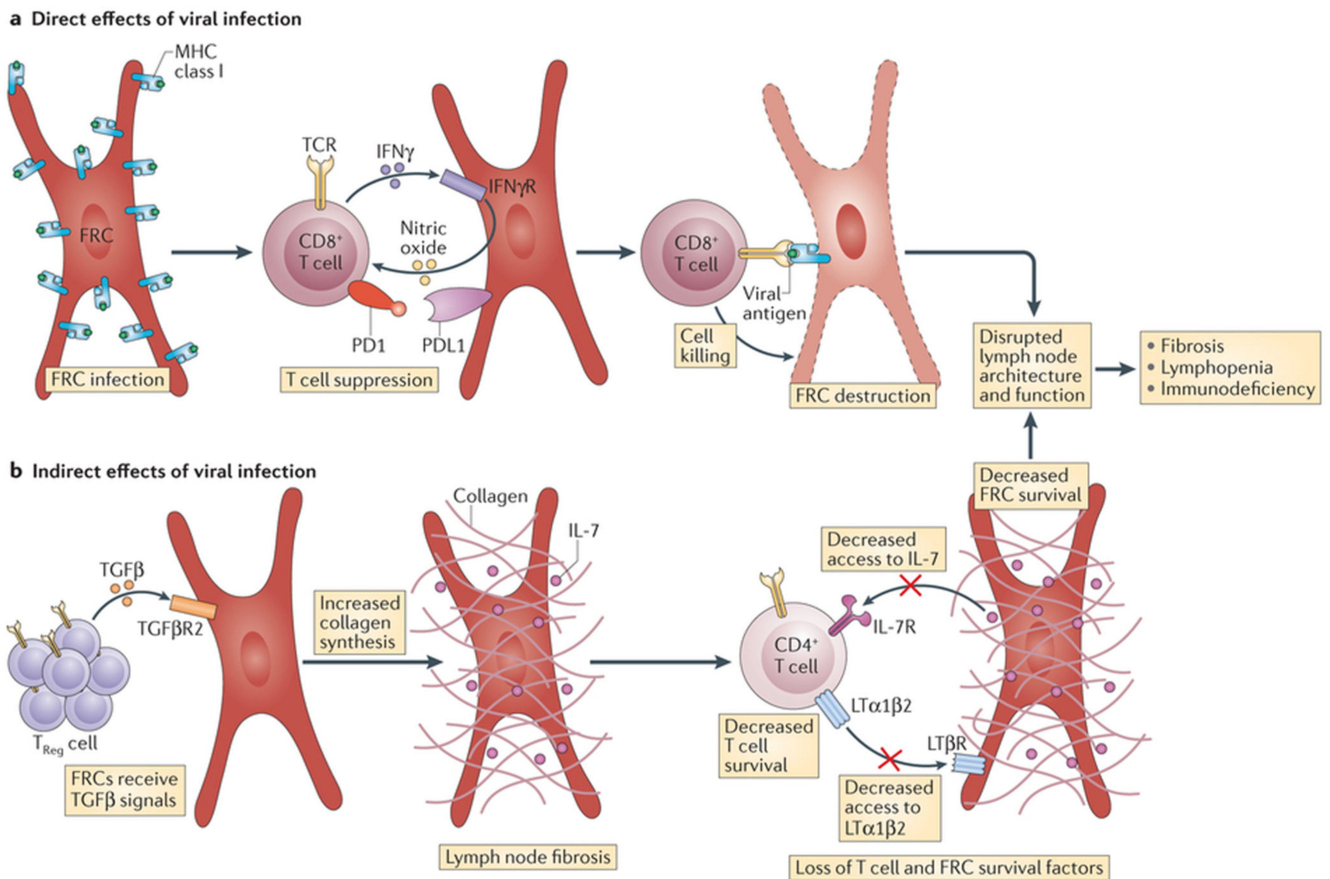

Figure 4. Viral pathology drives fibroblastic reticular cell (FRC)-mediated immunodeficiency.

a | FRCs are directly infected by Ebolavirus, LCMV and other viruses. They present viral antigens on MHC class I molecules and are likely to propagate infection by disseminating virus. Infected FRCs slow down the onset of fatal immunopathology through effects on activated T cells. This involves PD-L1 expression by FRCs and, we speculate, may also involve other known suppressive pathways such as NO release, which is produced in response to IFNγ produced by T cells. FRCs slow viral clearance through their use of such T cell-suppressive mechanisms. Eventually however, infected FRCs are destroyed with high efficiency, which disrupts lymph node architecture and function, causing systemic lymphopenia and crippling the immune response to new antigens40,63,64.

b | During chronic infection, the expansion of T_{Reg} cell populations results in increased output of immunosuppressive TGFβ. This signals to FRCs to increase their collagen output, causing lymph node fibrosis. T cell access to the FRC-produced survival factor IL-7 is impeded. Loss of T cells concomitantly reduces the access of FRCs to LTα1β2, which signals through LTβR. Hence, both FRCs and T cells are lost. This causes systemic, long-term lymphopenia, and loss of lymph node architecture and the conduit system22,62–64.

Abbreviations: FRC: fibroblastic reticular cell. IFNγ: interferon gamma. LCMV: lymphocytic choriomeningitis virus. LN: lymph node. LTα1β2: lymphotoxin alpha 1 beta 2. LTβR: lymphotoxin beta receptor. TGFβ:transforming growth factor beta

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Table 1

Subsets of FRCs reported in lymph nodes

Name	Defining features	Defining functions	Refs
T cell zone reticular cells	• $PDPN^{+}desmin^{+}$ $MADCAM1^{-}$ • CCL19, CCL21 and IL-7 secretion	• Maintaining the T cell zone • Constructing the conduit network	1,2,3,4,5,9
Marginal reticular cells	• Subcapsular location • $PDPN^{+}desmin^{+}MADCAM1^{+}IL\text{-}7^{hi}CXCL13^{+}RANKL^{hi}$ • Not found in tertiary lymphoid organs	• Rich source of IL-7 • Differentiation into FDCs	8,13,31
B cell zone reticular cells	• Resident cells: $PDPN^{+}CCL19^{+}BAFF^{+}$ and negative for FDC markers • Inducible cells: $PDPN^{+}$ subset of $CD21^{-}$ FRCs with a history of CD21 expression; convert into $CXCL13^{+}$ cells during the B cell response	• Maintaining B cell survival and follicle boundaries	30,36,78
FDCs	$CD21^{+}CD35^{+}MFGE8^{+}CXCL13^{+}ICAM1^{+}VCAM1^{+}BAFF^{+}$	• Maintaining germinal centres • Facilitating the production of high-affinity antibodies	6,13,32
Pericytic FRCs	• $PDPN^{+}$ • Located around HEVs • PDPN signals to CLEC2 on platelets	Preventing bleeding from HEVs into lymph nodes	40

BAFF, B cell-activating factor; CCL, CC-chemokine ligand; CLEC2, C-type lectin domain family 1 member B; CXCL13, CXC-chemokine ligand 13; FDCs, follicular dendritic cells; FRCs, fibroblastic reticular cells; HEVs, high endothelial venules; ICAM1, intercellular adhesion molecule 1; IL-7, interleukin-7; MADCAM1, mucosal vascular addressin cell adhesion molecule 1; MFGE8, lactadherin; PDPN, podoplanin; RANKL, receptor activator of NF-κB ligand; VCAM1, vascular cell adhesion molecule 1.