# Tumor Microenvironment Impact on Follicular Lymphomas

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Follicular lymphomas (FL) are neoplasms that resemble normal germinal center (GC) B-cells. Normal GC and neoplastic follicles contain non-neoplastic cells such as T-cells, follicular dendritic cells, cancer associated fibroblasts, and macrophages, which define the tumor microenvironment (TME), which itself is an essential factor in tumor cell survival. The main characteristics of the TME in FL are an increased number of follicular regulatory T-cells ($T_{reg}$) and follicular helper T-cells ($T_{fh}$), M2-polarization of macrophages, and the development of a nodular network by stromal cells that creates a suitable niche for tumor growth. All of them play important roles in tumor angiogenesis, inhibition of apoptosis, and immune evasion, which are key factors in tumor progression and transformation risk.

tumor microenvironment follicular lymphoma therapeutic targets

## 1. Quiet Bystanders: TME Impact on FL Development and Prognosis

Understanding the main differences between normal and neoplastic lymph nodes (LNs) is essential. There is now growing evidence that crosstalk between lymphoma cells and TME cells is crucial for disease onset and progression.

In the non-neoplastic setting, B-cells must generate enormous variability in the antigen (Ag) recognition sites of their immunoglobulins (Igs), also known as B-cell receptor (BCR). For this purpose, they developed a mechanism that, in three independent steps (VDJ recombination, somatic hypermutation (SHM), and class switching) enables the production of Igs of almost unlimited specificity, although there is a risk of pathological mutations arising during this process. Naïve B-cells leave the BM after V(D)J recombination and migrate through the bloodstream to secondary LNs. B-cells that have encountered Ag enter the GC, which is formed by the expansion of selected clones within the FDC meshwork [1][2]. The proliferating GC B-cells are known as centroblasts, and they undergo SHM of the Ig variable region (IGV) genes, which alters the antigen affinity of the Ig that will be produced by the cell [3]. On the other hand, when centroblasts stop dividing, they become medium-sized cells called centrocytes, and if the IGV gene mutations have resulted in increased affinity for antigen, they present it to the $T_{fh}$ cells via the major histocompatibility complex class II (MHCII). Centrocytes initially accumulate among the centroblasts and then migrate to the opposite pole of the GC. This causes the polarization of the GC into a “dark zone”, which contains centroblasts and closely packed centrocytes, and a “light zone”, which contains centrocytes, FDCs, and numerous T-cells. These two zones can be differentiated by the proliferation marker Ki67 as the “dark zone”

contains the highly proliferating centroblasts and the “light zone” the low proliferating centrocytes. The selected centrocytes reenter the dark zone, where clonal expansion occurs and cells may cycle between the dark and light zones multiple times [4][5]. Finally, centrocytes will become either plasma B-cells or memory B-cells. The interaction with CD23, which is expressed by FDCs, seems to be important in the differentiation into plasma cells, stimulating class switching. On the other hand, interaction with $T_{fh}$ cells through the CD40–CD40 ligand appears to be important for generating memory B-cells [6][7].

This GC reaction is highly dependent on the TME. T-cells are predominantly located in the light zone and most are $T_{fh}$ cells, which are important for selecting B-cells for entry and proliferation within the GC. $T_{reg}$ cells are present in low numbers; they exert negative regulatory effects on both B- and T-cells and are needed to stop the GC reaction, preventing excessive immune responses [8]. FDCs are large cells with delicate nuclear membranes that derive from mesenchymal origin. They have surface complement receptors (CD21) and Fc receptors (CD23) that bind free antigen and antigen–antibody complexes for presentation to B-cells. In normal lymph nodes, the role of FDCs and CAFs is to build a network to support the GC reaction. In contrast, macrophages (CD68+) are typically phagocytic, containing apoptotic debris from B-cells that have failed to express a surface Ig molecule [9]. Macrophages can be polarized into M1 (inflammatory phenotype) or M2 (anti-inflammatory), resulting in distinct cytokine production or T-cell function (Th1 and Th2).

Many of the components of the FL microenvironment mimic those present in normal GCs, but some important differences may contribute to tumor-cell survival. The role of TME in FL can be defined in two directions: supporting tumor growth and suppressing the antitumor immune response [10]. **Table 1** recapitulates the main cells involved in the TME of FL.

**Table 1.** Main cells involved in the tumor microenvironment of follicular lymphoma.

| Cells | Phenotype | Secreted Cytokine | Role in FL |
|---|---|---|---|
| $T_{fr}$ | CD4+ CD25+ FOXP3+ CXCR5high ICOShigh PD1high BCL6low BLIMP1+ | CCL4, IL-16 | $T_{reg}$ recruitment. More suppressive than normal $T_{regs}$<br>Inhibition of CD8+ T-cell activity |
| $T_{fh}$ | CD4+ CD25- CXCR5high ICOShigh BCL6+ PD1high TIM3- | IL-4, IL-17, IL-21, IFNγ | pSTAT6 ↑<br>$T_{reg}$-recruiting CCL17 and CCL22 production by FL cells<br>FL cell survival and proliferation<br>Inhibition of apoptosis |
| Stromal cells<br>• FDCs | CD21+, CD23+ | CXCL13<br>CXCL12, CXCL10 (ICAM-1↑), CCL2/19/21, BAFF | Creation of a neoplastic niche<br>Monocyte recruitment and M2-polarization<br>Migration and activation of FL B-cells<br>T-cell recruitment |

| Cells | Phenotype | Secreted Cytokine | Role in FL |
|---|---|---|---|
| • CAFs | | | |
| M2 TAM | CD163+, CD68- | IL-10, IL-15, VEGF | Th2 response<br>Angiogenesis |

$T_{fr}$: follicular regulatory T-cell; $T_{fh}$: follicular helper T-cell; FL: follicular lymphoma; FDC: follicular dendritic cell; CAF: cancer-associated fibroblasts; TAM: tumor-associated macrophage.

## 2. B-Cells

The histopathology of FL is closely related to key events in normal B-cell development and differentiation. As previously mentioned, in approximately 85% of FL cases, the malignant B-cells harbor a translocation t(14;18)(q32;q21) that occurs as a consequence of aberrant VDJ recombination in a progenitor B-cell in the BM, in contrast to normal GCs [11][12]. By juxtaposing BCL2 next to the immunoglobulin-heavy chain enhancer, this translocation leads to a constant expression of BCL2, which is observed in almost all FL (≥25% of FL cells) but not in normal GCs, resulting in the inhibition of apoptosis and favoring the selection of these clones in the GCs of secondary lymphoid organs [13]. The t(14;18) translocation in B-cells increases the risk of developing FL, although it is not sufficient and the acquisition of additional mutations is necessary for neoplastic transformation and disease progression, since the t(14;18) translocation has also been observed in GC B-cells of healthy individuals [14]. FL B-cells undergo repeated re-entry into GCs, so this leads to the acquisition of these additional aberrations that enable FL to develop.

Unlike normal GCs, in FL, there is no differentiation into a “dark zone” and a “light zone”, but there is a predominance of centrocytes interspersed with some centroblasts [15]. FL B-cells express many of the antigens that are found on normal GC B-cells and that are associated with interactions with T-cells and dendritic cells such as costimulatory molecules CD80, CD86, CD40/43/44, and CD70 [16]. Furthermore, they express surface Igs that have undergone SHM of the IGV genes and, in approximately 20–50% of cases, have undergone Ig class switching. In a high proportion of FL cases during SHM, new mutations can be acquired. These mutations introduce consensus sequences of unusual mannosylated glycans, which are not seen in normal B-cells and can bind to lectins on FDCs and macrophages, enabling them to survive in the GC environment in the absence of cognate antigen [17][18].

## 3. T-Cells

In the neoplastic follicles, T-cells are less numerous than in reactive follicles and they are randomly distributed, in contrast to the concentration in the light zone that characterizes normal lymph nodes [19]. CD4+ T-cells ($T_{reg}$ and $T_{fh}$) are predominant with a higher CD4:CD8 ratio, $T_{reg}$ cells being more numerous than $T_{fh}$ cells. Both types of cells are fundamental to providing tumor support and facilitating immune evasion.

FL-associated $T_{fh}$ lymphocytes are defined by the expression of the chemokine receptor CXCR5, the inducible T-cell co-stimulator (ICOS), the programmed cell death 1 (PD-1), and the transcription repressor BCL6. These cells produce an excess of chemokines (IL-4, IL-17, IL-21, and IFN-γ) and overexpression of CD40L, supporting B-cell viability, inhibiting apoptosis, and influencing the TME. Moreover, $T_{fh}$ cells expressing IL-4 and CD40L can also induce FL cells to produce CCL17 and CCL22, thereby promoting the active migration of $T_{regs}$ [7][19].

On the other hand, $T_{reg}$ cells are characterized by the expression of the transcription factor FOXP3 and they have a negative regulatory effect on B- and T-cells, playing a pro-tumor role due to their immunosuppressive activity, which hampers CD8+ T-cell activation [20]. Interestingly, FL patients are particularly rich in CD4+ T-cells that harbor $CXCR5^{high}$ $ICOS^{high}$ $PD1^{high}$ and FOXP3+/CD4+/CD25+ phenotypes, which are called follicular regulatory T-cells (Tfr). $T_{fr}$ cells have greater suppressive capacity than $T_{regs}$ in normal LNs because they upregulate cytotoxic T-lymphocyte antigen 4 (CTLA-4), IL-10, and the glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR). This pattern has been described as being a predictor of shortened overall survival (OS) [21].

## 4. Stromal Cells, Follicular Dendritic Cells and Macrophages

Stromal cells and macrophages are key factors in the pathogenesis of FL, but their association with prognosis remains controversial.

FDCs and CAFs are stromal cells that phenotypically and functionally differ from their normal counterparts, presenting a niche-based model of oncogenesis attributed to the dynamic coevolution of cancer and stromal cells. FDCs are particular Ag-presenting cells (APCs), and they present intact Ag–Ab complexes on their cell surface, which enable survival and induce the differentiation of FL cells. They can also trigger $T_{fh}$ recruitment by the production of CXCL13, which interacts with CXCR5. The FDCs of almost all FL express CD21, while only a small subgroup express CD23, which can be expressed by FDCs and B-cells. In contrast, CAFs secrete components of the extracellular matrix such as laminin, fibronectin, and collagen. The nodular network built in the neoplastic setting infiltrates follicles, creating a suitable niche even in extranodal sites and BM, suggesting that they are recruited by the neoplastic cells [7]. The crosstalk between FDCs, CAFs, and FL B-cells is crucial to promote tumor cell survival. They are responsible for B- and T-cell migration thanks to the upregulation of CXCL12, CXCL13, and BAFF signaling as well as adhesion through interactions between adhesive molecules such as VCAM-1 and VLA-4. Through these interactions, stromal cells protect malignant cells against apoptosis induced by chemotherapy or rituximab. They can also induce malignant monocyte recruitment and their differentiation to M2-protumoral macrophages through the secretion of CCL2 and CSF-1 [22][23].

TAMs are typically non-phagocytic, with a low proliferation fraction, and they do not undergo apoptosis. They express CD163+, the Fc fragment of IgG, and C-type lectin domains. These cells can display two different phenotypes: M1- and M2-polarization. This polarization is controlled by tumor cells within the TME and is dynamic throughout cancer development. M1-macrophages are typically tumor-suppressing cells that act in the TME by recruiting CD8+ T-cells and NK cells. These CD8+ and NK cells express high levels of cytokines and chemokines, recruiting other immune cells and favoring the signaling of anti-tumorigenic pathways [24]. On the other hand, the M2-phenotype is associated with tissue remodeling, angiogenesis, and progression, making them an attractive target for therapies [25]. These M2-macrophages secrete immunosuppressive molecules into the TME, suppress T-cell mediated anti-tumor responses and recruit $T_{reg}$ lymphocytes, facilitating tumor proliferation and immune evasion [24][26]. TAMs also play a key role in tumor angiogenesis by secreting VEGFA, which can stimulate the chemotaxis of endothelial cells and macrophages [27]. In addition, they promote the epithelial–mesenchymal transition process, which enables cancer cells to leave the tissue site, favoring the initiation of metastasis [28].

However, TAM's polarization state is not fixed. At early stages of tumor development, M1-macrophages are predominant, while the M2-phenotype is more frequent in the advanced setting where tumor proliferation and invasion increases [24]. For the time being, the role of TAMs for the prognosis of FL remains unclear. In the pre-rituximab era, some studies have suggested that macrophage infiltration was associated with lower survival, probably due to their M2-polarization. Nevertheless, the addition of rituximab to chemotherapy modified their prognostic impact, and, although the mechanism is still not well-known, it is likely to be associated with antibody-dependent cell-mediated toxicity (ADCC) [29][30]. The antitumoral activity of rituximab is dependent on interactions with the effector cells that have Fc receptors (neutrophils, natural killer cells, and macrophages). Because tissue macrophages are critical for B-cell depletion after anti-CD20 antibody therapy, it is plausible that there is a relationship between TAM content and the efficacy of rituximab [31]. The Finnish and French groups showed that the addition of rituximab to chemotherapy reversed the negative prognostic impact of high macrophage content to favorable, reporting a survival benefit in the rituximab arm [32].

## 5. Neutrophils

Little is known about the interactions between FL cells and neutrophils although they are important players in the innate immune system. Unlike other hematological cells, they are predominantly present in peripheral blood. In preclinical studies, tumor associated neutrophils (TANs) have demonstrated a reduced cytotoxic effect of chemotherapy through the interaction of CD11b/ICAM-1 with CD44 of malignant B-cells. An increased number of TANs in FL patients has been correlated with poor prognosis and weak treatment responses. These findings suggest that TANs could become a new targeted therapy [33].

In summary, FL B-cells are surrounded by a network of supportive cells that take part in the development of tumor cells, treatment resistance, prognosis, and histological transformation.

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