Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Immunology

Lineage specification and heterogeneity of T follicular helper cells

Di Yu[1], Marcel Batten[1,3], Charles R Mackay[1,2,3] and Cecile King[1,3]

T follicular helper (Tfh) cells were originally described as a non-polarized CD4$^+$ T cell subset with follicular homing capacity and a potent ability to induce antibody production from B cells. However, a number of studies published in the past year have revealed a degree of heterogeneity within the germinal center CD4$^+$ T cell population, which suggests additional complexity. The overzealous activities of Tfh cells, or inappropriate expression of certain cytokines, represent new pathways for the development of autoimmune diseases. This review focuses on current progress in unraveling the biology of Tfh cells in health and disease, and understanding the relationship of Tfh cells to other CD4$^+$ T cell lineages.

Addresses
[1] Immunology and Inflammation, Garvan Institute of Medical Research, Sydney, NSW, Australia
[2] Monash University, Faculty of Medicine, Nursing and Health Services, Wellington Rd., Clayton, Vic 3800, Australia
[3] St Vincent's Clinical School, University of NSW, Sydney, NSW, Australia

Corresponding author: Mackay, Charles R (c.mackay@garvan.org.au)

Current Opinion in Immunology 2009, **21**:619–625

This review comes from a themed issue on
Autoimmunity
Edited by Jeffrey Bluestone and Vijay Kuchroo

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2009.09.013

Introduction

The production of high affinity, class-switched antibody is important for both the clearance of pathogens following infection and for the establishment of long-term humoral immunity. In order for this to be achieved, antibody-producing B cells must receive instruction from CD4$^+$ T cells that recognize the same antigen in germinal centers (GCs) [1–4]. It is now known that this cognate help is mediated by a specialized subset of CD4$^+$ T cells, termed T follicular helper (Tfh) cells [5]. The seminal studies that identified Tfh cells as a phenotypically and functionally distinct T cell subset were published almost a decade ago. These cells are a non-Th1/Th2 CD4$^+$ T cell population that expresses high levels of the chemokine receptor CXCR5, and show a potent ability to stimulate antibody production in B cells [6–8]. However, questions regarding the origin of these cells, the signals that drive their formation and their relationship to other effector CD4$^+$ T cells remain unanswered. The past 12 months has enjoyed a resurgence of studies focused on this still controversial T helper subset.

Characteristics of Tfh cells

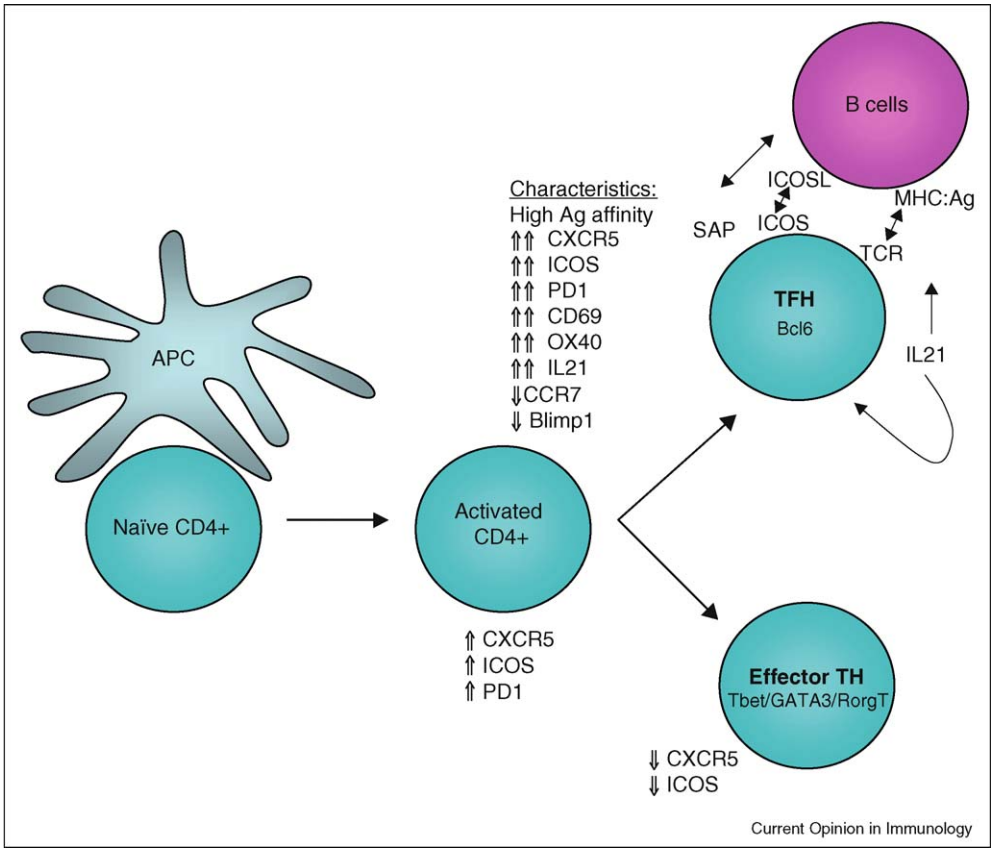
The fundamental features of Tfh cells are their unique localization within GCs and their ability to select B cells and support their differentiation into memory and plasma cells during the process of affinity maturation. The distinctive positioning of Tfh cells is facilitated by altered chemokine receptor expression and, as such, Tfh cells are defined by their expression of CXCR5, which in conjunction with the loss of CCR7, allows their migration into the CXCL13-rich B cell follicles of secondary lymphoid tissues [9–11]. CXCR5 is transiently expressed on CD4$^+$ T cells during their interaction with peptide-MHC but Tfh cells can be distinguished from other T cells by their sustained expression of high levels of CXCR5. The capacity of CD4$^+$ T cells to provide help to B cells depends upon the acquisition of molecules that are known to play functional roles in T–B cell collaboration, such as the costimulatory molecules CD40 ligand (CD40L) and inducible costimulator (ICOS), and the cytokine interleukin (IL)-21 [4] (Figure 1). Tfh cells have been shown to express the highest levels of these molecules and this heightened expression correlates with a superior ability to facilitate antibody production [6–8,12,13]. Tfh cells are important for normal humoral immune responses, but during aberrant or chronic responses, the unleashing of their potent helper activity can lead to malignancies and autoimmunity [14,15].

For other T helper cells, specific transcription factors have been identified that act as master-controllers of subset-specific gene expression programs [16]. Early microarray data from Tfh cells identified Bcl-6 as a transcription factor that was specifically expressed in Tfh but not other effector Th subsets [17]. Bcl-6 has since been used as a marker of Tfh cells and its expression is closely associated with their function. Activated CD4$^+$ T helper cells in the T cell zone have been shown to exhibit pronounced expression of the transcriptional repressor Blimp-1 and this expression was lost in favor of Bcl-6 expression in CXCR5^{hi} Tfh cells [18••]. Our recent studies, and those of others, demonstrate that induced expression of Bcl-6 is able to suppress alternative differentiation pathways and enhance features of Tfh cells, confirming that Bcl-6 indeed directs Tfh development [19••,20•,21•].

Differentiation of Tfh cells

One crucial aspect of Tfh cell biology that remains unresolved is the early events surrounding their conception. McHeyzer-Williams and colleagues recently

Figure 1



Differentiation of Tfh cells. Upon activation of naïve CD4$^+$ T cells by MHC:Ag on APCs (most probably DC), CXCR5, ICOS, CD69 and PD1 are upregulated; however, only cells that go on to become Tfh cells retain highest expression of these markers. Activated cells may produce cytokines, such as IL-4, however in cells that upregulate the transcriptional repressor Bcl-6, the differentiation of Th1, Th2 and Th17 is repressed. In these cells, Bcl-6 expression in conjunction with microenvironmental cues drives the differentiation of Tfh cells. The antigen specificity of the TCR appears to determine their differentiation into Tfh cells, versus terminally differentiated effector cells, with the CXCR5^{hi} ICOS^{hi} PD1^{hi} Tfh cell population showing higher pMHC binding affinity. The expression of the aforementioned surface markers may therefore reflect ongoing or enhanced antigenic stimulation. Indeed, the continuing interaction with B cells is a requirement for Tfh cells. This interaction is stabilized by SAP, without which B:T conjugates are short lived and Tfh development is poor. Delivery of ICOSL signals from the B cells is also essential. Tfh cells maintain high levels of expression of IL-21, and autocrine signals from this cytokine are necessary for expansion of the Tfh population.

showed that CD4$^+$ T cells with the highest specific binding of peptide-MHC II-ligand and the most restricted TCR junctional diversity are those that are selected to the Tfh cell pool [18••]. This study suggests that the generation of Tfh cells depends upon strong signals through the TCR or extended interaction with antigen-presenting cells. Differential TCR specificity of Tfh cells implies that they are a lineage distinct from other helper T cell types, which arise from different T cell precursor clones.

Following the activation of CD4$^+$ T cells in the T cell zone, Tfh cell precursors move to the T–B border where they interact with antigen-experienced B cells. Recent publications indicate that sustained interaction of T and B cells is necessary for Tfh cell generation. Signaling lymphocyte activation molecule-associated protein (SAP) is an adaptor molecule known to bind to signaling lymphocyte activation molecule (SLAM) and modulate both TCR signaling and Th2 differentiation [22]. In human X-linked lymphoproliferative disease and the analogous gene-targeted mouse model, a deficiency in SAP causes a profound defect in GC formation [22]. The mechanism underlying this defect has been recently brought to light by live imaging of the dynamics of the interaction of CD4$^+$ T cells with dendritic cells (DCs) and B cells following immunization [23••]. In the study by Qi *et al.*, SAP expression in T cells was found to be important for the stability of antigen-dependent T–B cell interactions but was dispensable for T-DC interactions. In other studies also supporting a crucial role for SAP in Tfh cell generation, Vinuesa and colleagues demonstrated that deficiency of SAP in sanroque mice, in which a point mutation in Roquin results in the constitutive overexpression of ICOS [24,25],

caused a reduction in Tfh cells and abrogated spontaneous formation of GCs, autoantibody formation, and renal pathology that normally occurs in sanroque mice [15]. These findings reveal the importance of sustained T-B interactions for the development of Tfh cells.

Additional signals from the microenvironment such as the cytokines IL-6 and IL-21 and the subsequent activation of STAT3 appear to reinforce the generation of B helper capacity [26,27] that, at this early stage, remains compatible with multiple differentiation programs [28]. Several studies using mice in which IL-21–IL-21R interactions have been disrupted have demonstrated an important role for IL-21 in differentiation of multiple T helper subsets [26,29–31]. For Tfh cells, IL-21 exhibits an autocrine role in Tfh cell differentiation [26,30] that precedes the acquisition of B cell follicle homing capacity [30]. Elevated expression of IL-21 is a characteristic feature of Tfh cells and recent studies have demonstrated greater levels of IL-21 in GC Tfh cells than in Th2 cells located in the lungs [13,17,18••,32••]. Activated $CD4^+$ T cells that are destined to become Tfh cells migrate beyond the T-B border into the GC, which distinguishes Tfh cells from other differentiated Th subsets that either facilitate antibody production in the extrafollicular compartment or enter the circulating lymphocyte pool.

Relationship of Tfh cells to other T helper subsets

Tfh and Th2

Several recent studies have utilized transgenic mice that express a reporter that enables the identification of cells producing IL-4 protein and/or IL-4 mRNA to analyze the relationship between Th2 cells and Tfh cells during infection [32••–34]. These studies confirm previous reports demonstrating IL-4 production in Tfh cells [8,11,35] and offer new insight into the phenotype of Tfh cells following infection with pathogens that induce a Th2 dominant response. Locksley and colleagues observed that IL-4-producing Tfh cells that were generated during Leishmania infection could be distinguished from effector Th2 cells by their high expression of CXCR5, IL-21, Bcl-6 and SAP [32••]. IL-4 is known to promote Ig isotype switching to IgG1 during antibody responses, whereas IFN-γ promotes isotype switching to IgG2a. The delicate isolation of T-GC B cell conjugates demonstrated that IgG1-producing B cells were those in contact with IL-4-producing T cells and that IgG2a-producing B cells were those in contact with IFN-γ-producing T cells *in vivo* [32••]. These findings indicate that Tfh cells are capable of producing cytokines in the GC that direct the class switch of antibody isotypes.

Two of these studies argue that the demonstration of IL-4 competent or IL-4 secreting $CD4^+$ T cells in the GC challenges the concept that Tfh cells are a separate lineage [33,34], but, as mentioned earlier, IL-4 production may be a feature of Tfh cells, and serve to simply skew antibody responses to a particular isotype. Archetypical Th2 cells also produce the eosinophilic cytokine IL-5, and induce expression of the chemokine Eotaxin, which is not observed in GCs. However elevated mRNA levels of the Th2 transcription factor GATA3 have been reported in PD-1^+ IL-4-expressing $CD4^+$ T cells [33]. It is important to note here that, like IL-21, ICOS and CXCR5, PD-1 expression *per se* does not distinguish Tfh cells from activated $CD4^+$ T cells, suggesting that PD-1^+ IL-4-producing $CD4^+$ T cells represent a mixed population [33]. These findings perhaps emphasize the limitations of the current phenotypic identification of Tfh cells, since all these markers also serve as T cell activation markers. Where possible, GC localization probably best defines Tfh cells. The future identification of new markers for Tfh cells, including good intracellular staining for Bcl-6, will hopefully enable a more accurate distinction and understanding of their function.

Tfh and Th1

In contrast to IL-4, the prototypical Th1 cytokine IFN-γ was not thought to be produced by Tfh cells. Nevertheless, some IFN-γ-producing Tfh cells were observed in GCs after Leishmania infection [32••]. These cells were present at a much lower frequency than IL-4-producing GC T cells, and B cells conjugated with IL-4-producing helper T cells had much higher levels of activation-induced cytidine deaminase (AID) compared with those conjugated with IFN-γ-producing helper T cells suggesting that IFN-γ-producing helper T cells have a reduced capacity for GC B cell help [32••]. In the systems tested thus far, IFN-γ-producing cells appear to make up a minority of T cells within GCs and IgG2a class switching can occur extrafollicularly [36] suggesting that IFN-γ need not be supplied in the context of a GC reaction. A caveat herein is that Th subsets are known to develop according to the type of pathogen encountered and the Leishmania and parasite infections used in the recent studies are expected to produce IL-4 dominant responses. Future studies will be needed to determine whether IFN-γ-producing Tfh cells constitute a major proportion of Tfh cells during an IFN-γ biased humoral response.

Tfh and Th17

Tfh-inducing cytokines including IL-6 and IL-21 also promote Th17 differentiation [26,37,38]. Therefore, it is surprising that IL-17 is normally detected at very low levels in Tfh cells [19,26,39]. Recently we and others showed that Bcl-6 overexpression suppressed IL-17 production by non-polarized $CD4^+$ T cells, and also by Th17-polarized cells *ex vivo* [19••,21•]. Bcl-6 is a well-defined transcriptional repressor and we found that it binds to the promoter region of RORγt in Tfh cells, presumably inhibiting the transcription of RORγt to prevent Th17 differentiation. However, as discussed

for IFN-γ-producing cells, IL-17 production by Tfh cells may depend on the antigenic stimulus. Numerous autoimmune diseases have been associated with Th17 responses and two recent publications show that during autoimmune disease, IL-17 expressing Tfh cells can be detected [39,40]. Moreover, enhanced IL-17 production accelerated GC formation and antibody production [39]. An intriguing question, yet to be answered, is whether Th17 cells can function as Tfh cells, or whether Tfh cells aberrantly produce IL-17 under certain conditions.

A pathogenic role for IL-17 in dysregulated antibody responses and autoimmunity is supported by a very recent report that IL-17, in synergy with B cell-activating factor (BAFF), enhances human B cell survival and promotes their proliferation and differentiation into antibody-secreting cells [41]. Thus, the production of IL-17 by Tfh cells would be expected to have a positive role in GC activity, potentially modulating the normal threshold for B cell survival and subverting normal tolerance mechanisms in the GC.

Tfh and Foxp3$^+$ Treg

Tfh cells and Tregs cells are phenotypically distinct subsets that express different transcriptional profiles and transcription factors [16]. However, there is now evidence that, in the unique context of the gut, Tfh cells can be generated from Treg cells. By adoptively transferring Foxp3$^+$ and Foxp3$^-$ cells from Foxp3^{EGFP} reporter mice into *Cd3ε*$^{-/-}$ mice, Fagarasan and colleagues showed the preferential generation of Tfh cells from Foxp3$^+$ rather than Foxp3$^-$ T cells in gut Peyer's patches (PPs). The resulting Tfh cells potently promoted GC reactions and IgA production in PPs [42••]. By contrast, Foxp3$^+$ T cells were unable to differentiate into Tfh cells or support GC formation in spleen or lymph nodes in the same experimental conditions, demonstrating a unique microenvironment in PPs that fosters Tfh cell differentiation from Treg cells.

Treg cells also represent a small but *bona fide* population of GC T cells in non-gut tissues [43,44]. Nevertheless, the incorporation of Treg into the Tfh pool is hard to reconcile with the fact that, in addition to their well-established role in suppression of T cell proliferation and effector functions, Treg cells directly suppress B cells via a cell contact-dependent mechanism [43,45]. Future investigation of the mechanisms controlling the relationship between Tfh and Treg will hopefully shed light on the physiological relevance of these observations.

The importance of Tfh cells to autoimmune disease

An elevated level of circulating autoantibodies is one classic feature of many autoimmune diseases [46,47]. The pathogenic role of autoantibodies in autoimmune diseases includes direct interference with normal cellular functions and induction of disease through the formation of immune complexes, Fc-mediated activation of the complement system and recruitment of inflammatory cells [48]. Autoantibodies can be produced in the absence of T cell help, for example in T-cell-deficient BAFF transgenic (Tg) mice [49]. However, pathogenic autoantibodies are predominantly produced by somatically mutated B cells [50,51] suggesting inappropriate helper signals are delivered to B cells by Tfh cells in autoimmune diseases.

There is strong evidence from murine models for the involvement of Tfh cells in systemic autoimmunity. The *N*-ethyl-*N*-nitrosourea-induced mutant *sanroque* strain possesses a homozygous point mutation in the *Roquin* gene, which normally limits gene expression including ICOS expression by promoting the degradation of messenger RNA [25]. Consequently, these mice have high levels of ICOS expression, which contributes to lupus-like autoimmune syndrome [24,25] with many features typical of Systemic Lupus Erythematosis (SLE) including high-affinity antibodies against double-stranded DNA (dsDNA), focal proliferative glomerulonephritis with deposition of IgG-containing immune complexes, anemia and autoimmune thrombocytopenia as well as other autoimmune manifestations such as lymphadenopathy, splenomegaly, necrotizing hepatitis and plasmacytosis [24]. As discussed above, deficiency in SAP in this strain reduced Tfh numbers and ameliorated disease, suggesting that Tfh cells play a pathogenic role [15]. Aberrant Tfh differentiation has also been witnessed in autoimmune BXD2 [39] and B6.*Sle1 yaa* mice [52].

Molecules associated with Tfh development and function are important for both human and murine systemic autoimmune disease. Genetic deletion or blockade of ICOS or IL21 signaling in mice ameliorates disease in multiple models of autoimmunity including NOD diabetes, Rheumatoid Arthritis (RA), and murine models of SLE [53–60]. In addition, a recent publication has demonstrated the importance of ICOS and IL21 in the extrafollicular T-dependent antibody response [61], thus the effects of these molecules may extend beyond the GC. With regard to human disease, a soon to be published report shows that a population resembling Tfh cells can be detected in the blood of SLE and Sjogren's syndrome patients and that the presence of this population correlates with increased diversity and titer of autoantibodies and the severity of end-organ involvement [62]. Analysis of genetic polymorphisms has shown an association of ICOS/CTLA4 locus variants with RA, primary billiary cirrosis and celiac disease [59,63] while variants in the 4q27 region, which contains the *IL2* and *IL21* genes, have been associated with a variety of autoimmune diseases. The interpretation of these data are confounded by potential changes in nearby gene expression, but more recently a specific *IL21R* SNP has been associated with

SLE [64], strengthening the evidence for a role of IL21 in human autoimmune disease. However, there is no evidence to date indicating that such associations affect Tfh cells. Tfh cells are one of the main cell types that produce IL-21, and GC B cells are one of the main cell types that express IL-21R [17]. We would predict that an *IL21R* SNP that associates with autoimmunity would enhance IL-21R signaling in B cells.

Concluding remarks

Recent studies have demonstrated a previously unappreciated degree of heterogeneity within the Tfh cell population. In the future, it will be important to determine whether the heterogeneity of cytokine production from Tfh is indicative of their pluripotency, or whether there are distinct Tfh cell subsets within a broader Tfh cell family. The identification of Bcl-6 as a Tfh cell transcription factor offers firm evidence for the lineage sovereignty of Tfh cells. However, the potential exists for an intriguing degree of plasticity for T helper cell differentiation. In the experimental models examined to date, Tfh cells produce a significant amount of IL-4 [32••–34], a detectable amount of IFN-γ [32••] and little IL-17 [19••,26], whereas IL-17 production is aberrantly increased in autoimmune models [39,40]. It has been reported that Bcl-6 alone suppresses IL-4 [65], IFN-γ [19••] and IL-17 [19••,21•] production. The heterogenecity of Tfh cytokine profiles suggests Bcl-6 might work with other antagonistic transcription factors to shape cytokine expression profiles. The field requires a better understanding of the microenvironmental cues that drive lineage specification of Tfh cells, and also the availability of reporter systems or reagents for transcription factor and cytokine expression by T cells temporally and spatially during immune responses. Understanding the genesis of Tfh cells and how they can be manipulated offers opportunities for the design of better vaccines, and for the development of new therapies for autoimmune diseases.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Claman HN, Chaperon EA, Triplett RF: **Thymus-marrow cell combinations. Synergism in antibody production**. *Proc Soc Exp Biol Med* 1966, **122**:1167-1171.
2. Miller JF, Mitchell GF: **Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes**. *J Exp Med* 1968, **128**:801-820.
3. Liu YJ, Malisan F, de Bouteiller O, Guret C, Lebecque S, Banchereau J, Mills FC, Max EE, Martinez-Valdez H: **Within germinal centers, isotype switching of immunoglobulin genes occurs after the onset of somatic mutation**. *Immunity* 1996, **4**:241-250.
4. King C, Tangye SG, Mackay CR: **T follicular helper (TFH) cells in normal and dysregulated immune responses**. *Annu Rev Immunol* 2008, **26**:741-766.
5. MacLennan IC: **Germinal centers**. *Annu Rev Immunol* 1994, **12**:117-139.
6. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B: **CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function**. *J Exp Med* 2000, **192**:1553-1562.
7. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, Forster R: **Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production**. *J Exp Med* 2000, **192**:1545-1552.
8. Kim CH, Rott LS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC: **Subspecialization of CXCR5$^+$ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5$^+$ T cells**. *J Exp Med* 2001, **193**:1373-1381.
9. Arnold CN, Campbell DJ, Lipp M, Butcher EC: **The germinal center response is impaired in the absence of T cell-expressed CXCR5**. *Eur J Immunol* 2007, **37**:100-109.
10. Hardtke S, Ohl L, Forster R: **Balanced expression of CXCR5 and CCR7 on follicular T helper cells determines their transient positioning to lymph node follicles and is essential for efficient B-cell help**. *Blood* 2005, **106**:1924-1931.
11. Haynes NM, Allen CD, Lesley R, Ansel KM, Killeen N, Cyster JG: **Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1 high germinal center-associated subpopulation**. *J Immunol* 2007, **179**:5099-5108.
12. Rasheed AU, Rahn HP, Sallusto F, Lipp M, Muller G: **Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression**. *Eur J Immunol* 2006, **36**:1892-1903.
13. Bryant VL, Ma CS, Avery DT, Li Y, Good KL, Corcoran LM, de Waal Malefyt R, Tangye SG: **Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5$^+$ T follicular helper cells**. *J Immunol* 2007, **179**:8180-8190.
14. Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG: **Follicular helper T cells: lineage and location**. *Immunity* 2009, **30**:324-335.
15. Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL, Schwartzberg PL, Cook MC, Walters GD, Vinuesa CG: **Follicular helper T cells are required for systemic autoimmunity**. *J Exp Med* 2009, **206**:561-576.
16. Zhou L, Chong MM, Littman DR: **Plasticity of CD4$^+$ T cell lineage differentiation**. *Immunity* 2009, **30**:646-655.
17. Chtanova T, Tangye SG, Newton R, Frank N, Hodge MR, Rolph MS, Mackay CR: **T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells**. *J Immunol* 2004, **173**:68-78.
18. •• Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG: **The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding**. *Nat Immunol* 2009, **10**:375-384.
TCR signal strength influences development of Tfh cells. Tfh cells, non-GC TH cells and emigrant effector cells can be defined in LN after immunization based on expression of CD62L, CXCR5, Blimp-1 and Bcl-6. TCR Tg cells with highest affinity for antigen preferentially become Tfh cells and tetramer-based detection of endogenous antigen specific cells indicated that Tfh cells have the highest antigen binding compared with other T cell populations.
19. •• Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL, Srivastava M, Linterman M, Zheng L, Simpson N *et al.*: **The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment**. *Immunity* 2009.
Bcl-6 is a key transcription factor controlling the Tfh expression program It's expression negatively regulates alternative Th differentiation pathways and suppresses IFN-γ and IL-17 cytokine production. This study showed that Bcl-6 repressed numerous microRNAs that accounted for the charachteristic Tfh expression signature.

20. Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B,
• Dent AL, Craft J, Crotty S: **Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation**. *Science* 2009, **325**:1006-1010.
Through studying the effects of overexpression of BCL-6 and its genetic deficiency, this transcrptional repressor was observed to be both sufficient and necessary for Tfh cell differentiation.

21. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S,
• Matskevitch TD, Wang YH, Dong C: **Bcl6 mediates the development of T follicular helper cells**. *Science* 2009, **325**:1001-1005.
Shows that Bcl-6 is a key transcription factor controlling the Tfh expression program.

22. Veillette A: **Immune regulation by SLAM family receptors and SAP-related adaptors**. *Nat Rev Immunol* 2006, **6**:56-66.

23. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN:
•• **SAP-controlled T–B cell interactions underlie germinal centre formation**. *Nature* 2008, **455**:764-769.
This study showed that SAP was crucial for stabilizing T–B cell interactions, but was not necessary for T-DC interactions. Without SAP, defective T–B conjugates resulted in insufficient help to B cells and poor generation of *bona fide* Tfh cells.

24. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, Yu D, Domaschenz H, Whittle B, Lambe T *et al.*: **A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity**. *Nature* 2005, **435**:452-458.

25. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG, Hutloff A, Giles KM, Leedman PJ, Lam KP *et al.*: **Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA**. *Nature* 2007, **450**:299-303.

26. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, Wang YH, Watowich SS, Jetten AM, Tian Q *et al.*: **Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages**. *Immunity* 2008, **29**:138-149.

27. Eddahri F, Denanglaire S, Bureau F, Spolski R, Leonard WJ, Leo O, Andris F: **Interleukin-6/STAT3 signaling regulates the ability of naive T cells to acquire B-cell help capacities**. *Blood* 2009, **113**:2426-2433.

28. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR: **IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways**. *Nat Immunol* 2007, **8**:967-974.

29. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK: **IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells**. *Nature* 2007, **448**:484-487.

30. Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C: **A fundamental role for interleukin-21 in the generation of T follicular helper cells**. *Immunity* 2008, **29**:127-137.

31. Frohlich A, Marsland BJ, Sonderegger I, Kurrer M, Hodge MR, Harris NL, Kopf M: **IL-21 receptor signaling is integral to the development of Th2 effector responses *in vivo***. *Blood* 2007, **109**:2023-2031.

32. Reinhardt RL, Liang HE, Locksley RM: **Cytokine-secreting**
•• **follicular T cells shape the antibody repertoire**. *Nat Immunol* 2009, **10**:385-393.
This paper shows that after parasite infection both IL-4 and IFNg expressing Tfh cells can be detected in the GC and these cells drive cytokine-specific isotype switching in T–B conjugates. Focusing on IL-4, they showed that the majority of IL-4 in the LN is produced by T cells located in the B cell follicle and GC. Although IL-4$^+$ Tfh cells comprised only 1–3% of T:B conjugates, the interacting B cells had high expression of GC B cell markers.

33. Zaretsky AG, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ: **T follicular helper cells differentiate from Th2 cells in response to helminth antigens**. *J Exp Med* 2009, **206**:991-999.

34. King IL, Mohrs M: **IL-4-producing CD4$^+$ T cells in reactive lymph nodes during helminth infection are T follicular helper cells**. *J Exp Med* 2009, **206**:1001-1007.

35. Fazilleau N, Eisenbraun MD, Malherbe L, Ebright JN, Pogue-Caley RR, McHeyzer-Williams LJ, McHeyzer-Williams MG: **Lymphoid reservoirs of antigen-specific memory T helper cells**. *Nat Immunol* 2007, **8**:753-761.

36. Pape KA, Kouskoff V, Nemazee D, Tang HL, Cyster JG, Tze LE, Hippen KL, Behrens TW, Jenkins MK: **Visualization of the genesis and fate of isotype-switched B cells during a primary immune response**. *J Exp Med* 2003, **197**:1677-1687.

37. Vogelzang A, King C: **The modulatory capacity of interleukin-21 in the pathogenesis of autoimmune disease**. *Front Biosci* 2008, **13**:5304-5315.

38. Dong C: **Mouse Th17 cells: current understanding of their generation and regulation**. *Eur J Immunol* 2009, **39**:640-644.

39. Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J, Yi J, Guentert T, Tousson A, Stanus AL *et al.*: **Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice**. *Nat Immunol* 2008, **9**:166-175.

40. Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH, Kuchroo VK: **The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells**. *Nat Immunol* 2009, **10**:167-175.

41. Doreau A, Belot A, Bastid J, Riche B, Trescol-Biemont MC, Ranchin B, Fabien N, Cochat P, Pouteil-Noble C, Trolliet P *et al.*: **Interleukin 17 acts in synergy with B cell-activating factor to influence B cell biology and the pathophysiology of systemic lupus erythematosus**. *Nat Immunol* 2009, **10**:778-785.

42. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O,
•• Honjo T, Hori S, Fagarasan S: **Preferential generation of follicular B helper T cells from Foxp3$^+$ T cells in gut Peyer's patches**. *Science* 2009, **323**:1488-1492.
Tracking of Foxp3-eGFP cells indicated that Tfh cells in the Peyer's patches are preferentially generated from Treg precursors, whereas those in the spleen and LN were not. Treg cells that became Tfh cells downregulated Foxp3 expression. This demonstrates a degree of plasticity for CD4 T cell development, although begs the question why Tregs in Peyer's patches and not elsewhere could differentiate to Tfh cells.

43. Lim HW, Hillsamer P, Kim CH: **Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses**. *J Clin Invest* 2004, **114**:1640-1649.

44. Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, Qin FX, Gilliet M, Liu YJ: **Two functional subsets of FOXP3$^+$ regulatory T cells in human thymus and periphery**. *Immunity* 2008, **28**:870-880.

45. Lim HW, Hillsamer P, Banham AH, Kim CH: **Cutting edge: direct suppression of B cells by CD4$^+$ CD25$^+$ regulatory T cells**. *J Immunol* 2005, **175**:4180-4183.

46. Tan EM: **Autoantibodies and autoimmunity: a three-decade perspective. A tribute to Henry G. Kunkel**. *Ann NY Acad Sci* 1997, **815**:1-14.

47. Davidson A, Diamond B: **Autoimmune diseases**. *N Engl J Med* 2001, **345**:340-350.

48. Martin F, Chan AC: **B cell immunobiology in disease: evolving concepts from the clinic**. *Annu Rev Immunol* 2006, **24**:467-496.

49. Groom JR, Fletcher CA, Walters SN, Grey ST, Watt SV, Sweet MJ, Smyth MJ, Mackay CR, Mackay F: **BAFF and MyD88 signals promote a lupuslike disease independent of T cells**. *J Exp Med* 2007, **204**:1959-1971.

50. Wellmann U, Letz M, Herrmann M, Angermuller S, Kalden JR, Winkler TH: **The evolution of human anti-double-stranded DNA autoantibodies**. *Proc Natl Acad Sci U S A* 2005, **102**:9258-9263.

51. Mietzner B, Tsuiji M, Scheid J, Velinzon K, Tiller T, Abraham K, Gonzalez JB, Pascual V, Stichweh D, Wardemann H *et al.*: **Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors**. *Proc Natl Acad Sci U S A* 2008, **105**:9727-9732.

52. Subramanian S, Tus K, Li QZ, Wang A, Tian XH, Zhou J, Liang C, Bartov G, McDaniel LD, Zhou XJ *et al.*: **A Tlr7 translocation accelerates systemic autoimmunity in murine lupus**. *Proc Natl Acad Sci U S A* 2006, **103**:9970-9975.

53. Bubier JA, Sproule TJ, Foreman O, Spolski R, Shaffer DJ, Morse HC 3rd, Leonard WJ, Roopenian DC: **A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-Yaa mice**. *Proc Natl Acad Sci U S A* 2009, **106**:1518-1523.

54. Herber D, Brown TP, Liang S, Young DA, Collins M, Dunussi-Joannopoulos K: **IL-21 has a pathogenic role in a lupus-prone mouse model and its blockade with IL-21R.Fc reduces disease progression**. *J Immunol* 2007, **178**:3822-3830.

55. Spolski R, Kashyap M, Robinson C, Yu Z, Leonard WJ: **IL-21 signaling is critical for the development of type I diabetes in the NOD mouse**. *Proc Natl Acad Sci U S A* 2008, **105**:14028-14033.

56. Jang E, Cho SH, Park H, Paik DJ, Kim JM, Youn J: **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* 2009, **182**:4649-4656.

57. Hu YL, Metz DP, Chung J, Siu G, Zhang M: **B7RP-1 blockade ameliorates autoimmunity through regulation of follicular helper T cells**. *J Immunol* 2009, **182**:1421-1428.

58. Young DA, Hegen M, Ma HL, Whitters MJ, Albert LM, Lowe L, Senices M, Wu PW, Sibley B, Leathurby Y *et al.*: **Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis**. *Arthritis Rheum* 2007, **56**:1152-1163.

59. Yong PF, Salzer U, Grimbacher B: **The role of costimulation in antibody deficiencies: ICOS and common variable immunodeficiency**. *Immunol Rev* 2009, **229**:101-113.

60. Datta S, Sarvetnick NE: **IL-21 limits peripheral lymphocyte numbers through T cell homeostatic mechanisms**. *PLoS ONE* 2008, **3**:e3118.

61. Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C, Flavell RA, Craft J: **ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity**. *J Exp Med* 2008, **205**:2873-2886.

62. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, Manku H, Vyse TJ, Huttley GA, Goodnow CC, et al.: **Expansion of circulating T cells resembling TFH cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus**. *Arthritis Rheum* 2009, in press.

63. Walker EJ, Hirschfield GM, Xu C, Lu Y, Liu X, Coltescu C, Wang K, Newman WG, Bykerk V, Keystone EC *et al.*: **CTLA4/ICOS gene variants and haplotypes are associated with rheumatoid arthritis and primary biliary cirrhosis in the Canadian population**. *Arthritis Rheum* 2009, **60**:931-937.

64. Webb R, Merrill JT, Kelly JA, Sestak A, Kaufman KM, Langefeld CD, Ziegler J, Kimberly RP, Edberg JC, Ramsey-Goldman R *et al.*: **A polymorphism within IL21R confers risk for systemic lupus erythematosus**. *Arthritis Rheum* 2009, **60**:2402-2407.

65. Kusam S, Toney LM, Sato H, Dent AL: **Inhibition of Th2 differentiation and GATA-3 expression by BCL-6**. *J Immunol* 2003, **170**:2435-2441.