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Minireview

Life of T Follicular Helper Cells

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Antibodies are powerful defense tools against pathogens but may cause autoimmune diseases when erroneously directed toward self-antigens. Thus, antibody producing cells are carefully selected, refined, and expanded in a highly regulated microenvironment (germinal center) in the peripheral lymphoid organs. A subset of T cells termed T follicular helper cells (Tfh) play a central role in instructing B cells to form a repertoire of antibody producing cells that provide life-long supply of high affinity, pathogen-specific antibodies. Therefore, understanding how Tfh cells arise and how they facilitate B cell selection and differentiation during germinal center reaction is critical to improve vaccines and better treat autoimmune diseases. In this review, I will summarise recent findings on molecular and cellular mechanisms underlying Tfh generation and function with an emphasis on T cell costimulation.

INTRODUCTION

T follicular helper cells (Tfh) are a subset of CD4 T cells that have ability to migrate into B cell follicles in the secondary lymphoid organ and facilitate germinal center (GC) reaction (Crotty, 2014; Victora and Nussenzweig, 2012). Within the GC, B cell clones that have specificity to foreign antigens expand, their antibody affinity is enhanced through somatic mutations, and their antibody isotype can be switched. Only these selected B cells become memory B cells or antibody-secreting plasma cells. Importantly, this process is highly regulated by antigen-specific Tfh cells which specifically deliver "help" signals to qualified B cells through cell-cell contact (Fig. 1A). Initially, some naïve T cells become precursor Tfh (pre-Tfh) during interaction with dendritic cells in the T cell zone of secondary lymphoid organ. Guided by chemokine gradients, the pre-Tfh cells migrate to T-B border where they meet cognate B cells (B cells sharing antigen specificity with Tfh cells) and resulting stable T-B conjugates migrate into the germinal center. Within the germinal center, Tfh cells make brief but intimate contact with cognate B cells during which key helper factors can be delivered (Fig. 1B). Since the number of antigen-specific Tfh cells is limited, only B cells that efficiently pick up antigens and present those to cognate Tfh cells survive, expand, and undergo differentiation. The number and activities of Tfh cells are highly regulated failure to do so leads to immunodeficiency or antibody-mediated autoimmune diseases (Pratama and Vinuesa, 2014). Not surprisingly, the generation and function of Tfh cells is controlled at multiple checkpoints along the process of early generation in T cell zone and throughout to the effector phase of T-B interaction within the GC. Since many aspects of Tfh biology have been covered by recent reviews (Crotty, 2014; Liu et al., 2013; Pratama and Vinuesa, 2014; Sweet et al., 2012), this review will focus on T cell costimulatory mechanisms and how they may maximize antibody diversity against foreign antigens while maintaining self-tolerance at each stage of their life: generation of pre-Tfh, guiding them into GC, effector functions of GC Tfh, and generation of potential memory pools.

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STAGE 1: GENERATION OF PRE-T_{fh}

After immunization or infection, a cohort of naïve CD4 T cells in the T cell zone obtain features of pre-Tfh cells after interacting with dendritic cells (Fig. 2A). Since T cells are primed during interaction with dendritic cells in T cell zone and B cells reside in the B cell follicle, antigen-specific T cells and their cognate B cells should migrate within a secondary lymphoid organ to meet each other. Thus, one of the hallmark of Tfh cells is their chemokine receptor profile: sustained expression of CXCR5 (homing receptor to B cell zone) and down regulation of CCR7 (homing receptor to T cell zone), combination of which allows migration of pre-Tfh cells away from T cell zone towards B cell follicle (Breitfeld et al., 2000; Haynes et al., 2007; Kim et al., 2001; Schaerli et al., 2000). Early studies found that Bcl6 is both necessary and sufficient (when ectopically overexpressed) for programming of Tfh including CXCR5 expression (Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009). However, recent work indicates that initial induction of CXCR5 and down regulation of CCR7 is directly controlled by the transcription factor Ascl2 (Liu et al., 2014b) and Bcl6 is crucial for the maintenance of Tfh program including CXCR5 (Liu et al., 2012).

The fate of CD4 T cells into Tfh lineage appears to be determined early during T-DC interaction with an increase of Bcl6 and downregulation of its antagonist Blimp1 (Baumjohann et al., 2011; Choi et al., 2011a). This process is taking place in the T cell zone within 3 days after immunization or infection under the influence of IL-6 and IL-21 (mice) or IL-12 (humans) and costimulation through ICOS and likely CD28 (Choi et al., 2011b; Ferguson et al., 1996; Nurieva et al., 2009; Schmitt et al., 2009). CD4 T cells harboring high affinity TCR appear to preferentially undergo pre-Tfh differentiation over other effector T helper cells

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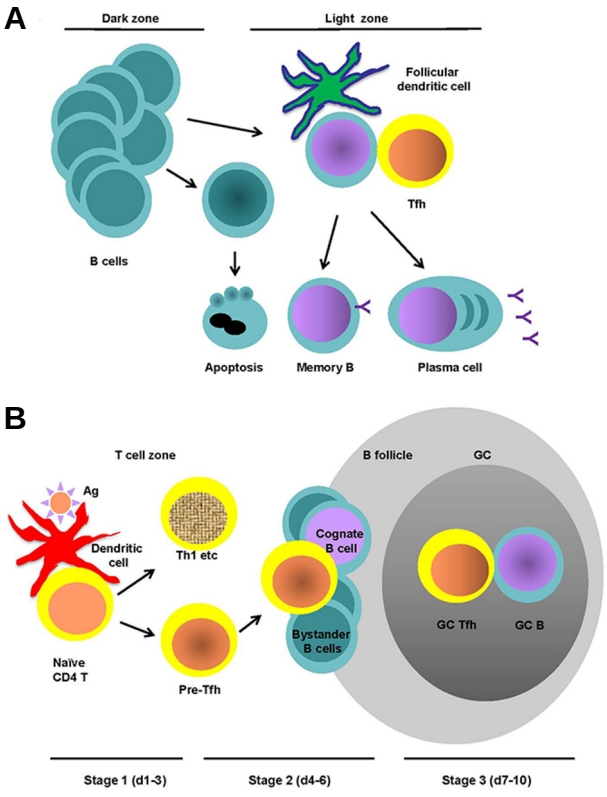

Fig. 1. (A) B cell selection in the GC is facilitated by competition for Tfh. B cells undergo clonal expansion in the dark zone of GC. B cells that have gained high affinity antibody on the surface compete better in the light zone to receive "help" form Tfh and further differentiate into memory B cells or antibody-secreting plasma cells; other clones undergo apoptosis. (B) Life of Tfh. Pre-Tfh cells arise during DC-mediated priming phase under optimal polarization conditions (Stage 1, day 1-3 post-immunization or infection). Pre-Tfh migrate to the B cell follicle guided by chemokine gradient and find cognate B cells in the plethora of non-cognate B cells (Stage 2, day 4-6). Only stable conjugates of T-B pairs sharing antigen-specificity move into the GC. Around day 7-10, mature Tfh cells are found in GC interacting with B cells (Stage 3). Some of the GC Tfh (and pre-Tfh cells) may get into circulation to form memory-like Tfh pool (Stage 4).

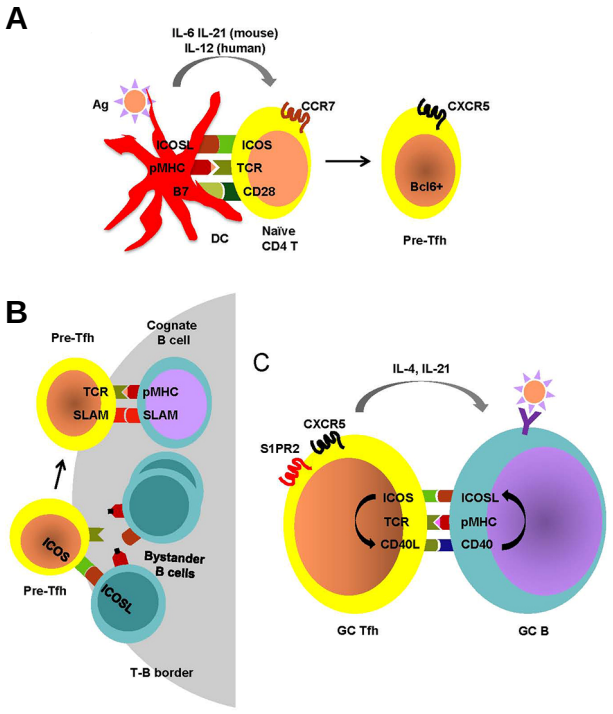

Fig. 2. Molecular components controlling Tfh generation and function. (A) CD4 T cells possessing higher affinity TCR under the influence of costimulation (CD28 and ICOS) and cytokine milieu (IL-6 and IL-21 in mice; IL-12 in humans) become pre-Tfh (Bcl6+, CXCR5+, CCR7low). (B) Pre-Tfh should continuously move around in the T-B border until they encounter cognate B cells. ICOSL-expressing non-cognate B cells facilitate the motile behavior of pre-Tfh at the T-B border in an antigen-independent manner. Afterwards, SAP-dependent signaling induced by SLAM family receptors (such as CD84) promote the formation of stable T-B conjugates. Defects in any of these two components stall pre-Tfh at the T-B border and GC reaction is severely affected. (C) Mature GC Tfh cells (Bcl6++, CXCR5++, PD-1++) make rather short-lasting yet entangled contact with cognate B cells in the GC. At this time, ICOS and CD40 signaling axes form a feedforward circuit that should allow focused delivery of key helper cytokines such as IL-4 and IL-21. GC Tfh cells can visit other GCs but the residence time of GC Tfh in an individual GC can be regulated by cooperative functions of CXCR5 and S1PR2.

(Fazilleau et al., 2009; Tubo et al., 2013).

However, establishment of Tfh program concomitant to higher expression of Bcl6 and PD-1 and establishment of IL-21 expression takes place at later times (day 4 -7) during T-B interaction; without B cells pre-Tfh cells disappear (Baumjohann et al., 2011; Choi et al., 2011b; Goenka et al., 2011; Haynes et al., 2007). Since augmenting antigen presentation through repeated peptide injections (Deenick et al., 2010) or chronic viral infection (Fahey et al., 2011) bypass the requirement of B cell for formation of Tfh population, this B cell dependency may simply reflect that B cells become the major antigen presenting cells in most cases. It is intriguing to see if this B-independent Tfh maturation, especially during chronic infection, may promote generation of autoantibodies.

CD28 and ICOS-mediated costimulation plays an important role for the formation of Tfh cells and GC reaction and likely to be involved in pre-Tfh generation (Choi et al., 2011b; Ferguson et al., 1996; Linterman et al., 2009; Suh et al., 2004). The impact of CD28 appears to be prominent in the early phase of Tfh generation (Walker et al., 2003). In contrast, ICOS is crucial for generation of pre-Tfh (Choi et al., 2011b) as well as later stages of Tfh (Liu et al., 2014a; Xu et al., 2013) (see below). One obvious prediction was that CD28-mediated costimulation promotes Tfh generation through augmented expression of IL-2 that promotes clonal expansion. However, recent results argue against this notion: strong IL-2-STAT5 signaling suppresses Tfh generation and GC reaction (Ballesteros-Tato et al., 2012; Johnston et al., 2012; Leon et al., 2014; Nurieva et al., 2012). Thus, it appears that Tfh generation will be favored by optimal CD28 costimulation with balanced clonal expansion (IL-2 dependent) as well as IL-2-independent CD28 costimulatory effects such as enhancement of CXCR5 and ICOS expression (McAdam et al., 2000; Walker et al., 1999). Normally, ICOS has a limited ability to compensate CD28-deficiency (Suh et al., 2004); however, ICOS can fully rescue Tfh defect in CD28-deficint mice if it is

excessively upregulated by a mutation in Roquin system which regulates steady-state level of ***Icos*** mRNA (Linterman et al., 2009).

It became clear that Tfh is a distinct T cell subset based on its distinct gene expression profiles (Chtanova et al., 2004; Kim et al., 2004; Rasheed et al., 2006) and the presence of "master" regulator Bcl6 which can drive Tfh formation independently of , and even competing with, other key regulators of T helper subsets: T-bet (for Th1), GATA-3 (for Th2), or RORγt (Th17) (Johnston et al., 2009; Nurieva et al., 2008; 2009; Yu et al., 2009). However, most helper T cell subsets maintain their diversity and plasticity by co-expression of mater regulators that interact with each other and Tfh is not an exception (Nakayamada et al., 2012). First, it was found that CD4 T cells undergoing early stage of Th1 polarization do express a low amount of Bcl6 and other Tfh markers but repressed by T-bet along the establishment of Th1 program (Nakayamada et al., 2011). Interestingly, during chronic viral infection, Th1 (CD4+ CXCR5- T-bet+ IFN-γ+) cells can convert to functional Tfh provided that they receive persistent TCR signaling (Fahey et al., 2011). Similarly, ***in vitro*** polarised Th2 cells (CD4+ CXCR5- PD-1- IL-4+) can convert to Tfh ***in vivo*** (Zaretsky et al., 2009) and IL-4 expressing Tfh are generated during parasite infection that are known to induce strong Th2 responses (King and Mohrs, 2009; Reinhardt et al., 2009; Zaretsky et al., 2009). Although, there is no direct evidence that polarised Th17 cells can convert to Tfh, Tfh and Th17 cells depend on IL-6 for differentiation and produce IL-21 as a signature cytokine suggesting their close relationship. Keeping in line with these, circulating Tfh-like cells in human blood can be divided into Th1, Th2, and Th17 subtypes based on master regulators and chemokine receptors they express (Morita et al., 2011).

In summary, during a protein immunization or a cute infection, pre-Tfh fate is determined early (within 3-days) during DC-mediated priming followed by establishment of Tfh program through interaction with B cells. However, persistent antigenic exposure or chronic infections may recruit polarized effector helper T cells into Tfh pathway which can bypass B cell-mediated checkpoint.

STAGE 2: GUIDING PRE-Tfh INTO GC

Primed Tfh cells and antigen-stimulated B cells migrate to T-B border where Tfh and B cells sharing antigenic specificity (i.e., cognate T-B pairs) make stable conjugate and move into the GC (Fig. 2B). Two T cell costimulatory mechanisms come into play to guide nascent Tfh cells into the GC: ICOS and SAP. First, ICOSL expressing bystander B cells keep pre-Tfh cells motile in the plethora of bystander B cells until they find cognate B cells (Xu et al., 2013). The motility of pre-Tfh cells depend on dynamic cytoskeletal remodeling induced by ICOS-mediated PI3K activation. Importantly, overexpression of CXCR5 or Bcl6 could not overcome lack of ICOS-ICOSL interaction indicating that the role of ICOS is not simply maintaining high levels of CXCR5 or Bcl6. Once T cells encounter cognate B cells in the T-B border, stable T-B conjugates are formed and move together into the GC but T cells that fail to find the B cell partner accumulate in T-B border. The formation of stable T-B conjugates is promoted by SLAM family receptors that signal through the adaptor protein SAP (Cannons et al., 2010; Qi et al., 2008; Schwartzberg et al., 2009). Thus, in the absence of SAP, pre-Tfh formation is intact but they fail to get into the GC due to reduced ability to make conjugates with cognate B cells (Qi et al., 2008).

A body of evidence indicate that the phosphoinositide 3-kinase (PI3K) plays crucial role in Tfh generation possibly by multiple mechanisms. We have shown that ICOS is a potent activator of PI3K and selective abrogation of ICOS-PI3K signaling drastically reduced Tfh formation and GC reaction (Gigoux et al., 2009). Consistent with this, T cell-specific ablation of p110δ catalytic subunit reduced Tfh numbers and deletion of PTEN gene in T cells did the opposite (Rolf et al., 2010). Mechanistically, ICOS-induced PI3K activity keeps pre-Tfh cell motile in the T-B border to facilitate cognate T-B pairing (Xu et al., 2013). ICOS-PI3K pathway also has additional role which can be more important in transition of pre-Tfh to GC Tfh as well as effector function of GC Tfh: it augments IL-4 and IL-21 mRNA in activated CD4 T cells (Gigoux et al., 2009; Rolf et al., 2010) and acutely promotes IL-4 protein synthesis through mTOR pathways (Gigoux et al., 2014).

The importance of PI3K signaling pathway in Tfh generation was reinforced by the finding that T-cell specific deletion of microRNA miR-17~92 severely reduced generation of Tfh and GC reaction (Kang et al., 2013). miR-17~92 is induced at the early stage of CD4 T cell activation and it regulates PI3K signaling intensity in Tfh cells through downregulation of PHLPP2, a phosphatase that negative regulates Akt activity.

STAGE 3: GC Tfh

Accumulating evidence suggest that there is a limited number of antigen-specific Tfh cells within the GC light zone and competition for T cell help is the key factor for the selection of high affinity B cells; B cells that efficiently present antigens to cognate Tfh cells get selected and others die (Allen et al., 2007; Victora and Nussenzweig, 2012; Victora et al., 2010). Two recent ***in vivo*** imaging studies revealed the nature of T-B interaction within the GC (Liu et al., 2014a; Shulman et al., 2014). Both studies show that a single T cell makes contact with multiple B cells with short durations, mostly 2-5 min, but can be extended up to 30 min when B cell antigen presentation is maximized (Shulman et al., 2014). When Tfh cells meet cognate B cells, they increase the contact surface area, prolong the contact time, and release intracellular calcium. This led to co-expression of key helper cytokines IL-4 and IL-21 at the mRNA level (Shulman et al., 2014) as well as relocation of CD40L to the cell surface (Liu et al., 2014a). ICOS on Tfh cells and ICOSL on GC B cells appear to provide important route for T-B communication during this interaction (Liu et al., 2014a) (Fig. 2C). Tfh cells release intracellular calcium in a B cell ICOSL-dependent manner to enhance surface CD40L, which in turn stabilizes ICOSL level on the surface of cognate B cells. This feedforward mechanism provides competitive advantage for GC B cells allowing generation of high affinity antibodies. Several early observations qualify ICOS-ICOSL signaling pairs to fulfill this conduit function. First, we and others have shown that ICOS can potentiate TCR-mediated calcium flux (Gigoux et al., 2009; Nurieva et al., 2007) in a PI3K-independent manner (Gigoux et al., 2009). Second, ICOSL is readily downregulated by B cell mitogenic signals or ICOS-binding unless CD40 signal (e.g., by CD40L from cognate Tfh cells) or TLR signals are given (Liang et al., 2002; Logue et al., 2006; Watanabe et al., 2008). Third, we have shown that ICOS can promote translation of IL-4 mRNA through PI3K-mTOR-4EBP signaling pathway in the context of short ***in vitro*** activation (Gigoux et al., 2014). Collectively, ICOS appears to be best suited to potentiate TCR-mediated effector functions in Tfh cells by acutely regulating relocation of CD40L and promoting immediate cyto-

kine synthesis for targeted delivery to cognate B cells.

In addition to ICOSL, CD80 (B7.1) and/or CD86 (B7.2) may play important role during T-B collaboration beyond pre-Tfh generation by dendritic cells. Initially, it has been shown that CD80 and CD86 have overlapping compensatory role in GC reaction and Ab class switch and this was thought to be due to impaired T cell priming through CD28-B7 interaction during T-DC interaction (Borriello et al., 1997). However, recent work demonstrated that B cell-intrinsic expression of CD80 reduces GC B cell survival and generation of long-lived plasma cells possibly through reduced Tfh number and function in a protein immunization model (Good-Jacobson et al., 2012). In a vaccinia virus infection model, CD86 deficiency (but not CD80 deficiency) in B cells reduced selectively Tfh generation without affecting Th1 response, decreased GC B cell and plasma cell differentiation (Salek-Ardakani et al., 2011). Importantly, transient activation of CD28 using agonist antibodies rescued Tfh generation but failed to reverse GC B cell defects supporting the role of CD86 in the later phase of T-B interaction in the GC. In keeping with this notion, CD86 is selectively upregulated on the surface of B cells of the GC light zone where GC B cells interact with GC Tfh (Victora et al., 2010). Thus, CD28-B7 interaction may provide base-line costimulatory signals and ICOS-ICOSL provides extra help for B cells destined to be selected. However, more work is required to define the relative contributions of ICOS and CD28 pathways in T-B interaction within the GC.

It has been established that GC B cells are clonally restricted within individual GC (Kuppers et al., 1993; Liu et al., 1991). This may prevent competition between GCs allowing diversity in differentiating B cells. Does a similar physical containment of Tfh clones within a GC boundary exist? ***In vivo*** imaging of photoconverted Tfh cells demonstrated that, unlike GC B cells, clonal Tfh cells can leave GC and visit B cell follicles and neighboring GCs but rarely get into circulation (Shulman et al., 2013). It seems likely that this exchange of Tfh cells between GCs within a draining LN facilitates diversification of antibody and memory B cell repertoire. The same study has shown that newly activated Tfh cells can enter existing GC and support B cell differentiation opening up a possibility that late-coming helper T cells during immune reaction boost or prolong established GC reaction possibly covering antigenic variants. Although Tfh cells freely move between GCs their mobility is much slower compared the bulk flow of naïve T cells (Lo et al., 2005) suggesting mechanisms that restrict premature egress of Tfh out of GC. One such a mechanism can be provided by sphingosine-1-phosphate receptor 2 (S1PR2), an inhibitory chemokine receptors highly expressed in Tfh cells and known to suppress CXCL12/13-mediated migration (Moriyama et al., 2014). In S1PR2-deficient mice, T cells obtained all the features of Tfh but their accumulation in the GC was substantially reduced. Early studies have shown that accumulation of CXCR5-deficient T cells is reduced approximately two-fold (Arnold et al., 2007; Junt et al., 2005; Haynes et al., 2007). This defect was further accentuated in CXCR5-S1PR2 double knockout mice (Moriyama et al., 2014). Therefore, it appears that there are overlapping chemotactic mechanisms that control GC entry/residence of Tfh. It is conceivable that dynamic changes of chemokine receptors may determine the GC residence time of Tfh cells and this may depend on the nature of adjuvant, pathogens, and GC B cell antigen specificity and affinity.

STAGE 4: MEMORY T_{fh}?

One intriguing open question is if Tfh cells remain as memory cells in the lymphoid organs or in blood after germinal centers are resolved. One study have shown that Tfh can remain in the draining LN of primary infection in mice and give rise to quicker and more robust response to a second challenge (Fazilleau et al., 2007). However, these Tfh cells may represent persistent Tfh cells - as opposed to true memory Tfh cells - since there was continuous antigen supply in the local environment in this study. It has been shown that Tfh cells as marked with IL-21-GFP reporter gene, when adoptively transferred to naive hosts give rise to more robust Tfh responses compared to non-Tfh cells (Liu et al., 2012; Luthje et al., 2012). However, "memory" Tfh cells showed remarkable plasticity to become conventional T helper subsets. Thus, it appears that CD4 T cells that underwent Tfh program preferential reactivate its Tfh program upon rechallenge but still maintain the capacity to become non-Tfh cells.

In an evolutionary point view, generating and maintaining memory Tfh cells may not provide significant advantage to host protection against secondary pathogenic infection. A successful round of GC reaction generates both long-lived plasma cells and memory B cells. Long-lived plasma cells home to bone marrow and supply circulating antibodies for protection (Oracki et al., 2010). Memory B cells of GC origin equipped with high affinity antigen receptors will remain in the secondary lymphoid organs as well as in circulation as sentinels (Shlomchik and Weisel, 2012). Importantly, upon antigenic rechallenge, these memory B cells can terminally differentiate into plasma cells or initiate GC reaction (Zuccarino-Catania et al., 2014). Furthermore, a population of memory B with germline B cell receptor can be generated outside of GC and co-exist with memory B cells of GC origin and participate in recall responses (Kaji et al., 2012; 2013). Potentially these unmutated memory B cells can better cope with antigenic variants than highly mutated memory B cells of GC origin. The observation that persisting CD4 T cells maintaining Tfh phenotypes after GC reaction can readily convert to conventional T helper subsets may reflect a loose selection pressure to maintain memory Tfh reservoir during evolution.

Whether human blood contains memory Tfh cells is an important question since it can provide biomarkers for vaccine efficacy and progression of autoimmune diseases. An early study showed that CD4+ CXCR5- cells contain tetanus toxin specific- memory cells (Breitfeld et al., 2000). In contrast, recent works indicate that subsets of CD4+ CXCR5+ T cells have phenotypic and functional equivalents of lymphoid Tfh cells and there abundance correlates with the concentration of protective or autoimmune antibodies (Bentebibel et al., 2013; He et al., 2013; Locci et al., 2013; Morita et al., 2011). However, whether these cells truly reflect memory Tfh cells or helper T cells that have been periodically exposed to antigens remain arguable and most studies failed to establish precursor-product relationship. It is reported that, both in mice and humans, early Tfh-like cells (CD4+ CXCR5+ PD-1hi CCR7low) that are generated in a Bcl6 and ICOS-dependent yet SAP-independent manner get into circulation and support GC reaction in distal draining LN (He et al., 2013). These cells may boost systemic production of protective antibodies in the face of rapidly spreading pathogens but may also promote progression of autoimmunity. Much work is required to test these ideas. Meanwhile, monitoring these circulating Tfh cells in human blood may facilitate vaccine development and diagnosis of autoimmune diseases.

CONCLUSION

Over the past decade, we gained a great deal of insight into

molecular and cellular mechanisms as to how Tfh cells arise and how they facilitate B cell selection and differentiation in the GC. It remains to be seen if Tfh cells play dominant roles in determining B cell fates into memory B vs. plasma cells, and if so, how. Also, it is yet to be established if a memory pool of Tfh cells is generated during GC reaction, and, if it is the case, to what extent they contribute to host defense and/or progression of autoimmunity.

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