CD10 delineates a subset of human IL-4 producing follicular helper T cells involved in the survival of follicular lymphoma B cells

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Key Points

- CD10 identifies a unique subset of fully functional germinal center TFH that are activated and amplified within the FL cell niche.
- FL CD10pos TFH specifically display an IL-4hiIFN-γlo cytokine profile and encompass the malignant B-cell-supportive TFH subset.

In follicular lymphoma (FL), follicular helper T cells (TFH) have been depicted as one of the main components of the malignant B-cell niche and a promising therapeutic target. Although defined by their capacity to sustain FL B-cell growth together with specific gene expression and cytokine secretion profiles, FL-TFH constitute a heterogeneous cell population. However, specific markers reflecting such functional heterogeneity are still lacking. In this study, we demonstrate that CD10 identifies a subset of fully functional germinal center T FH in normal secondary lymphoid organs. Importantly, this subset is amplified in the FL context, unlike in other B-cell lymphomas with a follicular growth pattern. Furthermore, whereas FL-TFH produce high levels of interleukin (IL)-21 and low levels of IL-17 irrespectively of their CD10 expression, CD10pos FL-TFH specifically exhibit an IL-4hiIFN-γlo cytokine profile associated with a high capacity to sustain directly and indirectly malignant B-cell survival. Altogether, our results highlight the important role of this novel functional subset in the FL cell niche. (Blood. 2015;125(15):2381-2385)

Introduction

The follicular lymphoma (FL) microenvironment is characterized by a strong infiltration of helper T cells displaying a complex phenotype, including an overexpression of both activation and exhaustion markers, and a specific gene expression profile (GEP), underlying altered T-cell activation, motility, and polarization.1-5 Recently, we demonstrated more precisely that genes related to follicular helper T cells (TFH), the specialized CD4pos T cells involved in normal germinal center (GC) B-cell survival and differentiation,6 represent a significant part of FL-specific microenvironment signature and revealed their unique capacity to support malignant B-cell growth.7,8 FL-TFH are regarded as a promising therapeutic target in this still incurable disease.9 FL-TFH were characterized by a specific cytokine profile, combining overexpression of interleukin (IL)-4, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, and decreased expression of helper T 17-related genes.8 However, specific markers associated with FL-TFH heterogeneity and identifying precisely the tumor-supportive FL-TFH subset are lacking.

In reactive lymphoid tissues, CD57 has been initially proposed as a marker of immature T and B cells and GC B cells virtually absent on circulating mature T cells,14 has been reported to display an IL-4hiIFN-γlo cytokine profile associated with a high capacity to sustain directly and indirectly malignant B-cell survival.11 Combining GEP, histology, phenotype, and functional approaches, we demonstrate that CD10 expression is restricted to a unique subset of GC-TFH, specifically amplified in the FL context. Moreover, CD10pos FL-TFH exhibit a peculiar IL-4hiIFN-γlo TNF-αhi cytokine profile associated with a strong capacity to sustain directly and indirectly malignant B-cell survival.

Study design

Details are provided in the supplemental Materials and Methods (available on the Blood Web site).

The online version of this article contains a data supplement.

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Samples
Subjects were recruited under institutional review board approval and the informed consent process according to the Declaration of Helsinki. Samples comprised LNs obtained from patients with FL, nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), and mantle cell lymphomas (MCLs); tonsils collected from children undergoing routine tonsillectomy; and reactive LNs with follicular hyperplasia. CD3posCD4posCXCR5hiICOShiCD25neg TFH, CD10pos TFH, and CD10neg TFH were sorted using a FACSAria (BD Biosciences) (purity .98%). Tonsil and FL B cells were purified using the human B-cell isolation kit II (Miltenyi Biotec).

Phenotypic study
Membrane and intracellular staining were performed using standard flow cytometry techniques. Data were acquired on a CyAn ADP flow cytometer and analyzed using Kaluza software (Beckman Coulter). Tissue sections were used for single immunohistochemical (Programmed cell death 1 [PD-1]), double immunohistochemical (CD10/PAX5), and double immunofluorescence stainings (CD10/CD3, CD10/inducible T-cell costimulator [ICOS], CD10/C-X-C motif chemokine ligand [CXCL] 13).

Microarray hybridization
GEP of 7 FL-TFH and 7 tonsil-TFH was analyzed using GeneChip HG-U133 Plus 2.0 microarrays (Affymetrix) and normalized using Partek software. Microarray data are registered to the Gene Expression Omnibus under accession number GSE66384.

Results and discussion
Scattered PAX5negCD10hi cells could be identified within neoplastic follicles in 16/19 FL samples and were characterized as CD3posCD4posCXCR5hiICOShiCD25neg T FH, CD10pos T FH, and CD10neg T FH were sorted using a FACSAria (BD Biosciences) (purity >98%). Tonsil and FL B cells were purified using the human B-cell isolation kit II (Miltenyi Biotec).

Statistical analyses
Statistical analyses were performed with the GraphPad Prism software using nonparametric Wilcoxon test for matched pairs, or Mann-Whitney U tests.

FL B-cell antiapoptotic assay
Purified FL malignant B cells were cultured alone, with an activation cocktail (CD40 ligand, IL-2, IL-4), or in the presence of purified CD10pos or CD10neg T FH (ratio 1:1). After 48 hours, B-cell apoptosis was assessed using active caspase-3 phycoerythrin apoptosis kit (BD Biosciences).

Figure 1. Characterization of CD10pos T cells in B-cell lymphomas. (A) Double staining revealed the presence of scattered strong CD10pos cells within FL neoplastic follicles. Numerous CD10pos (brown) PAX5neg (red) lymphocytes are evidenced (arrows). Original magnification: objective ×20 (left) and ×40 (right). (B) Representative characterization of ICOS, CD10, CXCR5, and PD-1 staining by flow cytometry on CD3posCD4pos viable cells from FL LNs. (C) In situ characterization of CD10pos T cells in RLH including reactive LNs and tonsils, and B-cell lymphomas with follicular growth pattern (MCL, NLPHL, and FL). For each sample, the numbers of CD10posPAX5neg and PD-1pos cells found inside GCs were counted in 10 high power fields (>40), and the median of these 10 values was calculated. Data are expressed as the median [range] of the different samples tested. ND, not done. (D) Expression of MME/CD10 in CD3posCD4pos CXCR5posICOSposCD25neg T FH, T FH, and CD10pos T FH were sorted using a FACSAria (BD Biosciences) (purity >98%). Tonsil and FL B cells were purified using the human B-cell isolation kit II (Miltenyi Biotec).
lymphomas with a follicular growth pattern. Whereas cells expressing PD-1 were rarely detected in MCL samples, in agreement with a lack of PAX5negCD10pos T cells, we confirmed the presence of numerous PD-1pos cells forming rosettes around neoplastic cells in NLPHL, but these cells were essentially CD10neg (data not shown). The lack of MCL-infiltrating TFH and the absence of CD10 expression on PD-1pos T cells in the NLPHL microenvironment were confirmed by flow cytometry (data not shown). By contrast, we identified PAX5negCD10pos T cells at a highly variable frequency within the GC of all RLHs. These cells tended to concentrate at the periphery of the GC and coexpressed CD3, ICOS, and CXCL13 (supplemental Figure 1A-B). To evaluate how CD10 expression could impact TFH function, we sorted paired CD10pos and CD10neg TFH from tonsils and demonstrated similar expression of canonical TFH genes (supplemental Figure 1C). Moreover, CD10pos and CD10neg TFH displayed the same capacity to sustain immunoglobulin production by autologous purified B cells (supplemental Figure 1D). We next checked whether CD10pos tonsil-TFH could be enriched for CD57- or Nrp-1-expressing cells (supplemental Figure 1E). As reported for Nrp-1, CD10 was expressed by a higher proportion of CD57pos than CD57neg T FH (22.2% [6.1% to 51.4%] vs 15.6% [3.4% to 35.2%], P < .001, n = 20). However, the expression of CD10 and Nrp-1 was mutually exclusive. Finally, CD10 was not expressed on CXCR5hiICOShiFoxp3hiCD25hi tonsil follicular regulatory T cells. Thus, CD10 identifies a distinct subset of fully functional GC-TFH in human RLH. Interestingly, conversely to NRP1 and CD57, CD10/MME was significantly upregulated in FL-TFH compared with tonsil-TFH, as revealed by GEP analysis. Accordingly, the percentage of CD10pos-TFH was increased in FL samples (6.17% [2.7% to 22.9%]) compared with tonsils (1.56% [0.2% to 10.1%], P < .001) (Figure 1D-E), raising the hypothesis that CD10pos-TFH expansion could play a specific role in FL pathogenesis.

CD10pos FL-TFH were essentially Ki-67neg but expressed high levels of HLA-DR indicating a nonproliferating but activated status.
more IL-4 than their CD10neg counterpart, indicating that CD10

similar levels of IL-21 and IFN-

vival, and production of the Treg-recruiting CC chemokine ligands

Figure 2A). Compared with tonsil-TFH, FL-TFH displayed an IL-

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-producing FL-TFH and IFN-γ-producing FL-TFH belonged to nonoverlapping cell subsets (Figure 2B). These results prompted us to evaluate the direct role of CD10pos vs CD10neg FL-TFH on malignant FL B cells. CD10pos FL-TFH were more efficient than their CD10neg counterpart to support autologous malignant FL B-cell survival in vitro (Figure 2C). Finally, the previously reported overexpression of TNFα by FL-TFH appeared uniformly supported by CD10pos and CD10neg FL-TFH subsets, containing both ~35% of TNFα-producing cells. Altogether, our data suggest that these 2 FL-TFH subsets may have different roles within the FL cell niche. CD10pos FL-TFH produce high amounts of IL-4 that could trigger B-cell activation, survival, and production of the Treg-recruiting CC chemokine ligands 17 and 22. In addition, CD10pos FL-TFH exhibit a TNFα IFNγ phenotype that could favor the induction and maintenance of a B-cell supportive lymphoma stroma network. 22 Conversely, overexpression of IFN−γ by CD10neg T FH could promote activation of cytotoxic CD8pos T cells displaying efficient antitumor activity 23 but also expression of stroma-derived indoleamine-2,3-dioxygenase that could inhibit not only T-cell but also malignant B-cell proliferation. 24

In conclusion, our study supports the current understanding of the FL cell niche as an intricate network of cell interactions in which each cell subset should be exquisitely characterized individually and in relationship with the other partners before being proposed as a biomarker or therapeutic target.

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Authorship

Contribution: P.-A.T. designed and performed research, analyzed data, and wrote the manuscript; S.H. designed and performed research, analyzed data, and corrected the manuscript; C.A., J.M., M.S.B., R.J., L.L.P., C.M., and N.M. performed research; and P.G. and K.T. supervised and designed research, analyzed data, and wrote the manuscript.

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