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Impact of B cell/lymphoid stromal cell crosstalk in B-cell physiology and malignancy

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Highlights

- Lymphoid stromal cells form a heterogeneous and plastic cell compartment
- Follicular and extrafollicular stromal cells contribute to normal B-cell activation
- FL-CAFs exhibit specific phenotypic and transcriptomic features
- FL B cells and their immune microenvironment trigger FL-CAF differentiation
- FL-CAFs support directly malignant B-cell growth and organize tumor cell niche

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Abstract

Stromal cells have been considered for a long time essentially as a structural component organizing tissue architecture, including those of secondary lymphoid organs. More recently, highly specialized stromal cell subsets were shown to differentially organize immune cell recruitment, survival, and differentiation within lymph nodes. In particular, mature B cells interact with different lymphoid stromal cell networks through bidirectional interactions involving cell-cell contact and soluble factors. Follicular lymphoma (FL) is the paradigm of a B-cell malignancy dependent on a lymphoid-like microenvironment supporting tumor cell growth, drug resistance, and clonal evolution. This review provides an overview of our current knowledge of lymphoid stromal cell heterogeneity and functions in normal B-cell activation. In addition, we also depict the dynamic and plasticity of FL cancer-associated fibroblasts, the mechanisms underlying their key role within FL permissive niches, and their potential as therapeutic targets in this still fatal malignancy.

Keywords: fibroblastic reticular cells, follicular dendritic cells, tumor niche, cell plasticity, cancer-associated fibroblasts

Abbreviations

BM: bone marrow; CAF: cancer-associated fibroblast; CRC: CXCL12-expressing reticular cells; DLBCL: diffuse large B-cell lymphoma; GC: germinal center; FDC; follicular dendritic cells; FL: follicular lymphoma; FRC: fibroblastic reticular cell; IDO: indoleamine-2,3 dioxygenase; LN: lymph node; LT: lymphotoxin-$\alpha 1\beta 2$; MRC: marginal reticular cell; MedRC: medullary reticular cell; TAM: tumor-associated macrophages; TNF: TNF-α; TRC: T-zone reticular cell

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1. Introduction

First identified in 1965 as a unique population of antigen-retaining reticular cells within germinal centers (GC) of secondary lymphoid organs, follicular dendritic cells (FDCs) are well known to play a central role in humoral immunity. However, more recent studies have shed new light on the different lymphoid stromal cell networks that collectively contribute to B-cell recruitment, motility, activation, selection, and differentiation during the initiation of adaptive immune response. Interestingly, these highly specialized stromal cell subsets are spatially, phenotypically, and functionally altered in various pathological settings, including autoimmune diseases, infections, and cancers. Follicular lymphoma (FL), the most frequent indolent lymphoma, is a B-cell malignancy strongly dependent on a GC-like permissive microenvironment exhibiting FL-specific features. This review highlights the phenotype, origin, and function of lymphoid stromal cells with a specific focus on their capacity to favor or restrain normal and malignant B-cell activation within secondary lymphoid organs.

2. Heterogeneity and origin of lymphoid stromal cells

Stromal cells organize the compartmentalization of lymph nodes (LNs) into discrete three-dimensional niches supporting lymphocyte activation and are essentially characterized by their common expression of podoplanin/gp38 in the absence of hematopoietic, epithelial, and endothelial markers [1]. The two classical stromal cell subsets are CXCL13-producing FDC, populating B-cell follicles, and CCL19/CCL21-producing fibroblastic reticular cells (FRCs), residing outside follicles. However, the diversity of lymphoid stromal cells has been recently better unraveled according to the development of new strategies of genetic targeting/high-resolution cell imaging and single-cell RNAseq (Table 1).

Besides mature CD21/CD35^{pos} FDCs, positioned in the light zone of the GC, B-cell follicles also include dark zone CXCL12-expressing reticular cells (CRCs), and TNFSF11/RANKL^{pos} marginal reticular cells (MRCs), residing at the edge of the follicle underneath the subcaspular sinus. In addition, a subset of non-FDC *cd21*-lineage tagged cells has been identified as dormant stromal cells that could be converted into CXCL13-secreting cells upon contact with activated B cells of the growing follicles and are thus called versatile stromal cells [2]. Within FRCs, at least two main functional subsets could be highlighted. The first one, found in the T-cell area and termed T-zone reticular cells (TRCs), attract CCR7^{pos} T, B, and dendritic cells, promote lymphocyte

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survival, and allow the diffusion of small molecules throughout the LN [1]. The second one, called medullary FRC (MedRCs), forms the major structural component of the plasma cell niche within LN medullary cords [3]. Of note, the landmark gene expression study of mouse LN stromal and endothelial cells performed by the Immgen consortium has defined the transcriptomic profile of podoplanin^{pos} CD31^{neg} cells, but TRCs, MedRCs, MRCs, CRCs, and FDCs all fall into this subset [4]. More recently, an elegant single-cell RNAseq analysis has described nine stromal cell subsets within mouse LN, including FDCs, MRCs, MedRC-like, and at least 3 TRC subsets, without a clear identification of versatile cells and CRCs [5]. However, these newly identified subsets need to be functionally explored to understand their role in immune response.

Lymphoid stromal cells are supposed to derive from local mesenchymal progenitors but the identity, localization, and differentiation potential of these progenitors remain controversial despite the development of extremely valuable cell-fate mapping strategies. Pre-adipocytic precursors have been shown to differentiate *in vivo* into several lymphoid stromal cell subsets including at least MRCs and FRCs [6]. MRC, the putative adult counterparts of embryonic lymphoid tissue organizer (LTo), could proliferate and differentiate into FDCs during inflammation-induced LN remodeling [7]. Moreover, perivascular cells have been proposed as precursors of lymphoid stromal cell subsets even if competing views exist concerning their specific features and origin, including podoplanin^{neg}CD31^{neg} double negative mural cells expressing smooth muscle actin and PDGFRβ *versus* podoplanin^{pos}CD34^{pos} adventitial cells [8-10]. Of note, splenic and LN stromal cells appear to rely on different developmental mechanisms and tissue-specific progenitors have been proposed [11]. Importantly, whatever their cell of origin, lymphotoxin-$\alpha 1\beta 2$ (LT) and TNF-α (TNF) are the two non-redundant key factors involved in lymphoid stromal cells differentiation and maintenance.

As a whole, heterogeneity and origin of lymphoid stromal cell subsets have recently emerged as highly challenging research fields with major impacts on our understanding of initiation and regulation of normal and pathological immune response. Noteworthy, very few data are available on human lymphoid stromal cell features and all of them have been obtained after long-term *in vitro* cell amplification without analyzing culture-related artifacts, including the general switch off of lymphoid chemokine expression and the loss of some cell sub-compartments such as mature FDCs.

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3. Interaction between stromal cells and B cells within normal LN

A remarkable feature common to all secondary lymphoid organs is the segregation of B cell- and T cell-zones defined by specific stromal cell networks. However, B cells, depending on their activation status and stage of differentiation, could be engaged in a bidirectional crosstalk with virtually all lymphoid stromal cell subsets (Figure 1A).

3.1 Follicular stromal cells

Functional follicles are based on the mutually dependent collaboration between B cells and FDCs. FDCs are crucial for follicular identity and B-cell organization [12] whereas the production of TNF and LT by B cells is required for proper FDC development and maintenance and is closely dependent on FDC-derived CXCL13 [13,14]. CXCL13 is also essential for sequestering CXCR5-expressing GC B cells and follicular helper T cells (Tfh) within GC light zone. One of the key FDC properties is their capacity to retain intact antigens captured as complement-coated immune complexes through Fc and complement receptors and protected from degradation for extended periods of time by continuous recycling [15]. Light zone GC B cells, also called centrocytes, pick up antigen from FDC surface within a unique synaptic architecture allowing stringent affinity discrimination [16] and present it as antigenic peptides to cognate Tfh cells, which in turn provide them with survival and differentiation signals [17]. The need to compete for limited T-cell help favors B cells with a high affinity BCR, whereas the others undergo apoptosis. Besides BCR activation, FDCs could contribute to GC B-cell survival through expression of adhesion molecules, in particular VCAM-1/CD106, Notch ligands, and soluble factors, including BAFF, IL-15, Shh, and Wnt5a [18-23]. However the role of each of these different molecules deserves further investigation involving targeted gene inactivation strategies. In addition, FDCs have been proposed to contribute to class switch recombination through the secretion of TGF-β1 [24]. Interestingly, FDCs also regulate apoptotic B-cell clearance through the production of MFGE8 (also called FDC-M1) that acts as a bridging molecule between apoptotic B cells exposing phosphatidylserine on their surface and tingible body macrophages expressing integrin $\alpha v\beta 3$ [25].

FDCs are not the only stromal cell subset present within GC and dark zone CRCs have been recently identified as a specific cell network. CRCs are distinguishable from FDCs and FRCs at the phenotypic level but they share with FDCs a high expression of SOX9

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transcription factor [5,26]. CRCs are involved in the recruitment of $CXCR4^{hi}$ centroblastic B cells in the dark zone, the pole of the GC closest to the T cell zone [27]. Dark zone centroblasts proliferate and undergo random somatic hypermutations in the variable regions of immunoglobulin genes, owing to their strong expression of the activation cytidine deaminase (AID) and the error-prone DNA polymerase eta [17]. Progressive downregulation of CXCR4 is then associated with centroblast migration towards the CXCL13 gradient produced by light zone FDCs for further affinity-based selection processes. Importantly, high affinity B cells selected into the light zone by contact with Tfh are induced to re-enter into the dark zone for further rounds of amplification and somatic hypermutation. Ultimately, GC B cells are triggered to differentiate into plasma cells or memory B cells, at least in part depending on their affinity for antigen that regulates the stringency of BCR- and Tfh-dependent signaling and the induction of specific differentiation molecular pathways [28]. Of note, all dark zone stromal cells do not produce CXCL12 supporting the presence of additional stromal cell types inside GC dark zone. As an example, hepatocyte growth factor (HGF) and its activator (HGFA) were detected in poorly characterized dark zone stromal cells and could contribute to proliferation, survival, and/or adhesion of MET-positive centroblasts [29].

Outside GC, MRCs form a thin layer of reticular cells directly under the subcapsular sinus and expressing CXCL13 and MadCAM-1, unlike CD21 [30]. Besides their role as putative stromal cell precursors and as a niche for innate lymphoid cells, the conduit network constructed by MRCs in the outer follicle could transport small soluble antigens to B cells and FDCs [31]. Very recently, MRCs were also proposed to act as nonprofessional phagocytes for apoptotic GC B cells in spleen [32]. However, MRCs remain a poorly characterized cell subset, in agreement with their very low number and the lack of specific tracking strategy in vivo.

Finally, FRC ablation *in vivo* in a *Ccl19*-Cre x iDTR mouse model was recently shown to reduce substantially B-cell viability within resting LN [33] whereas FDC loss has a minor impact on B-cell homeostasis [12]. This direct effect on naïve B-cell survival was assigned to a discrete subset of FRC found within and around follicles and producing high amounts of BAFF. The relationship between these cells and CXCL13-expressing versatile stromal cells remains unclear.

3.2 Extrafollicular stromal cells

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FRCs produce and enwrap extracellular matrix components including laminin, fibronectin, collagen IV, and tenascin to form a highly dynamic network regulating LN organization and immune cell trafficking [1,34]. Besides their initially identified crucial role on T-cell immune response, FRCs also impact B-cell behavior. First, TRCs recruit B cells through the release of CXCL12 and CCL19/CCL21 and ensure their guided trafficking from the paracortex to B-cell follicles [35]. Second, MedRCs generate different extracellular matrix structure than TRCs and specifically produce high amounts of CXCL12, BAFF, APRIL, and IL-6. They were shown to guide the migration of CXCR4^{pos} plasma cell within the medulla and to contribute, together with medullary macrophages, to plasma cell survival *in situ* [3]. At the earliest stage of GC response, both Tfh and a specific FRC subset producing APRIL have recently been involved in the output of plasmablasts at the GC-T zone interface before terminal maturation into plasma cells [36].

Importantly, FRCs undergo massive morphological changes and proliferative expansion to accommodate the increase of B and T lymphocyte numbers, and these modifications involved the triggering of podoplanin/gp38 on FRCs by CLEC-2 expressing dendritic cells, leading to a reduction of FRC contractility [37]. B cells were more recently suggested to contribute to FRC activation and maintenance in both LN and spleen via their inducible expression of LT [11,38,39].

Interestingly, FRCs are also involved in peripheral immune tolerance and might tolerize both CD4^{pos} and CD8^{pos} T cells through direct antigen presentation [40]. They were also shown to limit the proliferation of activated T cells independently of antigen presentation. In mice, this suppressive function was attributed to the expression of the inducible NO synthase [41] whereas in human, a recent study proposed that FRCs constrain T-cell activation via a combination of several mechanisms including indoleamine-2,3-dioxygenase (IDO), prostaglandin E2, TGF-βR, and adenosine A2 receptor signaling [42]. Since IDO has also been involved in the inhibition of B-cell proliferation by IFNγ-activated human stromal cells [43], it is tempting to speculate that FRCs could also regulate B-cell activation within inflamed secondary lymphoid organs.

4. Interaction between stromal cells and FL B cells

4.1 Follicular lymphoma microenvironment

FL B cells exhibit the general hallmark of centrocytes and are strongly dependent on direct interaction with a GC-like microenvironment including Tfh, myeloid cells, and

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stromal cells while developing efficient immune escape mechanisms [44,45]. The protumoral role of tumor-infiltrating lymphoid stromal cells in FL has been highlighted by the identification of FRC- and FDC-like cells ectopically induced within invaded bone marrow (BM) where they are found admixed with malignant B cells [46,47]. In addition, despite strong differences in cell composition and organization, BM- and LN-FL niches display common stromal cell phenotype and extracellular matrix composition features including deregulation of the matricellular protein secreted protein acidic and rich in cysteine (SPARC), involved in collagen deposition and organization [48]. We have initially identified FL supportive stromal cells as lymphoid-like stromal cells obtained by *in vitro* stimulation of mesenchymal precursors by TNF/LT or by contact with malignant B cells [49]. However, numerous questions remain open concerning in particular i) the direct and indirect mechanisms underlying the protumoral activity of FL cancer-associated fibroblasts (CAFs); and ii) the heterogeneity and dynamic of FL-CAFs *in situ*, including their putative precursors and differentiation pathways.

4.2 Mechanisms of stromal cell protumoral activity

Tumor-promoting activities of CAFs, including cancer cell survival, proliferation, and dissemination, immune escape, and drug resistance have been recently described in solid cancer. Similarly, FL-CAFs display pleiotropic tumor-supportive functions (Figure 1B). First, they could contribute to tumor B-cell recruitment and adhesion to protective cell niches. An upregulation of CXCL12 has been recently highlighted in LN and BM FL-CAFs and contributes to migration, adhesion, and activation of FL B cells that harbor a CXCR4^{pos}CXCR5^{pos}CCR7^{lo} phenotype [47]. Moreover, VCAM-1/CD106, the ligand of $\alpha4\beta1$ (VLA-4) integrin, has been involved in GC B-cell lymphoma growth and resistance to the anti-CD20 antibody Rituximab [50]. Interestingly, genetic loss-of-function alterations of the S1PR2 pathway, involved in the local confinement of normal GC B cells to the follicle center [51], was associated with FL transformation into aggressive diffuse large B-cell lymphomas (DLBCLs) [52]. Second, FL stromal cells could prevent FL B-cell apoptosis *in vitro* through a panel of mechanisms only partially solved including the production of hedgehog (Hh) ligands, BAFF, and TGF-β, the upregulation of ABC multidrug resistance transporters, and the activation of a C-MYC/HDAC6 loop in tumor cells [44,53]. In agreement, the gain of cell-autonomous activation of Hh pathway in DLBCLs likely contributes to their decreased stroma-dependence. Interestingly, it has been recently demonstrated that heparanase, an

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endoglycosidase triggering extracellular matrix disassembly and increasing the bioavailability of growth factors bound to heparin sulfates including CXCL12, is strongly expressed in about 50% of FLs and DLBCLs [54]. Finally, besides these direct B-cell supportive functions, FL-CAFs were proposed as organizers of FL cell niche. FL-CAFs overexpressed CCL2 and recruit monocytes, further converted into proangiogenic and anti-inflammatory macrophages [55]. Importantly, tumor-associated macrophages play a key role in malignant B-cell growth through the release of IL-15 and the triggering of FL BCR signaling [56,57]. FL-CAFs also favor the recruitment and survival of protumoral neutrophils, at least in part through the release of high amounts of IL-8 [58]. Interestingly, FL-infiltrating stromal cells overexpress the immunosuppressive molecule PGE2 [59], and stromal cells were proposed to favor B-cell lymphoma growth in a murine model of lacrimal gland lymphoma through the recruitment of Treg and myeloid-derived suppressor cells [60].

Of note, a majority of functional studies have been performed using stromal cells maintained after long-term *in vitro* cultures and a detailed *in situ/ex-vivo* characterization of FL-CAF heterogeneity and function is still lacking.

4.3 Heterogeneity and dynamic of FL-CAFs

As discussed above, lymphoid stromal cells form a highly heterogeneous and plastic landscape within normal lymphoid tissues raising the question of the nature of tumor-permissive stromal cells in FL. Mature gp38^{hi}CD21L^{pos} FDCs have been shown to progressively disappear from FL LN, in agreement with the strong decrease of LT production in FL B cells compared to normal GC B cells [47,61,62]. Whether this FDC loss results from their dedifferentiation or from their replacement by another stromal cell subset remains unknown. Interestingly, RANKL^{pos} MRCs, one of the putative FDC precursors, are virtually absent from FL LN (our unpublished data). Conversely, the specific downregulation of LT in FL B cells combined with the upregulation of CXCL12 in FL-CAFs argue for a CRC-like origin of at least some FL stromal cells since dark zone CRCs, unlike FRCs and versatile cells, do not require LT and TNF for maintenance of CXCL12 expression and network morphology [26]. Importantly, it is very likely that several subsets of FL-CAFs exist and organize different cell niches with specific functions as recently proposed in breast cancer [63]. Interestingly, cultured FL BM stromal cells retained numerous features of their native counterpart, including the

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overexpression of CCL2, IL-8, and CXCL12, suggesting an imprinting of stromal cells by the tumor context [47,55,58].

Whatever their origin, the mechanisms underlying the differentiation of FL-CAFs are of utmost significance considering their potential as therapeutic targets. FL B cells could directly contribute to the activation of the FRC meshwork within invaded LN and BM, as highlighted by their capacity to trigger *in vitro* the commitment of mesenchymal precursors into FRC-like phenotype and their overexpression of CCL2 and IL-8 in a TNF-dependent manner [49,55,58]. However, non-malignant surrounding cells could also participate to FL-CAFs polarization. First, neutrophils, recruited by IL-8-producing FL stromal cells, contribute in turn to their FRC-like differentiation through the activation of NF-κB pathway [58]. Moreover, FL-Tfh overexpress IL-4 that induces a transglutaminase^{hi}podoplanin^{lo}VCAM-1^{hi}CXCL12^{hi} phenotype in human mesenchymal progenitors and FRC-like cells, a profile close to that identified *in situ* within FL cell niche [47]. FL-Tfh produce also high amounts of TNF and LT and TNF/LT-primed stromal cells are more sensitive to IL-4-dependent CXCL12 upregulation, at least in part through an increased expression of STAT6 signaling molecule. Interestingly, some of the recurrent genetic alterations in FL regulate the tumor niche re-education by tumor cells. In particular, loss-of-function alterations of *HVEM/TNFRSF14* found in 50% of FL patients were shown, in a vavP-*BCL2* FL mouse model, to be associated with an amplification of Tfh producing high amounts of IL-4, TNF, and LT [64]. In agreement, lymphoid stromal cells exhibit an activated phenotype in *hvem*-deleted mice. Moreover, HVEM^{neg} FL patients exhibit an expansion of Tfh compared to HVEM^{pos} FL patients. Of note, the nature of FL-CAF precursors and the source of IL-4 within invaded BM remain unknown.

Finally, a very challenging question is to understand how FL-infiltrating stromal cell networks manage the continuous LN enlargement. FL LN are characterized by a disorganized collagen-based reticular network (our unpublished data) and a specific downregulation of podoplanin expression [47]. Moreover, the proportion of CLEC-2-expressing dendritic cell is strongly reduced within FL LN compared to inflamed lymphoid tissues [65], raising the question of the mechanisms engaged to sustain the expansion of malignant B cells in enlarged LN.

4.4 How to study stromal cell/ FL B cell interactions?

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Many technical limitations hamper the study of the B-cell/stromal cell crosstalk in FL including: i) the lack of true FL B-cell lines reflecting the indolent and microenvironment-dependent stage of the disease; ii) the increasing use of fine-needle aspiration biopsies instead of lymph node excision for FL diagnosis; iii) the difficulty to maintain functional lymphoid stromal cells *ex vivo*, requiring the set up and validation of relevant *in vitro* differentiation models; iv) the failure to establish FL xenografts in immunocompromised mice lacking T cells and mature lymphoid stromal cell niches. The development of new models recapitulating the complexity of normal and malignant GC reaction is thus a major unmet need.

A first interesting approach is the implementation of 3D *in vitro* models taking into account extracellular matrix organization and mechanical forces. In particular, recent studies adapted from the hanging drop method have proposed to evaluate some of these parameters using multicellular aggregates of lymphoma B cells and suggested that this 3D culture systems is more closely related to FL biology than classical cell suspensions [66]. However, this model does not include stromal cells and compressive forces thus limiting its potential impact. Interestingly, the role of both VLA-4/VCAM-1 crosstalk and lymphoid stromal cells in GC B-cell lymphoma growth was recently confirmed in an elegant gelatin-based 3D organotypic culture system functionalized with variable integrin specific ligands [67]. Moreover, since lymphoma growth exposed malignant B cells to an increased fluid flow, the same group developed a lymphoma micro-reactor platform, allowing to evaluate the impact of fluid shear stress and nutrient mass transport on lymphoma B-cells [68]. Such biophysical stimuli were shown to influence integrin and BCR signalling with functional consequences on cell proliferation and drug resistance. Interestingly, secretion of TNF by malignant B cells was also increased in these culture conditions making such model potentially useful to analyze B-cell/stroma interactions. Similarly, another lymphoma-on-chip system was recently proposed consisting of a hydrogel-based tumor model traversed by a vascularized, perfusable, round microchannel complex. This highly promising model is supposed to recapitulate the multifaceted cell interactions occurring within lymphoma microenvironment but is currently validated only for mouse aggressive lymphoma tumors [69].

Besides *in vitro* 3D models, the establishment of fully relevant FL mouse models remains the best strategy to study the kinetic and spatial co-evolution of tumor cells and their supportive microenvironment. The founder genetic hallmark of FL, *i.e.* the

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t(14;18) translocation associated with *BCL2* overexpression, is not sufficient for progression to overt FL and a low proportion of t(14;18)^{pos} B cells can be detected in the peripheral blood of most healthy individuals [70]. In agreement, Eμ-BCL2 transgenic mice, in which BCL2 is under the control of the early transcriptional enhancer of immunoglobulin genes, merely develop follicular hyperplasia and not FL, likely due to weak BCL2 expression, lack of mature B-cell specificity, and lack of additional oncogenic hit. Conversely, the vavP-*BCL2* model was reported to yield FL in mice over 12 months, and allowed to show that additional alterations frequent in FL patients could accelerate *BCL2*-driven lymphomagenesis [71-73]. However in this model BCL2 is overexpressed in all hematopoietic cells including FL-supportive T cells. Globally, there has been no report in mouse of a lymphoma model truly reproducing the main features of FL, *i.e.* a GC B-cell proliferation where B-cell survival is initially driven by BCL2, but later grown under supportive B/Tfh/stromal cell interactions. Nevertheless, the recent data showing that the suppression of the inhibitory receptor *hvem* in vavP-*BCL2* transgenic mice accelerate FL development in association with an activation of Tfh and stromal cells [64] clearly argue for further development of new models more closely related to FL biology.

5. Concluding remarks

Together with genetic heterogeneity, the complex landscape of pro-tumoral and anti-tumoral signals delivered by FL microenvironment is now widely considered as a key determinant of lymphoma development and evolution. Besides the long-lasting interest for immune ecosystem, FL-CAFs have recently emerged as a major and highly heterogeneous component of FL supportive cell niches.

Considering the role of CAFs in FL pathogenesis, two additional levels of heterogeneity should be considered. First rare distinct FL variants have been recognized in the 2016 revision of the World Health Organization classification of lymphoma neoplasms leading to the conclusion that FL is more than a single entity [74]. Duodenal-type FLs are characterized by low clinical stages and histological grades, and favorable outcome. Interestingly FDC network is disrupted in duodenal compared to nodal FLs with a specific redistribution at the periphery of neoplastic follicles [75]. Predominantly diffuse FLs are usually BCL2-negative and often carry a 1p36 deletion including HVEM but tumor B cells produce very low amounts of LT and mature FDCs are poorly detectable [62]. Another important issue is the level of tumor cell intratumoral

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heterogeneity in this disseminated disease. FL B cells display different cytological grades, proliferation rates, phenotypes, and subclonal genetic profiles depending on their localization with LN *versus* BM, suggesting that trafficking within various microenvironments could differentially impact FL pathogenesis and clonal selection [76-79]. In this context, the nature of the specific stromal niches supporting FL proliferative cells *versus* the FL ancestral common precursor clone responsible for disease relapse and transformation remains to be explored.

The identification of the main features and the dynamic of FL B cell/stromal cell crosstalk could be of great relevance for the design of new therapeutic strategies. An appealing strategy would be to target malignant B cell retention within their supportive niches. Interestingly, both the disruption of CXCL12/CXCR4 and the use of BCR inhibitors, such as ibrutinib or idelalisib, have been shown to impair B-cell interactions with stromal cells [53]. Another approach consists of the inhibition of CAF network differentiation, activation, and organization. As a proof-of-concept, anti-TGFβ1 antibodies were recently proposed to reprogram CAFs and increase T-cell infiltration in urothelial tumors, thus synergizing with anti-PD-L1 antibodies [80]. Altogether, such data suggest that deciphering FL-infiltrating stromal cell heterogeneity, plasticity, and origin will provide new CAF-targeted therapies with interesting potential to bolster the effectiveness of anti-tumor cytotoxic drugs and immunotherapies.

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Figure Legend

Figure 1: B-cell/stromal cell interactions within normal and malignant follicles

(A) Within normal follicles, dark zone is characterized by the accumulation of $CXCR4^{hi}$ proliferating centroblasts (CB) retained by CXCL12-expressing reticular cells (CRC). Conversely, light zone centrocytes (CC) exhibiting high affinity BCR are engaged in a "ménage-a-trois" with Tfh and follicular dendritic cells (FDC) that provide them with survival and differentiation signals. Low affinity CC are eliminated by tingible body macrophages (TBM). Subcapsular marginal reticular cells (MRC) also contribute to B-cell activation through delivery of antigens and as putative precursors of FDC. Versatile stromal cells (VSC) are localized at the border of B-cell follicles and are converted into CXCL13-secreting cells upon contact with activated B cells of the growing follicles. Finally T-zone reticular cells (TRC) contribute to naïve B cell recruitment and guided trafficking outside the follicles.

(B) In FL infiltrated LN, FL cancer associated fibroblasts (FL-CAF) form a heterogeneous compartment overexpressing factors involved in direct FL B-cell activation and in the recruitment or activation of other components of tumor supportive cell niche such as tumor associated macrophages (TAM).

Figure 1

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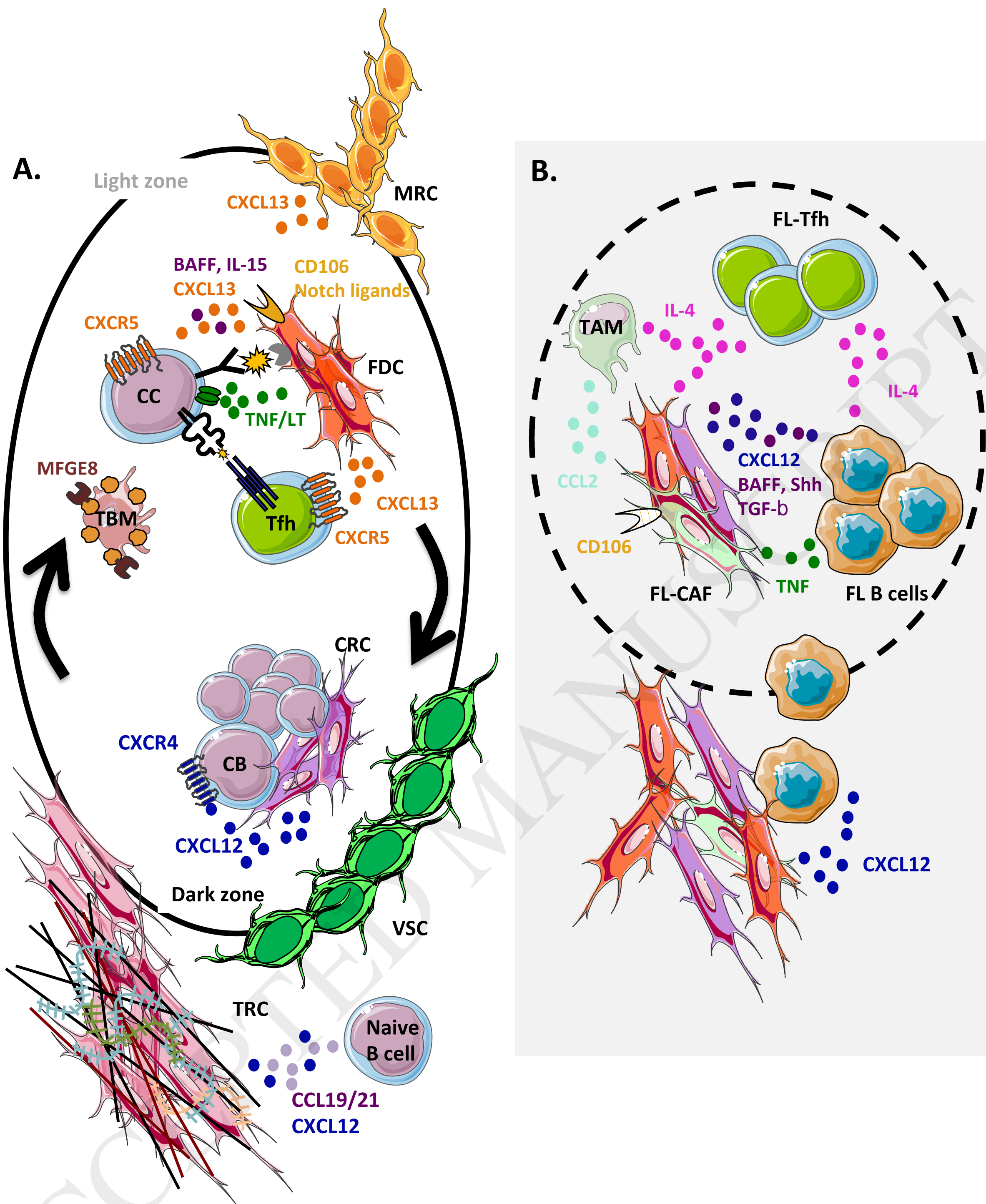

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Table 1. Main mature lymphoid stromal cell subsets

Stromal cell type	Localization	Phenotype	Function
Follicular dendritic cells (FDC)	GC light zone	GP38pos CD21/CD35pos	B-cell selection and differentiation GC organization
CXCL12-expressing reticular cells (CRC)	GC dark zone	GP38pos CD21/CD35neg	Organization of GC dark zone
Marginal reticular cells (MRC)	Beneath the subcaspular sinus	GP38pos CD21/CD35neg RANKLpos MAdCAMpos	Stromal cell precursors? Ag delivery
Versatile stromal cells (VSC)	T-cell zone of resting lymph node adjacent to follicles	GP38pos CD21/CD35neg but have expressed CD21	Could be converted to CXCL13-expressing cells by LT-expressing B cells
T-zone reticular cells (TRC)	T-cell zone	GP38pos CD21/CD35neg	Ag delivery Recruitment of B-cell, T-cell and DC T-cell and B-cell survival Peripheral immune tolerance HEV integrity Heterogeneous cell subset with distinct functions
Medullary fibroblastic reticular cells (MedRC)	Medullary zone	GP38pos CD21/CD35neg	Plasmablast migration and survival

GC: germinal center; HEV: High endothelial veinule; LT: lymphotoxin

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