T follicular helper cells: a potential therapeutic target in follicular lymphoma

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ABSTRACT

Follicular lymphoma (FL), the most common indolent B-cell non-Hodgkin lymphoma (B-NHL), is a germinal center (GC)-derived lymphoma. The mechanisms underlying B-cell differentiation/maturation in GCs could be also involved in their malignant transformation. Moreover, the non-malignant cell composition and architecture of the tumor microenvironment can influence FL development and outcome. Here, we review recent research advances on CD4 helper T cells in FL that highlight the pivotal role of T follicular helper (TFH) cells in a complex multicellular system where they interact with B cells during GC dynamics. After describing the mechanism of FL lymphomagenesis, we discuss the emerging evidence about TFH cell enrichment and involvement in FL tumorigenesis and in B-T cell interaction, TFH regulation by T follicular regulatory cells (TFR) and its potential effect on FL. Then, we provide an overview on the flexible interplay between the different CD4 T-cell subtypes and how this may be predicted in normal and pathologic contexts, according to the cell epigenetic state. Finally, we highlight the importance of targeting TFH cells in the clinic, summarize the main outstanding questions about TFH and TFR cells in FL, and describe strategies to potentiate FL therapy by taking into account TFH cells.

INTRODUCTION

Most B-cell non-Hodgkin lymphomas (B-NHLs) originate from GC B cells and are associated with B-cell deregulation [1–4]. Follicular lymphoma (FL) is the second most common form of B-NHLs. FL gene expression profiling has revealed that the molecular characteristics of non-malignant tumor–infiltrating immune cells have a major influence on patient survival [5, 6]. However, the role of CD4+ helper T cells, and particularly of T follicular helper (TFH) cells, in FL is still unclear [7–11]. TFH cells are the specialized helper T cells in the germinal center (GC), and their differentiation is controlled by BCL6 [12–14]. They contribute to GC formation and maintenance, to GC B-cell differentiation into plasma cells and to immunological memory. However, TFH cells also have an important role in human pathology. Indeed, their deregulation can lead to various diseases, such as autoimmune diseases, immunodeficiency disorders, and lymphoma [15–17]. Increasing evidence shows a link between TFH cells and FL [8, 11, 18–20]. Their constant crosstalk with B cells and their increased count in FL suggest that they might represent a novel therapeutic target in FL. Treatment with rituximab alone, or in combination with chemotherapy or radiotherapy, has markedly improved the overall survival of patients with advanced-stage FL. Moreover, more aggressive treatment approaches with high-dose chemotherapy and stem cell transplantation can be proposed to patients with more resistant disease, but with good performance status [21–25]. Nevertheless, the persistence of TFH cells following rituximab treatment and their enrichment in FL [26] highlight the need to precisely control TFH cell function and production in this cancer. In this review we will discuss recent findings on helper...
T cells in FL pathology and their prognostic significance by positioning TFH cells as the main CD4+ T helper cells that infiltrate FL, and the recently identified T follicular regulatory (TFR) cells as their regulator in GC responses. Specifically, we describe the intriguing interplay between TFH and TFR cells, their finely modulated interaction and spectacualr plasticity with other helper T cells. We then discuss possible future research directions on TFH cells. Finally, we raise outstanding questions that need to be answered to understand how modulation of TFH cell function could improve FL therapeutic options.

**FL pathogenesis**

Germinal centers (GC) are sites within lymph nodes where mature B lymphocytes rapidly proliferate and differentiate to produce plasma cells that secrete high-affinity antibodies during the humoral immune response to protect against infections [27]. This process includes somatic hypermutation (SHM) of the genes encoding the immunoglobulin variable region (IgV) [28–30] and class switch recombination (CSR) (Figure 1A). The rapid division, B cells are called centroblasts, and after they have stopped proliferating and started selection, they are known as centrocytes [31, 32]. Thus, GCs are an important part of the B cell-based humoral immune response. However, the beneficial role of GC B cells in immunity is somewhat weakened by their genomic instability than can lead to lymphomagenesis. Indeed, most B-cell lymphomas originate from GC B cells [1–3]. The best examples are B-NHLs, a large group of lymphomas that includes also FL. Each B-NHL subtype is characterized by distinct genetic alterations that are often major determinants of their phenotype. About 90% of patients with FL harbor the t(14;18) translocation in which the immunoglobulin heavy chain (IGH) enhancer region and the B-cell lymphoma 2 (BCL2) gene are juxtaposed [33]. This translocation occurs early during B cell development, but contributes to lymphomagenesis at later stages during the GC reaction, possibly as a consequence of antigen stimulation (Figure 1A). The juxtaposition of the IGH regulatory regions leads to BCL2 ectopic expression and activation of the anti-apoptotic program [34, 35]. However, the detection of BCL2 genetic aberration at low frequency (40%) also in healthy individuals is one of the many evidences supporting the hypothesis that this translocation is necessary, but not sufficient for FL and that other events are required for tumor progression [36–38]. Moreover, BCL2 deregulation provides a survival advantage that might favor the acquisition of additional genetic aberrations during repeated transits of BCL2-overexpressing B cells through the GC [39].

Among the secondary alterations frequently observed in FL (Figure 1B), the genetic inactivation of the histone methyltransferase MLL2 (KMT2B) (in ~89% of cases) seems to be an early event that leads to the deregulation of the B cell transcriptional program. Moreover, mutations in the genes encoding other chromatin remodelers, such as EZH2 (in ~27% of cases) and the acetyltransferases CREB-binding protein (CREBBP) and E1A-binding protein P300 (EP300) (in ~40% of cases), are often observed in FL [40–44]. Rearrangements of the transcriptional repressor BCL6 also are detected in 10% of FL [36, 45], suggesting that BCL6 physiological function (prevention of premature activation and differentiation of GC B cells) could be diverted during B-cell malignant transformation [4]. BCL6 targets BCL2, TP53 and BLIMP1 that are involved in key pathways (apoptosis, cell cycle and B-cell activation and differentiation) [3, 4, 34, 46–56]. Therefore, despite the low percentage of BCL6 rearrangements in FL, its deregulation may be critical specifically for the DNA damage response, leading to increased tolerance against genetic alterations and to their accumulation [3, 4, 10, 57, 58]. Finally, a recent analysis of candidate genes in FL highlighted loss of EPHA7 (a receptor tyrosine kinase and potential tumor suppressor) [59] and inactivating mutations in the TNFRSF14 gene in 72% and 18–46% of cases, respectively [60, 61, 62]. As TNFRSF14 interacts with molecules that control T cell biology (for example B- and T-lymphocyte attenuator, BTLA), this finding provides the first insight into the link between FL and its microenvironment.

**CD4+ T cells in FL microenvironment: friends or foes?**

In 2004, Dave et al demonstrated that the molecular features of non-malignant immune cells within FL (i.e., the tumor microenvironment) at diagnosis are correlated with patient survival. Specifically, by gene expression analysis of tumor biopsy specimens from untreated patients with FL, they discovered two different signatures (immune response-1 and immune response-2) that reflect the gene expression profiles of the non-malignant immune cells that infiltrate the tumor. The immune response-1 signature is broadly enriched in T cells and is associated with good prognosis. Conversely, the second signature is enriched in monocytes/macrophages and is associated with bad prognosis [5, 6]. In the immune response-1 signature, the presence of CD8+ T cells (known as cytotoxic T lymphocytes, CTLs) in FL microenvironment is clearly associated with good prognosis and longer survival. Indeed, CTLs efficiently kill tumor cells upon contact, through the release of cytokines and perforin/granzyme [63–67]. However, the specific role of CD4+ T cells has not been fully defined. This could be partially explained by the fact that there are various CD4+ T cell populations with specific and also overlapping functions, such as helper T cells and regulatory T cells (Tregs). These multifunctional cells can express transcription factors
that are specific of different cell subpopulations and can elicit multiple cytokine-mediated effector functions simultaneously (see below). Therefore, their function could be influenced by their microenvironment. Moreover, several studies have shown an association between the number and the histologic pattern of FL infiltration by helper CD4\(^+\) T cells, Tregs and TFH cells [7, 8, 11, 66, 68-70]. These two characteristics are a strong predictor of survival and risk of histological transformation to aggressive B-NHL. Specifically, a follicular pattern (i.e., predominant intrafollicular or perifollicular localization of cells in the lymph nodes) is associated with poor survival compared with a distribution pattern outside the follicle [7–9]. Finally, transcription factor analysis showed differences within FL in the distribution and anatomical localization of regulatory and helper T cell populations that express CXCR5, the receptor for the chemokine CXCL13, including a population of T helper

Figure 1: FL lymphomagenesis. (A) The germinal center (GC) is the main source of mature B cells that produce high-affinity antibodies. In a physiological context, during the initiation step, precursor B cells that have successfully undergone V(D)J recombination and that express functional B-cell receptors become naive B cells and migrate to the T-cell zone (a T cell-rich area in lymphoid organs), where they will be fully activated as a result of their interaction with CD4\(^+\) T cells and antigen presenting cells (APCs). Then, they differentiate into centroblasts that undergo clonal expansion in the GC dark zone. During this intense proliferation step, somatic hypermutation (SHM) allows the diversification of the B-cell receptor repertoire. They then differentiate into centrocytes and move to the light zone, where they improve antigen binding with the help of CD4\(^+\) T cells (specifically, T follicular helper (TFH) cells) and follicular dendritic cells (FDCs). Centrocytes that produce an unfavorable antibody are eliminated by apoptosis (cells in brown), whereas successful centrocytes undergo immunoglobulin class-switch recombination (CSR), a biological mechanism whereby their immunoglobulin production is changed. The GC reaction finally generates memory B cells and plasma cells. In follicular lymphoma (FL), during the early initiation step, a t(14;18) chromosomal translocation occurs that involves the immunoglobulin IgH locus and the proto-oncogene BCL2. This leads to BCL2 overexpression in tumor B cells (FL B) that later activate an anti-apoptotic program to promote their survival (dashed arrows). However, the finding that this translocation is also observed at low frequency in normal B cells indicates that the BCL2 genetic alteration is necessary, but not sufficient to cause FL. (B) Accumulation of secondary events during the GC reaction is necessary for tumor progression. All these alterations will accumulate during somatic hypermutation (SHM) and class-switch recombination (CSR) and will contribute to dysfunctional B-T cell interactions (illustrated within the dashed circle; see Figure 2 for more details) that favor tumor growth, escape and dissemination.
cells known as TFH cells that play a critical role in GC reaction [8, 11, 13, 71, 72]. Moreover, it was reported that programmed death-1 (PD-1) expression in T cells located within follicles of secondary lymphoid organs, including TFH cells, is correlated with FL clinical outcome. In lymphoma, PD-1 is involved in T cell exhaustion (reduced cell differentiation, proliferation, and effector function), thus its expression can reduce the anti-tumor response of effector T cells [73–76]. Hence, there is a real need to better understand the place and prognostic value of helper T cell populations, specifically of TFH cells in this pathology.

New non-malignant players in FL

Early work in human cell systems supported the hypothesis that FOXP3, a master regulator of Treg development and function, is only expressed in CD4+CD25+ T cells with in vitro immunosuppressive activity [77–79]. However, later studies suggested that T cell receptor (TCR) stimulation of CD4+CD25+/FOXP3− T cells leads to the induction of FOXP3 expression [80–82]. Moreover, a population of CD4+CD25+/FOXP3+ T cells was identified at the tumor site of human B-NHLs [83–85], suggesting that chronic stimulation of T cells might alter T cell differentiation and FOXP3 expression, independently of CD25. This could be even more likely in cancer, where inflammation is often the starting point. Taken together, these findings suggest that the antitumor response can be increased by targeting CD25+ Treg cells. However, the residual CD25+/FOXP3 or FOXP3+ T cells that can mediate immune suppression would still remain and inhibit the host antitumor response. In this context, it is important to take into account TFH cells, a major sub-population of CD4+ T cells that express PD-1, but not CD25, and their main regulators TFR cells [12, 13, 85, 86].

TFH cells

TFH cells have been the subject of intense investigation, but their true identity remains elusive. These cells are considered to be a distinct T helper cell subset with an essential role in adaptive immunity. Specifically, they contribute to GC formation, B cell development and maturation, and immunoglobulin class switching. Several cell surface molecules, such as CXCR5, PD-1 and inducible T-cell co-stimulator (ICOS), have been used as TFH cell markers [12, 13, 87]. In addition to their anatomical location in GC, TFH cells display features of specialized helpers of B cells, such as expression of interleukin 21 (IL-21), an inducer of B cell functions (activation, proliferation and differentiation), and of costimulatory molecules, for instance CD40 ligand (CD40L) and ICOS [87–90].

Despite this confined functional definition, the TFH compartment complexity and involvement in cancer, and more especially in hematological malignancies, are starting to emerge [91–95].

Indeed, a recent study has found significantly higher plasma levels of TFH cells that also strongly express ICOS, PD-1, and IL-21 at pretreatment in patients with acute lymphoblastic leukemia, multiple myeloma or NHL, compared with healthy donors [96]. Moreover, comparison of TFH cell count and ICOS, PD-1, and IL-21 expression in these three groups of patients showed that they were the highest in patients with NHL. These authors also observed a negative correlation between TFH cell number and therapeutic effect, implying that TFH might have a prognostic value in the clinic [96].

TFH cells with strong PD-1 expression (a marker correlated with FL outcome) are expanded and active in FL microenvironment (lymphomatous follicles or residual GCs) and might result in T cell inhibition and loss of effective anticancer immune function [69, 74, 97, 98]. FL-infiltrating TFH cells display a specific gene expression profile with overexpression of IL-6 and IL-21 [11, 18] (Figure 2). Moreover, high levels of IL-4, mostly produced by TFH, have been associated with Signal Transducer and Activator of Transcription protein 6 (STAT6) and ERK-dependent FL B-cell activation [13]. Through IL-4 and CD40-L production, TFH cells might contribute to FL B-cell survival [19, 20, 99]. In turn, the FL niche, which includes mesenchymal stromal cells (MSCs), supports the viability of FL-infiltrating TFH cells through an IL-6-dependent mechanism [18]. Interestingly, MSCs in the tumor microenvironment can induce FOXP3 expression in TFH cells, thus mediating their conversion to TFR cells. This suggests that TFR can be derived from TFH cells in FL [18]. Altogether, these evidences stress the need to take into account and to control TFH cell function and production in FL.

TFR cells

TFR cells are also located in the GC and share phenotypic characteristics of TFH and classical Treg cells [18, 100–103]. They show a subtle and flexible balance between their opposite TFH and Treg identities, and should be considered as a separated cell subset. Although many aspects of their biology and origin are still unclear, TFR cells have been identified as a distinct subpopulation of CD4+ T helper cells that express FOXP3, acquire a follicular phenotype and migrate into GCs, where they exert their specific regulatory role by modulating TFH number and function [101, 102]. In comparison with the classical Treg cells that express CD25 (an IL2 receptor with regulatory activity), TFR cells might downregulate CD25, but still retain their regulatory function, while upregulating genes associated with the TFH cell phenotype [85].

Concerning TFR role in FL, an excessively elevated number of follicular FOXP3+ Tregs that express CXCR5, a chemokine receptor with an important role in GC B
cell migration, is associated with poor patient survival [18, 104]. Moreover, the proportion of these CD4+ T cells is higher in FL tissues than in normal lymph nodes. Differently from what observed in mice, FL TFR cells are partially derived from TFH. In addition, several findings suggest that in FL, Treg differentiation is directed toward the TFR subpopulation. Consequently, TFR cells are accumulated within the FL microenvironment (malignant lymph node) and preferentially localized and retained in malignant GCs [18, 105].

In FL, helper T cell differentiation is skewed in the direction of inflammatory and activation signals that could shape an adequate tumor microenvironment [11] in which Treg and TFH cells might functionally interact [11, 106]. Therefore, given that TFH and TFR cells are reciprocal and antagonistic regulators of GC responses, the TFH-TFR balance is critical for immune homeostasis and could influence FL biology.

**TFH-TFR dichotomy**

The complex relationship between TFH and TFR cells is dictated by a dichotomy driven by specific signals, in which the TFR/TFH ratio is defined according to their anatomical location and the inflammatory response.

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**Figure 2: B-T synapse in FL.** In GCs, at the B-T cell border, TFH cells interact with different cell types and secrete several molecules and cytokines that play a critical role in lymphomagenesis. Within altered GCs, TFH cells provide a precious support to FL B cells by displaying a specific molecular signature: upregulation of IL-6, IL-21, FOXP3 and, most importantly, IL-4 that is associated with STAT-6 and ERK-dependent FL B cell activation. Through IL-4 and CD40-L production, TFH cells might contribute to FL B cell survival. Following interaction with TFH cells, FL B cells might acquire additional pro-tumor features. Moreover, mesenchymal stromal cells (MSCs) in the FL niche support the viability of FL-infiltrating TFH cells via an IL-6-dependent mechanism. This fine interplay between FL B and TFH cells could be the consequence of **BCL6** deregulation, which mediates CD80 and PD-L1 modulation. Within this abnormal synapse, follicular dendritic cells (FDCs), as exclusive providers of both co-stimulatory signals and antigens driven to B cells, could also be subverted by tumor B cells and the tumor niche. Their ability to secrete CXCL13, BAFF, IL-6 and CD40 give them a pivotal role in TFH and FL B cell survival [159–164].
strength. Basically, B7 molecules and the PD-1/CTLA-4 couple are the most important factors that regulate GC dynamics through TFR/TFH modulation (Figure 3) [107, 108]. Little is known on how the PD-1/CTLA-4 couple contributes to regulating the TFR/TFH ratio. PD-1 is highly expressed by TFH and TFR cells, exerts an inhibitory signal on TFR function and differentiation, and restrains TFH function. However, in a pro-inflammatory context, PD-1 limits TCR stop signal and consequently could increase the number of B cells that TFH cells might encounter, thus preventing the quick proliferation of TFH cells, giving them time to correctly activate B cells [107, 109]. In FL, where tumor B cells alter T cells by upregulating PD-1 expression, this could easily degenerate into an uncontrollable proliferation of B cells, favoring tumor progression. CTLA-4 is highly expressed by TFR cells and negatively controls their differentiation and expansion and thus TFH cells fate. In addition, the high

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**Figure 3**: CD4⁺ T cell plasticity. T helper (TH) cell subtypes and Treg lineages were previously classified simply as TH1, TH2, TH17, Treg and TFH on the basis of their specific cytokine signature (in black) and the expression of a single transcription factor. However, recent research shows that CD4⁺ T-cell subsets have an incredible inter-convertibility and trans-differentiation ability. Emerging evidence indicates that Treg and TFH cells, at least, are in the center of a flexible, interconnected system in which cells are in a multifunctional poised state and might be responsive to a multitude of cytokines and transcription factors that influence their differentiation and function. TFH cells can differentiate into TH1 and TH2 cells that express two transcription factors (BCL6 and T-bet or GATA3, respectively) and secret specific molecules, such as STAT-4 and IL-12 (TFH/TH1 hybrid cells) and CNS2 and IL-4 (TFH/TH2 hybrid cells). Likewise, Treg cells can differentiate into TH1, TH2 and TH17 by expressing two transcription factors (FOXP3 and T-bet or GATA3 or ROR γt, respectively) and by modulating the expression/activity of various molecules, such as TGF-β (Treg/TH1 and Treg/TH2 hybrid cells) and IL-2 and IL-1β (Treg/TH17 hybrid cells). This fine adaptation to the microenvironment is possible through a complex epigenetic remodeling process regulated by cell-specific transcription factors. In this complex cellular system, the mechanisms of Treg and TFH cell interplay are not fully known. A recent hypothesis reported by Sage et al on the molecular interaction between TFH and T follicular regulatory (TFR) cells is illustrated here. The PD-1/CTLA-4 couple contributes to the regulation of the TFR/TFH ratio. PD-1, which is highly expressed in TFH and TFR cells, exerts an inhibitory effect on TFR cell function/differentiation and limits TFH cell function. By limiting the TCR stop signal, PD-1 increases the number of B cells that TFH cells encounter, thus preventing their too fast proliferation and allowing proper B-cell activation. CTLA-4 is highly expressed by TFR cells and negatively controls their differentiation and expansion and consequently, also TFH cell fate. The high affinity interaction of CTLA-4 on TFR cells with B7 on B cells might prevent, through mechanical disruption of TFH-B cell contacts, TFH cell binding to B cells. According to the nature of the stimulus and the microenvironment (for example, a pro-tumor context), PD-1 and CTLA-4 might modulate differently the follicular program. Green, activation; red, inhibition; dashed arrows, interactions that have not been confirmed experimentally.
affinity interaction of CTLA-4 at the surface of TFR cells with B7 on B cells may prevent TFH binding to B cells through mechanical disruption of TFH-B cell contacts [100, 109, 110].

In conclusion, PD-1 and CTLA-4, through their multifaceted roles in TFH and TFR biology, may drive the predominance of the TFR or TFH cell phenotype, depending on the nature of the stimulation and the microenvironment. However, it is not-known how PD-1 and CTLA-4 modulate the follicular program and most importantly how these molecules act in FL. Although it is known that TFH and TFR cells are strongly interconnected in FL, it is now important to understand how they can be specifically modulated and the possible consequences of these targeted approaches.

The incredible plasticity of CD4+ T cells

It is now clear that helper T cells do not fit into a restrictive model consisting of mutually exclusive T cell lineages. For instance, the recently described conversion of T helper-17 (TH17) into T helper-2 (TH2) cells [111] highlights the flexibility of the different CD4+ T lymphocyte subsets. Moreover, the notion of CD4+ T cell plasticity has been strengthened by the ex vivo identification of CD4+ T lymphocyte hybrids that co-express transcription factors of two distinct subsets [112]. T cell fate plasticity can be advantageous for the host defense against pathogens, but can also play a determinant role in tumor development.

Several evidences highlight TFH cell flexibility and interplay with other helper T lineages. First, both TFH and TH2 cells produce IL-4 that has a major role in humoral immunity [113]. Moreover, the conserved non-coding sequence 2 (CNS2) distal IL-4 enhancer, while dispensable for TH2 cell function, plays an important role in IL-4-mediated humoral responses that are mainly regulated by TFH cells [114, 115]. This relationship was confirmed by the finding that TFH cells can develop from TH2 cells in vivo after helminth infection [116]. As IL-4 can also promote tumorigenesis through alteration of the tumor suppressing activity of monocytes and macrophages [117–119], the overlapping phenotypical and functional characteristics of THF and TH2 cells might also favor the tumor niche in FL. Second, both TFH and TH1 cells produce interferon gamma (IFNγ) that has a main role in immune responses. Through STAT4, IL-12 induces a transitional stage of TFH-TH1 cells that express IL-21 and BCL6, promoting both phenotypes. STAT4 also induces T-bet expression that, together with IFNγ, represses the TFH cell phenotype and functionalities, promoting full TH1 cell differentiation [120]. Moreover, human B cells might induce prominent and stable co-expression of the TH1 and TFH cell signatures during priming and antigen recall [121]. This suggests that human B cells exploit CD4+ T cell plasticity to render the effector T cell response more flexible. Moreover, increasing evidence indicates that transcription factors thought to be master regulators of other helper T cell lineages can also be expressed by TFH cells [101, 121–126]. This suggests that TFH cells have the potential to express a number of different lineage-defining factors, depending on the environmental conditions (Figure 3). Therefore, it is important to determine whether in FL, tumor B cells might influence this plasticity.

Likewise, Tregs can convert into TH1, TH2 or TFH lymphocytes [127, 128] (Figure 3). Mouse FOXP3+ Tregs express also T-bet, a TH1 cell-transcription factor [129] [17, 112, 130–132]. Similarly, human memory FOXP3+ Tregs express also RORyt, the main transcription factor of TH17 cells [133], and they can convert into TH17 upon IL-2 and IL-1β stimulation, or in the presence of IL-6 and TGF-β [112, 134–136].

Many aspects of T-cell plasticity still need to be elucidated, including the identification of the expression patterns and the mechanistic distinction between stable cells and more flexible subsets within a given cell lineage during physiological immune responses and in FL. Importantly, the ability of transcription factors to redefine or to modulate cell fate depends on whether they can regulate epigenetic events in a given setting. Recent data suggest that the helper T cell lineage-defining transcription factors T-bet, GATA3, and FOXP3 act in part by regulating the epigenetic environment of the cells in which they are expressed [137–141]. Along with lineage-specific transcription factors, epigenetic regulation (histone modifications and DNA methylation) plays an important role in helper T cell plasticity, and consequently in cancer initiation and development [140, 148–149]. Indeed, H3K4 methylation and H3K27 methylation are associated with permissive and repressive epigenetic states, respectively, and are two histone modifications that are important during helper T cell differentiation. In progenitor cells, the co-localization of these two histone modifications, which is called bivalent chromatin, represents an epigenetic state whereby genes are poised to be expressed, but with the potential to be turned on or off during the next cell developmental stage, depending on which signaling pathway(s) will be activated in response to environmental cues [150–152]. As a consequence, the stability of helper T cells may be challenged by abnormal environmental stimuli that lead to the expression of poised genes in a not expected cell lineage, as it may be the case in the highly deregulated tumor microenvironment. Moreover, recent reports suggest a role for DNA methylation and histone modifications in TFH-cell differentiation [153], supporting the high potential of TFH cells for reprogramming into other TH cell subsets [123, 124].

For all these reasons, it is important to elucidate the mechanisms by which each transcription factor functionally regulates gene expression both alone and in combination, as well as the global epigenetic background of each T cell lineage. This could allow predicting the
consequences of their overlapping expression patterns in normal and pathogenic settings.

**Future direction of research**

It is increasingly clear that some tumor-infiltrating lymphocytes may be friends, while others may be foes. Indeed, the overall increase in their number may not reflect a better prognosis, if this does not concern the right cell subsets with the right topography and at the right timeline. This may help explaining, in part, the disappointing clinical results of cancer vaccination strategies [154]. Therefore, therapies should act not only on FL B cells, but also on specific T cell populations to achieve acceptable results. For that, each subpopulation must be more precisely identified.

In the complex set of cellular interactions within the FL microenvironment, tumor-infiltrating CD4+ T cells display overlapping functions with critical roles. Among them, TFH cells are strongly enriched in the FL microenvironment and support malignant FL B cells, partly through CD40-L and IL-4. TFH cells are tightly regulated by and interact early with B cells in the GC. This FL-infiltrating T cell population plays a major role in FL biology and in follicular lymphomagenesis. Their precise mechanism of action and the timeline of their action need to be determined. TFR cells, which share both Treg and TFH features, exert a suppressive function on GC responses. The interplay between TFH and TFR cells has an impact on FL biology because of their reciprocal regulation. Most importantly, the persistence of TFH and TFR cells following rituximab treatment [26] shows the limit of the current therapies and is a valuable reason to strongly support the development of combinatory treatments in the clinic. Indeed, rituximab treatment leads to depletion of circulating naive B cells that express CD20, but this does not necessary reflect a B-cell deficiency in tissues [26, 155, 156], thus leaving the memory B-cell compartment intact and resistant to treatment. Moreover, pre-B and mature plasma cells do not express CD20 and are consequently not targeted by rituximab. Contrary to what reported in mice where GC B-cell depletion results in TFH cell loss [26, 157], Wallin et al showed that human TFH and TFR cells are not affected by GC B-cell depletion, suggesting that human TFH and TFR cells require B cells for their formation but not for their maintenance. Therefore, TFH and TFR subsets also should be targeted, to reduce relapse following rituximab treatment and to prevent the rapid reconstitution of the pathological GC response, once the B-cell pool begins to recover. Despite the interesting data on TFH enrichment and involvement in FL (Box 1), there is no clinical trial targeting these cells in FL yet. On the other hand, some clinical studies in the USA are assessing in patients with lupus the association of rituximab (after its unexpected failure on its own) with belimumab, an inhibitor of B-cell activating factor (BAFF, known to promote TFH cell formation) to reduce the abnormal GC activity orchestrated by TFH cells. These trials include the open-label CALIBRATE study (rituximab followed by belimumab for lupus nephritis) and the SYNBIoSe study (Synergetic B cell Immodulation in systemic lupus erythematosus). Moreover, a very recent study on the dynamic changes of peripheral TFH cells in patients with malignant lymphoid disease during treatment showed a negative correlation between TFH numeration, therapeutic effect and remission [96].

Also, it remains unclear whether TFH cells can switch phenotype and the exact nature of their relationship with the other helper T cells in physiological and

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**Box 1: Summary of what is known about TFH cells in FL**

1) TFH cells are strongly enriched in FL.
2) TFH cells contribute to the FL niche by expressing IL-6, IL-21, IL-4 and CD40-L.
3) TFH and TFR cells support FL viability, partly through an IL-6 dependent mechanism.
4) In FL, TFH cells could convert to TFR cells by expressing FOXP3.
5) TFH-TFR balance could partly regulate FL biology.
6) Overall, there is a negative correlation between TFH numeration and the therapeutic effect in patients with malignant lymphoid disease.

**Box 2: Outstanding questions**

1) What are the limits of T helper cell plasticity in FL?
2) Should T helper hybrid populations be considered to be specialized cells with a particular function or a differentiation/conversion intermediate?
3) Could TFR cells derive from Treg and/or TFH cells? Are they inter-convertible or fully separated?
4) Which mechanisms dictate TFH/TFR dichotomy in FL?
5) Are TFH and TFR cells the only follicular T-cell subsets present in the FL niche?
pathological settings. Novel therapeutic strategies that target the relevant transcription factors and epigenetic modifications may help to identify and control their mechanisms of action. The ultimate goal would be exploiting T-cell plasticity for reprogramming T-cell populations towards a specific phenotype, for instance, to reduce inflammation or improve the anti-tumor immune response.

More specifically, a better understanding of the key TFH cell markers (CD28, CD80, CXCR5...) and cytokine/chemokine signaling (IL-6, IL-21, CXCL13...) would offer a large panel of novel therapeutic options for targeting these cells. In addition, given the important role of PD-1/CTLA4 and of PD-L2/ICOS-L expression in regulating the quality, quantity and consequently the balance of TFH/TFR and B cells in GC, strategies to modulate the expression/function of these molecules could be useful in FL to reverse CTL anergy. Furthermore, as it is mostly expressed by TFH cells, PD-1 upregulation in FL microenvironment could represent a potential prognostic factor. Lastly, additional functional studies on TNFRSF14, a gene mutated in FL, are needed to elucidate its specific role and interaction with TFH cells via BTLA, which is highly expressed by TFH cells only in B-cell small lymphocytic lymphoma and chronic lymphocytic leukemia [158]. Preclinical studies are required to determine whether some of these molecules show possible efficacy and their safety before contemplating clinical trials.

Finally, several outstanding questions on the different T cell subtypes present in the FL microenvironment remain (Box 2). As TFH and TFR cells are both affected in FL and somehow inter-connected, understanding whether they can be interchangeable and how they are regulated would be helpful for their specific exploitation as therapeutic targets. FOXP3 induction raises the question of whether its expression is a common activation marker of the Treg and TFR cell subsets.

Ultimately, the future seems to belong to therapeutic strategies that do not only destroy directly cancer cells, but also modify the tumor niche/environment to deprive them of the indispensable support for their survival. To this aim, it is necessary to combine molecular epigenetic and immunological studies to predict the potential of key transcription factors to over-ride the epigenetic changes imposed by the tumor. Thus, a better understanding of the early and late epigenetic events might help developing specific drugs for each cancer stage and typology.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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