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Regulation of T follicular helper cell formation and function by antigen presenting cells

Elissa K Deenick[1,2], Cindy S Ma[1,2], Robert Brink[1,2] and Stuart G Tangye[1,2]

CD4$^+$ T cells can differentiate into numerous subsets characterized by expression of a suite of cytokines and effector molecules that endow them with specialized functions. By mediating the differentiation of B cells into memory and plasma cells following exposure to T-dependent antigens (Ag), T follicular helper (T_{FH}) cells have emerged as the predominant subset of CD4$^+$ T cells responsible for regulating humoral immunity. The generation of T_{FH} cells from naïve precursors typically involves sequential cognate interactions with distinct populations of Ag-presenting cells (APCs): dendritic cells within the T-cell zone of lymphoid tissues, and activated B cells at the border of the T-zone and follicle, and then within a germinal center. Recent studies have illuminated the key roles of APCs in T_{FH} development, and have also re-defined the role of B cells in this process.

Addresses
[1] Immunology Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, 2010, NSW, Australia
[2] St Vincent's Clinical School, University of NSW, Kensington, 2033, NSW, Australia

Corresponding author: Tangye, Stuart G (s.tangye@garvan.org.au)

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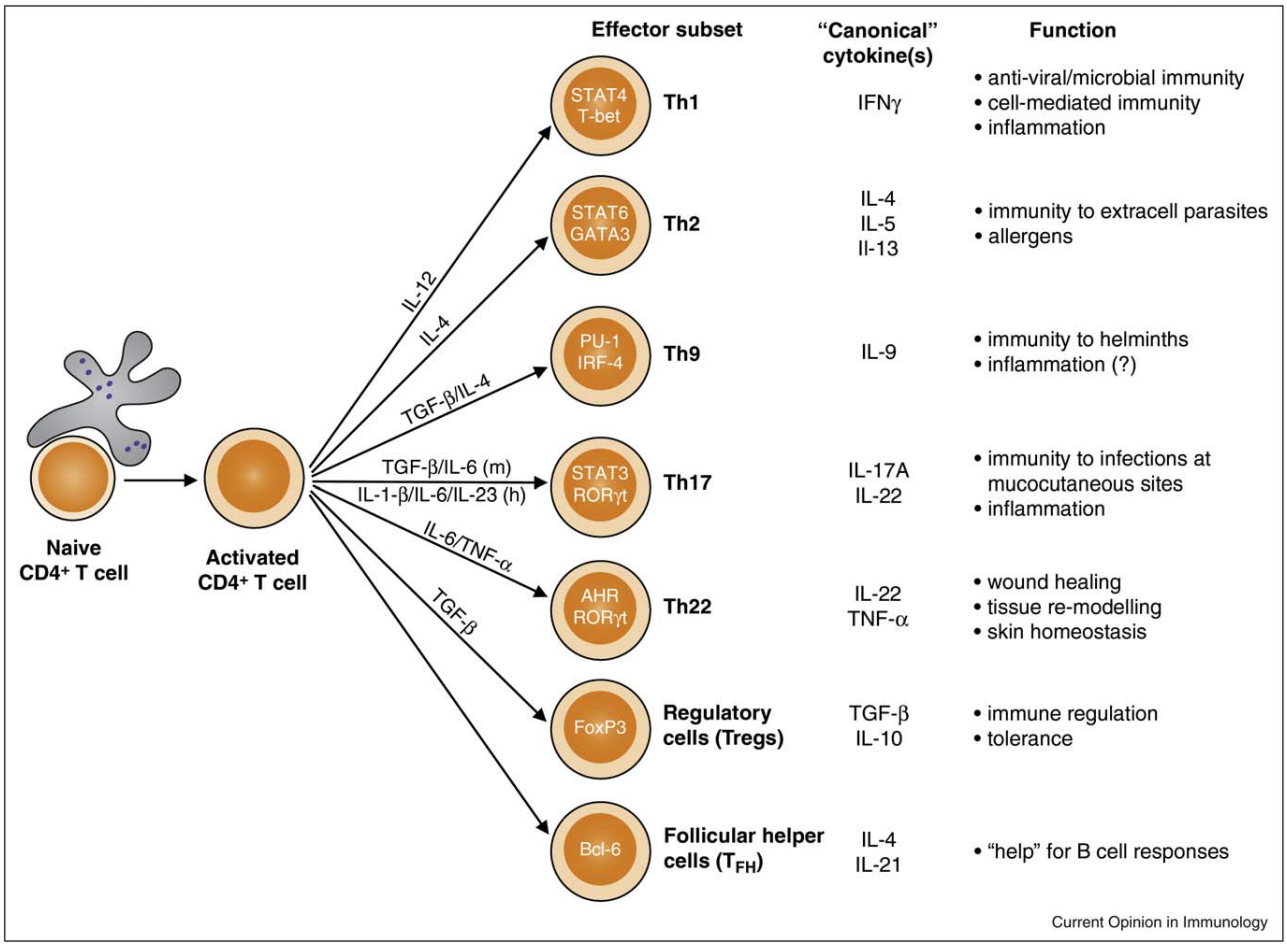
Functional specialization of CD4$^+$ T cells

CD4$^+$ T helper cells have the remarkable capacity of developing into distinct lineages that, depending on the array of effector molecules they produce, exhibit unique functional properties. Thus, Th1 cells secrete IFN-γ and are primarily involved in immune responses against intracellular pathogens; Th2 cells produce IL-4, IL-5 and IL-13, are generated in response to parasites and helminths, and may contribute to humoral immunity by inducing Ig class switching; Th17 cells produce IL-17A/F, IL-21, IL-22 and IL-26, and are crucial for protection against extracellular pathogens at mucocutaneous sites; and regulatory T cells (Tregs) suppress the action of inflammatory cells to limit tissue damage elicited during the course of protective immune responses [1,2] (Figure 1).

The generation of effector CD4$^+$ T cells is regulated by cytokines produced within a stimulatory microenvironment that co-ordinate the actions of specific transcription factors. Thus, IL-12 activates STAT4 and induces T-bet that specifies Th1 cells. Similarly IL-4 induces Th2 cells through STAT6, GATA3 and c-maf, while TGF-β induces Tregs via induction of FoxP3 [1,2]. IL-6, which activates STAT3 and induces RORγt and RORα, co-operates with TGF-β to guide differentiation of murine CD4$^+$ T cells into Th17 cells [1,2], while combinations of IL-6, IL-21and IL-23, which activate STAT3 to induce RORγt, commit naïve human CD4$^+$ T cells to the Th17 lineage [3,4] (Figure 1). More recently, additional 'lineages' of CD4$^+$ T cells – Th9 and Th22 – have been reported, with the transcription factors PU-1 and IRF-4 being implicated in the development of murine Th9 cells [5,6] (Figure 1). One of the most intensely studied classes of CD4$^+$ T cells in recent years has been T follicular helper (T_{FH}) cells, which play a central role in supporting protective Ab responses derived from Ag-specific B cells (Figure 1).

Interactions between Ag-specific B cells, CD4$^+$ T cells and dendritic cells (DCs) in response to foreign Ag lead to the formation of germinal centers (GCs), specialized structures within B-cell follicles of secondary lymphoid tissues where selection of high-affinity B cells occurs [7–9]. CD4$^+$ T cells support the differentiation of Ag-selected GC B cells into memory or plasma cells (PCs), which are responsible for ensuring long-lived humoral immunity following infection or vaccination with T-dependent (TD) Ag [7–9]. Although the requirement for CD4$^+$ T cells in generating TD immune responses is well established, the exact nature of the 'help' provided has been enigmatic. Indeed, while early studies implicated Th2 cells, mice lacking key regulators of Th2 development – IL-4/IL-4R and STAT6 – can still form GCs and elicit TD Ab responses [10–12]. T_{FH} cells have emerged as a specialized CD4$^+$ T cell lineage that provides help to B cells for their selection and differentiation into memory and PC [13,14]. Despite this, our understanding of the molecular requirements for the development and function of T_{FH} cells has lagged behind that of other T helper lineages. Here, we will discuss recent advances and controversies in our understanding of the mechanisms underlying the induction and maintenance of T_{FH} cells and the

Figure 1



Naïve CD4$^+$ T cells can differentiate into multiple subsets of effector cells. Cytokines provided by the stimulatory microenvironment activate specific transcription factors that regulate naïve CD4$^+$ T cell differentiation. Distinct subsets of effector CD4$^+$ T cells can be identified by the production of a set of 'canonical' cytokines that endow the cells with specific functions. AHR: aryl hydrocarbon receptor; (h): human; (m): mouse.

important role played by Ag-presenting cells (APCs) in this process.

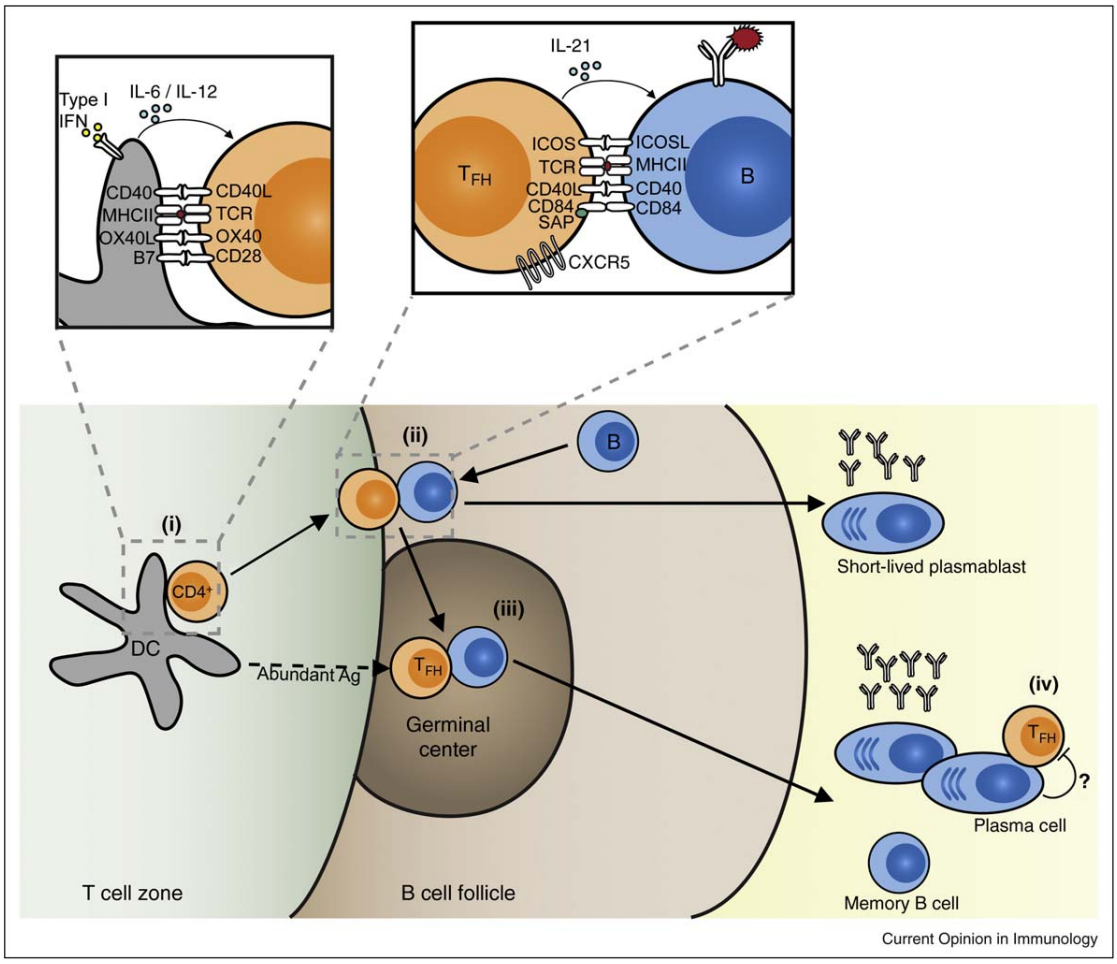
T follicular helper cells – a distinct subset of effector CD4$^+$ T cells

The term 'T_{FH} cell' was coined in 2000 to describe a subset of CD4$^+$ T cells in human tonsils that expressed the B-cell follicle homing chemokine receptor CXCR5 [15,16]. Acquisition of CXCR5, and concomitant loss of CCR7, allows T_{FH} cells to re-locate to follicles [17–20] that, together with acquisition of numerous effector molecules, allows them to directly support B-cell expansion and differentiation [15,16,21,22,23••]. Over the past decade, several cardinal features of T_{FH} cells have been determined. For the purpose of this review, we consider these defining features to be (1) a unique phenotype, with increased expression of CXCR5, ICOS, PD-1, CD200, BTLA, OX40 and SLAM-associated protein (SAP), and downregulation of CCR7 and CD127 (IL-7Rα); (2) production of high amounts of the B-cell stimulatory cytokine IL-21; (3) expression of the transcription factor *Bcl6*; and, probably most importantly, (4) localization within B-cell follicles [15,16,19–22,23••,24••,25–27] (Figure 1).

Cellular interactions underlying T_{FH} cell development

Ag-specific CD4$^+$ T cells undergo initial activation following receipt of signals usually provided by DCs within the T-cell zone of secondary lymphoid tissues. Activated CD4$^+$ T cells can then localize to follicles where they interact with rare Ag-specific B cells [8]. The initial commitment and subsequent differentiation of naïve CD4$^+$ T cells to the T_{FH} lineage is dependent upon multiple signals provided by these populations of APCs. Thus, as well as engagement of the T cell receptor (TCR), numerous receptor/ligand interactions between

Figure 2



T_{FH} cell generation. (i) Naïve $CD4^+$ T cells are activated when they recognize peptide–MHC class II complexes on DCs within the T-cell zone. DCs provide co-stimulatory signals such as CD28/B7 and OX40/OX40L and the cytokines IL-6 (in mice) and IL-12 (in humans). CD40/CD40L interactions and type I IFN production result in enhanced activation of DCs and increased expression of molecules such as IL-6, IL-12 and OX40L. These activating signals provided by the DCs upregulate CXCR5 and downregulate CCR7 on the $CD4^+$ T cells allowing them to migrate to the follicle. (ii) At the T–B border T cells interact with activated B cells presenting cognate Ag. This results in the $CD4^+$ T cells delivering help to the B cells via CD40L and IL-21, as well as T-cell co-stimulation via ICOS–ICOS-L interactions. The delivery of these signals is dependent on the formation of stable T–B conjugates, which requires CD84–SAP interactions. Ongoing Ag stimulation provided by the B cells drives the development of T_{FH} cells. In the presence of abundant Ag, however, continual Ag stimulation can be provided by DCs circumventing the requirement for B-cell presentation. (iii) Following interactions at the T–B border B cells can differentiate into short-lived extrafollicular plasmablasts or enter into a GC. Within the GC T_{FH} cells continue to provide help to the B cells, supporting the GC reaction and allowing for the generation of long-lived plasma cells and memory B cells. Presumably, reciprocal signals provided by the B cells are also crucial for sustaining the T_{FH} cells. (iv) Plasma cells can also present Ag to $CD4^+$ T cells, however this presentation seems to inhibit – rather than promote – T_{FH} cells. This may act to downregulate the T_{FH} response although when this would operate and the molecular mechanisms involved remains to be determined.

$CD4^+$ T cells and APCs, as well as APC-derived cytokines, have been implicated in T_{FH} cell generation (Figure 2).

Receptor/ligand interactions

Initial studies demonstrated that OX40 signaling in $CD4^+$ T cells upregulated CXCR5 expression [18,28,29] resulting in migration of activated $CD4^+$ T cells to the border of the T-zone and the B-cell follicle [28–30] (Figure 2). Movement of activated $CD4^+$ T cells to the follicle was exaggerated in mice transgenic for OX40L expression on $CD11c^+$ DCs, highlighting the importance of OX40/OX40L interactions between $CD4^+$ T cells and DCs [30]. Follicular localization of activated $CD4^+$ T cells also requires CD40 expression on DCs [28]. Interestingly, impaired trafficking of $CD4^+$ T cells to B-cell follicles in the absence of CD40 on DCs could be overcome by an OX40 agonist [28], suggesting that upregulation of

OX40L on DCs is secondary to CD40/CD40L interactions [30,31]. The effects of OX40 were abrogated in *Cd28*-deficient mice, indicating the importance of CD28 signaling in the early phase of T_{FH} cell formation [29]. These findings are consistent with decreased numbers of T_{FH} cells in *Cd28*$^{-/-}$ and *Cd40*$^{-/-}$ mice [32] and *CD40LG*-deficient humans [33]. Notably, dependence on OX40/OX40L during T_{FH} formation can be influenced by the mouse strain, lymphoid organ involved and route of immunization [32], thereby suggesting some redundancy in the requirement for OX40 signaling in this process. Thus, defects observed in the absence of CD28 and CD40 probably reflect effects in addition to regulating OX40 expression. Nevertheless, these studies highlight the importance of CD28/B7 interactions in the initial activation of CD4$^+$ T cells, and conversely CD40/CD40L interactions in the activation and 'licensing' of APCs. Abrogation of these signaling pathways are likely to result in general defects in CD4$^+$ T cell activation regardless of their ultimate effector fate.

B cells are also important for T_{FH} cell formation, because a marked deficiency is observed in B-cell deficient mice [20,26]. The central role for B cells in mediating T_{FH} generation is supported by studies reporting that the conversion of Tregs into T_{FH}-like cells in Peyer's patches [34], or Th2 cells into T_{FH} cells in the lymph nodes of mice infected with parasites [35], was abolished in B-cell deficient mice. Similarly, *in vitro* culture of human activated CD4$^+$ T cells with autologous B cells induced features of T_{FH} cells, including sustained ICOS and OX40 expression, and heightened production of IL-10 [36]. T_{FH} formation was also disrupted in mice whose B cells lacked key molecules involved in mediating their function or cognate interactions with CD4$^+$ T cells (i.e. CD19, CD40, MHC class II and ICOS-L) [12,20,24••]. These findings demonstrated that T_{FH} cell genesis was critically dependent upon interactions between activated CD4$^+$ T cells and Ag-presenting B cells via molecules that underpin TD immune responses and GC formation. Interestingly, while CD28/B7 interactions are required early during DC-priming of pre-T_{FH} cells and the initiation of TD Ab responses, they are not necessary at later time points to maintain a GC [37]. Instead, ICOS/ICOS-L interactions appear to be central to B-cell mediated T_{FH} cell induction [12], rather than initial modulation of expression of trafficking receptors and recruitment of CD4$^+$ T cells to follicles [38•] (Figure 2). This finding probably explains the deficiency of T_{FH} cells in ICOS/ICOS-L-deficient mice [32,33], and of recirculating CD4$^+$CXCR5$^+$ T cells in patients with *ICOS* mutations [33]. The dependence of T_{FH} cell formation on ICOS/ICOS-L interactions reflects the T-cell intrinsic requirement for ICOS-mediated activation of the phosphoinositide-3-kinase (PI3K) signaling pathway, since mutating the PI3K binding site in the cytoplasmic domain of ICOS [39•], deleting the p110δ isoform of PI3K from T cells, or inhibiting its function [38•] severely impeded T_{FH} cell differentiation, GC formation and humoral immune responses. These studies also suggested that ICOS/PI3K-signaling was important for the acquisition of key T_{FH} features, including *IL4*, *IL21* and *cmaf* [38•,39•].

While the critical roles of ICOS and CD40L in TD B cell differentiation and T_{FH} cell formation are well established [13,14], it is clear that interactions between Ag-specific CD4$^+$ T cells and B cells mediated by the SLAM family of surface receptors are also crucial for humoral immunity. The SLAM family includes SLAM, CD84, 2B4, Ly9 and NTB-A (Ly108 in mice), and these receptors elicit intracellular signaling by recruiting the cytoplasmic adaptor molecule SAP [40]. SAP-deficient mice have impaired GC formation accompanied by defective T_{FH} cell development in many, but not all, situations [24••,41,42,43••,44••,45,46]. The gene encoding SAP is mutated in X-linked lymphoproliferative disease [40] and T_{FH}-like function is impaired in these individuals [47]. It is now apparent that the T_{FH} deficiency in the absence of SAP is secondary to its requirement in CD4$^+$ T cells to form stable conjugates with B cells, but not DCs [43••,44••], and thus receive ongoing stimulation from Ag-presenting B cells [24••]. Recent work demonstrated that CD84 – possibly in concert with Ly108 – is the primary SLAM family member expressed by CD4$^+$ T cells responsible for mediating stable conjugate formation with B cells, which then promotes efficient T_{FH} development *in vivo* [44••] (Figure 2).

Cytokines

There is also evidence to suggest a key role for APC-derived cytokines in T_{FH} cell generation. Abolition of type I IFN-mediated signaling reduced T_{FH} formation. Intact IFN signaling was required in CD11c$^+$ DCs, rather than other APCs (B cells, macrophages) or CD4$^+$ T cells themselves [48•], and functioned by inducing IL-6 production [48•] (Figure 2). These findings are consistent with independent studies demonstrating that: (1) type I IFNs promote humoral immune responses in a manner requiring IFN signaling in DCs [49]; (2) IL-6 supported the differentiation of murine T_{FH}-like cells *in vitro* [27,50•,51•,52•] and (3) deletion of IL-6, or STAT3 which signals downstream of the IL-6R, hindered T_{FH} development *in vivo* [12]. However, the effect of IL-6/STAT3-deficiency on T_{FH} development in these studies was not absolute, since low numbers of these cells were still observed in gene-targeted mice [12]. Furthermore, other groups have reported intact T_{FH} cell formation in the absence of IL-6 or STAT3 [50•,53]. Curiously, one of these studies did report a deficit in B-cell helper function in the absence of IL-6, however this defect was independent of CD4$^+$CXCR5$^+$ T cells, which were generated normally in IL-6-deficient mice [50•].

Investigation of factors mediating the differentiation of human $CD4^+$ T cells into T_{FH}-like cells also revealed an important role for cytokines [23••,54••,55••]. IL-12, produced by activated DCs, was identified as the most efficient inducer of IL-21 expression in human naïve $CD4^+$ T cells [23••,54••] (Figure 2). IL-12-derived IL-21^+ human $CD4^+$ T cells resembled T_{FH} cells inasmuch that they expressed elevated levels of CXCR5 and ICOS, supported Ab production *in vitro* [23••,54••,55••] and produced CXCL13, a feature of human T_{FH} cells [55••]. IL-6 and IL-23 also induced IL-21 expression by human $CD4^+$ T cells, but to a much lesser extent than IL-12 [23••,54••]. These studies confirmed, and clarified the interpretation of, previous studies that found IL-12 and IL-23 maintained expression of ICOS and CD40L on human naïve $CD4^+$ T cells [56,57]. Interestingly, IL-12 is not a strong inducer of IL-21 in murine T cells, highlighting potential species-specific differences in IL-12 function on human and murine $CD4^+$ T cells [12,51•,52•].

T_{FH} cells are also reduced in mice lacking IL-21 or IL-21R [12,58], which is consistent with the ability of IL-21 to induce its own expression in naïve human [23••,54••] and murine [12,27,51•] $CD4^+$ T cells. However, the extent of the T_{FH} deficiency is variable (i.e. 2–5-fold reduction to no effect) depending on the experimental system [59]. Since IL-21 is largely produced by T cells, and not APCs, it is likely to function in an autocrine manner to further instruct DC-primed $CD4^+$ T cells to become a T_{FH} cell. For this reason, the involvement of IL-21 in APC-mediated T_{FH} cell generation will not be further discussed here (see [59] for recent review).

Taken together, these studies provided a foundation for a model whereby cytokines – predominantly IL-6 for mice and IL-12 (and to a lesser extent IL-23) for humans – produced by DCs, together with specific receptor/ligand interactions (CD40/CD40Land OX40/OX40L) provide a stimulatory *milieu* for the initial priming of naïve $CD4^+$ T cells to re-locate from the T-zone to the B-cell follicle to become pre-T_{FH} cells (Figure 2). Notably, activation of human DCs through CD40 or OX40L induced impressive production of IL-6 and IL-12 [31,54••], thereby providing a source of T_{FH}-inducing cytokines in addition to that mediated by type I IFNs. These activated $CD4^+$ T cells undergo subsequent commitment to the T_{FH} lineage following interactions with B cells via the delivery of CD84/SAP-dependent, CD40/CD40L-dependent and ICOS/ICOS-L-dependent signals (Figure 2). Naïve B cells, but not GC B cells, also produce IL-6 [60], raising the possibility that cytokines released by activated B cells at the T/B border, rather than those in the GC, can shape $CD4^+$ T cell differentiation to a T_{FH} fate. While this model places B cells at the center of T_{FH} cell formation, as discussed below it is possible to circumvent the need for B cells by altering the delivery of Ag [24••].

Prolonged TCR signaling by Ag-presenting cells preferentially induces T_{FH} cell formation

While studies of mice and humans with deficiencies in specific genes have provided evidence for important roles for cell/cell interactions and cytokines in regulating T_{FH} cell formation, Ag affinity and/or prolonged TCR signaling have emerged as important determinants for the differentiation of Ag-primed $CD4^+$ T cells into T_{FH} cells. McHeyzer-Williams and colleagues reported that cells with the highest affinity for Ag are preferentially selected into the T_{FH} compartment [61•]. Similarly, we recently found that boosting ovalbumin-immunized mice with the immunodominant peptide resulted in the generation of an increased frequency of activated T_{FH} cells, associated with greater downregulation of CD127 expression [24••]. Strikingly, this prime-boost regime overcame the obligatory requirement for B cells in generating Ag-specific T_{FH} cells [24••]. The mechanism underlying such B-cell independent T_{FH} generation was prolonged Ag presentation by DC [24••] (Figure 2). Collectively, these findings suggest that $CD4^+$ T cells receiving the strongest or most prolonged Ag-dependent signals are most likely to differentiate into T_{FH} cells.

A major implication of these finding is that activated B cells do not possess a specific signal or combination of signals that uniquely drive T_{FH} differentiation. Rather, other APCs can deliver the requisite signals for T_{FH} generation under certain circumstances. This indicates that it is the context of the responding B cells that underlies their critical role in T_{FH} cell generation – that is they are likely to be the major source of Ag in the microenvironment where specific T cells reside as the immune response progress. Thus, not only are B cells abundant as the T cells move to the T–B border, but their ability to specifically capture Ag via their BCRs may render them the predominant source of peptide/MHC class II, particularly as Ag levels wane.

It is likely that within GCs B cells also play a crucial role in the maintenance of T_{FH} cell responses. T_{FH} cells that persist within the GC will have sustained exposure to Ag on the surface of responding GC B cells, which continue to capture and process Ag displayed on follicular DCs [8,9]. These GC B:T_{FH} cell interactions could promote GC B cell selection as well as T_{FH} cell survival. Dynamic 2-photon intravital microscopy revealed that T_{FH} cells complex not only with responding GC B cells, but also with small 'blebs' derived from apoptotic GC B cells [62]. Since this interaction can have no direct effect on GC B cells, it may provide ongoing survival or stimulatory signals to T_{FH} cells, particularly as Ag becomes more limiting in the GC.

The generation of T_{FH} cells in response to protein Ag is maximal within 5–7 days, and rapidly contracts at subsequent times [24••]. This suggests that the size of the

T_{FH} population is strictly regulated. The requirement for such stringent control is consistent with the involvement of dysregulated T_{FH} cells in the development of autoimmune conditions and T-cell malignancies (reviewed in [14]). One way in which T_{FH} cells may be controlled is via production of specific Ab by PCs that would clear the immunizing/infectious Ag, thereby resulting in termination of Ag presentation as well as dissolution of a GC reaction. Interestingly, recent evidence has proposed a direct role for PCs in inhibiting the T_{FH} cell response [63]. Intriguingly, although PCs retain MHC class II expression and can present Ag to $CD4^+$ T cells, presentation by PCs does not induce features characteristic of T_{FH} cells (e.g. *Bcl6* and *IL21*) (Figure 2). Instead, it appears to extinguish expression of these molecules in existing T_{FH} cells [63]. However, exactly where – within the context of T_{FH} cell positioning – PCs would interact with T_{FH} cells and mechanistically how they would deliver such inhibitory signals remains to be determined.

Conclusions

Studies from the past 5 years have identified some of the critical requirements for regulating the formation and function of T_{FH} cells, and the importance of distinct populations of APCs in providing the requisite stimuli during this process. A picture has now emerged that Ag-specific B cells at different stages of differentiation regulate the T_{FH} compartment in accordance with the requirements of the overall response. Despite this, the role of B cells in generating T_{FH} cells can be circumvented by altering the delivery of Ag and thereby prolonging presentation by DCs, indicating that extended Ag presentation is the critical determinant of T_{FH} generation. That T_{FH} development should be regulated via Ag presentation, a sensitive readout of the progression and effectiveness of the response, makes teleological sense. Confusion still exists, however, as to the relative contributions of cytokines (e.g. IL-6 and IL-21) to T_{FH} generation, as several discrepancies have been reported. It is probable that control of T_{FH} numbers involves the integration of multiple semi-redundant signals, controlling both generation and survival, that are in turn influenced by the initiating immunization. Thus, the differences observed are probably a result of the different experimental systems used, such as the nature of the immunizing Ag or adjuvant. Therefore, it will be important for future studies to systematically compare the requirement for specific cytokines and signaling pathways on T_{FH} cell formation under a diverse range of immunization regimens to more fully dissect requirement for each signal and the level of redundancy that exists in controlling T_{FH} cell formation.

Elucidating the roles of APCs in T_{FH} generation, and identifying surface receptors, soluble molecules and signaling pathways involved in this process, has greatly improved our understanding of the molecular and cellular biology of T_{FH} cells. The next challenge will be to harness this knowledge to enable manipulation of humoral immune responses, such that B-cell differentiation can be improved in settings of vaccine development or immune deficiency, or T_{FH} function attenuated in cases of humoral autoimmunity.

Note added in proof

Marcel Batten and colleagues [64] have recently reported that the heterodimeric cytokine IL-27 potently induces production of IL-21 by murine and human $CD4^+$ T cells in a STAT3-dependent manner. In vivo IL-27R is required on $CD4^+$ T cells for normal TFH cell generation, germinal center formation and Ab responses, thereby revealing a non-redundant role for IL-27 in regulating T-dependent humoral immune responses. These findings may explain the detection of residual TFH cells in the absence of IL-6 signalling.

Conflict of interest

The authors declare no conflict of interest.

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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. Weaver CT, Hatton RD, Mangan PR, Harrington LE: **IL-17 family cytokines and the expanding diversity of effector T cell lineages**. *Annu Rev Immunol* 2007, **25**:821-852.
2. Dong C: **TH17 cells in development: an updated view of their molecular identity and genetic programming**. *Nat Rev Immunol* 2008, **8**:337-348.
3. Boniface K, Blom B, Liu YJ, de Waal Malefyt R: **From interleukin-23 to T-helper 17 cells: human T-helper cell differentiation revisited**. *Immunol Rev* 2008, **226**:132-146.
4. Ma CS, Chew GY, Simpson N, Priyadarshi A, Wong M, Grimbacher B, Fulcher DA, Tangye SG, Cook MC: **Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3**. *J Exp Med* 2008, **205**:1551-1557.
5. Chang HC, Sehra S, Goswami R, Yao W, Yu Q, Stritesky GL, Jabeen R, McKinley C, Ahyi AN, Han L *et al.*: **The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation**. *Nat Immunol* 2010, **11**:527-534.
6. Staudt V, Bothur E, Klein M, Lingnau K, Reuter S, Grebe N, Gerlitzki B, Hoffmann M, Ulges A, Taube C *et al.*: **Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells**. *Immunity* 2010, **33**:192-202.
7. Tangye SG, Tarlinton DM: **Memory B cells: effectors of long-lived immune responses**. *Eur J Immunol* 2009, **39**:2065-2075.
8. Goodnow CC, Vinuesa CG, Randall KL, Mackay F, Brink R: **Control systems and decision making for antibody production**. *Nat Immunol* 2010, **11**:681-688.
9. Vinuesa CG, Linterman MA, Goodnow CC, Randall KL: **T cells and follicular dendritic cells in germinal center B-cell formation and selection**. *Immunol Rev* 2010, **237**:72-89.

10. Kopf M, Le Gros G, Coyle AJ, Kosco-Vilbois M, Brombacher F: **Immune responses of IL-4, IL-5, IL-6 deficient mice**. *Immunol Rev* 1995, **148**:45-69.

11. Cunningham AF, Serre K, Toellner KM, Khan M, Alexander J, Brombacher F, MacLennan IC: **Pinpointing IL-4-independent acquisition and IL-4-influenced maintenance of Th2 activity by CD4 T cells**. *Eur J Immunol* 2004, **34**:686-694.

12. 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.

13. Vinuesa CG, Tangye SG, Moser B, Mackay CR: **Follicular B helper T cells in antibody responses and autoimmunity**. *Nat Rev Immunol* 2005, **5**:853-865.

14. King C, Tangye SG, Mackay CR: **T follicular helper (TFH) cells in normal and dysregulated immune responses**. *Annu Rev Immunol* 2008, **26**:741-766.

15. 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.

16. 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.

17. Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG, Cyster JG: **In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines**. *J Exp Med* 1999, **190**:1123-1134.

18. Flynn S, Toellner KM, Raykundalia C, Goodall M, Lane P: **CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1**. *J Exp Med* 1998, **188**:297-304.

19. 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.

20. 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-1high germinal center-associated subpopulation**. *J Immunol* 2007, **179**:5099-5108.

21. 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.

22. 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.

23. •• Ma CS, Suryani S, Avery DT, Chan A, Nanan R, Santner-Nanan B, Deenick EK, Tangye SG: **Early commitment of naive human CD4(+) T cells to the T follicular helper (T(FH)) cell lineage is induced by IL-12**. *Immunol Cell Biol* 2009, **87**:590-600.

Together with [54••], these studies were the first to show that the cytokine IL-12 is capable of inducing the differentiation of human naïve CD4 into T_{FH} cells. Since IL-12 may not have the same importance in the development of murine T_{FH} cells, these papers highlight potential species-specific differences that exist between humans and mice.

24. •• Deenick EK, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R, Tangye SG: **Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling**. *Immunity* 2010, **33**:241-253.

This paper demonstrates that the requirement for B cells in the generation of T_{FH} cells reflects Ag availability rather than unique B-cell signaling. Thus, when Ag is limited B cells become the primary APCs; however, when Ag is abundant prolonged Ag presentation can be provided by DCs in order to drive T_{FH} development.

25. 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, **31**:457-468.

26. Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B, Dent AL, Craft J, Crotty S: **Bcl6 Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation**. *Science* 2009, **325**:1006-1010.

27. 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.

28. Fillatreau S, Gray D: **T cell accumulation in B cell follicles is regulated by dendritic cells and is independent of B cell activation**. *J Exp Med* 2003, **197**:195-206.

29. Walker LS, Gulbranson-Judge A, Flynn S, Brocker T, Raykundalia C, Goodall M, Forster R, Lipp M, Lane P: **Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers**. *J Exp Med* 1999, **190**:1115-1122.

30. Brocker T, Gulbranson-Judge A, Flynn S, Riedinger M, Raykundalia C, Lane P: **CD4 T cell traffic control: in vivo evidence that ligation of OX40 on CD4 T cells by OX40-ligand expressed on dendritic cells leads to the accumulation of CD4 T cells in B follicles**. *Eur J Immunol* 1999, **29**:1610-1616.

31. Ohshima Y, Tanaka Y, Tozawa H, Takahashi Y, Maliszewski C, Delespesse G: **Expression and function of OX40 ligand on human dendritic cells**. *J Immunol* 1997, **159**:3838-3848.

32. Akiba H, Takeda K, Kojima Y, Usui Y, Harada N, Yamazaki T, Ma J, Tezuka K, Yagita H, Okumura K: **The role of ICOS in the CXCR5+ follicular B helper T cell maintenance in vivo**. *J Immunol* 2005, **175**:2340-2348.

33. Bossaller L, Burger J, Draeger R, Grimbacher B, Knoth R, Plebani A, Durandy A, Baumann U, Schlesier M, Welcher AA *et al.*: **ICOS deficiency is associated with a severe reduction of CXCR5+CD4 germinal center Th cells**. *J Immunol* 2006, **177**:4927-4932.

34. 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.

35. 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.

36. Ebert LM, Horn MP, Lang AB, Moser B: **B cells alter the phenotype and function of follicular-homing CXCR5+ T cells**. *Eur J Immunol* 2004, **34**:3562-3571.

37. Walker LS, Wiggett HE, Gaspal FM, Raykundalia CR, Goodall MD, Toellner KM, Lane PJ: **Established T cell-driven germinal center B cell proliferation is independent of CD28 signaling but is tightly regulated through CTLA-4**. *J Immunol* 2003, **170**:91-98.

38. • Rolf J, Bell SE, Kovesdi D, Janas ML, Soond DR, Webb LM, Santinelli S, Saunders T, Hebeis B, Killeen N *et al.*: **Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction**. *J Immunol* 2010, **185**:4042-4052.

This paper along with [39•] demonstrates the critical role of phosphoinositide 3-kinase signaling downstream of ICOS in the induction of T_{FH} cells and support of germinal centers.

39. • Gigoux M, Shang J, Pak Y, Xu M, Choe J, Mak TW, Suh WK: **Inducible costimulator promotes helper T-cell differentiation through phosphoinositide 3-kinase**. *Proc Natl Acad Sci U S A* 2009, **106**:20371-20376.

This paper along with [38•] demonstrates the critical role of phosphoinositide 3-kinase signaling downstream of ICOS, but not CD28, in the induction of T_{FH} cells and support of germinal centers.

40. Ma CS, Nichols KE, Tangye SG: **Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules**. *Annu Rev Immunol* 2007, **25**:337-379.

41. Crotty S, Kersh EN, Cannons J, Schwartzberg PL, Ahmed R: **SAP is required for generating long-term humoral immunity**. *Nature* 2003, **421**:282-287.

42. Kamperschroer C, Roberts DM, Zhang Y, Weng NP, Swain SL: **SAP enables T cells to help B cells by a mechanism distinct from Th cell programming or CD40 ligand regulation**. *J Immunol* 2008, **181**:3994-4003.

43. •• 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 elegant study employed 2 photon *in vivo* imaging to identify a defect in sustained T-B cell interactions as a mechanism for impaired T_{FH} cell function, trafficking and retnetion within germinal centers, and subsequent defective humoral immune responses, in the absence of SAP. Importantly, initial activation of SAP-deficient $CD4^+$ T cells on DCs and interactions between these cells was unaffected, highlighting distinct requirements for cognate interactions between $CD4^+$ T cells and either DCs (ie SAP-independent) or Agspecific B cells (ie SAP-dependent).

44. •• Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, Wakeland EK, Germain RN, Schwartzberg PL: **Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84**. *Immunity* 2010, **32**:253-265.
This study demonstrated that the SLAM family receptor CD84, upregulated on T_{FH} cells relative to naïve cells, was the predominant SAP-associating receptor required for SAP-dependent adhesion between $CD4^+$ T cells and B cells, and subsequent formation of T_{FH} cells. It was also demonstrated that an additional SLAM family member Ly108 probably acted in concert with CD84 to ensure optimal SAP-dependent interactions between $CD4^+$ T cells and B cells for T_{FH} cell generation.

45. 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.

46. Yusuf I, Kageyama R, Monticelli L, Johnston RJ, Ditoro D, Hansen K, Barnett B, Crotty S: **Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150)**. *J Immunol* 2010, **185**:190-202.

47. Ma CS, Hare NJ, Nichols KE, Dupre L, Andolfi G, Roncarolo MG, Adelstein S, Hodgkin PD, Tangye SG: **Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+ T cells**. *J Clin Invest* 2005, **115**:1049-1059.

48. • Cucak H, Yrlid U, Reizis B, Kalinke U, Johansson-Lindbom B: **Type I interferon signaling in dendritic cells stimulates the development of lymph-node-resident T follicular helper cells**. *Immunity* 2009, **31**:491-501.
This paper implicates type I interferons in the development of T_{FH} cells in the lymph node. Mechanistically, type I interferon signaling in DCs was shown to induce IL-6 production that in turn induced the differentiation of murine $CD4^+$ T cells into T_{FH} cells.

49. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF: **Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo**. *Immunity* 2001, **14**:461-470.

50. • Eddahri F, Denanglaire S, Bureau F, Spolski R, Leonard WJ, Leo O, Andris F: **Interleukin-6/STAT3 signalling regulates the ability of naive T cells to acquire B cell help capacities**. *Blood* 2008, **113**:2426-2433 This study demonstrated that $CD4^+$ T cells that were either obtained from IL-6-deficient mice or lacked STAT3 were impaired in providing help to B cells in vitro. It was subsequently established that IL-6 derived from DCs was required for intact humoral immune responses *in vivo*, and this was achieved through induction of IL-21. Interestingly though this study did not observe a defect in T_{FH} cells, as defined by expression of CXCR5..

51. • Suto A, Kashiwakuma D, Kagami S, Hirose K, Watanabe N, Yokote K, Saito Y, Nakayama T, Grusby MJ, Iwamoto I *et al.*: **Development and characterization of IL-21-producing CD4+ T cells**. *J Exp Med* 2008, **205**:1369-1379 This was the first study to demonstrate that IL-6 and IL-21 induced expression of IL-21 in murine $CD4^+$ T cells, and to characterize IL-21-producing cells at the single cell level and with reference to Th1 and Th17 cells..

52. • Dienz O, Eaton SM, Bond JP, Neveu W, Moquin D, Noubade R, Briso EM, Charland C, Leonard WJ, Ciliberto G *et al.*: **The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells**. *J Exp Med* 2009, **206**:69-78.
Like Ref [50•], this study also demonstrated that the mechanism of action of IL-6 in supporting humoral immune responses *in vivo* was its ability to induce IL-21 production by $CD4^+$ T cells, which acted directly on B cells to induce Ab secretion.

53. Poholek AC, Hansen K, Hernandez SG, Eto D, Chandele A, Weinstein JS, Dong X, Odegard JM, Kaech SM, Dent AL *et al.*: **In vivo regulation of Bcl6 and T follicular helper cell development**. *J Immunol* 2010, **185**:313-326.

54. •• Schmitt N, Morita R, Bourdery L, Bentebibel SE, Zurawski SM, Banchereau J, Ueno H: **Human dendritic cells induce the differentiation of interleukin-21-producing T follicular helper-like cells through interleukin-12**. *Immunity* 2009, **31**:158-169.
Together with [23••], this study was the first to implicate IL-12 in the differentiation of human, but not murine $CD4^+$ T cells into T_{FH} cells. In addition, Schmitt *et al.* convincingly demonstrated that the initial source of IL-12 was likely to be activated (e.g. CD40Land TLR) DCs, thereby providing a context for the induction of IL-12 secretion and subsequent T_{FH} generation.

55. •• Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L, Briere F, Chaussabel D, Zurawski G, Palucka AK *et al.*: **Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells**. *Immunity* 2008, **29**:497-510.
This study systemically dissected the functional differences between distinct subsets of human DCs. It was found that $CD14^+$ dermal DCs more efficiently induced co-cultured $CD4^+$ T cells to provide 'help' to human B cells *in vitro* compared to Langherans cells or $CD1a^+$ DCs also isolated from the dermis. Although this paper did not actually demonstrate that $CD14^+$ DCs directed $CD4^+$ T cells to become T_{FH} cells with respect to expression for CXCR5, Bcl-6 or IL-21, it was reported that these cells produced the highest levels of CXCL13, a feature of human T_{FH} cells (see Refs. [21,22]). Importantly, this study paved the way for Ma *et al.* (Ref. [23••]) and Schmitt *et al.* (Ref. [54••]) to subsequently establish that IL-12 derived from activated DCs was a potent inducer of T_{FH} cell generation from human naïve $CD4^+$ T cells.

56. Peng X, Remacle JE, Kasran A, Huylebroeck D, Ceuppens JL: **IL-12 up-regulates CD40 ligand (CD154) expression on human T cells**. *J Immunol* 1998, **160**:1166-1172.

57. Wassink L, Vieira PL, Smits HH, Kingsbury GA, Coyle AJ, Kapsenberg ML, Wierenga EA: **ICOS expression by activated human Th cells is enhanced by IL-12 and IL-23: increased ICOS expression enhances the effector function of both Th1 and Th2 cells**. *J Immunol* 2004, **173**:1779-1786.

58. 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.

59. Yu D, Vinuesa CG: **The elusive identity of T follicular helper cells**. *Trends Immunol* 2010, **31**:377-383.

60. Burdin N, Galibert L, Garrone P, Durand I, Banchereau J, Rousset F: **Inability to produce IL-6 is a functional feature of human germinal center B lymphocytes**. *J Immunol* 1996, **156**:4107-4113.

61. • 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.
This paper demonstrated that TCR binding affinity is a critical determinant of T_{FH} development. Together with [24••] this supports a picture where continued strong TCR stimulation is central to the generation of T_{FH} cells.

62. Allen CD, Okada T, Tang HL, Cyster JG: **Imaging of germinal center selection events during affinity maturation**. *Science* 2007, **315**:528-531.

63. Pelletier N, McHeyzer-Williams LJ, Wong KA, Urich E, Fazilleau N, McHeyzer-Williams MG: **Plasma cells negatively regulate the follicular helper T cell program**. *Nat Immunol* 2010, In Press.

64. Batten M, Ramamoorthi N, Kljavin NM, Ma CS, Cox JH, Dengler HS, Danilenko DM, Caplazi P, Wong M, Fulcher DA, Cook MC, King C, Tangye SG, de Sauvage FJ, Ghilardi N: **IL-27 supports germinal center function by enhancing IL-21 production and the function of T follicular helper cells**. *J Exp Med*, In Press.