Insights into the Role of Follicular Helper T Cells in Autoimmunity

Hong-Jai Park1,2#, Do-Hyun Kim1,2#, Sang-Ho Lim1,2, Won-Ju Kim1,2, Jeehee Youn3, Youn-Soo Choi4 and Je-Min Choi1,2*

1Department of Life Science, 2Research Institute for Natural Sciences, Hanyang University, Seoul 133-791, Korea, 3Department of Anatomy & Cell Biology, College of Medicine, Hanyang University, Seoul 133-791, Korea, 4Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037, USA

Follicular helper T (TFH) cells are recently highlighted as their crucial role for humoral immunity to infection as well as their abnormal control to induce autoimmune disease. During an infection, naïve T cells are differentiating into TFH cells which mediate memory B cells and long-lived plasma cells in germinal center (GC). TFH cells are characterized by their expression of master regulator, Bcl-6, and chemokine receptor, CXCR5, which are essential for the migration of T cells into the B cell follicle. Within the follicle, crosstalk occurs between B cells and TFH cells, leading to class switch recombination and affinity maturation. Various signaling molecules, including cytokines, surface molecules, and transcription factors are involved in TFH cell differentiation. IL-6 and IL-21 cytokine-mediated STAT signaling pathways, including STAT1 and STAT3, are crucial for inducing Bcl-6 expression and TFH cell differentiation. TFH cells express important surface molecules such as ICOS, PD-1, IL-21, BTLA, SAP and CD40L for mediating the interaction between T and B cells. Recently, two types of microRNA (miRNA) were found to be involved in the regulation of TFH cells. The miR-17-92 cluster induces Bcl-6 and TFH cell differentiation, whereas miR-10a negatively regulates Bcl-6 expression in T cells. In addition, follicular regulatory T (TFR) cells are studied as thymus-derived CXCR5+PD-1+Foxp3+ Treg cells that play a significant role in limiting the GC response. Regulation of TFH cell differentiation and the GC reaction via miRNA and TFR cells could be important regulatory mechanisms for maintaining immune tolerance and preventing autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Here, we review recent studies on the various factors that affect TFH cell differentiation, and the role of TFH cells in autoimmune diseases.

INTRODUCTION

CD4 helper T cells play a significant role in regulating adaptive immune responses against foreign antigens. Once activated by the antigen, they differentiate into various types of T cells, including Th1, Th2, Th17, Th9, and Treg cells, depend on environmental cytokines to control antigen-specific immune responses. IL-6 and IL-21 contribute to follicular helper T (TFH) cell differentiation when naïve T cells are stimulated with T cell Receptor (TcR) and co-stimulatory molecules such as ICOS and CD28 (1). TFH cells are a distinct subset of T cells by expressing Bcl-6 and are localized to B cell follicle in lymphoid organs with critical roles in the mediation of humoral adaptive immunity (2,3).

Various cytokines, surface molecules, and transcription factors are reported to be involved in TFH cell differentiation (Fig. 1). IL-6 and IL-21 are critical cytokines for TFH cell differen-
Figure 1. Molecular mechanisms of Bcl-6 expression in T cells. Bcl-6, the master regulator of T FH cell differentiation is controlled by a complex signaling pathway. Co-stimulatory molecules such as CD28 and ICOS activate PI3K to induce Bcl-6 expression. PTEN, PHLPP2 inhibit Bcl-6 expression through interfering PI3K signaling and Foxo1 directly inhibits Bcl-6 expression. Various cytokines, such as IL-6, IL-21, IL-12, and IFN-γ induce Bcl-6 expression through JAK-STAT signaling pathway while high level of IL-2 in combination with IL-12 induces T-bet to inhibit Bcl-6. Blimp-1 and Bcl-6 is reciprocally regulating each other to make a decision of effector T cell fate between T FH and non-T FH effector cells. Some miRNA such as miR-17-92 induces Bcl-6 expression by interfering phosphatases, which inhibit PI3K signaling pathway while miR-10a directly inhibits Bcl-6 expression.

Figure 2. Germinal center reaction controlled by T FH and T FR cells. Naïve T cells following stimulation with TcR and co-stimulatory molecules with IL-6 and IL-21 by dendritic cells can differentiate into T FH cells and migrate to the CXCL13-rich B cell follicle region. In the B cell follicle, T FH cells interact with B cells via TcR and co-stimulatory molecules such as ICOS and CD40L. Upon interaction between T FH cells and B cells, IL-4 and IL-21 from T FH cells allow B cells to differentiate into memory B cells or plasma cells, which are involved in long-lasting antibody production. T FR cells derived from nTreg precursor cells from the thymus by expressing Bcl-6 and CXCR5 migrate to B cell follicle and inhibit both T FH cell and B cell function.

entiation (4). Surface molecules, including ICOS, CD40L, PD-1, BTLA, and SAP are also important for T FH cell differentiation and their functions (5). Inhibiting the interaction between CD40 and CD40L, or deficiency of ICOS or its ligand causes defects in formation of the germinal center (GC) (6) and T FH cell differentiation (7,8). In addition, SAP contributes to T FH cell differentiation by maintaining stable T and B cell interaction (6,9). Cytokine- and co-stimulatory molecule-mediated signaling pathways are essential for expression of the transcription factor B cell lymphoma-6 (Bcl-6), which is the master regulator of T FH cell differentiation and is inhibited by the antagonizing transcription factor Blimp-1. Expression of Bcl-6 and Blimp-1 is reciprocally regulated during T cell differentiation (1).

Bcl-6-deficient T cells failed to differentiate into T FH cells and the GC responses are hardly developed, demonstrating the absolute requirement for Bcl-6 (2,3). T FH cell differentiation program involves a dramatic change in surface expression of chemokine receptors. Reciprocal up-regulation of CXC-chemokine receptor 5 (CXCR5) and down-regulation of CCR7 enables T FH cells to migrate into B cell follicles by responding to CXCL13, the ligand of CXCR5 (10-12). Inside of B cell follicles, T FH cells provide B cell help signals by expressing co-stimulatory molecules and secreting cytokines such as IL-4 and IL-21, which are essential for germinal center B cells to undergo class switch recombination, somatic hyper-mutation, affinity maturation, and differentiation of plasma cells and memory B cells in the GC (13-15).

Recently, it was reported that some microRNAs (miRNAs) have a regulatory role in T FH cell differentiation and the GC reaction. The miR-17-92 cluster acts as a positive regulator of T FH cell differentiation via suppression of phosphatases that inhibits ICOS-mediated PI3K signaling pathways (16). In addition, the miR-17-92 cluster represses the expression of ROR α, which induces inappropriate gene expression during T FH cell differentiation (17). By contrast, miR-10a directly inhibits Bcl-6 expression (18), which strongly indicating that miRNAs are involved in dynamic regulation of T FH differentiation.

If the GC reaction mediated by T FH cells is dysregulated or if autoreactive T and B cells are activated, high levels of autoantibody can be accumulated through abnormal GC formation, which contributes to the development of autoimmune
diseases (19). Thus, T_{FH} cells should be tightly regulated to prevent autoimmunity by limiting germinal center reactions to self antigen (20). Recently, follicular regulatory T (T_{FR}) cells expressing CXCR5 were demonstrated to limit the GC reaction and reduce antibody production by migrating into B cell follicles (21). The regulation of germinal center reaction by T_{FH} and T_{FR} cells for normal immunity is summarized as figure (Fig. 2).

In this review, we discuss the function of cytokines, transcription factors, and signaling pathways related to the differentiation or characteristics of T_{FH} cells. Additionally, we discuss the role of the GC reaction related to T_{FH} and T_{FR} cells in the maintenance of immune homeostasis and provide both a better understanding of the importance of T_{FH} cells in autoimmunity and their clinical relevance in human autoimmune diseases.

**SIGNALING PATHWAYS REQUIRED FOR T_{FH} CELL DIFFERENTIATION**

ICOS, PI3K, and Foxo1
It has been reported that a strong interaction between the TcR and major histocompatibility complex (MHC) class II molecules triggers T_{FH} cell differentiation, which indicates that a strong TcR signal is essential for T_{FH} cell differentiation (22). In addition, among surface co-stimulatory molecules being expressed by T_{FH} cells, ICOS is induced when CD4 T cells become activated by recognizing antigen through TcRs, which then interact with ICOS-L that is expressed on B cells (7,11,23,24). Its binding to the ligand ICOS-L triggers activation signals in a similar way to other members of CD28 family co-stimulatory receptors (25,26). ICOS plays a significant role in increasing T cell proliferation and the production of cytokines, including IL-21 and IL-4 (11,27,28).

ICOS-mediated PI3K activation is crucial for T_{FH} cell differentiation, as a point mutation on the cytoplasmic tail of ICOS, where PI3K binds to and activates, led to a severely impaired T_{FH} cell differentiation of CD4 T cells (28). In contrast, over-expression of ICOS is sufficient to maintain T_{FH} cells in CD28-deficient mice (7). Among PI3K subunits p110γ appears to convey ICOS-mediated T_{FH} cell differentiation signaling pathway, as p110γ deficiency resulted in a defective T_{FH} cell differentiation, further strongly indicating that ICOS and PI3K are important for either differentiation or survival of T_{FH} cells. These results imply that ICOS-mediated PI3K signaling is crucial for the differentiation of T_{FH} cells (25). Moreover, Heping et al, reported that ICOS signaling is critical for mobility of T_{FH} cells into the B cell follicle in a Bcl-6 independent manner (29).

PI3K signaling pathways following TcR and co-stimulation regulate the phosphorylation of Foxo1 to relocate it from the nucleus to the cytoplasm (30,31). A recent study revealed that Foxo1 negatively regulates T cell activation and contributes to T cell tolerance (32). Foxo1-deficient CD4 T cells contribute to the development of autoimmune phenotypes including increased autoantibody production with reduced Foxp3+ regulatory T cell development and function, and augmented generation of T_{FH} cells and GC formation. In addition, the presence of Foxo-binding elements has been identified in the promoter region of Bcl-6 (33), which suggests that Foxo1 might act as a transcriptional repressor of Bcl-6 and, if so, Foxo1 might negatively regulate T_{FH} differentiation. Thus, ICOS and PI3K signaling could be a regulator of GC reaction.

**IL-21, IL-6, and STATs**
IL-6 and IL-21 are well-known pro-inflammatory cytokines with important roles in Bcl-6 expression and T_{FH} cell differentiation (4). IL-21 induces B cell proliferation and class switch recombination and IL-21R is required for antibody response and GC formation (44). The IL-6-mediated STAT3 activation is also important for IL-21 expression in human and mouse naïve CD4 T cells upon TcR stimulation (35,36). STAT3 is phosphorylated by JAK upon IL-6 stimulation, and activated STAT3 was shown to bind to Bcl-6 in T cells (33). IL-6 is an important factor for Bcl-6 induction in CD4 T cells during dendritic cell priming stage of CD4 T cell activation (37). However, other signaling pathways could compensate for IL-6 dependent T_{FH} differentiation pathway, as T_{FH} cells are normally found at the peak of the immune response to infection and immunization (38,39). IL-6 signaling is required for IL-21 expression via c-Maf (40-42). Once being produced, IL-21 further increases its own production through a positive feedback mechanism (43).

Augmented IL-21 was reported to induce the expression of the master regulator for T_{FH} cell differentiation, Bcl-6 (44), while controversies exist whether IL-21 is a critical factor for Bcl-6 induction in CD4 T cells (37,39). At a downstream level, Choi et al, showed that IL-6-mediated STAT1 signaling can also prime T_{FH} cells by compensating for STAT3 and inducing Bcl-6 expression. Another recent study demonstrated that IL-12-mediated STAT4 signaling can induce expression of...
both Bcl-6 and T-bet, and T-bet inhibits the function of Bcl-6 (45). The balance between T-bet and Bcl-6 expression might be regulated by IL-2 concentration (33). Furthermore, IFN-γ was accounted to lead to abnormal T FH cell differentiation in the sanroque mouse model (46). Given that IFN-γ induced Bcl-6 via pSTAT1 which binds to an IRE in an exon of Bcl-6 (47), IFN-γ could function as a positive regulator by directly inducing Bcl-6 expression in CD4 T cells. This supported by recent study by Lee et al., which demonstrated that T cell specific deletion of IFN-γ resulted in decreased T FH cell differentiation in sanroque mice (46). Further studies are needed to clarify how this complex cytokine network regulates Bcl-6 expression and T FH cell differentiation.

**Bcl-6 and Blimp-1**

The zinc-finger-containing transcriptional repressor Bcl-6 was originally described as a key molecule in GC formation and B cell response (48,49). Bcl-6-deficient mice cannot develop somatic hyper-mutation in B cell, result impaired GC formation (50,51). In addition, B cells from these mice do not undergo affinity maturation, somatic hyper-mutation, and class switch recombination of immunoglobulin (49). Recently, Bcl-6 was identified as a crucial factor for T FH cell differentiation (3). Bcl-6-deficient mice show impaired T FH cell differentiation (2) and non-T FH CD4 T cells do not express increased levels of Bcl-6 (2,52). Bcl-6 directly inhibits a number of transcription factors, including T-bet and ROR γt, which are key modulators of differentiation of Th1 and Th17 cells, respectively (3). Bcl-6 also inhibits expression of CCR7 and PSGL-1, which negatively regulate the migration of T cells into B cell follicles (39,53). Moreover, Bcl-6 regulates the expression of various T FH cell-related molecules, including ICOS, PD-1, PTLA, CD200, and SAP (23). Turner et al, identified the mouse form of Blimp-1, which is induced by cytokine-mediated B cell differentiation (54). Recent studies reported that the transcription factor Blimp-1 has an antagonistic role of Bcl-6 (1,52,55) and inhibits T FH cell differentiation (1), Blimp-1 is highly expressed in non-T FH effector T cells such as Th1, Th2, and Th17 cells (1,52), whereas Bcl-6 is highly expressed only in T FH cells. Moreover, constitutive expression of Blimp-1 inhibited T FH cell formation (1) and Blimp-1 is important for terminal differentiation of both CD4+ and CD8+ T cells, which is characterized by high levels of effector molecule secretion and low proliferative potential (52). IL-2 mediated STAT5 signaling in activated CD4+ T cells induces expression of Blimp-1, which suppresses Bcl-6 and T FH cell differentiation (56). High level of IL-2, especially in effector Th1 cells, induces T-bet, which also inhibits Bcl-6 expression and T FH cell differentiation (33). Th1 cells might have the flexibility to regulate the expression of T-bet and Bcl-6 depending on environmental conditions (33). IL-6 and IL-21-mediated STAT3 signaling can also induce Blimp-1 or Bcl-6 (57) through the participation of additional transcription factors (5). To summarize, effector T cell fate seems to rely on the expression of Bcl-6 or Blimp-1 and they are reciprocally inhibit each other via complex signaling pathway, eventually act as a decision maker between T FH cell and other effector T cell differentiation.

**REGULATION OF T FH CELL DIFFERENTIATION VIA T FR CELL AND miRNA**

**Follicular regulatory T cells**

Foxp3-expressing regulatory T (T reg) cells contribute to the maintenance of immune tolerance by suppressing the dysregulated immune response to self-antigens (58). Scurfy mice without Foxp3+ T cells demonstrate severe systemic autoimmune phenotype. In addition, CD4 T cells isolated from scurfy mouse are hyper-responsive to TcR stimulation (59,60). It has been recently reported that the mice with CCR5-deficient T reg cells have more GC with augmented immunoglobulin production owing to the limited capability of these cells to migrate into B cell follicular region. This suggests that CCR5 expression of T reg cells is crucial for regulation of the GC reaction (61). In addition, T reg cells expressing Bcl-6 and CCR5, which originate from CCR5- natural T reg cell precursors, are found in GC (21). In the absence of CCR5-Bcl-6+ T reg cells, the GC reaction was not controlled efficiently leading to enhanced immunoglobulin production and increased B cell population in GCs. This result implies that T reg cells expressing CCR5 have important roles in regulation of the GC reaction. T reg cells in GC are called follicular regulatory T (TFR) cells, which share characteristics of both T FH and T reg cells since Bcl-6, CD28 and SAP also affect development of T FR cells. 5~25% of T FH cells expressing CCR5 and PD-1 are also Foxp3+ T FR cells and are located in the B cell follicle region (62). Recent study demonstrated that lack of the PD-1-PD-L1 pathway induced increase of T FR cells and its suppressive ability, suggesting the regulatory role of PD-1 in the differentiation of T FR cells (63). Co-transfer experiments with thymus-derived Foxp3+ CD4 T cells and Foxp3- CD4 T cells into recipient demonstrated that Foxp3+...
miR-17-92-deficient mice show impaired T FH cell differentiation, and Blimp-1 down-regulates the number of T FR cells, including plasma cell production and affinity maturation. By contrast, Bcl-6 overexpression is down-regulated in these iT reg cells and over-transgenic mice demonstrate spontaneous Bcl-6 expression, β ICOS-PI3K pathway. The miR-17-92 cluster also directly inhibits the expression of PTEN and PHLPP2 expression, which regulate the ICOS-PI3K pathway. The miR-17-92 cluster induces TFH cell differentiation through suppression of PTEN and PHLPP2 expression, which regulate the ICOS-PI3K pathway. The miR-17-92 cluster also directly inhibits expression of RORγt, which is involved in gene expression of non-TFH effector T cell differentiation (17). In addition, miR-10a, which is specifically expressed in T reg cells by TGF-β and retinoic acid, directly suppresses Bcl-6 expression (18). Some induced-Treg (iTreg) cells migrate to GC in Peyer’s patch and have TFH-like phenotypes, miR-10a expression is down-regulated in these iTreg cells and over-expression of miR-10a significantly inhibits the conversion of iTreg into TFH-like cells. More studies on the role of Trf cells and miRNA in TFH differentiation are needed to improve our understanding on dynamic regulation of germinal center reaction.

**miRNAs**

miRNAs are functional single stranded RNAs (ssRNAs), which are encoded endogenously, and are involved in immune cell development and differentiation (64,65). Recent study reported that the miR-17-92 cluster was regulated by Bcl-6 in CD4 T cells (3). T cells overexpressing Bcl-6 demonstrated diminished expression of the miR-17-92 cluster, as do TFH cells, which, suppresses the expression of CXCR5. However, several studies have shown that the miRNA-17-92 cluster induces TFH cell differentiation (16,17). T cell specific miR-17-92 transgenic mice demonstrate spontaneous Bcl-6 expression, TFH cell differentiation, and GC formation (16). In contrast, miR-17-92-deficient mice show impaired TFH cell differentiation during acute and chronic virus infection. The miR-17-92 cluster induces TFH cell differentiation through suppression of PTEN and PHLPP2 expression, which regulate the ICOS-PI3K pathway. The miR-17-92 cluster also directly inhibits expression of RORγt, which is involved in gene expression of non-TFH effector T cell differentiation (17). In addition, miR-10a, which is specifically expressed in T reg cells by TGF-β and retinoic acid, directly suppresses Bcl-6 expression (18). Some induced-Treg (iTreg) cells migrate to GC in Peyer’s patch and have TFH-like phenotypes, miR-10a expression is down-regulated in these iTreg cells and over-expression of miR-10a significantly inhibits the conversion of iTreg into TFH-like cells. More studies on the role of Trf cells and miRNA in TFH differentiation are needed to improve our understanding on dynamic regulation of germinal center reaction.

**TFH CELLS IN AUTOIMMUNE DISEASES**

**Systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) is an autoimmune disease with a complex phenotype that includes systemic inflammation, fever, fatigue, and chills (66). Diagnosis of SLE is very difficult because its phenotype overlaps with other diseases. Recent studies have suggested that the pathogenesis of SLE is profoundly related to TFH cells (44,67,68). Spontaneous GC formation and autoantibody production have been reported in many mouse models of SLE (44,67), suggesting that TFH cells might be associated with pathogenesis of SLE. Indeed, recent studies demonstrated that TFH cell differentiation is spontaneously induced in these mouse models (44,67,68). Also, dysregulated TFH cell activity contributes to the pathogenesis of SLE through aberrant GC formation and massive production of autoantibodies, such as anti-dsDNA and ANA. TFH cells induce these phenomena via cytokines and co-stimulatory molecules which stimulate B cells (69,70). Autoimmune phenotypes were alleviated when TFH cell differentiation was inhibited in sanroque mice, which have increased GC formation and TFH cell differentiation (70). Linterman et al. crossed sanroque mice with IL-21- or SAP-deficient mice, or mice heterozygous for Bcl-6 to examine the role of Bcl-6 in development of the lupus-like phenotype (71). They found that the deficiencies of Bcl-6 or SAP ameliorate the lupus-like phenotype in sanroque mice IL-21 independently. However, lupus-like autoimmune phenotypes were reduced in another study when IL-21 signaling is not present in BXSB-Yaa mice, another mouse model of human SLE (72), recapitulating the complexity of pathogenesis of SLE in human. Remarkably, IL-21 expression was up-regulated in SLE patients than in healthy controls (73), and elevated production of TFH relating factors such as CXCL13, BAFF were reported in human SLE patients (74). These results suggest that abnormal TFH cell differentiation strongly related to SLE pathogenesis.

**Rheumatoid arthritis**

Rheumatoid arthritis (RA) is an autoimmune disorder, which is recently studied that it is associated with dysregulated TFH cell differentiation. Deborah et al, found that blockade of IL-21 signaling by IL-21R-Fc fusion protein treatment reduces disease severity in mouse and rat RA models (75). Furthermore, IL-21 blockade in animal models results in decreased IL-6 expression. A recent study by Victoratos et al, found that
TFH cells have a critical role in the maintenance of follicular dendritic cell (FDC)-mediated GC formation and autoantibody production in KRN/B mice that spontaneously develop RA (76,77). In addition, Jang et al. reported that IL-21 receptor-deficient KBx/N mice have less severe RA with reduced TFH cell population in draining lymph node (43). An IL-21R-Fc fusion protein that inhibits IL-21 signaling can delay disease onset and progression. Platt et al. also found increased TFH cells and antibody production in an OVA-induced RA mouse model (78). In this study, they showed that abatacept, a fusion protein composed of the Fc region of IgG and the extracellular domain of CTLA-4 has a role in regulation of TFH cell differentiation in OVA-induced RA mouse models (79). This increased TFH-like cells population correlated with enhanced 28-joint count disease activity score and anti-CCP antibody, which indicates disease severity.

Synthetically, TFH cells seem to be involved in the pathogenesis of human autoimmune diseases such as SLE, RA, etc., therefore, regulation of TFH cell differentiation could be an important strategy for the suppression of autoimmune diseases.

CONCLUSION

Recently, characterization of TFH cells and germinal center reaction has been highlighted in immunology field that TFH cells have crucial roles in B cell response in adaptive immunity, IL-6 and IL-21 signaling induce expression of CXCR5, which enables the migration of T cells into B cell follicles by expressing Bcl-6, a master transcription factor for TFH cell differentiation. These characteristics of TFH cells distinguish them from other helper T cells. TFH cells can induce affinity maturation, somatic hyper-mutation which mediate memory B cells and long-lived plasma cells with increased germinal centers. However, aberrantly activated TFH cell function give rise to an immune reaction against auto-antigens, and subsequently could trigger autoimmune diseases such as SLE, RA, etc. There are several ways including miRNAs, TFR cells, and IL-21 blockade to potentially correct abnormal germinal center reaction by negatively regulating aberrant TFH cell differentiation. Through better understanding of current knowledge of TFH cell mediated dynamic germinal center reaction, we hope to discover novel therapeutic approaches by targeting TFH cells in human autoimmune diseases.

ACKNOWLEDGEMENTS

This study is supported by Basic Science Research Program through National Research Foundation of Korea grants (NRF-2011-0012859 and NRF-2013R1A 1A2 10060048).

CONFLICT OF INTEREST

The authors have no financial conflicts of interest to declare.

REFERENCES

1. Johnston, R. J., A. C. Poholek, D. DiToro, I. Yusuf, D. Eto, B. Burnett, A. I., Dent, J. Craft, and S. Crotty. 2009. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science 325: 1006-1010.
2. Nurieva, R., I., Y. Chung, G. J. Martinez, X. O. Yang, S. Tanaka, T., D. Matskevitch, Y. H., Wang, and C. Dong. 2009. Bcl6 mediates the development of T follicular helper cells. Science 325: 1001-1005.
3. Yu, D., S. Rao, I., M. Tsai, S. K. Lee, Y. He, E. L. Sutcliffe, M. Srivastava, M. Linterman, I. Zheng, N. Simpson, J. I., Ellyard, I., A. Parish, C. S., Ma, Q. J., Li, C. R., Parish, C., R, Mackay, and C. G. Vinuesa. 2009. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity 31: 457-468.
4. Nurieva, R., I., Y. Chung, D. Hwang, X. O. Yang, H. S. Kang, L., Ma, Y. H., Wang, S. S., Watowich, A., M. Jetter, Q. Tian, and C. Dong. 2008. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 29: 138-149.
5. Crotty, S. 2011. Follicular helper CD4 T cells (TFH). Annu. Rev. Immunol. 29: 621-663.
6. Qi, H., J. I., Cannon, K. Klauschen, P. I., Schwartzberg, and R. N., Germain. 2008. SAP-controlled T-B cell interactions underlie germinal centre formation, Nature 455: 764-769.
7. Dong, C., U., A. Temann, and R. A., Flavell, 2001. Cutting edge: critical role of inducible costimulator in germinal center reactions. J. Immunol. 166: 3659-3662.
8. Iwai, H., M., Abe, S. Hirose, F. Tsushima, K. Tezuka, H. Akiba, H., Yagit, K. Okumura, H. Kobesaka, N. Miyasaka, and M., Azuma. 2003. Involvement of inducible costimulator-B7 homologous protein costimulatory pathway in murine lupus nephritis. J. Immunol. 171: 2848-2854.
9. Lu, K. T., Y. Kanno, J. I., Cannons, R. Handon, P. Bihe, A. G., Elkhahlon, S. M., Anderson, L., Wei, H. Sun, J. J., OShea, and P. L., Schwartzberg. 2011. Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. Immunity 35: 622-632.
10. Breitfeld, D. L. Ohl, E. Kremmer, J. Ellwart, F. Sallusto, M. Lipp, and R. Förster. 2000. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J. Exp. Med. 192: 1545-1552.
11. Schaefer, P., K. Willimmann, A. B. Lang, M. Lipp, P. Loetscher, and B. Moser, 2000. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function, J. Exp. Med. 192: 1532-1562.

12. Balkwill, F., 2004. Cancer and the chemokine network, Nat. Rev. Cancer 4: 540-550.

13. Jacobs, J., G. Kehoe, K. Rajewsky, and U. Weiss, 1991. Intrachromosomal generation of antibody mutants in germinal centres, Nature 354: 389-392.

14. Berek, C., A. Berger, and M. Apel, 1991. Maturation of the immune response in germinal centers, Cell 67: 1121-1129.

15. Liu, Y. J., F. Malisio, O. de Bouteiller, C. Guret, S. Lebecque, J. R. Teijaro, and C. Xiao. 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

16. Kang, S. G., W. H. Liu, P. Lu, H. Y. Jin, H. W. Lim, J. Shepherd, D. Fremgen, E. Verdin, M. B. Oldstone, H. Qi, J. R. Teijaro, and C. Xiao, 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

17. Baumjohann, D., R. Kageyama, J. M. Clingan, M. M. Morar, J. R. Teijaro, and C. Xiao. 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

18. Takahashi, H., T. Kanno, S. Nakayamada, K. Hirahara, G. S. Rawal, Y. H. Wang, H. Lim, J. M. Reynolds, X. H. Zhou, S. Patels, D. de Kouchkovsky, O. Bannard, J. A. Bluestone, M. Matlokhian, K. M. Ansel, and L. T. Jeker, 2013. The microRNA cluster miR-17-92 promotes TFH cell differentiation and represses subset-inappropriate gene expression, Nat. Immunol. 14: 849-857.

19. Takakushi, H., T. Kanno, S. Nakayamada, K. Hinahara, G. Sciumè, C. S. A. Muljo, S. Kuchen, R. Casellas, L. Wei, Y. Kanno, and J. J. O'Shea. 2012. TGF-beta and retinoic acid induce the microRNA miR-10a, which targets Bcl-6 and constrains the plasticity of helper T cells, Nature 496: 587-595.

20. Vinuesa, C. G., M. C. Cook, 2001. The molecular basis of lymphoid architecture and B cell responses: implications for immunodeficiency and immunopathology, Curr. Mol. Med. 1: 690-725.

21. King, C., S. G. Tangye, and C. R. Mackay, 2008. T follicular helper (TFH) cells in normal and dysregulated immune responses, Annu. Rev. Immunol. 26: 741-766.

22. Chung, Y., S. Tanma, F. Chu, R. I. Nurieva, G. J. Martinez, S. Rawal, Y. H. Wang, H. Lim, J. M. Reynolds, X. H. Zhou, H. M. Fan, Z. M. Liu, S. S. Neelapu, and C. Dong, 2011. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions, Nat. Immunol. 12: 983-988.

23. Fazilleau, N., L. J. McHeyzer-Williams, H. Rosen, M. G. Greenberg, and W. K. Suh. 2009. Inducible costimulator promotes helper T-cell differentiation through phosphoinositide 3-kinase, Proc. Natl. Acad. Sci. USA 106: 20371-20376.

24. Hutloff, A., A. M. Dittrich, K. C. Beier, B. Eljaschewitsch, R. Stüber, and C. R. Mackay. 2005. A fundamental role for interleukin-21 in the generation of T follicular helper cells, Immunity 25: 127-137.

25. Vogelzang, A., H. M. McGuire, D. Yu, J. Sprent, C. R. Mackay, and C. King, 2008. A fundamental role for interleukin-21 in the generation of T follicular helper cells, Immunity 29: 127-137.

26. Kageyama, R., M. J. Ying, S. Mor, P. Lu, H. Y. Jin, H. W. Lim, J. Shepherd, D. Fremgen, E. Verdin, M. B. Oldstone, H. Qi, J. R. Teijaro, and C. Xiao, 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

27. Vogelzang, A., H. M. McGuire, D. Yu, J. Sprent, C. R. Mackay, and C. King, 2008. A fundamental role for interleukin-21 in the generation of T follicular helper cells, Immunity 29: 127-137.

28. Kageyama, R., M. J. Ying, S. Mor, P. Lu, H. Y. Jin, H. W. Lim, J. Shepherd, D. Fremgen, E. Verdin, M. B. Oldstone, H. Qi, J. R. Teijaro, and C. Xiao, 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

29. Kageyama, R., M. J. Ying, S. Mor, P. Lu, H. Y. Jin, H. W. Lim, J. Shepherd, D. Fremgen, E. Verdin, M. B. Oldstone, H. Qi, J. R. Teijaro, and C. Xiao, 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

30. Kageyama, R., M. J. Ying, S. Mor, P. Lu, H. Y. Jin, H. W. Lim, J. Shepherd, D. Fremgen, E. Verdin, M. B. Oldstone, H. Qi, J. R. Teijaro, and C. Xiao, 2013. MicroRNAs of the miR-17 approximately 92 family are critical regulators of TFH differentiation, Nat. Immunol. 14: 849-857.

31. Yuan, T. L. and L. C. Cantley. 2008. PI3K pathway alterations for immunodeficiency and immunopathology. Curr. Mol. Immunol. 1: 241-250.

32. Kerdiles, Y. M., E. L. Stone, D. R. Beisner, M. A. McGargill, N. Malenkovich, C. Jabs, V. K. Kuchroo, V. Ling, M. Collins, A. H. Sharpe, and G. J. Freeman. 2000. Mouse inducible cos-}
differenciation, *PloS One* 6: e17739.

39. Poholek, A. C., K. Hansen, S. G. Hernandez, D. Ero, A. Chandelee, J. S. Weinstein, X. Dong, J. M. Odegard, S. M. Kaech, A. I. Dent, S. Crotty, and J. Craft, 2010, *In vivo* regulation of Bcl6 and T follicular helper cell development, *J. Immunol.* 185: 313-326.

40. Takeda, K., T. Kaisho, N. Yoshida, J. Takeda, T. Kishimoto, and S. Akira, 1998, Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis generation and characterization of T cell-specific Stat3-deficient mice, *J. Immunol.* 161: 4652-4660.

41. Nurieva, R., X. O. Yang, G. Martinez, Y. Zhang, A. D. Ye, B. H., G. Cattoretti, Q. Shen, J. Zhang, N. Hawe, R. de Waard, C. Leung, M. Nouri-Shirazi, A. Orazi, R. S. Chaganti, C. Almeida, V. and S. Akira, 1998, Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis generation and characterization of T cell-specific Stat3-deficient mice, *J. Immunol.* 161: 4652-4660.

42. Yang, Y., J. Ochando, A. Yopp, J. S. Bronnberg, and Y. Ding, 2005, IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation, *J. Immunol.* 174: 2720-2729.

43. Jang, E., S. H. Cho, H. Park, D. J. Paik, J. M. Kim, and J. Youn, 2009, A positive feedback loop of IL-21 signaling provoked by homeostatic CD4+CD25+ T cell expansion is essential for the development of arthritis in autoimmune K/BxN mice, *J. Immunol.* 182: 4649-4656.

44. Oake, K., R. Spolski, R. Ettinger, H. P. Kim, G. Wang, C. F., Qi, P., Huw, D. J. Shaffer, S. Akleshe, D. C. Roopenian, H. C. Morse, 3rd, J. E. Lipsky, and W. J. Leonard, 2004, Regulation of B cell differentiation and plasm cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6, *J. Immunol.*, 173: 5361-5371.

45. Nakayamada, S., Y. Kanno, H. Takahashi, D. Jankovic, K. T. Liu, T. A. Johnson, H. W. Sun, G. Vahedi, O. Hakim, R. Handon, P. I., Schwartzberg, G. L. Hager, and J. J. O'Shea, 2011, Early Th1 cell differentiation is marked by a Th1 cell-like transition, *Immunity* 35: 919-931.

46. Lee, S. K., D. G. Silva, J. J. Martin, A. Pratama, X. Hu, P. P. Chung, G. Walters, and C. G. Vinuesa, 2012, Interferon-gamma excess leads to pathogenic accommodation of follicular helper T cells and germinal centers, *Immunity* 37: 880-892.

47. Zhou, G., and S. J. Ono, 2005, Induction of BCL-6 gene expression by interferon-gamma and identification of an IRE in exon I, *Exp. Mol. Pathol.* 78: 25-35.

48. Dent, A. L., A. L. Shaffer, X. Yu, D. Allman, and I. M. Staudt, 1997, Control of inflammation, cytokine expression, and germinal center formation by BCL-6, *Science* 276: 589-592.

49. Klein, U., and R. Dalla-Favera, 2008, Germinal centres: role in B-cell pathology and malignancy, *Nat. Rev. Immunol.* 8: 22-33.

50. Ye, B. H., G. Cattoretti, Q. Shen, J. Zhang, N. Haye, R. de Waard, C. Leung, M. Nouri-Shirazi, A. Oruzu, R. S. Chaganti, P. Rothman, A. M. Stall, P. P. Pandolfi, and R. Dalla-Favera, 1997, The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation, *Nat. Genet.* 16: 161-170.

51. Toyama, H., S. Okada, M. Hatano, Y. Takahashi, N. Takeda, H. Ichii, T. Takemori, Y. Kuroda, and T. Tokuhisa, 2002, Memory B cells without somatic hypermutation are generated from Bcl6-deficient B cells, *Immunity* 17: 320-339.

52. Crotty, S., R. J. Johnston, and S. P. Schoenberger, 2010, Effectors and memory: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation, *Nat. Immunol.* 11: 114-120.

53. Haynes, N. M., C. D. Allen, R. Lesley, K. M. Ansel, N. Killeen, and J. G. Cyster, 2007, Role of CXC85 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-high germinal center-associated subpopulation, *J. Immunol.* 179: 5099-5108.

54. Turner, C. A. Jr., D. H. Mack, and M. M. Davis, 1994, Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells, *Cell* 77: 297-306.

55. Martins, G. and K. Kalame, 2008, Regulation and functions of Blimp-1 in T and B lymphocytes, *Annu. Rev. Immunol.* 26: 133-169.

56. Johnston, R. J., Y. S. Choi, J. A. Diamond, J. A. Yang, and S. Crotty, 2012, STAT3 is a potent negative regulator of ThF cell differentiation, *J. Exp. Med.* 209: 243-250.

57. Kwon, H., D. Thierry-Mieg, J. Thierry-Mieg, H. H. Kim, J. Oh, C. Tunyaplin, S. Carotta, C. E. Donovan, M. L. Goldrnan, P. Tailor, K. Ozato, D. E. Levy, S. L. Nutt, K. Kalame, and W. J. Leonard, 2009, Analysis of interleukin-21-induced Pdml gene regulation reveals functional cooperation of p38 and IRF4 transcription factors, *Immunity* 31: 941-952.

58. Sakaguchi, S. 2004, Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses, *Annu. Rev. Immunol.* 22: 531-562.

59. Karangat, S., P. Blair, R. Reddy, M. Dabeshia, V. Godfrey, B. T. Rouse, and E. Wilkinson, 1996, Disease in the scurfy (sf) mouse is associated with overexpression of cytokine genes, *Eur. J. Immunol.* 26: 161-165.

60. Clark, L. B., M. W. Appleby, M. E. Brunikow, J. E. Wilkinson, S. F. Ziegler, and F. Ramsdell, 1999, Cellular and molecular characterization of the scurfy mouse mutant, *J. Immunol.* 162: 2546-2554.

61. Wollenberg, I., A. Agua-Doce, A. Hernández, C. Almeida, V. G. Oliveira, J. Faro, and I. Gracu, 2011, Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells, *J. Immunol.* 187: 4533-4540.

62. Linterman, M. A., W. Pierson, S. K. Lee, A. Kallies, S. Kawamoto, T. F. Rayner, M. Srivastava, D. P. Divekar, L. Beach, J. J. Hogan, A. Liston, K. G. Smith, and C. G. Vinuesa, 2011, Foxp3+ follicular regulatory T cells control the germinal center response, *Nat. Med.* 17: 975-982.

63. Sage, P. T., I. M. Francisco, C. V. Carman, A. H. Sharpe, 2013, The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood, *Nat. Immunol.* 14: 152-161.

64. Cobb, B. S., T. B. Nesterova, E. Thompson, A. Hertweck, E. O'Connor, J. Godwin, C. B. Wilson, N. Brockdorff, A. G. Feger, S. T. Smale, and M. Merkenschlag er, 2005. T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer, *J. Exp. Med.* 201: 1367-1373.

65. Muljo, S. A., K. M. Ansel, G. Kanellopoulou, D. M. Livingston, A. Rao, and K. Rajewsky, 2005, Aberrant T cell differentiation in the absence of Dicer, *J. Exp. Med.* 202-
Insights into the Role of Follicular Helper T Cells in Autoimmunity
Hong-Jai Park, et al.

261-269.

66. Doria, A., M. Zen, M. Canova, S. Bettio, N. Bassi, I. Nalotto, M. Rampudda, A. Ghirardello, and L. Iaccarino. 2010. SLE diagnosis and treatment: when early is early. Autoimmun. Rev. 10: 55-60.

67. Luzina, I. G., S. P. Atamas, C. E. Storrer, L. C. daSilva, G. Keloe, J. C. Papadimitriou, and B. S. Handwerger. 2001. Spontaneous formation of germinal centers in autoimmune mice. J. Leukoc. Biol. 70: 578-584.

68. Simpson, N., P. A. Gatenby, A. Wilson, S. Malik, D. A. Fulcher, S. G. Tange, H. Manku, T. J. Vyse, G. Roncador, G. A. Huttley, C. C. Goodnow, C. G. Vinuesa, and M. C. Cook. 2010. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. Arthritis Rheum. 62: 234-244.

69. Daikh, D. I., B. K. Finck, P. S. Linsley, D. Hollenbaugh, and D. Wofsy. 1997. Long-term inhibition of murine lupus by brief simultaneous blockade of the B7/CD28 and CD40/gp39 costimulation pathways. J. Immunol. 159: 3104-3108.

70. Vinuesa, C. G., M. C. Cook, C. Angelucci, V. Athanasopoulos, L. Rui, K. M. Hill, D. Yu, H. Domaschenz, B. Whittle, T. Lambe, I. S. Roberts, R. R. Copley, J. I. Bell, R. J. Cornall, and C. C. Goodnow. 2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 435: 452-458.

71. Linterman, M. A., R. J. Rigby, R. K. Wong, D. Yu, R. Brink, J. L. Cannons, P. L. Schwartzberg, M. C. Cook, G. D. Walters, and C. G. Vinuesa. 2009. Follicular helper T cells are required for systemic autoimmunity. J. Exp. Med. 206: 561-576.

72. Babier, J. A., T. J. Sproule, O. Foreman, R. Spolski, D. J. Shaffer, H. C. Morse 3rd, W. J. Leonard, and D. C. Roopenian. 2009. A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-Yaa mice. Proc. Natl. Acad. Sci. USA 106: 1518-1523.

73. Dolf, S., W. H. Abdulahad, J. Westra, B. Doombos-van der Meer, P. C. Limburg, C. G. Kallenberg, and M. Bijl. 2011. Increase in IL-21 producing T-cells in patients with systemic lupus erythematosus. Arthritis Res. Ther. 13: R157.

74. Wong, C. K., P. T. Wong, I. S. Tan, E. K. Li, D. P. Chen, and C. W. Lam. 2010. Elevated production of B cell chemokine CXCL13 is correlated with systemic lupus erythematosus disease activity. J. Clin. Immunol. 30: 45-52.

75. Young, D. A., M. Hegen, H. I. Ma, M. J. Whitters, I. M. Albert, I. Lowe, M. Senices, P. W. Wu, B. Sibley, Y. Leathurby, T. P. Brown, C. Nickerson-Nutter, J. C. Keith Jr, and M. Collins. 2007. Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis. Arthritis Rheum. 56: 1152-1163.

76. Kouskoff, V., A. S. Korganow, V. Duchatelle, C. Degott, C. Benoist, and D. Mathis. 1996. Organ-specific disease provoked by systemic autoimmunity. Cell 87: 811-822.

77. Victoratos, P. and G. Kollias. 2009. Induction of autoantibody-mediated spontaneous arthritis critically depends on follicular dendritic cells. Immunity 30: 130-142.

78. Platt, A. M., V. B. Gibson, A. Patalas, R. A. Benson, S. G. Nadler, J. M. Brewer, I. B. McInnes, and P. Garside, 2010. Abutacceptor limits breach of self-tolerance in a murine model of arthritis via effects on the generation of T follicular helper cells. J. Immunol. 185: 1558-1567.

79. Liu, R., Q. Wu, D. Su, N. Che, H. Chen, L. Geng, J. Chen, W. Chen, X. Li, and L. Sun, 2012. A regulatory effect of IL-21 on T follicular helper-like cell and B cell in rheumatoid arthritis. Arthritis Res. Ther. 14: R255.