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A novel subset of T-helper cells: follicular T-helper cells and their markers

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It has been known for a while that T-helper (T_H) cells are involved in the regulation of B-cell responses. The characterization of a precise subset of these specialized cells, called follicular T-helper cells (T_{FH}), was first reported in 2000.[1] T_{FH} cells are characterized by their homing capacity in CXCL13-rich areas, such as B-cell follicles, through CXCR5 expression and their ability to support immunoglobulin production. The differentiation and homing of T_{FH}, as well as their interactions with B cells, have been the subject of intense research and several markers have been identified as components of the T_{FH} signature. Thanks to gene expression profiling carried out on peripheral T-cell lymphomas, a T_{FH} signature has been reported in angioimmunoblastic T-cell lymphoma (AITL) and in follicular T-cell lymphoma.[2-4] Several markers that identify T_{FH} cells are now used in hematopathology to reinforce the diagnostic criteria of AITL. In this issue of the Journal, Marafioti *et al.*[5] describe that inducible T-cell co-stimulatory molecule (ICOS), a CD28 homolog implicated in the regulation of T-cell differentiation and function, appears to be a relevant marker for the diagnosis of T_{FH}-derived lymphoma such as AITL and follicular T-cell lymphoma. This review first summarizes the recent evidence on T_{FH} cell differentiation and the present state of knowledge on molecules expressed by T_{FH} cells. Then, from this perspective, the potential implications of T_{FH} cells in lymphomagenesis and as part of the tumor microenvironment are covered.

Lineage and differentiation of follicular T-helper cells

T_H cell responses are heterogeneous and effector function choice is imposed during initial activation of the naïve T_H cells through the expression of transcription factors. It is now clear that T_{FH} cells belong to a subset of T_H cells that differ from the other T_H cell subsets. Indeed, T_H1 cells express STAT4, STAT1 and T-box transcription factor/T-bet, T_H2 cells express GATA3, T_H17 cells express retinoid related orphan receptor (RORγt) and regulatory T cells (Treg) express FOXP3. It has recently been shown that the T_{FH} cell differentiation program is controlled by BCL6.[6,7] T_{FH} cells upregulate BCL6 which in turn blocks T_H1, T_H2 and T_H17 cell differentiation by repressing their selective transcription factors.[6] Moreover, BCL6 antagonizes the expression of Blimp-1 transcription factor, which is preferentially expressed by T_H cells in the T zone area.[6,8] BCL6 also represses the expression of miR-17-92, a micro RNA that down-regulates CXCR5 expression, which is essential for T_{FH} cell function. Finally over-expression of BCL6 in activated T_H cells induces expression of interleukin (IL)-6 receptor and IL-21 receptor, which are both required for T_{FH} cell generation as shown by the role of IL-6 and IL-21 in T_{FH} cell differentiation.[9] ICOS is also implicated in the regulation of T_{FH} cell differentiation. Once engaged with its ligand (ICOS-L), expressed on antigen-presenting cells including B cells, ICOS induces the production of helper cytokines such as IL-2, IL-4 and especially IL-10 and IL-21.[10] ICOS deficiency is associated with a reduction of germinal center formation and fewer T_{FH} cells, suggesting that ICOS signaling is essential in T_{FH} cell generation.[10] Recent reports described that T_{FH} cells can produce significant amounts of IL-4, interferon-γ and IL-17 which are normally associated with T_H2, T_H1 and T_H17 cells, respectively.[1,11] Thus, T_{FH} cells share phenotypic features with other T_H cell lineages raising the question of whether T_{FH} cells really do constitute a distinct lineage or whether they result from a conversion of effector T_H cells that acquire T_{FH} cell functions.

The development of follicular homing ability by activated T_H cells is the first event in the process of T_{FH} generation. Indeed, naïve T cells that express CD62L and CCR7 enter secondary lymphoid organs in the T-cell zones.[8,11] Up-regulation of CXCR5, the receptor for CXCL13, a chemokine produced by follicular dendritic cells which promotes B-cell entry into the follicle, occurs after the interaction between antigen-presenting cells and naïve T_H cells.[8] Thanks to the expression of CXCR5 and down-regulation of CCR7, T_{FH} cells move into the B follicle where they regulate antigen-specific B-cell responses via co-stimulatory signals delivered through CD28, OX40 and ICOS.[11]

Cell functions and markers of follicular T-helper cells

In addition to CXCR5, T_{FH} cells express markers such as CD25, CD69, CD95, CD57 (in humans only), OX40 (CD134) and CD40L (CD154) and induce over-expression of activation-induced cytidine deaminase in B cells. T_{FH}

also produce cytokines such as IL-10 and IL-21 that promote B-cell survival and antibody production.[10] An antigen-dependent T-B cell interaction is a critical initiating event in the development of effective B-cell immunity. This cognate interaction induces B cells to become short-lived plasma cells or to enter the germinal center where B cells will undergo maturation processes (immunoglobulin class-switching and somatic hypermutation) which will lead in the end to the development of high-affinity long-lived plasma cells and memory B cells.[1,2,8] Mutations of *CD40L* or *ICOS* genes in mice or humans are associated with T_{FH} cell deficiency and affect B-cell differentiation and humoral responses, indicating the important role of the co-stimulatory molecules in regulating B-cell responses. Of note, T_{FH} cells express some molecules which play a key role in the stability of the antigen-dependent T cell-B cell interaction such as programmed cell death 1 (PD1), an immunoreceptor which favors strong cognate interactions with B cells in the germinal center, and the signaling lymphocytic activation molecule-associated protein (SAP) which is required to form stable contacts between B and T cells and supports T_{FH} cell generation and germinal center formation.[10,12] A recent study demonstrated that T_{FH} can express high levels of B- and T-lymphocyte attenuator (BTLA), an inhibitory receptor of the CD28 superfamily which negatively regulates the immune response in synergy with the PD1/PDL1 inhibitory pathway.[13]

T_{FH} cells seem to play an important role in systemic autoimmunity. Mice with systemic autoimmune diseases such as systemic lupus erythematosus show a constant increase of T_{FH} cells.[2] Furthermore, drugs that block ICOS or IL-21 signaling pathways improve autoimmune disorders such as diabetes, rheumatoid arthritis and murine models of systemic lupus erythematosus.[11] T_{FH} cells could, therefore, represent an interesting therapeutic target in autoimmune diseases.

Implications of follicular T-helper cells in lymphoma

AILT is an aggressive lymphoma characterized by a polymorphic infiltrate, marked proliferation of endothelial venules and a dense meshwork of follicular dendritic cells.[14] Gene expression profiling studies have identified a specific signature corresponding to T_{FH} cells.[2-4] Furthermore, these studies have shown that T_{FH} cells represent the putative cell of origin of AILT. T_{FH} cells seem to be responsible for the clinico-biological aspects of AILT, including B-cell activation, hypergammaglobulinemia and proliferation of follicular dendritic cells. Typically the tumor cells of AILT have a T_{FH} phenotype with expression of CD3, CD4, CXCR5, CD57 and BCL6. CD10, which is not expressed in normal T_{FH} cells, but rather in a distinct subset of GC T_H cells, is frequently present at the cell membrane of lymphoma cells.[3,4] In addition, the neoplastic T cells frequently lose CD5 and CD7.[14] Most interestingly, CXCL13, PD1 and SAP are also markers of malignant cells in AILT.[15,16] This signature is not completely specific to AILT since it has also been reported in peripheral T-cell lymphoma not otherwise specified.[15,17] Rare cases of peripheral T-cell lymphoma with a follicular growth pattern display a T_{FH} phenotype and show some overlapping features with AILT, raising the question of a possible relationship between the two lymphoma types.[5,18] In addition, in the study by Marafioti *et al.*,[5] ICOS appeared to be expressed by tumor cells of both AILT and peripheral T-cell lymphoma not otherwise specified.

Nodular lymphocyte-predominant Hodgkin's lymphoma is a monoclonal B-cell neoplasm characterized by nodular or nodular and diffuse proliferation of scattered

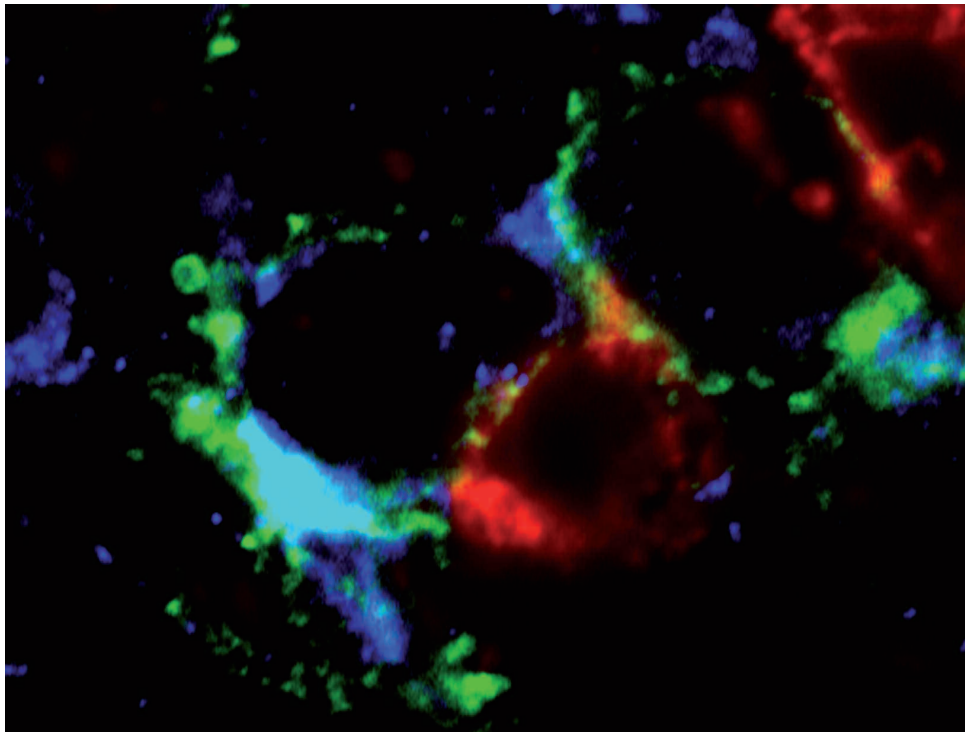

Figure 1. Human T_{FH} in a lymph node from a patient with angio-immunoblastic T-cell lymphoma. Note the interactions between PD-1$^+$ (in green) and CD4$^+$ (in blue) T_{FH} cells and CD20 (in red) B cells (confocal microscopy x500).

large neoplastic B cells referred to as popcorn or lymphocyte-predominant cells.[14] These cells interact with aggregates of follicular dendritic cells and immune cells such as small naïve B cells and T cells. Furthermore, nodules of this form of lymphoma are characterized by an increase of germinal center-derived CD57$^+$ T cells which are closely associated with neoplastic lymphocyte-predominant cells.[19] These CD57$^+$ T cells also express markers reminiscent of germinal center T_H cells such as PD1, BCL6, IRF4/MUM1 and c-MAF.[19] Altogether these data suggest that some of these reactive cells could be T_{FH} cells, but a more precise micro-dissection of the microenvironment is required to confirm this.

In follicular lymphoma, derived from germinal center B cells, tumor cells reside and proliferate in follicular structures in close association with T_H cells and follicular dendritic cells.[19] In order to proliferate, follicular lymphoma cells require cellular interactions such as those in normal germinal centers, including contacts with germinal center T_H cells which express CD3, CD4, CD57, PD1 and CXCL13.[19] Proliferation is stimulated by CD40-CD40L interaction, markers expressed on malignant B cells and T_{FH} cells, respectively.[19] This is further supported by the presence of significant amounts of PD1 in the germinal centers of patients with follicular lymphoma, as shown in a recent study.[20] As for FOXP3$^+$ cells, high numbers of PD1$^+$ cells seem to have a favorable impact on progression in follicular lymphoma.[20] Nevertheless, the exact nature of those PD1$^+$ cells remains unclear and further studies are needed to determine whether or not they correspond to T_{FH} cells.

The increasing attention paid to T_{FH} cells has revealed notable plasticity in the differentiation pathways of these cells. Beside canonical T_H markers, the minimal T_{FH} signature requires expression of CXCR5, PD1, BCL6, SAP and ICOS. These markers can also be individually expressed by other T_H cell subsets. The use of immunohistochemistry to detect T_{FH} cells on tissue sections is, therefore, questionable since only one or two markers can be labeled simultaneously. Hence, identification of T_{FH} cells relies on both phenotype and function, which are closely related to the specific microenvironment in which T_{FH} cells localize, requiring further studies based on multicolor cell imaging.

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