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Review

The yin and the yang of follicular lymphoma cell niches: Role of microenvironment heterogeneity and plasticity

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ABSTRACT

Follicular lymphoma (FL) results from the malignant transformation of germinal center B cells and is characterized by recurrent genetic alterations providing a direct growth advantage or facilitating interaction with tumor microenvironment. In agreement, accumulating evidences suggest a dynamic bidirectional crosstalk between FL B cells and surrounding non-malignant cells within specialized tumor niches in both invaded lymph nodes and bone marrow. Infiltrating stromal cells, macrophages, and T/NK cell subsets either contribute to anti-tumor immune response, or conversely form a tumor supportive network promoting FL B cell survival, growth, and drug resistance. This review depicts the phenotypic heterogeneity and functional plasticity of the most important FL cell partners and describes their complex interplay. We also unravel how malignant B cells recruit and subvert accessory immune and stromal cells to trigger their polarization toward a supportive phenotype. Based on these observations, innovative therapeutic approaches have been recently proposed, in order to benefit from local anti-tumor immunity and/or to selectively target the protective cell niche.

1. Introduction

Follicular lymphoma (FL) is the most frequent indolent lymphoma and is considered virtually incurable with high response rates to therapy but frequent relapses [1]. Progression to aggressive diffuse large B-cell lymphoma (DLBCL) occurs in about 35% of cases, an event associated with poor outcome [2]. Malignant FL B cells express germinal center (GC) B-cell markers such as BCL6 and CD10, have somatically mutated immunoglobulin variable genes with ongoing intraclonal diversification, and display a gene expression profile of centrocytes, indicating that FL results from the malignant transformation of GC-derived B cells [3]. The genetic hallmark of FL is the t(14;18) translocation associated with an overexpression of the anti-apoptotic protein BCL2, actively repressed in normal GC B cells. However, this founder genetic event is detected at a low frequency in most healthy individuals within peripheral blood IgM memory B cells, the so-called FL-like cells (FLLC) [4], suggesting that additional driver genetic events are required to complete cell transformation. Accordingly, genome-wide profiling has recently shed new lights on the mutational landscape in FL and delineated a hierarchical model of successive genetic events supporting FL tumorigenesis [1,5].

Besides the failure of primary FL cells to survive and grow autonomously *in vitro*, the major role of the microenvironment in FL development and evolution has been highlighted by several seminal observations. First, like their normal counterpart, malignant FL B cells are found admixed with lymphoid stromal cells, macrophages, and follicular helper $CD4^{pos}$ T cells (T_{FH}) in GC-like follicles within invaded lymph nodes (LN) [6]. In addition, bone marrow (BM) infiltration found in up to 70% of patients at diagnosis is characterized by an ectopic differentiation of lymphoid-like stromal cells [7] and local enrichment in $CD4^{pos}$ T cells [8] suggesting a critical dependence of malignant B cells to this specific supportive cell niche. Despite these similarities, some differences in cell composition and organization exist between LN and BM niches [8,9]. In agreement, different subclones could be detected within BM and LN, and BM FL cells are characterized by a lower cytological grade and proliferation [9–11]. These data support the hypothesis that trafficking in various specific microenvironments could contribute to FL clonal selection and molecular heterogeneity [12]. Second, several highly frequent genetic alterations are not oncogenic *per se* but favor the crosstalk of FL cells with neighboring cells. Among them, mutations in *TNFRSF14/HVEM* affecting its expression or binding to the inhibitory receptor BTLA could contribute to the maintenance and

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supportive activity of BTLA^{hi} FL-infiltrating T_{FH} [13,14]. Moreover, more than 90% of FL cases display unusual sites for N-linked glycosylation within their immunoglobulin variable regions introduced during the somatic hypermutation process (SHM) [15]. Added glycans contain oligomannoses that might interact with C-type lectins expressed by myeloid cells in the microenvironment, allowing FL cells to receive antigen-independent but cell contact-dependent survival signals through their BCR [16]. Finally, several studies based on expression profiling and immunohistochemistry have proposed a panel of prognostic biomarkers reflecting the number, activation, and/or spatial organization of infiltrating immune cells, further emphasizing the central role of FL microenvironment [17]. In the landmark study performed on whole tumor biopsies, the clinical outcome of FL patients was primarily predicted by molecular features of non-malignant cells and not by specific genetic characteristics of tumor B cells [18]. However, these studies led to highly contradictory results, in part due to treatment heterogeneity, and remained essentially descriptive without transposition of the data into more functional and mechanistic approaches.

Our current knowledge of the relationship between FL B cells and their microenvironment has been hindered by four main technical pitfalls: (i) the lack of true FL B-cell lines; (ii) the lack of relevant transgenic mouse model of FL; (iii) the difficulty to establish FL xenografts in immunocompromised mice in the absence of T-cell help and mature secondary lymphoid organs; (iv) the heterogeneity and plasticity of the numerous cell subsets involved in FL cell growth, associated to their limited survival and proliferation *in vitro*. Nevertheless, several recent studies have provided interesting clues illustrating the two faces of FL microenvironment; *i.e.* its capacity to exert anti-tumor activity by itself or by potentiating the efficacy of FL-targeting drugs *versus* its capacity to favor directly and indirectly FL B-cell growth. This review will try to integrate them in a comprehensive view of the intricate FL cell niche. A related interesting question is how malignant B cells co-opt and divert their microenvironment to create a conducive niche in LN and BM and how this niche is modified after treatment and support FL relapse. A better understanding of the ambivalent role of FL microenvironment would be useful to select the more relevant biomarkers for patient stratification and prognosis. It will also make it possible to design new microenvironment-targeted treatments, a field that recently gained increasing attention in B-cell lymphomas.

2. Microenvironment can inhibit FL cell growth

FL has long been considered as particularly immune responsive based on reports of spontaneous regressions, high response rates to monoclonal antibodies (mAb) associated with a long-lasting vaccinal effect, and good biological responses to vaccination using tumor-specific idiotype or immunogenic neoplastic cells [19–22]. Several immune cell subsets could contribute to this anti-tumor activity and provide useful biomarkers and potential therapeutic targets.

2.1. Cytotoxic lymphoid cells

CD8^{pos} T cells are major actors of anti-tumor immunity and an increased CD8^{pos} T-cell infiltrate is correlated to a better FL prognosis [23]. Similarly, high levels of blood CD3^{pos}, CD4^{pos}, and CD8^{pos} predict favorable outcome in patients treated with rituximab [24]. Using 3-D tissue imaging, Laurent et al. described a rich infiltrate of functional CD8^{pos} cells containing granzyme B^{pos} lytic granules in the interfollicular spaces [25]. T cells at the follicular border form lytic synapse-like structures with FL B cells, suggesting a tonic control of malignant cell trafficking and FL progression. However, a global CD8^{pos} T-cell exhaustion as well as dysfunctional synapses with FL B cells has been reported in biopsy specimens [26,27]. In addition, intratumoral regulatory T cells (Treg) have been shown to inhibit *in vitro* degranulation and cytotoxic activity of infiltrating CD8^{pos} T cells exposed to lymphoma B cells [28].

Beside antigen-driven cytotoxicity of CD8^{pos} T cells, innate anti-tumor cytotoxicity involved essentially NK cells and γδ T lymphocytes (Fig. 1). Our knowledge of *in situ* NK cells in FL is limited, and the low frequency of CD56^{pos} cells on malignant tissue sections has not been associated with the progression of the disease [29]. However, we could hypothesize an induction of NK-DC crosstalk by therapeutic mAb, which could trigger tumor antigen-specific T cell immunity [30]. Considering γδ T cells, Vγ9δ2 T cells recognize tumor phosphoantigens, like isopentenyl pyrophosphate (IPP), and are able to kill *in vitro* a wide variety of tumor cell lines, as well as primary FL B cells [31]. Whereas γδ T cells could migrate into GC within normal secondary lymphoid organs [32], immunohistochemistry studies revealed that these cells display mainly perifollicular localization and are represented at lower density in FL LN tissues, compared to reactive LN [33]. Moreover, those FL B cells retaining HVEM expression could inhibit proliferation of BTLA^{pos} infiltrating Vγ9δ2 T cells [34]. In agreement, we found a lower *in vitro* expansion capacity of FL infiltrating Vγ9δ2 T cells in response to a combination of their pharmacological agonist bromohydrin pyrophosphate (BrHPP) with IL-2 (our unpublished data). FL B cells were shown to express ULBP proteins, the ligands for NKG2D activating receptor and an increase in circulating ULBP-responsive Vδ1 T lymphocytes have been described in FL patients [35]. However, the role of Vδ2^{neg} γδ T cells in FL pathogenesis remains poorly understood.

Finally, NK cells, γδ T lymphocytes, and a subset of CD8^{pos} T cells share the capacity to mediate antibody-dependent cellular cytotoxicity (ADCC), an important mechanism of anti-tumor immune response. The association between Rituximab clinical efficiency and a specific polymorphism in CD16/FcγRIIIa resulting in a modulation of affinity for IgG1 revealed the critical role of CD16-expressing cells in the activity of this anti-CD20 mAb [36].

Overall, cytotoxic cells of both innate and adaptative immunity could efficiently kill lymphoma B cells but this antitumor immune response is actively counteracted by tumor escape mechanisms affecting immune cell recruitment and activation.

2.2. Myeloid cells

Tumor-associated macrophages (TAM) exhibit a dual role in FL pathogenesis, as underlined by the opposite predictive value of a high TAM content, depending on treatment schedule. In fact, high numbers of CD68^{pos} or CD163^{pos} TAM are associated with adverse outcome in FL patients treated with conventional chemotherapy, whereas this prognosis value is abrogated or even inversed when Rituximab is combined with chemotherapy [37–39]. These data suggest that FL TAM could favor tumor progression but also contribute to the clinical efficacy of antibody-based anti-lymphoma drugs (Fig. 2). Accordingly, B cell depletion with anti-CD20 mAb in mouse models prominently depends on macrophages, and more specifically on their expression of activating FcγR [40]. In addition, for rituximab-mediated tumor clearance in human, antibody-dependent cellular phagocytosis (ADCP) mediated by macrophages probably plays a key role beside that of NK-mediated ADCC [41]. In particular, Rituximab and Ofatumumab show high direct ADCP capacities *in vitro* and elicit TNF-α release by macrophages, which could indirectly contribute to NK cell activation [42]. Interestingly, alternatively activated M2 macrophages were shown to display *in vitro* a greater phagocytic capacity toward Rituximab-opsonized B cells from chronic lymphocytic leukemia (CLL), when compared to M1 proinflammatory macrophages [43]. This was associated with a differential regulation of FcγR expression by polarizing cytokines. In agreement, several reports demonstrate that IL-4 decreases

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