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HIGHLIGHTS

REVIEW

Compartmentalized multicellular crosstalk in lymph nodes coordinates the generation of potent cellular and humoral immune responses

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Distributed throughout the body, lymph nodes (LNs) constitute an important crossroad where resident and migratory immune cells interact to initiate antigen-specific immune responses supported by a dynamic 3-dimensional network of stromal cells, that is, endothelial cells and fibroblastic reticular cells (FRCs). LNs are organized into four major subanatomically separated compartments: the subcapsular sinus (SSC), the paracortex, the cortex, and the medulla. Each compartment is underpinned by particular FRC subsets that physically support LN architecture and delineate functional immune niches by appropriately providing environmental cues, nutrients, and survival factors to the immune cell subsets they interact with. In this review, we discuss how FRCs drive the structural and functional organization of each compartment to give rise to prosperous interactions and coordinate immune cell activities. We also discuss how reciprocal communication makes FRCs and immune cells perfect compatible partners for the generation of potent cellular and humoral immune responses.

Keywords: immune crosstalk · fibroblastic reticular cells · germinal center · lymph nodes · adaptive immunity

Introduction

Lymph nodes (LNs) constitute an important crossroad where immune cells circulate and interact supported by a dynamic 3-dimensional network of stromal cells, which includes endothelial cells and fibroblastic reticular cells (FRCs). Many terms have been used in the past to describe FRCs, including myofibroblasts and pericytes [1]. The term "reticular cell" was introduced for the first time in the 1960s, with the description of a peculiar reticular antigen-bearing cell with filiform processes located in the B cell follicles, today known as follicular dendritic cells (FDCs) [2, 3]. The "reticular cell" denomination is now extended to the entire family of fibroblastic cells that occupy secondary lymphoid organs (SLOs). The description of the SLO cellular environment, as well as the definition of its key cell types, was the first important step toward its functional understanding. These discoveries formed the basis to identify mechanisms involved in GC B cell differentiation and adaptive immune response initiation [2]. In the 1990s, the development of new *in vitro* culture systems, such as the long-term culture of human tonsillar B cells has allowed studying functional features of the crosstalk between B cells and other LN cellular compartments [4]. LNs have a unique microarchitecture organized into subanatomically separated areas, underpinned by particular FRC subsets: the subcapsular sinus (SSC), the paracortex, the cortex, and the medulla. Strong evidence exists that lymphoid organ stromal cells, *i.e.*, FRCs and endothelial cells, exhibit immunological properties that are crucial to immune cell survival and regulation, as well as LN organization. The strategic segregation and distribution of the FRCs create specific

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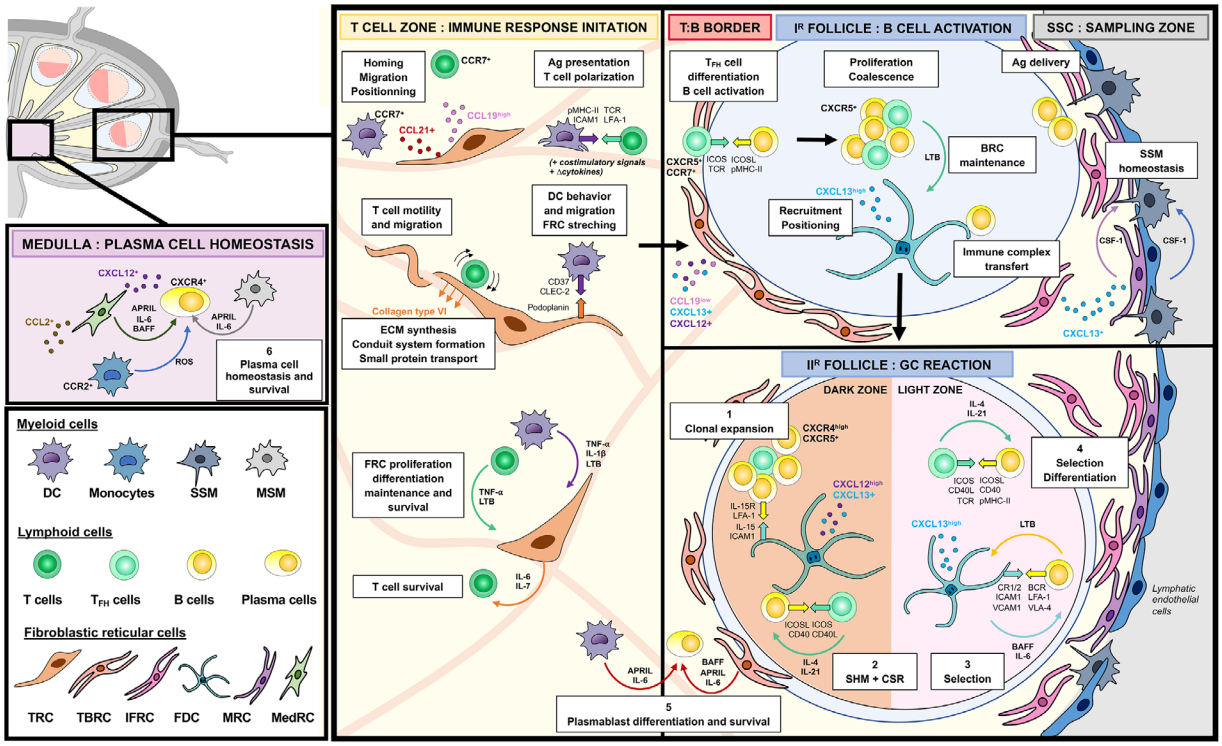

Figure 1. *Coordinated crosstalk between FRCs and immune cells govern the establishment of adaptive immune response in LN. Schematic representation of interactions occurring between FRCs and immune cells, upon antigen challenge. The names of the 4 major LN compartments are shown in different colors in each part of the figure: Paracortex/T cell zone in orange, Cortex/B cell zone in blue, SSC in grey, and medulla in purple. The different LN cell types are detailed in the bottom left panel of the figure. Direct cell/cell interactions are represented by large arrows, while the main interactions occurring through soluble molecules are represented by curved arrows. Different arrows colors refer to the color of the cell type providing the signal. Chemokines are represented by colored circles. The sequential events that drive B cell differentiation from the GC toward a plasma cell phenotype in the medulla are numbered from 1 to 6. In the T cell zone, immune response initiation results in simultaneous interactions between DCs, T cells, and TRCs. Immune cells induce TRC stretching and differentiation that support LN expansion. Conversely, TRCs influence DCs and T cells positioning and behavior and promote their encounter by producing CCL19 and CCL21 and survival signals such as IL-6 and IL-7. Reciprocally, immune cells produce IL1b, LTB, and TNF-α and promote TRCs survival. In parallel to T cell zone activities, B cells activation occurs close to SSC and B cells zone boundaries. Pre-TfH cells and newly activated B cells migrate at the T:B border to complete their activation before reaching the primary follicle. The coalescence and proliferation of both T_{FH} cells and B cells induce the remodeling of the primary follicle into a secondary follicle with a GC. They also secrete LTB that induces FDC maturation. Mature FDCs organize GC into a dark and a light zone by generating CXCL12 and CXCL13 gradients. In the dark zone, B cells clonally expand and undergo class switching recombination and somatic hypermutation, before migrating to n the light zone, attracted by CXCL13 from FDCs. In the light zone, FDCs and T_{FH} cells alternatively select B cells with the highest BCR affinity. Selected B cells received IL-6 and BAFF from FDCs and are rescued from apoptosis. Conversely, they maintain FDC in a mature state by providing them LTB. At the end of the light zone cycle, T_{FH} cells decide B cell fate by modulating IL-4 and IL-21 production and CD40L expression and orient them toward a return to a centroblast phenotype for dark zone recycling or induce their differentiation into antibody-secreting cells. Plasmablasts transit toward the T/B border and differentiate into plasma cells. The newly differentiated plasma cells reach the medulla, in which MedRCs and myeloid cells create a privileged IL-6/APRIL- rich environment for their homeostasis.

microenvironments, or niches, that guide cell-cell interactions and broadly organize these anatomical compartments into functional areas, each of them hosting specific immune cell subsets [5]. The paracortex is considered as a T cell zone, as it is mainly composed of T cells and dendritic cells (DCs) routed from the periphery to the LN through high endothelial venules (HEVs) and lymphatic vessels, respectively, and supported by T cell zone reticular cells (TRCs) [5]. Interactions between T cells, DCs, and TRCs occur in a synchronized manner. TRCs are an important source of nutrients, survival factors, and antigens for DCs and T cells and provide the scaffold and the molecular cues that facilitate cellular migration and interactions [6–8]. Conversely, TRCs receive from DCs and T cells all the signals and survival factors needed for their maintenance and their expansion [9, 10]. The cortex is referred to as the B cell zone, as it contains many follicles composed of B cells, follicular T helper (T_{FH}) cells and it is underpinned by B cell interacting-reticular cells (BRCs), such as FDCs [11]. There, interactions between these cellular effectors occur in a spatiotemporal regulated manner to support each step of naive B cell differentiation into antibody-secreting cells and memory B cells [12]. These two compartments are notably connected by the T-B border region, which forms an active interface allowing communication between the two zones. Lining the cortex, the SSC is a strategic sampling zone where SSC macrophages (SSMs) and marginal reticular cells (MRCs)

*Correction added on 28 October 2021 after first online publication: figure legend was wrongly placed at the end of the article.

are continuously exposed to lymph-borne antigens and deliver them to B cells [13, 14]. The medulla, for its part, constitutes a privileged niche in which medullary reticular cells (MedRCs) support plasma cell survival and homeostasis [15].

T cell-dependent antibody responses rely on tightly regulated and coordinated intercellular crosstalk within each of these areas. During the past decades, growing knowledge on stromal cell biology has demonstrated the immune-regulatory functions of FRCs, beyond their long-described role as architectural scaffold [16]. More recently revealed, their startling diversity gave a considerable impulse to our understanding of the complex spatiotemporal events taking place within LNs upon antigen challenge [11, 17, 18].

This review aims to describe how FRCs orchestrate immune cell maturation, distribution, and activation and how immune cell-derived signals tightly regulate FRC functions, maintenance, and survival. Here, we highlight the role of FRCs and immune cells interactions, which delineate a specific functional environment in the LN, and consequently support the ongoing immune response (Figure 1).

Simultaneous crosstalk between TRCs, DCs, and T cells drive adaptive immune response initiation

DC/T cell/TRC interactions promote T cell zone expansion

Immune responses initiate within the T cell zone where DCs play a dual role: (i) they present antigens to T cells inducing their differentiation into T effector cells and (ii) they trigger LN expansion through direct interactions with TRCs, the structural backbone of the T cell zone (Figure 1).

During a primary immune response, both DCs and T cells crawl at the surface of the TRC network. Activated DCs overexpress CLEC-2, which binds to podoplanin (PDPN), constitutively expressed by TRCs [19]. PDPN is expressed by most of the FRCs in the LN and is classically used to identify them, in association with the lack of expression of CD31, an endothelial cell marker [20]. However, the classical definition of FRCs as $CD31^{-}PDPN^{+}$cells is undergoing reevaluation since a subset of $PDPN^{-}CD31^{-}$ cells has been recently identified in mice [21, 22]. In vitro, these double-negative cells have a fibroblastic shape similarly to $PDPN^{+}$ FRCs, but differ by their chemokines pattern expression [21]. In addition, the lack of PDPN expression suggests that these double-negative cells (DNCs) likely exhibit morphodynamic characteristics distinct from $PDPN^{+}$FRCs and are differentially involved in LN architecture. Further investigations are needed to identify specific DNC markers, and to establish their location and functions. Thus, the use of PDPN as an FRC marker needs to be carefully considered according to the studied cell type. CLEC-2/PDPN physical interaction not only induces DC migration along the TRC network, but also allows TRC network stretching [19, 23]. CLEC-2/PDPN binding subsequently leads to PDPN inhibition and loss of tension of the TRC actomyosin cytoskeleton [23]. FRC network stretching is necessary for the LN expansion in order to receive the increasing number of lymphocytes recruited to the LN under antigen challenge, and is associated with inhibition of lymphocyte egress [24, 25]. In addition to stretching, immune cell recruitment and accumulation results in FRC proliferation and maintenance as well as their differentiation from mesenchymal precursors [9, 26, 27]. The signals controlling FRC development are still unclear, but some studies highlighted the potential role of $CD11c^{+}$ myeloid cell-derived IL-1β as well as ligands of the lymphotoxin-α1β2 (LTB) and TNF-α receptors, produced by DCs and lymphocytes [9, 10, 27–31]. DC depletion reduces $PDPN^{+}$FRCs number. First described in the spleen, the signal regulatory protein-α (SIRP-α) is also an important contributor to FRC development and differentiation in the LN, as surface SIRP-α is expressed on $CD11c^{+}$ DCs and increases TNF-α production in mice [32, 33]. Conversely, TRCs contribute to immune cell homeostasis by providing physical support and secreting survival factors such as IL-7 and providing T cells with antiapoptotic and proliferative signals [34]. It has been recently suggested that IL-7 was crucial for central memory T cell survival but dispensable for naive T cells [6]. Further investigations are needed to determine whether the level of IL-7 dependence that differs between the T cells subsets is associated with their respective sensitivity to IL-7, or with their location in particular regions more or less enriched in IL-7-producing FRCs.

Direct contact between T cells and differentiated TRCs stimulates the production of collagen type VI, a fibrous component of the extracellular matrix (ECM) recognized by the ER-TR7 antibody [35, 36]. ECM synthesis by FRCs is responsible for intertwining them to the reticular network contributing to T cell egress and immune cell migration within the LN [36]. FRCs organize and wrap themselves around a reticular network of collagen fibers that acts as a conduit system for the transport of antigens, nutrients, small proteins, and survival factors [37]. The topologically robust FRC network integrity is critical for immune cell aggregation in the LN and for the activation of adaptive immune responses [38]. The conduit system exhibits lower resilience to perturbation compared to the FRC network, while the robust topological organization of both networks is maintained in a lymphotoxin-β-receptor-independent manner [39]. The FRCs continue to maintain a minimal degree of connectivity in order to re-establish the LN network after the inflammation is resolved, while the conduit system readily becomes more fragmented and permeable during LN expansion [39, 40]. The conduit system network remodeling and reorganization that occur upon acute LN expansion are also influenced by CLEC-2 from DCs, which stimulates ECM production by FRCs when binding to PDPN [40].

TRCs drive DCs and T cells trafficking and positioning within the T cell zone

DCs are divided into several subtypes depending on morphological, phenotypical, and functional features. DCs are known as specialized sentinels that detect different types of danger signals

[41]. Both plasmacytoid pre-DCs (pDCs) and conventional DCs (cDC1 and cDC2) are present within the T cell zone. pDCs are specialized in the production of type I IFN, while cDCs are professional antigen-presenting cells which prime T cells and initiate the adaptive immune responses. DCs can be either LN-resident or migratory [42]. The LN-resident or migratory status of cDCs mainly depends on their steady-state location and the way they reach LNs and access antigens. LN-resident DCs continuously circulate and enter the LN from the blood and receive antigen on-site, while migratory DCs reach the LN from the lymphatic system following antigen recognition in the peripheral tissues [42]. As extensively characterized, pDCs, cDC1, and cDC2 exhibit different phenotypes and functions, respond to different stimuli, and secrete various cytokines upon stimulation. cDC1 are recognized by the expression of the surface markers BTLA, CD141, CADM1, CLEC9A [43]. They can sense viral nucleic acids through TLR3 and TLR8, secrete the cytokines TNF-α, CXCL10, IFN-λ, and IL-12, and are capable of antigen cross-presentation to $CD8^+$T cells [43]. cDC2 express the surface markers CD2, CD1c, and CD11c [43–46], they are equipped with TLR1 and TLR8, as well as CLEC7a and CLEC6a that allow them to recognize a wide panel of bacterial and fungal-derived elements. Upon stimulation cDC2 can produce the cytokines TNF-α, IL-8, IL-10, IL-12, and IL-23; they are very good $CD4^+$T cell stimulators and are capable of cross-presenting to CD8 T cells [47]. Finally, pDCs are described by the combination of surface markers $CX3CR1^-CD33^-CD123^+CD303^+CD304^+$, they are specialized in recognizing foreign nucleic acids through the expression of TLR7 and TLR9, and are professional IFN-α producers [43].

To ensure the proper functioning of the T cell zone, immune cells should display a correct spatial distribution on the FRC network throughout the lymphoid organ. The entire process of compartmentalization within the T cell zone is imposed by TRCs, which generate chemokine gradients, mainly CCL19 and CCL21, ligands for the CCR7 receptor expressed on naive and central memory T cells and DCs [8]. Different parameters modulate the CCR7-CCL19/CCL21-mediated trafficking: CCR7 chemotaxis has different levels of complexity and is sensitive to environmental cues. For example, polysialic acid (PSA) promotes DC chemotaxis toward CCL21. Indeed, PSA-mediated polysialylation of neuropilin 2 expressed at the DC surface facilitates the exposure of CCL21 to CCR7 [48].

An important role of TRCs is to provide the appropriate directional signals to ensure that naive T cells will encounter their cognate antigen [49]. In mice, it has been shown that loss of the FRC network integrity impedes immune cell aggregation and leads to depletion of both T cells, and resident as well as migratory DCs in the LN [50, 38]. Gremlin1 (GREM1)-expressing FRCs localization in the proximity of pre-DCs and cDCs at T-B cell junctions is essential for homeostatic proliferation and survival of resident cDCs [51]. Although CCL19 and CCL21 are known to retain DCs and T cells within the T cell zone, they also keep them in a continuous moving process that favors antigen presentation [49]. As the frequency of antigen-specific T cells is low, it is important that individual DCs can easily scan a large number of T cells to find their antigen-specific counterpart. Thus, specialized DC subsets have been identified in key compartments of the T cell zone. cDC2, which are professional in MHC-II antigen presentation colocalize with naive $CD4^+$ T cells, while cDC1, which are preferentially involved in MHC-I antigen cross-presentation, colocalize with naive CD8 T cells [52, 53]. Both naive $CD4^+$ T cells and cDC2 express the oxysterol receptor Ebi2, which directs them towards the periphery of the T cell zone. Conversely, $CD8^+$ T cells and cDC1, which do not express Ebi2, meet in the center of the T cell zone [54]. These oxysterol ligands, downstream metabolites of cholesterol oxidation and of yet unclear cellular source, depend on CH25H and CYP7B1, two enzymes abundantly expressed and active in $CXCL13^+$ and $CCL21^+$FRCs [55]. DC localization is also dependent on the DC antigen status. Antigen-bearing DCs preferentially prowl around HEVs to scan newly arrived lymphocytes before they return to the paracortex [56]. LN-resident DCs strategically localize around lymphatic sinus lumen to take over the lymph-borne antigens and rapidly initiate T cell responses [57].

TRCs influence DC and T cell behavior and promote their encounter

DC and T cell motility is a key element for an efficient T cell repertory scanning [58]. Physical interactions with TRCs, and changes in the density of their 3D framework, profoundly impact the morphodynamic behavior of DCs and T cells and place TRCs in a central position as important contributors of the immune response initiation [58]. More specifically, DCs are anchored to the TRC network to facilitate their scanning by T cells [31]. Besides contributing to TRC expansion and maintenance, the CLEC-2/PDPN axis also enhances DC motility through actin polymerization and protrusion extension [19]. This mechanism is further reinforced by the tetraspanin CD37, also expressed by DCs, which enhances and optimizes CLEC-2 expression at their surface [59]. Altered CD37 or CLEC-2 expression on DCs is associated with a deficiency in their trafficking and T cell priming [19, 59].

Both DCs and TRCs regulate T cell velocity by producing CCL19 and expressing immobilized integrins on their surface [60–62]. Except for chemokines and adhesion molecules, there are other factors contributing to lymphocyte movement in the LN, which remain to be characterized. The densely packed environment imposed by TRCs also seems to act as a determinant for T cell dynamics [49]. Indeed, TRCs constitute physical obstacles that impact T cell speed and organize their trajectory into small and dynamic "streams", enhancing their probability to meet their DC counterparts [38, 63]. Activated DCs increase surface expression of costimulatory molecules, change morphology, and can therefore efficiently activate T cells. DC-T cell interactions occur in distinct phases [64]. Initially, motile T cells interact briefly and repeatedly with DCs [64]. Then, the processed antigens are presented through the MHC molecules and allow T cell receptor (TCR) stimulation leading to the lymphocyte function-associated antigen 1 (LFA-1) upregulation at the T cell surface [62, 64]. In turn, T cells adopt a stationary phenotype with enhanced

adherent properties, allowing prolonged and more stable interactions with DCs and resulting in immunological synapse formation [64]. In a second phase, recently activated T cells have an intrinsic defect in establishing stable contacts with DCs. This transient T cell hypo-responsiveness promotes disengagement from antigen-presenting cells and favors effective clonal expansion [65].

TRCs promote T cell activation by facilitating access to antigens for migratory DCs, through the conduit system [7, 66]. Depending on the antigen, migratory DCs directly activate T cells but also transfer antigens to resident DCs to optimize $CD8^+$ T cell priming [67]. Following their activation by DCs, T cells undergo rapid clonal expansion in the LN and polarize into effectors subsets, defined by their cytokine production pattern and specific transcription factor expression. In response to activated T cells, TRCs upregulate CD40 and secrete IL-6 [68, 69]. CD40 was shown to promote $CD4^+$ T cell survival and proliferation [68]. IL-6 has the same effect on $CD8^+$ T cells, through epigenetic remodeling [69]. TRC-derived IL-6 also enhances IL-2 and TNF-α production by $CD8^+$ T cells that in turn may contribute to TRC maintenance [69]. Furthermore, polarized T cells, such as Th17, can also act on TRC survival and proliferation by inducing their metabolic reprogramming [70].

In a negative feedback loop, TRCs can prevent exacerbated T cell activation. Indeed, IFN-y and TNF-α stimulation, DC-derived signals, and direct contacts with activated T cells induce the release of nitric oxide (NO) by TRCs, moderating T cell expansion and reducing T cell priming by DCs [71, 72]. In general, FRCs act as key regulators of immune responses. In Peyer's patches (PP) and mesenteric LNs, FRCs regulate antiviral immunity by controlling local ILC1 activation and subsequent Th differentiation. FRCs build and maintain an exclusive IL-15-dependent niche for the group 1 ILCs and/or NK cells [73]. Furthermore, FRCs prevent exaggerated immune reactions to intestinal microbiota through the innate immunological sensing adaptor MyD88. Since the gut immune system represents a unique microenvironment, it remains to be determined whether the stromal cells exert such regulation on the ILC1 subset, outside of PPs and mesenteric LNs [73].

Although most of the data from mouse model studies stand in line with data from human FRCs, some differences have been observed. For example, in a culture model of human LN derived-FRCs, NO production after IFN-y stimulation has not been detected [74]. In this context, the use of an in vitro model could constitute a limitation for FRC maintenance as they are very fragile and sensitive to their environment. Overall, despite the studied model, the current literature demonstrates that the importance of FRCs in regulating immune cell homeostasis is no longer to be debated. The simultaneous crosstalk between immune cells and FRCs is crucial to their survival and differentiation, which determine the initiation of appropriate immune responses.

Maturation and migration of T_{FH} cell precursors

As already discussed, the differentiation of naive $CD4^+$ T cells into specialized effector T helper cells takes place within the T cell zone and at the T:B border, while further activation in the GC promotes their differentiation and function [11]. Among differentiated T cells, T_{FH} cells were shown to be essential in providing help to B cells for differentiation, proliferation, and affinity maturation [75].

In the T cell zone, DCs, and especially cDC2 efficiently initiate T_{FH} cell development through the production of inflammatory cytokines such as IL-6 and IL-2, as well as cognate interactions through the inducible T cell costimulatory factor (ICOS) and its ligand (ICOSL), respectively expressed by T cell and DCs [76, 77]. At this early stage of differentiation, ICOS engagement, but not the SLAM-associated protein (SAP) or CD40L, is required for inducing the expression of the transcription factor BCL-6, CXCR5, and the production of IL-21, all being canonical T_{FH} cell features [77, 78]. In addition to costimulatory molecules, a link between TCR signal strength, IL-2 production, and non-T_{FH} vs T_{FH} cell fate decisions was established [79, 80]. IL-2-producing T cells have stronger TCR signaling and preferentially express T_{FH}-associated genes such as BCL-6 and CD40L [80]. T cells with a weak TCR signaling do not produce IL-2 and receive paracrine IL-2 signaling that induces their differentiation toward a non-T_{FH} effector phenotype [80]. Even though studies of the past decade have provided new insights into our understanding of T_{FH} biology, some molecular mechanisms underlying key events that drive T_{FH} cell development such as CXCR5 induction remain elusive [81]. CXCR5 expression enables the so-called pre-T_{FH} cells to migrate to the T: B border, attracted by a CXCL13 gradient generating from FRCs that underpin the B cell zone [18]. The T cell zone chemokine environment inversely supports pre-T_{FH} cell transit. In 2018, three subsets of $CCL19^+$TRCs have been described in mice: (i) $CCL19^{high}$ TRCs, localized in the center of the T cell zone, (ii) $CCL19^{low}$ TRCs, more peripheral, located in interfollicular areas and at the T:B border, and (iii) $CXCL9^+$TRCs with a more scattered distribution [17]. In 2020, TRC nomenclature has been revised and four subsets have been identified according to their location and their molecular signature: $CCL19^{high}$TRCs, T:B border reticular cells (TBRCs) previously referred to as $CCL19^{low}$TRCs, $CXCL9^+$TRCs, and interfollicular reticular cells (IFRCs) located between B cell follicles [11, 82]. A recent study has even identified two murine TBRC subsets, that mainly differ by the percentage of CXCL13 and CXCL1-expressing cells and their CXCL10 expression level [82]. This further emphasizes the continuous changes in the FRC heterogeneity definition, in line with the increasing knowledge. The decreasing CCL19 gradient generated by the distinct TRC subsets from the T cell zone center to the B cell zone boundaries, in association to CCR7 downregulation by pre-T_{FH} cells, facilitate their movement out of the T cell zone and their placement at the T:B border to complete their differentiation, under the control of B cell-derived signals [83].

$CCL19^{high}$ TRC and $CXCL9^+$TRC molecular signatures mainly differ by a variation in the expression level of some genes rather than by their exclusive expression [17]. Thus, the identification of several CCL19-expressing FRC subsets in silico does not exclude the possibility that some of them are part of the same subset

adopting distinct identities to create local favorable immune niches in response to environmental changes. CXCL9$^+$ TRCs molecular signature is mainly associated with IFN-response associated genes, including the IFN-inducible chemokines CXCL9 and CXCL10 [17]. IFN receptor signaling in FRCs regulates LN myeloid cell composition and contributes to the generation of protective immunity against viral infections [82]. Furthermore, a role for CXCL9$^+$ TRCs in supporting CD8$^+$ T cell fate has recently emerged [84]. Upon viral infection, naive CD8$^+$T cells migrate at the LN periphery in a CXCR3-dependant manner to complete their differentiation and acquire their effector functions. In this context, both DCs and CXCL9$^+$ FRCs have been shown to be the main sources of the CXCR3 ligands, CXCL9 and CXCL10 [41, 84]. Depending on their location either in the T cell zone periphery or in interfollicular regions, DCs and TRCs balance their CXCL9/CXCL10 production to allow differential positioning of CD8$^+$T cells. This influences their differentiation toward either an effector or a central memory profile [84]. CXCL9$^+$ TRCs more likely represent TRCs in an activated state than a specific stable subset. In addition to chemokines, it is not excluded that FRC may provide other costimulatory signals to influence immune cell differentiation. The unrevealed plasticity of FRCs in response to a given inflammatory context also explains the difficulties to find a "consensus description" of their diversity. Moreover, the difficulty in studying FRC diversity is increased by the growing use of high throughput technologies that offer a great wealth of data, which can be very complex to analyze. Indeed, the number of subsets and their definition depend on the in silico-clustering strategy used, which can vary according to the technical parameters defined during the analysis. Further investigations using different experimental approaches and in distinct inflammatory conditions are needed to better appreciate the extent of the phenotypic spectrum of TRCs, and FRCs in general.

Sequential interactions between T_{FH}, B, myeloid cells, and BRCs orchestrate B cell differentiation

BRCs cooperate with SSMs for naive B cell activation

After entering the LN through HEVs, naive B cells remain only for a short time in the T cell zone before being distributed in primary B cell follicles. They are attracted by the interaction affinity between CXCR5, constitutively expressed on the B cell surface, and CXCL13, mainly produced by FDCs in the B cell follicle [85, 86]. Even though mainly associated with FDCs, CXCL13 is also expressed by other FRC subsets. The CXCL13$^+$ FRCs are confined to B-cell follicles and medullary cords and thus are collectively called "B-cell interacting reticular cells" (BRCs) as they guide B cell trafficking and follicle formation. BRCs can be subdivided into two broad categories: (i) CXCL13$^+$ CD21/35$^+$ FDCs that include two FDC subsets: dark zone FDCs (DZ-FDCs) and light zone-FDCs (LZ-FDCs) underpinning the GC, and (ii) CXCL13$^+$ CD21/35$^-$ BRCs that comprise MRCs, TBRCs, IFRCs, located underneath the subcapsular sinus, and two subsets of MedRCs in the medulla [11, 18]. BRCs together generate microenvironments that shape both immobilized and soluble CXCL13 gradients [18]. The subset specification is predetermined at steady state. Poised BRC microenvironments accommodate GC formation, rather than on-demand evolution of specialized niches upon inflammation [11].

In contrast to the TRC network, FDCs constitute a sparse network that restricts the number of possible directions for B cells. FDCs are crucial for cell organization, retention, and homogeneity in the primary B cell follicle [86]. Their network may act as a guide for B cell migration and probably optimizes FDC-B cell interactions. Interestingly, similar to results obtained for the role of Ebi2 on T cells and DCs, Ebi2 is also important for B cell placement [87]. In mice, Ebi2-deficient B cells failed to move to the outer B cell follicle and remain in the B cell follicle center [87]. In these settings, Ebi2 overexpression was sufficient to promote B cell localization to the outer B cell follicle. The distribution of CXCL13$^+$ cells expressing enzymes responsible for Ebi2 ligand synthesis, oxysterol, determines the concentration of the oxysterol ligand within the different regions of the B cell follicle possibly explaining how FDCs direct Ebi2-dependent B cell positioning [55].

CXCL13 binds to ECM components creating short sharp gradients proximal to the CXCL13-secreting cells, which is a key determinant of B-cell trafficking patterns within the B cell follicle. CXCL13 can be solubilized by cathepsin B (Cath-B) allowing the formation of soluble CXCL13 gradients that are essential for the formation of primary follicles within the LN [18]. The truncated molecule is capable of binding and signaling through CXCR5, but displays reduced affinity for the ECM. Moreover, the soluble CXCL13 is more potent than the truncated one, in attracting CXCR5$^+$B cells [18]. The availability of the immobilized and soluble CXCL13 forms is fine-tuned by the reticular-cell environment and by the enzyme Cath-B. Mice lacking Cath-B display aberrant follicular architecture, a phenotype associated with effective B cell homing to but not within LNs. Soluble CXCL13 may drive chemotactic homing behaviors while immobilized CXCL13 promotes haptokinetic scanning within the B cell follicle [18].

While T cell zone reorganization is mainly limited to its expansion under antigen stimulation, B cell zone reorganization is associated with architectural changes induced by the formation of the GC, a highly organized structure with strong mitotic activity [88]. This drastic remodeling is initiated by B cell activation after the binding of foreign antigens to the B cell receptor (BCR). How B cells access antigens is being extensively studied with new mechanisms continuously emerging. Antigens can be either in a soluble form or presented at the membrane of various cell types to be subsequently captured by B cells through BCR cross-linking.

Proximal to B cell follicles, the SSC is a privileged antigen sampling zone for B cell response initiation (Figure 1). There, SSMs capture soluble lymph-borne antigens and deliver them in

a complement-dependent manner to follicular B cells through their processes that extend into B cell follicles. Then, follicular B cells carry the complexes throughout follicles and deliver them to FDCs [89, 90]. Following interactions at the B cell follicle-SSC boundary, SSMs receive from B cells LTB that is needed for their survival and proliferation [90]. MRCs and lymphatic endothelial cells (LECs) that line the SCS are also crucial for SSMs maintenance that highly depend on RANKL stimulation and CSF-1. Mice deficient in stromal RANKL or CSF-1 show reduced numbers of SSMs and medullary sinus macrophages (MSMs). Consequently, they are compromised in antigen transport to B cells, and antiviral immune response [13, 14]. Moreover, RANK activation of LECs has been shown to be required during LN development and after inflammation, for the formation of both sinusoidal macrophage subtypes [13]. Through RANK-RANKL signaling and CSF-1, MRCs with LECs create a niche environment for sinusoidal macrophages to home, complete their differentiation program, and fulfil their innate immune function.

Low-molecular-weight antigens can be alternatively routed by a sparse follicular conduit system that originates from the SCS and permeates the B cell follicles [91, 92]. This conduit network forms during embryogenesis, partially disappears in adult LN, and also dramatically changes during B cell follicle expansion, simultaneously to the formation of the FDC network [91, 93]. In addition, the persistence of such structures in the B cell follicle allows antigen deposition on FDCs enwrapped around these residual conduits, and constitutes another way for B cells to access soluble antigens [91, 93]. As FDCs were mainly described for their key role in GC-B cell selection, their role in B cell activation in primary B cell follicles was long underestimated [94]. However, membrane-bound antigens provided by FDCs or SSM are believed to be more effective than the soluble ones at activating B cells [95]. B cells can also acquire antigens directly from the T cell zone, either by cDC2 located close to HEVs rapidly after they entered the LN, or during their transit toward the B cell zone following contact with DCs anchored in the TRC network [95, 96].

T_{FH} and B cell interactions at the T:B border initiate GC formation supported by a local TBRC network

To move in different LN areas according to their stage of activation and differentiation, B cells can balance their responsiveness to both T and B cell zone chemokines. Antigen recognition in the primary B cell follicle notably induces rapid upregulation of CCR7 at the B cell surface allowing them congregating to the T:B border and complete their activation under T cell help [97]. The T:B border is marked by TBRCs. TBRCs exhibit both T and B cell zone features, as they produce CCL19, CCL21, CXCL12, GREM1, B cell activating factor (BAFF), and at a lower level, CXCL13. This hybrid phenotype creates a connective interface where B cells meet their cognate T cells [11, 82]. Among those T cells, pre-T_{FH} cells, which are essential for the thymus-dependent immune response, are waiting for additional activation signals. The limited antigen access at the T:B border is critical for pre- T_{FH} cell maturation [98]. B cells are required for this process, as they are the only antigen-presenting cells readily available in this area [98]. Prolonged and repeated interactions between those 2 cell types further enhance BCL-6 expression by pre-T_{FH} cells, thus licensing terminal T_{FH} cell differentiation [99]. BCL-6 is also the master regulator of the GC-B cell program and is required to initiate, enhance, and stabilize the GC reaction [99, 100]. The important role of BCL-6 has been validated in mouse studies where BCL-6 deficiency was associated with failure in developing GC and specific antibody responses [101, 102].

T_{FH}/B cell interactions lead also to both ICOS and SAP engagement which is needed to supervise T_{FH} cell persistence at the T:B border and their subsequent entry within the GC [103, 104]. When T_{FH} cells meet B cells, CD40-CD40L and SAP interactions together with BCR signaling activation make B cells differentiate either into extra-follicular low-affinity antibody-producing plasma cells, or specialized long-lived plasma cells located in the center of the B cell follicle [105, 106].

The T_{FH}-B cell communication is also coordinated by soluble factors such as IL-4 and IL-21. IL-4 is known to be produced mainly by Th2 cells whereas IL-21 is considered as the key cytokine secreted by T_{FH} cells. Both act as checkpoints to modulate B cell affinity maturation and differentiation throughout the GC reaction [107]. More recently, it has been reported the existence of 3 T_{FH} subtypes sharing functional properties with Th1, Th2, and Th17 cells; co-producing IL-21 with either IFN-g ($T_{FH}1$), IL-4 ($T_{FH}2$), and IL-17 ($T_{FH}17$) [108]. The exact role of these three T_{FH} subsets in B cell proliferation and differentiation is still under investigation.

FDCs govern GC organization and polarization under T_{FH} and B cell help

At the T:B border, activated pre-GC B cells undergo a strong clonal expansion [109]. They also become highly motile and reach the center of the primary B cell follicle influenced by FDC-derived CXCL13 [18, 110, 111]. Only B cells with the highest amount of peptide-MHC-II (pMHC) complexes receive T cell help at the T:B border and are capable of entering the nascent GC [112]. At this stage, the early GC is not fully organized. The coalescence of these proliferative B cells initiates the formation of an early GC and shapes the primary B cell follicle into a secondary follicle [109]. Once mature, GC are polarized and divided into two compartments that support distinct phases of B cell maturation: a dark zone and a light zone, distinguishable by their chemokine environment and their cellular density [88]. Dark zone and light zone also house two distinct populations of B cells named centroblasts and centrocytes respectively. The differences that separate centroblasts from centrocytes are more associated with distinct migratory activities than to their maturation stage, to support B cell iterative transit between dark zone and light zone

[113]. Centroblasts are CD83^{low} CD86^{low} CXCR4^{high} and group in the dark zone enriched in CXCL12^{+}-producing FDCs. Centrocytes are CD83^{high} CD86^{high} CXCR4^{low}, confined in the light zone according to the CXCR5/CXCL13 axis [113–115]. Dark zone and light zone formation, which is mainly governed by FDCs, requires the production of LTB and TNF-α by activated B cells [111].LTB signaling is required for GC maintenance and organization but not for GC formation [116]. Indeed, the LTBR signaling pathway critically determines BRC development. In absence of LTB, MRCs and FDCs failed to mature and showed an altered CXCL13 production [11]. Conversely, CXCL13 binding at the B cell surface induces a mutual positive feedback loop leading to an upregulation of the LTB by B cells [85]. During helminth infection, it has been observed in mice that LTB-producing B cells can induce the expansion of CXCL13^{+} FRCs related to MRCs, around and within B cell follicles, as well as CCL19^{+}FRC enlargement for de novo B cell follicles formation [117, 118]. Primary and secondary B cell follicles are underpinned by MRCs, LZ- and DZ-FDCs, and TBRCs. LZ-FDCs express high levels of complement and Fc receptors as well as genes promoting intracellular stiffness, in line with the mechanical properties that support antigen sampling by GC-B cells. The cells underpinning the dark zone are a discrete subset of FDCs that exhibit a hybrid phenotype between LZ-FDCs and TBRCs. They express lower levels of CR1/2 than LZ-FDC and produce less CXCL12 compared to TBRCs [11].

CXCL12 is important for the chemotactic polarization of GC-B cells [11]. Upon deletion of *CXCL12* in CXCL13^{+} cells, naive LN retains normal development and hematopoietic cell distribution, B cell follicle formation, CXCL13^{+} cell numbers, and FDC and MRC maturation. However, the topological organization of the GC-BRC network after immunization is altered, reflecting perturbed spatial positioning of LZ- and DZ-FDCs. CXCL12-dependent topological remodeling of the GC was mediated through interaction with CXCR4^{+} GC- B cells. In control mice, the proliferating centroblasts were enriched in the GC dark zone, while they were dispersed throughout the GC in mice with *Cxcl12*-deficient Cxcl13^{+} cells, reflecting the topological disorganization of DZ-FDC upon inflammation [11]. Together these results show the importance of the BRC-B cell crosstalk for establishing the homeostasis of humoral immunity.

FDCs and T_{FH} cells sequentially drive B cell selection during GC reaction

A unique feature of the GC reaction is its dynamic nature. B cells continuously circulate between the dark zone and light zone in a cyclic process where short and repeated interactions with FDCs and T_{FH} cells determine future B cell fate decisions [12] (Figure 1). Along with the GC reaction, T_{FH} cells receive a wide range of B-cell-derived signals (e.g., ICOSL, CD40) that gradually modulate their functions. These signals depend on the antigen, the T_{FH} cell location, and the phase of the GC reaction. In this process, T_{FH} cells balance their CD40L expression and IL-4/IL-21 secretion in order to appropriately support each step of B cell differentiation [119].

In the dark zone, centroblasts diversify their Ig repertoire before being selected in the light zone, until they display a sufficient antigen-affinity to differentiate and get specialized into memory B cells or plasma cells. B cell specialization results from an intrinsic transcriptional program regulated by perpetual rearrangements and mutations of Ig coding genes. Initiated in the bone marrow, these transcriptional modifications occur all along B cell ontogenesis and end with class switching recombination and somatic hypermutation [107, 120]. Somatic hypermutation results in BCR affinity maturation and Ig repertoire diversification. Class switching recombination consists of Ig isotype modification to appropriately protect against a large variety of pathogens [120]. Class switching recombination and somatic hypermutation mainly involve DZ-T_{FH} cells, as their costimulatory molecules, ICOS and CD40L together with IL-21 are needed for the induction of the activation-induced cytidine deaminase (AID) that catalyzes them [121–123]. The Ig class resulting from class switching recombination is also regulated by DCs and other T cell subsets, which depending on the availability of IL-4, IL-10, TGF-b, IFN-g, a proliferation-inducing ligand (APRIL) and BAFF, create different molecular microenvironments [124–126]. Class switching recombination has been considered a post-somatic hypermutation event for a long time [4]. However, single-cell RNA sequencing studies have recently suggested that class switching recombination infrequently occurs outside the GC, before somatic hypermutation, presumably at the T:B border [127].

After somatic hypermutation, IL-4- and IL-21-derived signals, and CD40/CD40L interactions allow the newly mutated centroblasts to differentiate into centrocytes and migrate to the light zone to undergo selection [128]. Only centrocytes with sufficient antigen affinity are selected and receive survival signals, such as BAFF, needed to pursue their development. In the light zone, two selection steps will determine B cell fate. First, a pre-selection initiated by LZ-FDCs results in the elimination of B cells with a very low BCR affinity. Then, a T_{FH} cell-dependent selection ensures the survival of B cells with the highest antigen affinity. FDCs have the unique capability of retaining antigens in their native form and to present them to B cells either through complement (CD21/CR2, CD35/CR1) or Fc (CD23/FCeRII, CD32/ FcgRII) receptors expressed on their surface [94]. The antigen recognition by B cells induces the release of BAFF by FDCs, and LTB by B cells [129, 130]. This crosstalk is mutually beneficial. FDCs promote the survival of B cells, which in turn maintain FDCs in a mature state [129, 130]. Additionally, BAFF combines with CD44, 8D6 and FDC membrane-bound IL-15 to regulate B cell proliferation [130–132]. This prosperous immunological synapse between FDCs and B cells is reinforced and stabilized by the binding of the very late antigen 4 (VLA-4) and LFA-1 on B cells to their respective ligands VCAM-1 and ICAM-1 expressed on FDC surface [133, 134]. These adhesive interactions between FDCs and B cells also take place in the dark zone, independently of antigens, to protect centroblasts

from apoptosis and reinforce their proliferation [134, 135]. This provides adhesion molecules VCAM-1 and ICAM-1 with a still unappreciated role in the support of B cell affinity maturation. B cells that successfully go through this first selection process can then compete for T_{FH} cell help. LZ- T_{FH} cells are present in a limited number and discriminate B cells based on their pMHC density [136]. B cells that have internalized the largest amount of FDC-bound antigens have the highest BCR affinity, which gives them a competitive advantage [111]. This interaction is also critical for T_{FH} cell homeostasis, as T_{FH} cells with the strongest TCR signaling are preferentially selected and clonally expanded [137]. T_{FH} cells also respond to this affinity-dependent interaction by secreting soluble factors (IL-4, IL-21, and BAFF) and increasing integrins and CD40L expression [75]. Although the mechanisms involved in T_{FH} cell help are not fully elucidated, it is clear that CD40L and T_{FH} cell-derived cytokines in concert with adhesion molecules act as key modulators of B cell positive selection and fate decision [75]. B cells that trigger high CD40 signaling engage more stable interactions with T_{FH} cells and preferentially evolve into antibody-secreting cells (ASC) [138, 139]. B cells with less stable interactions return to their previous centroblast phenotype for additional maturation cycles in the dark zone [139]. In addition to its role in rescuing B cells from apoptosis, BAFF also influences the centroblast/ASC outcome by differentially interacting with its 3 known receptors expressed on the B cell surface: BAFF-R, TACI, and BCMA [140]. Hence, ASC engagement towards a plasmablast or a memory B cell profile results from a balance between signals from CD40, BAFF, and ICOS. This process is also modulated by other cytokines such as IL-2, IL-4, IL-10, and IL-21 [141, 142].

The efficiency of T_{FH} /B cell interaction is mainly conditioned by the CXCL12/CXCL13 gradient generated by BRCs [11]. The maturation and topology of $CXCL12^+$ FDCs determine the magnitude and efficiency of antigen-specific GC responses to complex protein antigens and haptens by limiting extrafollicular B cell responses and impacting GC-specific processes, such as somatic hypermutation. In the absence of CXCL12, the gene expression of GC-B cells is mainly unchanged by the perturbed FDC topology. However, Bach2 transcription and protein expression, which is inversely proportional to the magnitude of T cell help, is increased. This leads to an altered positioning of T_{FH} cells. Whereas normally localized in the light zone, in LN with CXCL12-deficient $CXCL13^+$ cells T_{FH} cells are homogeneously distributed throughout the GC, with significant localization in the regions corresponding to the dark zone [11]. As LZ- and DZ-FDC undergo limited transcriptional maturation upon inflammation, the CXCL12-orchestrated topological remodeling of BRC predominantly determines the efficiency of the GC response and humoral immunity by steering the interaction of B and T cells in the GC. Poised expression of CXCL12 by BRCs ensures the efficacy of early and peak stages of the GC response by limiting extrafollicular GC responses and positioning proliferating cells at the T:B border, which in turn is reciprocated through the competent remodeling of the BRC network [11].

GC-light zone output of selected B cells

Following T_{FH} cell selection, ASC and centroblasts alter their chemokines expression to exit the light zone, following distinct trajectories. Centroblasts migrate to the dark zone, to undergo additional cycles of maturation. Dark zone events can also be regulated by antigen-dependent light zone activities. LZ- T_{FH}/B cell interactions guide the number of centroblast divisions within the dark zone [143, 144]. DZ-FDCs may support additional cycles of somatic hypermutation by periodically arranging antigens which they are able to retain and store for a long period of time [145, 146]. Furthermore, their ability to produce IL-6 following immune complex stimulation also contributes to class switching recombination, somatic hypermutation, T_{FH} cell maintenance, and B cell differentiation [147].

Surprisingly, plasmablasts and memory B cells may have distinct GC-outputs. Memory B cells exit from the light zone toward the LN medulla, while plasmablasts appear to cross the dark zone and position themselves at the T:B border [148]. There, DCs, macrophages, and $CXCL12^+CCL19/21^+$ TBRCs create an IL-6, BAFF, and APRIL-enriched niche for plasmablast to plasma cell transition [82, 148, 149].

MedRCs and myeloid cells create a survival niche for newly differentiated plasma cells

Finally, the newly differentiated plasma cells can either migrate to the LN medulla or reach the bone marrow to finalize their differentiation into short- or long-lived plasma cells (Figure 1). In the medulla, myeloid cells such as macrophages and MedRCs create a local IL-6/APRIL environment for plasma cell homeostasis [15, 149]. MedRCs are phenotypically and functionally distinct from other FRC subsets. Notably, they generate different ECM structures from those of TRCs and organize a very dense meshwork to closely interact with plasma cells and promote their migration and survival by producing IL-6, BAFF, APRIL, and CXCL12 [15]. In the medulla, monocytes have a regulatory role in plasma cells survival by producing reactive oxygen species [150]. MedRCs indirectly contribute to this regulation by balancing their CCL2 production, thus modulating monocyte accumulation within the medulla [150]. Two MedRCs subsets have been identified in silico (MedRC1 and MedRC2). Their molecular signatures are very similar but differ by their CCL19 and CCL21 expression, higher in MedRC1 [11]. This suggests that MedRC2 may be located deeper in the medulla, while MedRC1 are more peripheral. In the future, transcriptomic analysis on a higher number of MedRCs may be useful to further characterize MedRC1 and MedRC2 and identify discriminant markers allowing their spatial identification. Close to MedRCs, an additional subset of FRCs in the deep cortex periphery (DCP) have been identified [22]. DCP seems to be a unique compartment as it differs from cortex and medulla by its FRC network organization and its cellular composition in that B cells and T cells are intermingled [22]. Thus, DCP reticular cells

(DRCs) can be regarded as a new FRC subset that delineates the B cell zone/medulla boundary. Compared to MedRCs, DRCs express higher levels of CXCL12 and CCL21 but only rarely interact with plasma cells, making MedRCs the major FRC subset involved in plasma cell homeostasis [22].

Concluding remarks and perspectives

Overall, this review summarizes the role of multicellular crosstalk occurring at the LN level. We paid particular attention to the description of their sub-anatomical localization, the mutual interactions between FRC subtypes and immune cells, and the tight regulation of those processes. The role of FRCs in inducing and regulating the initiation of an appropriate immune response has been widely described at the tissue level, as wells at the mechanistic level. We have also emphasized the role of different immune cells in regulating homeostasis and functions of FRCs to give a comprehensive view of LN functionality. In recent years, high throughput technologies such as RNA sequencing, highly multiplexed imaging, and in silico approaches, have enabled the generation of a systematic description of the cellular compartmentalization and crosstalk within LNs. This induced a shift from the study of individual components of different SLO anatomical compartments, towards a more global and integrated view of the immune and stromal microenvironment. This led, for example, to the concept of BRCs, which correspond to FRCs performing and sharing a particular type of function (CXCL13 production) within specialized niches.

Importantly, the cellular composition and context may have a critical impact on the function of immune regulating factors. For example, BAFF promotes B cell survival and proliferation when associated with IL-15 and CD44 from FDCs, but rather influences ASC differentiation when combined with CD40L and ICOS on T_{FH} cell surface. In addition, BAFF can exist either in a membrane-bound or a soluble form and bind to 3 receptors BAFF-R, BCMA, TACI, which result in distinct biological activities. Last, the T:B border is an important communication interface between the two zones, enabling interactions between T_{FH} and B cells, which contribute to the subsequent GC reaction.

Specifying the dynamics of intercellular communication networks in SLOs is an important challenge, especially in the human system. Organoids offer interesting perspectives as a controlled in vitro system reproducing some key features of primary LN [122]. Along that line, a recently developed tonsil-organoid was able to recreate in vitro the GC-B cell diversity found in vivo [123]. Integrating FRCs into such organoids will be a key step to further increase the physiological relevance of these models. Differences between LNs, spleen, and mucosa-associated lymphoid tissues such as tonsils, in terms of cellular compartments and microbial-host interface, should be taken into account in order to develop the most representative organoid models for each type of SLOs. Lastly, some key questions remain on the contribution of the various FRC subsets and their interactions with immune cells on the regulation of lymph filtration. This process can condition the cellular and antigenic composition of SLOs.

Deciphering cellular crosstalk and chemokine gradients in normal SLO could enable to re-program pathological SLO towards a homeostatic structure, for example by targeting the CXCL12/CXCL13 axis in lymphoma [151]. This could also open new avenues to exploit the formation of SLO-like structures in various locations to promote local immune responses. In tumors, for example, tertiary lymphoid structures (TLS) have been shown to be of potential interest as prognostic and predictive factors, several research groups are currently studying how to induce TLS neogenesis to ameliorate cancer treatment [152]. Last, educating FRCs to perform specific functions, create defined adaptive microenvironments, or present predefined antigens, may lead to antigen-specific immunomodulation in SLOs.

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Abbreviations: **AID**: activation-induced cytidine deaminase · **APRIL**: a proliferation-inducing ligand · **ASC**: antibody-secreting cell · **BAFF**: B-cell activating factor · **BCR**: B cell receptor · **BRC**: B-cell interacting reticular cell · **DC**: dendritic cell · **DCP**: deep cortex periphery · **DNC**: double-negative cell · **DRC**: deep cortex periphery reticular cells · **DZ**: dark zone · **FDC**: follicular dendritic cell · **FRC**: fibroblastic reticular cell · **GC**: germinal center · **GREM1**: gremlin1 · **HEV**: high endothelial venule · **ICOS**: inducible T cell costimulatory factor · **ICOSL**: inducible T cell costimulatory factor ligand · **IFRC**: interfollicular reticular cell · **LFA-1**: lymphocyte function-associated antigen 1 · **LN**: lymph node · **LTB**: lymphotoxin-$\alpha 1\beta 2$ · **LZ**: light zone · **MedRC**: medullary reticular cell · **MRC**: marginal reticular cell · **NO**: nitric oxide · **PDPN**: podoplanin · **PP**: Peyer's patches · **PSA**: polysialic acid · **SAP**: SLAM-associated protein · **SLO**: secondary lymphoid organ · **SSC**: subcapsular sinus · **SSM**: SSC macrophage · **TBRC**: T:B border reticular cell · **TCR**: T cell receptor · **TRC**: T cell zone reticular cell · **TFH**: follicular T helper cell · **TLS**: tertiary lymphoid structures · **VLA-4**: very late antigen 4

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