The Tumor Microenvironment in Follicular Lymphoma

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Abstract: Like other B-cell lymphomas, the development and progression of follicular lymphoma (FL) involves complex interactions between the neoplastic B cells and the surrounding microenvironment. Malignant B cells can manipulate the microenvironment by skewing the differentiation of immune cells, attracting regulatory T cells or suppressive monocytes, or secreting cytokines that promote an immunosuppressive environment. The importance of the microenvironment in FL has been demonstrated using methodologies such as gene expression profiling, which has shown that the nature of the tumor microenvironment predicts survival in patients with FL and may influence the response to immunotherapy and risk of transformation. Strategies that both enhance an effective antitumor response and reverse immunosuppression and dysfunction will be essential in the development of effective immunotherapeutic approaches in this disease.

Keywords
Follicular lymphoma, follicular helper T cells, follicular regulatory T cells

Introduction

Follicular lymphoma (FL), the second most common type of non-Hodgkin lymphoma (NHL), is a serious and often fatal illness.[1] The clinical course of this disease is variable, and the molecular and cellular mechanisms responsible for the clinical heterogeneity of follicular B-cell NHL are largely unknown. However, it is becoming increasingly clear that the tumor microenvironment in FL plays an important role in disease severity, clinical outcome, and response to therapy.[2-4]

The tumor microenvironment is comprised of the normal cells, molecules, and blood vessels that surround and feed a tumor cell. A tumor can change its microenvironment, and the microenvironment can affect how a tumor grows and spreads. The structure and composition of the tumor microenvironment varies among different types of cancers and from patient to patient. For example, the specific structure of secondary lymphoid organs from which most lymphomas originate (eg, the lymph nodes and spleen) makes the tumor microenvironment of hematologic malignancies significantly different from that of solid tumors. There are abundant immune cells in secondary lymphoid organs, which distinguish hematologic malignancies from solid tumors where the immune cells infiltrate in limited numbers. The frequency, distribution, and function of immune cells differ consider-

ably among patients with the same type of cancer, which has been shown to impact patient outcomes. This article will focus on the immune cells and cytokines in the tumor microenvironment of FL.

Composition of Lymph Nodes

In most patients with FL, the lymph node is the main organ in which lymphoma cells reside. The microenvironment of FL is complex in terms of structure and cellular composition because the lymph node has a unique architecture and function. Structurally, the lymph node can be divided into 3 compartments: the cortex (outer region), paracortex, and the medulla (inner region). Follicles containing germinal centers (GCs) are located within the cortex. Different types of cells preferentially reside in separate areas within these compartments. B cells are usually found in the follicles within the outer cortex, while T cells are mainly present within the paracortex and medulla. Upon antigen (Ag) stimulation, T and B cells home to T-cell zone and follicles in lymph nodes, interact with Ag-presenting cells (APCs), and undergo clonal expansion. Dendritic cells (DCs) are potent APCs that collect and process Ag from tissues, carry them to the lymph nodes, and present them to T cells to initiate primary immune responses. Follicular dendritic cells (FDCs) are the APCs that present Ag to B cells and are present only in follicles of primary and secondary lymph organs. Follicles with germinal centers are specifically important for normal B-cell maturation. Before Ag confrontation, the follicle is composed of unstimulated, naïve B cells and does not have a germinal center. Upon Ag stimulation, follicles develop germinal centers, where B cells differentiate and become immunoglobulin-secreting plasma cells. Although most T cells reside outside of the follicle, an important subset of CD4+ T cells known as follicular T helper (T_{FH}) cells reside specifically within germinal centers and are responsible for Ag-dependent activation of B cells in the follicle. CD8+ T cells are rarely seen in the germinal center of follicles. Scattered macrophages are sparsely present inside the germinal center and are responsible for removing debris from apoptotic cells.

In FL, the structural architecture and cellular composition within follicles differs from normal lymph nodes. While follicles are kept intact in FL, the germinal centers become larger due to increasing numbers of lymphoma B cells. An altered cellular composition is commonly seen in biopsies of FL when compared to that of normal lymph nodes. For example, increased regulatory T (T_{reg}) cells and decreased effector T cells, such as T_H1 and T_H17 cells, are found in FL. Many of the effector T cells present in the tumor microenvironment of FL have an exhausted phenotype and exhibit limited function. In addition, immature immune-suppressive macrophages are present in increased numbers in FL. These alterations have all been shown to potentially affect antitumor immunity and support lymphoma cell survival and growth in FL.

Intratumoral T Cells

Follicular lymphoma is characterized by the presence of a significant number of T cells, up to 50% of the cell mixture, in the tumor microenvironment. These intratumoral T cells have a substantial impact on antitumor immunity and patient outcome.[5] T cells are generally heterogeneous and influence tumor immunity both positively and negatively, depending on the prevalence of various T-cell populations within the microenvironment. Generally, elevated numbers of intratumoral T cells are associated with favorable prognosis in patients with FL,[6-8] but further studies have found that specific subsets of T cells correlate with patient outcome.[9,10]

CD8+ T Cells

T lymphocytes can be divided into 2 populations: CD4+ and CD8+ T cells. Generally, CD4+ T cells provide help to other immune cells, and CD8+ T cells are mainly engaged in target cell killing through the secretion of perforin and granzyme B (GrzB). In FL, the frequency of intratumoral CD8+ T cells varies, ranging from 10–50% in different patients when determined by immunohistochemistry.[11] Unlike CD4+ cells that are seen both inside and outside the follicles, CD8+ cells are mostly perifollicular. It could be speculated that these perifollicular CD8+ cells protect the follicle; however, 3-dimensional confocal laser scanning microscopy showed that most CD8+GrzB+ cells appeared to target FL B cells, as contact between cytotoxic CD8+ T-lymphocytes (CTLs) and FL B cells was observed.[11] Supporting this finding, in vitro-generated CD8+ T-cell clones show specific cytotoxicity directed against autologous lymphoma cells.[12-14] Clinically, higher intratumoral CD8+ T-cell numbers correlate with longer overall survival (OS) and disease-specific survival (DSS), independent of the Follicular Lymphoma International Prognostic Index (FLIPI) and all other prognostic factors,[11,15] suggesting that these cells are part of a lymphoma-specific immune response.

CD4+ T Cells

CD4+ T cells play a central role in the immune response by helping B cells make antibodies; promoting enhanced antimicrobial activity by macrophages; recruiting neutrophils, eosinophils, and basophils to sites of inflammation; and, through their production of cytokines and chemokines, orchestrating an effective immune response. Since it was recognized that CD4+ cells could be separated into

cells that made interferon (IFN)-γ and those that produced interleukin (IL)-4,[16] it has been realized that CD4+ T cells are not a single set of cells, but represent a series of cell populations with distinct functions.

T_{reg} Cells A notable advancement in T-cell biology was the identification of CD4+CD25+ T_{reg} cells.[17] T_{reg} cells are a small subset of CD4+ T cells expressing CD25 (naturally occurring T_{reg} cells) and represent approximately 5–10% of peripheral CD4+ T cells in both mice and humans. Inducible T_{reg} cells are T_{reg} cells that are generated by antigenic stimulation. It has been demonstrated that the forkhead/winged helix transcription factor family member p3 (Foxp3) is a master transcriptional regulator for the development and function of T_{reg} cells; as such, Foxp3 is now used as a specific marker for T_{reg} cells.[18-20] T_{reg} cells were originally identified as able to suppress T cells. Subsequent studies have found that T_{reg} cells are able to suppress other types of immune cells, such as B cells and natural killer (NK) cells, thereby influencing various types of immune responses, including autoimmune, antimicrobial, and antitumor immune responses. Elevated numbers of T_{reg} cells are generally found in the peripheral blood and biopsy specimens of patients with various cancers. In FL, T_{reg} cells are highly represented and have been found to efficiently suppress intratumoral CD4+ and CD8+ T cells, resulting in suppressed antitumor immunity.[21-23] The mechanisms accounting for elevated numbers of T_{reg} cells in B-cell NHL include increased recruitment and de novo generation of T_{reg} cells. It has been shown that chemokine receptor 4 (CCR4) and chemokine ligand 22 (CCL22) play a crucial role in attracting T_{reg} cells into the tumor site.[21] In addition to recruitment of T_{reg} cells, de novo generation of T_{reg} cells is another important mechanism in FL.[24-26] Tumor-derived, transforming growth factor (TGF)-β induces Foxp3 expression and contributes to tumor-mediated conversion of CD4+CD25- T cells into T_{reg} cells.[27] Interaction between CD27-CD70 is involved in lymphoma B-cell–mediated generation of T_{reg} cells, as CD70-expressing lymphoma B cells are efficient in inducing Foxp3 expression in intratumoral CD4+CD25- T cells.[24]

In addition to biological relevance, T_{reg} cells have also shown significant clinical relevance in FL. By immunohistochemistry, Carreras and associates[9] studied a cohort of 97 FL patients and found that Foxp3+ T_{reg} cells were present in all patients, with frequency varying from patient to patient. Low numbers of T_{reg} cells correlated with refractory disease, transformation, and aggressive histology. They noted that high numbers of T_{reg} cells predicted improved survival in FL, a finding that has been supported by other studies.[28] However, subsequent studies failed to produce consistent results and found that the number of infiltrating Foxp3 cells did not correlate with overall survival in FL.[29,30] Such a discrepancy may be due to heterogeneous therapies administered to patients in these studies. In this regard, Farinha and colleagues[30] conducted a study involving 105 advanced-stage FL patients enrolled in a phase II clinical trial who were treated uniformly with multi-agent chemotherapy and radiation. By tissue microarrays, the authors found that T_{reg} cell content did not impact survival, but the distribution pattern of Foxp3+ T_{reg} cells correlated with patient outcome. Patients with Foxp3+ cells localized in a follicular pattern (intrafollicular or perifollicular) displayed a significantly elevated risk of transformation and shorter survival than patients with Foxp3+ cells present in a diffuse pattern, a finding that is supported by other reports.[7] Taken together, these results suggest that the architecture of the microenvironment, and particularly the location of T_{reg} cells in the architecture, has an impact on patient outcome in FL.

T_H1 and T_H2 Cells CD4+ T cells were initially defined as helper cells (T_H) in order to distinguish them from cytotoxic CD8+ T cells. Based on their cytokine-secreting profiles, T_H cells were initially divided into T_H1[16] and T_H2[16] cells. Along with the recent identification of BCL-6 as a master transcriptional factor for follicular helper T cells,[31,32] the T_H family has expanded into 4 major lineages: T_H1,[16] T_H2,[16] T_H17,[33,34] and T_{FH}[35,36] cells. CD4+CD25+ T_{reg} cells, as discussed above, form the other major lineage of CD4+ T cells.[37] T_{reg} cells and T_H cells constitute 2 opposing immune responses and are critically involved in the modulation of immune responses in lymphoma. T_H1 cells normally produce cytokines such as IFN-γ, TGF-β, and IL-2, and mediate cellular immune response by enhancing the killing capacity of macrophages and cytotoxic CD8+ T cells. T_H2 cells secrete cytokines such as IL-4, IL-5, and IL-6, and mediate humoral immune responses by stimulating B-cell proliferation and antibody production. T_H17 cells produce cytokines such as IL-17 and IL-22, and induce inflammation by stimulating inflammatory cytokine production. T_{FH} cells are a subpopulation of CD4+ T cells and specifically help germinal center B-cell maturation and differentiation. Before the addition of T_H17 and T_{FH}, studies had been focused on the T_H1 or T_H2 immune response, as the balance between the 2 is critically skewed in many human diseases. For example, immune responses in acute bacterial infection, such as tuberculosis, are often T_H1 type dominant, and inflammation in atopy/allergy, such as asthma, is mediated by T_H2 type immunity. In lymphoma, skewing between a T_H1 and T_H2 phenotype has not been definitively supported by the literature. Jones

and coworkers[38] investigated 44 B-cell NHL patients and found that both T_H1 and T_H2 cytokines were expressed at high mRNA levels, measured by reverse transcriptase-polymerase chain reaction (RT-PCR). Although it is generally believed that the T_H1 immune response is more effective than T_H2 for antitumor immunity, and that T_H2 immune response actually favors tumor growth by both promoting angiogenesis and inhibiting T_H1 immune response, this study[38] observed that high IL-4 levels correlated with longer survival duration. In contrast, our group[39] has found that elevated serum levels of IL-12, the major cytokine in T_H1 immunity, are associated with a poor prognosis in FL. These findings therefore do not support the traditional concept of T_H1/T_H2-mediated antitumor immunity; in fact, this concept has been challenged, as the biological effects of T_H1 and T_H2 cells are often found to be inconsistent with clinical observations.

T_H17 Cells Due to the fact that T_H17 cells were identified and characterized only recently, data on T_H17 cells are limited in FL. The frequency of T_H17 cells is low in FL when compared to other types of B-cell NHL,[40] and malignant B cells play an important role in suppressing T_H17 cell differentiation, thereby leading to the reduced presence of T_H17 cells in FL. Costimulatory molecules participate in this suppression because the use of a blocking antibody against CD70, CD80, or CD86 reversed lymphoma B cell–mediated inhibition of T_H17 cells.[24] This inhibition also correlated with enhanced T_{reg} cell differentiation.[40] The low frequency of T_H17 cells and high representation of T_{reg} cells leads to inhibition of inflammation and may account for the lack of an inflammatory immune response in the tumor microenvironment in FL patients.

T_{FH} Cells While the biology of T_{FH} cells has been intensively investigated under normal physiological conditions, T_{FH} cells have not been assessed under pathological circumstances. Because of its specific localization, T_{FH} can be detected in biopsies of FL.[39,41] The intratumoral T_{FH} cells express high intensity staining for PD-1 and CXCR5, as well as BCL-6.[42] A recent study[41] quantified CD4+CXCR5+ICOS+ T cells and found that the frequency of this population was higher in lymph nodes from FL than from diffuse large B-cell lymphoma (DLBCL) or reactive lymph nodes. This population of cells is heterogeneous in terms of CD25 and Foxp3 expression. CD25 and Foxp3 expression divides this population into 2 subsets: follicular T helper (T_{FH}) cells and follicular regulatory T (T_{FR}) cells. Both subsets display unique gene expression profiles. T_{FH} cells support FL B-cell activation and rescue autologous malignant B cells from spontaneous apoptosis.[41] In contrast, T_{FR} cells exert a strong regulatory function by inhibiting CD4+CD25- effector T-cell proliferation. Furthermore, purified FL-derived T_{FH} cells overexpress several genes potentially involved (directly or indirectly) in lymphomagenesis (in particular, IL-4 or CD40L), which efficiently rescue malignant B cells from spontaneous and rituximab (Rituxan, Genentech/Biogen Idec)-induced apoptosis.[41,43]

Factors Regulating T-Cell Function

T-cell function is regulated by many factors, including suppressive signals from costimulatory molecules. One such set of signals is via the PD-1/PD-L1 signaling pathway. Programmed death 1 (PD-1), a member of the CD28/CTLA-4 family, is induced in activated T cells. PD-1, interacting with its receptor PD-L1, has been shown to negatively regulate T-cell receptor (TCR) signaling and decrease proliferation and cytokine production in T cells. We[21] observed that PD-1 is highly expressed on intratumoral CD4+CD25- T cells, and that CD4+CD25+ T_{reg} cells express PD-L1 upon TCR activation. This expression pattern suggested a role for PD-1 and PD-L1 interaction in intratumoral T_{reg} cell–mediated immune suppression. In fact, blocking the interaction between PD-1 and PD-L1 with a neutralizing antibody attenuated T_{reg} cell–mediated inhibition of CD4+CD25- T cells. A study of 100 FL samples confirmed the expression of PD-1 on T cells and also found that PD-1 expression was rarely found on Foxp3+ T_{reg} cells.[10]

TIM-3, a family member of T-cell immunoglobulin and mucin domain proteins, has been shown to inhibit T_H1-mediated auto- and allo-immune responses, and to promote immunologic tolerance.[44,45] Recently, a growing number of studies have suggested that, instead of functioning as an inhibitor for T_H1 cells, TIM-3 actually plays a crucial role in mediating T-cell exhaustion and contributing to negative immune responses in both viral infections and tumors.[46-50] We have found that TIM-3–expressing T cells were frequently detected in biopsy specimens of FL patients and displayed exhausted phenotypic and functional characteristics.[39] The intratumoral TIM-3+ T cells also express PD-1 and have impaired function. Of note, PD-1^{high} CD4+ T cells in FL biopsies have also been found to co-express CXCR5 and have been characterized as T_{FH} cells, suggesting that TIM-3 may be more specific as a marker for exhausted T cells than PD-1. Importantly, the numbers of CD4+TIM-3+ T cells were associated with a poor survival in FL patients. We found that IL-12 plays a key role in upregulating TIM-3 expression, inducing T-cell exhaustion, and contributing to the high number of TIM-3+ T cells in FL.[39]

In contrast to TIM-3 expression, the prognostic significance of PD-1 expression has been controversial. In initial studies, the number of PD-1+ cells was significantly lower in patients with a poor performance status

and high serum lactate dehydrogenase (LDH), and high numbers of PD-1+ T cells were associated with improved overall survival in FL patients.[10,51] However, subsequent studies[52] have challenged this finding and have identified PD-1+ T cells as an independent prognostic risk factor for decreased overall survival in FL. The disparate findings may be due to differences in the study populations, patient selection criteria, or clinical management, as well as the varying methodologies used to enumerate PD-1+ T cells. The finding that PD-1+ T cells are heterogeneous cells with phenotypes of both T_{FH} and exhausted T cells may contribute to the discrepant observations.

Intratumoral Monocytes/Macrophage/ Dendritic Cells

Tumor-Associated Macrophages

Cells of the monocyte lineage comprise different types based on maturation status, and their progeny includes macrophages and dendritic cells. Differentiation of these cells is defined by a variety of markers expressed on the cell surface, including CD11c, CD14, and CD68. Monocytic cells play an essential role in the innate immune response as a first line of resistance against pathogens and in activating adaptive immune responses. Depending on stimulation, macrophages may undergo classical M1 activation (stimulated by lipopolysaccharide and IFN-γ) or alternative M2 activation (stimulated by IL-4 and IL-13). The resulting M1 and M2 macrophages produce distinct cytokines and play different roles in the innate immune response. For example, M1 macrophages produce IL-12 and promote T_H1 cell development, and M2 macrophages secret IL-10 and facilitate the development of T_H2 cells.[53] In the early stage of tumors, M1 macrophages are recruited and infiltrate into the tumor microenvironment in response to inflammatory signals, and then release proinflammatory cytokines and chemokines to promote T- and NK-cell development and differentiation. In the later stages of tumor development, macrophages differentiate into a subpopulation called *tumor-associated macrophages* (TAMs). TAMs may polarize to M2 cells and release cytokines to encourage T_H2 differentiation and recruitment. It has been shown that TAMs inhibit antitumor immunity by secreting suppressive cytokines such as TGF-β, by promoting angiogenesis, and by expressing growth factors that support tumor growth.[53] Clinically, it has been observed that increased numbers and/or density of intratumoral macrophages correlate with both progression and prognosis in the majority of cancers, including B-cell non-Hodgkin and Hodgkin lymphoma.[54] Similar findings have been observed in FL. Farinha and associates[55] sought to determine the role of multiple biomarkers in determining outcome in FL, with a specific focus on the role of macrophages. Using tissue microarrays, CD68 staining was the only biomarker that correlated significantly with the overall survival of FL patients. Increased CD68+ cell content, termed *lymphoma-associated macrophages* (LAM), was associated with diminished survival. This finding supported a previous gene-profiling study in FL that found a macrophage-related immune signature correlated with a poor prognosis.[5] Other studies have confirmed that CD68+ LAMs are associated with an adverse outcome, but have also shown that a therapeutic regimen, such as rituximab, can circumvent this association.[56,57]

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of immature myeloid cells that have not yet differentiated into macrophages, dendritic cells, or granulocytes, and are normally present in the bone marrow of healthy individuals. In this regard, MDSCs are generally divided into 2 distinct subpopulations: monocytic and granulocytic MDSCs. While it is easy to define MDSCs in mice by using CD11b and Gr-1, the phenotypes to define this population in humans are quite divergent in studies employing different types of tissues. Thus, the lack of consensus in defining MDSCs makes it difficult to quantify this population in patient samples.[58,59] Various studies, however, have shown that MDSCs are highly represented in a variety of cancers and suppress NK and T cells through either direct cell contact, cytokines, or byproducts of metabolic pathways. MDSCs have also been shown to regulate expansion and activation of T_{reg} cells, support angiogenesis, and promote tumor cell metastasis.

MDSCs have been intensively investigated in solid tumors; however, few studies have been performed in hematologic malignancies. Recently, Lin and colleagues[60] identified a CD14+ subpopulation with low or absent human leukocyte antigen DR (HLA-DR) expression in the peripheral blood of patients with B-cell NHL, including FL. These CD14+DRlow/- monocytes inhibit T-cell function with a mechanism involving arginine metabolism. An elevated frequency of this suppressive monocytic subpopulation correlated with disease progression and overall survival. Studies in multiple myeloma[61] and T-cell lymphoma[62] support these findings and highlighted the role of HLA-DR expression in defining MDSCs in hematologic malignancies.

Because MDSCs are immature monocytic or granulocytic cells with immunosuppressive properties, it may be beneficial to promote differentiation to mature cells, such as macrophages and dendritic cells, and thereby decrease MDSCs. Thus far, 2 different agents have been shown to facilitate this process: 25-hydroxyvitamin D3 (VD3) and all-trans-retinoic acid (ATRA). Therapeutic administration of either VD3 or ATRA has been found

to be associated with decreased numbers of MDSCs and increased numbers of mature HLA-DR+ monocytes in cancer patients.[63-65] In agreement with these findings, recent studies found that VD3 insufficiency significantly correlates with a poor outcome in patients with hematologic malignancies.[66,67]

Other Intratumoral Cells

Natural Killer Cells

NK cells are a type of cytotoxic lymphocyte critical to innate immunity that do not require recognition of the major histocompatibility complex (MHC) presented on infected cell surfaces. NK cells comprise 2 main subsets: less mature CD3-CD56^{bright} and more mature CD3-CD56^{dim} cells, based on functional and phenotypical characteristics.[68] CD56^{bright} cells are mainly present in lymph nodes, express dim or negative CD16, and produce abundant cytokines, such as tumor necrosis factor (TNF)-α and IFN-γ. In contrast, CD56^{dim} cells are more commonly found in peripheral blood with bright expression of CD16, and have a cytotoxic function.[68] While most studies emphasize the role of intratumoral T cells and macrophages, few studies have focused on NK cells in B-cell NHL. Gibson and coworkers[69] analyzed the total number of NK cells, as well as NK subsets, and correlated the number with disease progression in B-cell NHL. They found that the total number of NK cells varied based on tissue site, and the proportions of NK cells were similar based on Ann Arbor stage. However, the proportions of NK cells did correlate with the FLIPI score.[69] Data obtained from patients with other B-cell lymphomas found that higher proportions of peripheral blood NK cells were associated with a favorable prognosis.[70,71]

Follicular Dendritic Cells

Follicular dendritic cells (FDCs) are the stromal cells located in the GC of follicles. FDCs comprise 1% of all GC cells.[72] Fully differentiated FDCs express low-affinity IgE receptor CD23, complement receptors CD21 and CD35, and lymphocyte chemoattractant CXCL13. Unlike other APCs, FDCs do not internalize, process, and present Ag, but present intact Ag-antibody (Ab) complexes on their cell surface. B cells that bind to these Ag-Ab complexes survive and differentiate into memory B cells or plasma cells. In vitro studies[73] using FDC cell lines such as HK cells showed that FDCs preferentially bind GC to B cells, providing them with survival signals, while the majority of unbound B cells undergo apoptosis. CD40 ligand and cytokines such as IL-15 are critically involved in FDC-mediated, GC B-cell proliferation and survival.[73] In FL patients, FDCs may be immature and show partial or complete absence of surface markers, such as CD23, CD21, or CD35.[74-76] FL patients with mature FDCs in the lymph nodes have increased numbers of FL-associated T cells, and the mature FDCs are associated with a higher clinical stage at the time of diagnosis.[74] In contrast, Cui and associates[77] found that atypical immature FDCs, which have diminished or absent CD21 staining, are present in 11% of FL patients. The majority of patients with a predominance of mature FDCs in the tumor showed advanced clinical stage (III or IV), whereas cases demonstrating an atypical immature FDC network showed localized clinical stage (I or II).[77]

Cytokines

In addition to the cellular compartment, cytokines (including chemokines) form another molecular compartment that critically influences tumor immunity and patient outcome in cancer. Among the cytokine family, interleukin (IL)-2 and its signaling components have been investigated in B-cell NHL, including FL. IL-2 was originally identified as a T-cell growth factor and was later found to promote the function of a variety of other immune cells, such as B cells, NK cells, and macrophages. Administration of IL-2 to cancer patients achieves a promising outcome, although the duration of benefit is often short.[78] Based on experimental and clinical results from other cancers, recombinant IL-2 (rIL-2) has been administered to enhance the efficacy of standard therapy regimens, especially rituximab, in FL patients.[79,80] Several clinical trials observed that, although rIL-2 boosts immune responses determined by enhanced NK-cell numbers and antibody-dependent cellular cytotoxicity (ADCC) activity, response rates were low,[80] suggesting that enhanced immune responses do not directly translate into meaningful clinical benefit for FL patients. Additional studies have found that IL-2 is essential to the development of T_{reg} cells that suppress tumor immunity, which may account for the limited benefit of rIL-2 in FL patients.

To identify cytokines and cytokine receptors that may be important in FL, our group performed a multiplex ELISA (Luminex) assay on serum specimens obtained from 30 previously untreated patients and compared the levels of 30 cytokines in these patients to those in normal controls. We observed elevated serum levels of a number of cytokines that included soluble IL-2 receptor alpha (sIL-2Rα).[81] Higher serum IL-2Rα levels before treatment were associated with a shorter progression-free survival in FL patients treated with rituximab alone as initial therapy, which is consistent with the findings in other aggressive lymphomas.[82-84] Biologically, sIL-2Rα, instead of blocking, actually maintains IL-2 signaling and induces Foxp3 expression in T cells, resulting in a regulatory phenotype.

These results indicate that sIL-2R plays an active biologic role in FL by binding IL-2 and sustaining IL-2 signaling rather than depleting IL-2 and blocking its function.

In FL, the data collected from in vitro assays or animal models do not consistently translate to clinical benefit. An example is the use of IL-12. IL-12 induces IFN-γ production and promotes the function of T and NK cells, which contributes to antitumor immunity. However, administration of IL-12 to boost antitumor immunity in cancer patients has shown minimal or no clinical benefit.[85] In fact, a clinical trial of IL-12 in combination with rituximab in B-cell NHL showed a lower response rate in patients treated with the combination than in patients treated with rituximab alone.[86] Supporting this finding, in FL patients, elevated IL-12 levels at diagnosis were associated with an inferior outcome.[39] These results suggested that, in contrast to the observations made in vitro or in vivo in mice, IL-12 actually plays a detrimental role in FL patients. Further study has revealed that IL-12 upregulates TIM-3 expression and induces T-cell exhaustion, which accounts for inferior outcome in FL patients treated with IL-12.[39]

Serum or intratumoral levels of other cytokines, including TGF-β, vascular endothelial growth factor (VEGF), IL-10, and TNF-α, have been shown to correlate with survival in patients with FL.[87-89] All of these cytokines regulate immune cell differentiation and function, and may play a role in promoting malignant B-cell growth and survival. Additional research is needed to define the role of intratumoral cytokines, as regulation of cytokine production may present a therapeutic opportunity for patients with lymphoma.

Conclusions

FL has a unique tumor microenvironment compared to solid tumors, and the presence of a substantial number of intratumoral immune cells has been shown to influence antitumor immunity and patient survival. Immune cells, such as T cells and macrophages, can reside within follicles and have direct contact with lymphoma B cells. Malignant B cells can manipulate the microenvironment by skewing the differentiation of immune cells, attracting regulatory T-cells or suppressive monocytes, or secreting cytokines that promote an immunosuppressive environment. All of these mechanisms promote immune suppression and immune exhaustion, as well as immune dysfunction within sites involved by lymphoma, making it difficult for tumor-specific effector cells to kill malignant cells. Therefore, to develop effective immunotherapeutic approaches in lymphoma, we will need to apply strategies that both enhance an effective antitumor response and reverse immunosuppression and dysfunction.

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