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FOLLICULAR HELPER T CELLS: LINEAGE AND LOCATION

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Abstract

Follicular helper T (T_{FH}) cells are the class of effector T_H cells that regulates the stepwise development of antigen-specific B cell immunity in vivo. Deployment of $CXCR5^+$ T_{FH} cells to B cell zones of lymphoid tissues and stable cognate interactions with B cells are central to the delivery of antigen-specific T_{FH} function. Recent advances help to unravel distinctive elements of developmental programming for T_{FH} cells and unique effector T_{FH} functions focused on antigen-primed B cells. Understanding the regulatory functions of T_{FH} cells in the germinal center and the subsequent regulation of memory B cell responses to antigen recall represent the frontiers of this research area with the potential to alter fundamentally the design of future vaccines.

INTRODUCTION

Clonal selection and the programmed development of antigen-specific immune function are the central defining characteristics of adaptive immunity. Classical studies identified the cells of the immune system as the fundamental unit of clonal selection (Burnet, 1957; Talmage, 1957) and evidence for the production of antibodies (Fagraeus, 1948) with single specificities by individual lymphocytes (Nossal, 1959; Raff et al., 1973) as the operative effector mechanism. At least two types of lymphocytes (Miller, 1961) comprised the responding cellular compartment with T cells enhancing the production of antibodies by B cells (Claman et al., 1966; Miller and Mitchell, 1967). Early studies using hapten-carrier conjugates (Katz et al., 1970; Mitchison, 1971; Paul et al., 1966) postulated the existence of an antigen-bridge for T–B co-operation (Rajewsky et al., 1969) and helped to establish the basic tenets of ‘cognate’ help for antigen-specific B cell immunity.

It is now clear that antigen-specific T_H cell development can proceed in multiple directions depending on the nature of the antigenic assault. The original T_H1/T_H2 paradigm (Mosmann and Coffman, 1989) identified distinguishable T_H cell functional programs based on differential cytokine production with distinct cellular targets of action in vivo (Zhu and Paul, 2008). More recently, multiple subsets of regulatory T_H (T_{reg}) cells have been described as negative regulators of immune responsiveness to inhibit self-reactivity or guard against over-reactivity to pathogens (Sakaguchi, 2004). The T_H17 cell subset adds a new layer to this complex system of immune regulation identifying separable developmental programs and cytokine profiles associated with chronic inflammatory disease and autoimmunity (Korn et al., 2009). There also exist less well-defined T_H cell subsets capable of modifying DC maturation in ways that impact the development of effective $CD8^+$ T cell memory (Janssen et al., 2003). In this context, follicular helper T (T_{FH}) cells can be considered a separable T_H cell subset specialized to regulate the evolution of effector and memory B cell responses (Fazilleau et al.,

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2007c; King et al., 2008; Vinuesa et al., 2005b). How the T_{FH} cell compartment develops in vivo and differs from other subsets of effector T_H cells is the subject of the current review.

Recent evidence suggests that T_{FH} cells constitute a separate lineage of effector T_H cells with distinct developmental programming and distinguishable effector function. There is also evidence to suggest that deployment of all effector T_H cell subsets to appropriate follicular locations defines a unique set of effector T_{FH} cell functions. We will present both positions and suggest that T_H lineage differentiation and the programming of follicular location define multiple subsets of effector T_{FH} cells needed to regulate antigen-specific B cell immunity. The regulation of antibody isotype across both effector and memory B cell development is one heterogeneous facet of T_{FH} function that will be discussed in more detail. Furthermore, the distinction between pre-germinal center (GC) effector T_{FH} and GC T_{FH} cell function needs more clarity and is an important area of current research that will be discussed below. Finally, the maintenance and function of antigen-specific memory T_{FH} cells that regulate memory B cell responses is a new emerging area of research that will be discussed in the last section of the review.

T_H CELL REGULATED B CELL IMMUNITY

It is important to consider the temporal and spatial cellular dynamics that accompanies T_H cell regulated B cell immunity (MacLennan, 1994; McHeyzer-Williams and McHeyzer-Williams, 2005). Specific recognition of peptide MHCII (pMHCII) complexes with threshold TCR affinity and adequate co-stimulation (Checkpoint IA) controls antigen-specific T_H clonal selection, responder T_H cell expansion and effector T_H cell differentiation. Naive B cells will also encounter antigen in draining LNs very early after initial antigen priming (Checkpoint IB) (Batista and Harwood, 2009). Antigen-specific B cells will internalize antigen, process and present pMHCII complexes and move to the T–B borders of LNs to receive help from pMHCII-specific T_H cells (Checkpoint II) (Allen et al., 2007a). Under the cognate control of effector T_H cells, antigen-specific B cell development then divides into two major pathways, plasma cell (PC) versus germinal center (GC) development. The spectrum of effector T_H cell activities delivered at this major developmental juncture belongs to the pMHCII-specific effector T_{FH} cell compartment. The GC supports somatic diversification of BCR and selection of high affinity variants into the memory B cell compartment (MacLennan, 1994; McHeyzer-Williams and McHeyzer-Williams, 2005). Follicular dendritic cells (FDC) present antigen to survey variant BCR for effective antigen binding affinity (Checkpoint IIIA) while pMHCII-specific GC T_{FH} cells form stable cognate conjugates with antigen-specific GC B cells (Checkpoint IIIB) (Allen and Cyster, 2008; Hauser et al., 2007b). The functions of pMHCII-specific GC T_{FH} cells are fundamentally distinct from pre-GC effector T_{FH} cells and thought to regulate clonal selection GC B cells and entry into the memory B cell compartment.

$CXCR5^+$ FOLLICULAR HELPER T CELLS

As the name implies, the cardinal characteristic of all T_{FH} cells is their re-positioning into the follicular regions of secondary lymphoid tissues. The chemokine receptor CXCR5 is highly expressed on B cells and is largely responsible for B cell partitioning into CXCL13 rich follicular areas in LNs (Ansel et al., 2000; Forster et al., 1996; Gunn et al., 1998a). In contrast, naive T_H cells express high levels of CCR7, promoting extra-follicular placement into CCL19 and CCL21 rich areas in lymphoid tissue (Forster et al., 1999; Gunn et al., 1998b). CXCR5 was first reported on T_H cells as the expression of an orphan receptor BLR1 on a small sub-population of $CD4^+CD45RO^+$ resting memory phenotype T_H cells ($CD44^{hi}CD62L^{lo}IL2R^{lo}$) in the peripheral blood of humans (Forster et al., 1994). Cells with this phenotype are also greatly enriched in human tonsils and distributed in the follicular areas and germinal centers (GC) of these inflamed tissues (Forster et al., 1994). In vitro, co-stimulation of $CD4^+$ T cells

through OX-40 and CD28 also induced CXCR5 expression and IL-4 secretion (Flynn et al., 1998). Importantly, blocking T_H cell activation in vivo with CTLA4-Ig or in CD28 deficient animals also blocked OX-40, CXCR5-expressing T_H cells and the development of germinal centers (Walker et al., 1999). At the same time, rapid CXCR5-expression was demonstrated directly on antigen-specific T_H cells following priming in vivo (Ansel et al., 1999). These antigen-specific $CXCR5^+$ T_H cells also lost CCL19/21 responsiveness and migrated to follicular areas and GCs after priming. Hence, up-regulation of CXCR5 and down-regulation of CCR7 help to increase the probability of antigen-specific contact between pMHCII-specific T_H cells and antigen-primed $pMHCII^+$ B cells in draining lymphoid tissue.

T_{FH} function

In these earliest reports of T_{FH} cells, B helper cell function was assessed using $CXCR5^+$ T_H cells from human tonsils (Breitfeld et al., 2000; Schaerli et al., 2000). The $CXCR5^+$ T_H cell compartment contained all the capacity to support IgG and IgA production when co-cultured with B cells from the same tonsils. Functional assessment of cytokine production by in vitro re-stimulated $CXCR5^+$ T_H cells from human peripheral blood indicated some IL-2, IFN-γ and IL-10 production with negligible T_H2 cytokines IL4, IL-5 or IL-13 (Schaerli et al., 2000). Antigen-specific TCR transgenic transfer experiments first demonstrated the concurrent and divergent development of tissue homing versus antibody promoting effector T_H cells (Campbell et al., 2001). In contrast, to the tissue homing T_H cells, the B cell helper subset was responsive to CXCL13 and not CCL21 with higher levels of IFN-γ and IL-4 upon re-stimulation in vitro. Both effector T_H cell subsets expressed similar levels of ICOS and CD40L indicating the presence of shared effector potential (Campbell et al., 2001). Hence, $CXCR5^{hi}CCR7^{lo}$ antigen-primed T_H cells define a distinguishable subset of T_H cells now called follicular B helper T cells or more commonly, follicular helper T cells (T_{FH}).

T_{FH} CELLS AS A SEPARATE T_H LINEAGE

The range of effector functions associated with T_H cells is extensive and the organization and developmental programming of these functions remains a focus of intense research. Changes in co-stimulation and cytokine delivery upon first contact with pMHCII-expressing DC have a major impact on T_H cell fate and function (depicted in Figure 1). Manipulating these signals in vitro and in vivo have provided important insights into the derivation of different effector T_H lineages and is the basis for most studies on T_H cell lineage development (Zhu and Paul, 2008). Antigen-specific naive T_H cells use initial instructive signals to drive cell-intrinsic developmental programs in their clonal progeny to define separable effector T_H cell lineages.

T_H1/T_H2 cells

The original T_H1/T_H2 paradigm identified differential programs of cytokine production thought to regulate cell-mediated immunity versus the production of antibody by B cells (Mosmann et al., 1986; Mosmann and Coffman, 1989). T_H1 cells mediate immune responses to intracellular pathogens (Paul and Seder, 1994). IL-12 and IFN-γ are the principle inducers of the T_H1 program with a synergistic role for IL-18. Induction of IL-12Rβ2 upon TCR activation (Szabo et al., 1997) increases Stat4-dependent IL-12 responsiveness in this pathway (Kaplan et al., 1996c). Stat1-dependent IFN-γ signals induce the T_H1 master regulator T-bet that not only induces further IFN-γ production, but also inhibits IL-4 expression (Szabo et al., 2003). In contrast, T_H2 cells mediate immune responses to extracellular parasites through the production of IL-4, IL-5, IL-13 and IL-25. Amplification of the T_H2 program occurs through expression of IL-4R and Stat6 transduction of IL-4 signals that lead to the expression of the T_H2 master regulator, GATA-3 (Kaplan et al., 1996b; Zheng and Flavell, 1997). GATA-3 is required for T_H2 differentiation but also blocks the T_H1 program. IL-2 can also potentiate T_H2 cytokine production in a Stat5a–dependent mechanism (Zhu et al., 2003). In this manner,

T_H1 and T_H2 can be considered alternate and non-overlapping cell fates that define separate T_H lineages with each program also extinguishing the alternate cell fate.

T_H17/T_{reg} cells

More recently, a separable T_H17 cell lineage has been described (Bettelli et al., 2006; Harrington et al., 2005; Mangan et al., 2006; Park et al., 2005; Veldhoen et al., 2006). T_H17 cells were initially associated with autoimmune disease and chronic inflammation but have also been shown to mediate responses to extracellular bacteria and fungi through the production of IL17, IL-17F and IL22 (Korn et al., 2009). TGF-β and IL-6 were shown to induce IL-17 production from naive T_H cells (Bettelli et al., 2006; Veldhoen et al., 2006). Importantly, TGF-β alone promoted T_{reg} cell differentiation while addition of IL-6 not only induced high levels of IL-17 but also blocked the FoxP3 dependent development of T_{reg} cells. Addition of IL-6 promoted expression of the orphan nuclear receptor RORγt in a Stat3-dependent manner. RORγt was necessary for T_H17 development (Ivanov et al., 2006) with a role for IRF-4 (Brustle et al., 2007) and IRF4-binding protein (Chen et al., 2008) in the control of its expression. RORγt and the related RORα can bind FoxP3 and antagonize its function as one means for the reciprocal regulation of these two cell fates (Yang et al., 2008; Zhou et al., 2008). IL-6 also induces IL23R to promote survival and maintenance of T_H17 cell function in the presence of the innate cytokine IL-23 (Yang et al., 2007; Zhou et al., 2007). Hence, T_H17 and Treg cells constitute separate lineages of T_H cells with unique transcriptional programs of development and distinct sets of effector cytokines from each other and the original T_H1/T_H2 options.

T_{FH} cells

IL-21 is an interesting new member of the IL-2 family of cytokines that plays an important role in T_H lineage development (Ozaki et al., 2000; Parrish-Novak et al., 2000; Spolski and Leonard, 2008). T_H17 cells, T_{FH} cells and T_H2 cells are all known to produce IL-21. In T_H17 development, IL-6 is a strong inducer of IL-21 in a Stat3 dependent mechanism (Nurieva et al., 2007; Zhou et al., 2007). Both IL-21 and IL21R deficient animals are defective in T_H17 development suggesting an autocrine loop for T_H17 cells with potential to amplify this particular lineage decision (Korn et al., 2007; Nurieva et al., 2007). IL21 and IL21R deficiency also impacts the development of B cell immunity with impaired isotype switch and deficient GC development (Ozaki et al., 2002; Spolski and Leonard, 2008). In this context, T_{FH} cells are known to produce abundant levels of IL-21 (Nurieva et al., 2008; Vogelzang et al., 2008) creating potential autocrine action with concomitant expression of IL21R (Chtanova et al., 2004; Rasheed et al., 2006; Vinuesa et al., 2005a). Importantly, IL-21R expression only on T_H cells partially rescued the B cell defect in IL21R deficient animals (Vogelzang et al., 2008). These studies also demonstrate a role for IL21 in the development of $CXCR5^+ICOS^{hi}$ T_{FH} cells that was further enhanced by contact with ICOSL on B cells in vitro (Vogelzang et al., 2008) and in vivo (Nurieva et al., 2008). At the same time, IL-6 was also shown to induce IL-21 in T_{FH} cells in a Stat3-dependent manner (Nurieva et al., 2008). TGF-β was not required for effector T_{FH} induction and RORγt was not expressed by T_{FH} cells or needed for T_{FH} development in vivo (Nurieva et al., 2008). Together, these studies provide strong evidence for the differential programming events that control T_H17 and T_{FH} development into separable T_H cell lineages.

Earlier microarray analyses of T_{FH} cells also indicated separable gene expression programs for the T_{FH} compartment. Using CD57 and CXCR5 expression to distinguish human tonsillar GC T_{FH} cells (Kim et al., 2001) emphasized high levels of the chemokine CXCL13 as a major attribute of T_{FH} cells (Kim et al., 2004). A separate study compared $CD57^+$ and $CD57^-$ $CXCR5^+$ tonsillar T cells to T_H1 and T_H2 cells polarized from cord blood in vitro, with central and effector memory T_H cells from human peripheral blood (Chtanova et al., 2004). This comprehensive analysis highlighted ICOS and IL-21 as an abundant product of $CD57^+$ T_{FH}

cells. The SLAM family member CD84 (Schwartzberg et al., 2009) and CD200 were highlighted as preferentially expressed by the T_{FH} compartment with evidence for the differential expression of the transcriptional repressor, Bcl-6 (King et al., 2008; Vinuesa et al., 2005b). The use of CD57 was later called into question by a separate study that indicated $CXCR5^{hi}ICOS^{hi}$ cells had the greatest B cell helper activity in the human tonsil (Rasheed et al., 2006). Nevertheless, this latter study also highlighted CXCL13 expression, IL21 and IL21R expression as well as low levels of GATA3 as distinctive in T_{FH} cells (Rasheed et al., 2006). This study implicated T_H2-associated transcription factors such as c-maf as being involved in T_{FH} programming. In mouse, gene expression studies of T_{FH} cells associated with over-expression of ICOS also identified IL-21 and CD200 as preferentially abundant in the dysregulated T_{FH} cells (Vinuesa et al., 2005a) with T_{FH} gene expression clustering distinctly when compared to T_H1, T_H2 and T_H17 cells (Nurieva et al., 2008). These expression studies strongly supports the existence of a unique developmental program that leads to effector T_{FH} cell functions separable from the other known T_H cell subsets.

T_{FH} LOCATION AS A DEVELOPMENTAL PROGRAM

While the recent data on separable lineage development are compelling, there are exceptions to the notion of a separate T_H cell lineage for the T_{FH} compartment. Both T_H1 and T_H2 cytokines are known to regulate antibody class switch (McHeyzer-Williams and McHeyzer-Williams, 2005; Vinuesa et al., 2005b) with IL-4 and IFN-γ being the earliest examples inducing IgG1/E and IgG2a respectively (Kuhn et al., 1991; Snapper and Paul, 1987; Takeda et al., 1996). TGF-β and IL-10 are implicated in the switch to IgA (Cazac and Roes, 2000; Defrance et al., 1992; Dullaers et al., 2009) although the cellular source of these factors during an immune response has not been well described. In vitro polarized T_H1 and T_H2 cells can both support B cell responses upon adoptive transfer (Secord et al., 1996; Smith et al., 2000). Furthermore, both T_H1 and T_H2 cells primed in vivo can enter follicular areas and interact with antigen-specific B cells (Pape et al., 2003; Smith et al., 2004; Toellner et al., 1998). IL-17 has also been implicated in the induction of autoreactive antibody responses (Nakae et al., 2003). A recent study localized IL-17 producing T_H cells to the spontaneous and exaggerated splenic GC responses of BXD2 autoimmune prone mice, implicating the T_H17 cells in the regulation of autoantibody (Hsu et al., 2008). Further to this notion, IL-17 production was induced preferentially from $CXCR5^{+}ICOS^{hi}$ T_{FH} cells following MOG peptide in CFA immunization (Bauquet et al., 2008). ICOS regulated IL-21 production through c-Maf was implicated in this pathway to IL-17 production. These data contrast the lack of IL-17 production from TCR transgenic T_H cells exposed to protein antigen in CFA (Nurieva et al., 2008) and may reflect the inherent plasticity in T_{FH} programming or the impact of antigen specificity and TCR signal strength in this process. Finally, $CD25^{+}$ T_{regs} have been reported within GC of human tonsils that interfere with T cell help and the induction of antibody (Lim et al., 2004). There are also reports of direct suppression of antibody production by $FoxP3^{+}$ GC T_{regs} (Lim et al., 2005). Thus, all known T_H lineages can migrate to follicular areas and into the GC to participate in the regulation of B cell immunity.

Polyclonal antigen-specific T_{FH} cells

Directly monitoring antigen-specific T_H cells in polyclonal immune systems (McHeyzer-Williams and Davis, 1995) and the advent of pMHCII tetramer technology (Altman et al., 1996; McHeyzer-Williams et al., 1996) provides experimental access to the polyclonal immune system. These new technologies allow direct assessment of antigen-specific T_H cell responses to model antigens (Fazilleau et al., 2005; Rees et al., 1999; Savage et al., 1999), infectious disease (Malherbe et al., 2000; Stetson et al., 2002) and local antigen persistence (McLachlan et al., 2009). Our early studies using these strategies allowed access to antigen-specific T_H cell repertoire (Fazilleau et al., 2007a; Malherbe et al., 2000; Malherbe et al., 2004; Malherbe et

al., 2008; McHeyzer-Williams et al., 1999), TCR responsiveness (Bikah et al., 2000), effector T_H cell function in vivo (Panus et al., 2000).

Our recent studies focused on the local development of antigen-specific $CXCR5^+$ $CD44^{hi}CD62L^{lo}$ effector T_{FH} in the draining LNs (Fazilleau et al., 2007b). Examining the mechanism underlying this assortment of effector T_H cell function, we demonstrated that the strength of TCR-pMHCII binding influenced the migratory behavior of antigen-specific effector T_H cells (Fazilleau et al., 2009). The highest pMHCII-binding TCR assorted with the differentiation of effector T_{FH} cells and localization to the B cell areas ($CXCR5^+CCR7^{lo}$). Under the same priming conditions, intermediate pMHCII binding promoted emigrant T_H cells ($CCR7^{lo}CD62L^{lo}$) that left the LN and low pMHCII binding promoted T zone localized T_H cells ($CCR7^{hi}CD62L^{hi}$)(depicted in Figure 1). In this polyclonal model, IL-2, IFN-γ, IL-10 and T-bet were expressed at equivalent levels by all subsets of pMHCII-specific effector T_H cells at day 7 after priming. Cytokines more typically associated with T_{FH} cells, IL-4 and IL-21 were present in all T_H subsets but at higher levels in the T_{FH} compartment. OX-40, CD69 and Bcl-6 were uniquely present in the effector T_{FH} compartment. Hence, there was evidence for multiple sub-types of T_H cells with separable developmental programs associated with the pMHCII-specific effector T_{FH} cell compartment in this model system.

Therefore, deployment of separable T_H cell lineages to follicular regions may represent an over-riding secondary developmental program for the antigen-specific regulation of distinct B cell fates. In this context, the nature of the innate stimulus would dictate the balance of effector T_H cell subsets and a subset of these responders can relocate to the follicular regions to regulate effector and memory B cell functions. For example, $CXCR5^+CCR7^-$ IFN-γ producing, T-bet expressing pMHCII-specific T_H1 cells could be considered to be a $T_{FH}1$ subtype of effector T_{FH} cell that regulate B cell fate at Checkpoint II in vivo.

PRE-GERMINAL CENTER EFFECTOR T_{FH} CELLS

Pre-GC effector T_{FH} function is delivered in an antigen-specific manner to pMHCII-expressing B cells but how these differential functions develop in vivo remains poorly resolved. Under static conditions in vitro, successful pMHCII-TCR interactions involve the ordered formation of a stable immunological synapse (Bromley et al., 2001; Grakoui et al., 1999; Monks et al., 1998). Inter-cellular adhesion facilitates the scanning for adequate TCR-pMHCII interactions, central clustering and rearrangement of the cellular interface into a stable synapse (Bromley et al., 2001; Grakoui et al., 1999; Monks et al., 1998). Real-time imaging using two-photon laser scanning technology has provided outstanding clarity to these early cellular interactions in vivo (Miller et al., 2002). Studies labeling antigen-primed DC and pMHCII-specific T_H cells indicate an early phase of transient 5–10 min contacts followed by longer duration interactions lasting many hours. Altered cell motility promotes serial DC engagement that leads to cell clustering and swarming behavior (Miller et al., 2004). The duration of DC-T cell contact during this later phase impacts cell fate (Celli et al., 2005; Celli et al., 2007; Shakhar et al., 2005) with at least 6hr of contact required to induce clonal expansion in $CD4^+$ T_H cells. Nevertheless, the impact of these early events on the development of effector and memory T_H functions is less clear and remains an important focus of current research.

Effector T_{FH} contact duration

First contact between antigen-specific T_H and antigen-primed B cells has also been captured through dynamic imaging (Okada et al., 2005). Stable ‘monogamous’ interactions between one antigen-specific T_H cell and one B cell can last for 10–60 min duration in the follicular regions of the LNs. These interactions were accompanied by highly dynamic movements with the B cells migrating extensively leading the T_H cells (Okada et al., 2005). A more recent study demonstrated that expression of the adaptor molecule SAP was needed to form long duration

contacts with antigen-primed B cells (Qi et al., 2008). There was no role for SAP in early T_H-DC contacts and the SAP deficient T_H cells reached the follicular regions and expressed all the hallmarks of effector T_{FH} cells ($CXCR5^{hi}$, $CD40L^+$, $ICOS^{hi}$ and $OX40^{hi}$) (Qi et al., 2008). However, in the absence of SAP, T_{FH} cells were still capable of cytokine production (Cannons et al., 2006), but unable to promote GC formation (Crotty et al., 2003; Schwartzberg et al., 2009). Importantly, effective contact duration between pre-GC effector T_{FH} cells and pre-GC B cells promoted the entry of T_{FH} cells into the GC reaction (Qi et al., 2008). Cognate interactions were also required for effective contact duration and would not occur if the T_H cell antigen and the B cell antigen were unlinked (Qi et al., 2008). These studies clearly establish that duration of pre-GC pMHCII-specific effector T_{FH} contact with pre-GC B cells is needed to promote normal GC formation. Furthermore, pre-GC effector T_{FH} cell contact with pMHCII-expressing antigen-primed B cells may also be required for aspects of GC T_{FH} functional developmental.

EFFECTOR T_{FH} CELL FUNCTION

While location and cognate contact are important, it is important to consider in more detail the driving forces and consequences of Checkpoint II interactions (depicted in Figure 2). Antigen-activated B cells up-regulate CCR7 while retaining CXCR5 levels to counterbalance migration to the T–B borders (Ebert et al., 2004;Ohl et al., 2003;Reif et al., 2002). $CXCR5^+$ effector T_{FH} cells must lose CCR7 expression to achieve follicular placement. Further, CXCR5 expression on T_H cells is not required for follicular placement but aspects of T_{FH} activity such as isotype switch and GC formation are decreased in its absence (Arnold et al., 2007;Haynes et al., 2007). Increased CXCL13 expression by effector T_{FH} cells must serve to attract $CXCR5^+$ B cells (Kim et al., 2004). There is also evidence for IL-21 producing extra-follicular T_H activity that controls self-reactive IgG production and is associated with CXCR4 expression and the loss of P-selectin ligand 1 binding (PSGL-1)(Odegard et al., 2008). Hence, B cell helper function may not only reside in the follicular areas but with pMHCII-specific T_H cells that balance chemokine receptor expression for appropriate placement in vivo.

Modifying cognate interactions

Cognate contact between effector T_{FH} cells and pMHCII-expressing B cells defines a critical initiating event in the development of effective B cell immunity that may be modified by a multitude of cell surface expressed molecules (depicted in Figure 2). CD28 deficiency or treatment with blocking anti-CD28 antibodies leads to profound defects in B cell immunity (Shahinian et al., 1993). CD28 is required early to initiate naive T_H cell responsiveness to $pMHCII^+$ CD80 and CD86 expressing DC (Janeway and Bottomly, 1994;Matzinger, 1994). In contrast, CD40-CD40L interactions are central to the delivery of T cell help to B cells (Armitage et al., 1992;Lederman et al., 1992;Noelle et al., 1992). Mice and humans deficient in CD40 or CD40L are unable to efficiently class switch, form GCs or promote memory B cell responses (Kawabe et al., 1994;Xu et al., 1994). Interfering with OX40-OX40L interactions prevents T_{FH} accumulation in follicular regions of LNs suggesting that early engagement with OX40L on activated DC (Brocker et al., 1999) or on a $CD3^-CD4^+$ accessory cell population (Kim et al., 2003) was needed to facilitate appropriate T_{FH} migration. The continued expression of OX40 on effector T_{FH} cells (Fazilleau et al., 2009;Qi et al., 2008) also suggests a role in the modulation of cell fate at effector T_{FH}-B cell interactions that may extend to within the GC reaction. There are reports of other TNFR/L expression such as CD30 (Kim et al., 2003) and BTLA (Nurieva et al., 2007) that can play a role in the development of T_{FH} cells and the delivery of T_{FH} functions in vivo.

The CD28 family member inducible co-stimulator molecule ICOS (Hutloff et al., 1999) has been implicated in T_{FH} cell function in pre-GC and the GC interactions with pMHCII-expressing B cells (Fazilleau et al., 2007c; King et al., 2008; Vinuesa et al., 2005b). ICOS

deficient humans (Grimbacher et al., 2003) and mice (Dong et al., 2001; McAdam et al., 2001; Tafuri et al., 2001) and ICOS-L deficient mice (Mak et al., 2003) have marked deficits in antibody production, isotype switch and GC formation. ICOS deficiency is associated with decreased T_{FH} cell development (Bossaller et al., 2006) and ICOS is considered an important molecule in the delivery of effector T_{FH} function (Nurieva et al., 2008; Vogelzang et al., 2008). The highest levels of ICOS are expressed on GC T_{FH} cells with the greatest capacity to support antibody production in vitro (Rasheed et al., 2006). Conversely, over-expression of ICOS in mice with a regulatory defect in ICOS expression (Yu et al., 2007) over-produced $CXCR5^+$ T_{FH} cells, displayed exuberant GC reactions with breakthrough autoimmune disease (Vinuesa et al., 2005a). Recent studies indicate that ICOS can substitute for CD28 and rescue the T_{FH} defects and B cell defects in CD28 deficient mice (Linterman et al., 2009a). Furthermore, the abundant $CXCR5^+$ T_{FH} cells in this model act in a T cell autonomous manner to promote autoantibody production (Linterman et al., 2009b). Another CD28 family member PD-1 that has been implicated in the negative regulation of chronically activated T cells has also been demonstrated on GC T_{FH} cells in human tonsil (Iwai et al., 2002) and mouse (Haynes et al., 2007). Positive and negative influences of co-stimulation are balanced in ways that remain poorly understood in vivo.

Overall, the precise function of different co-stimulatory molecules and their combinatorial impact on antigen-specific B cell fate remains an exciting area of current interest. Quantitative differences in cell surface molecules in combination with mixtures of cytokines will likely synergize in pre-determined ways to skew pMHCII-expressing B cells into separate pathways of B cell immunity. Unraveling these molecular combinations will help to define the rules of molecular control for antigen-specific B cell immunity.

PC versus GC development

Effectively regulated pMHCII-expressing B cells will either enter the PC or GC pathway at this juncture in development. The signaling lymphocyte activation molecule (SLAM) family of molecules plays an important role at the effector T_{FH}-B interface revealed through the action of the signal transducer SAP (Schwartzberg et al., 2009). Adoptive transfer studies indicate that the SAP defect in B cell immune responsiveness is primarily a T cell defect (Veillette et al., 2008). When effective duration T_{FH}-B contact cannot be sustained (Qi et al., 2008), the GC reaction and memory B cell development is compromised. In contrast, the extra-follicular pathway to plasma cell development remains intact in the SAP deficient animals with evidence for acute antibody responses and isotype switch (Crotty et al., 2003). SAP deficient T_H cells reconstituted with a Fyn recruiting mutant of SAP can promote cytokine production but still do not support GC formation (Cannons et al., 2006). Taken together, these data suggest that different subsets of effector T_{FH} cells may regulate entry into the extra-follicular pathway to short-lived PCs versus the GC pathway to memory B cell development. Alternatively, these separate developmental ‘decisions’ may require separable signals from the same cohort of effector T_{FH} cells (such as distinct affinity TCR-pMHCII interactions). Understanding and controlling the balance of early antibody responses versus affinity-matured memory B cell responses may provide useful and separate means for modifying the adaptive immune response in future immunotherapeutic strategies.

Antibody Class Switch

Developmental progression in antigen-specific PCs and GC B cells both involve the switch to downstream antibody isotypes (McHeyzer-Williams and McHeyzer-Williams, 2005)(depicted in Figure 2). Early studies indicated that switch occurred in extra-follicular sites and within the GC at the same time (Jacob et al., 1991). Thus, it is possible that some systemic factor controlled these events in a co-ordinate way. Alternatively, pMHCII-specific T_{FH} cells within both environments may actively and simultaneously instruct these developmental outcomes.

It is also plausible, that first contact with effector T_{FH} cells induced the commitment to switch to a particular antibody isotype in antigen-primed B cells, a process that requires multiple cell divisions to complete (Tangye et al., 2003). In the latter model, commitment to isotype switch would be imprinted by the cytokine signals delivered in a cognate manner at first contact with effector T_{FH} cells. As a corollary of this model, commitment to a particular antibody isotype may also define a separable lineage of antigen-experienced B cells with distinct origins and requirements for survival, function and long-term propagation in vivo.

Based on what is understood about the cytokine control of isotype switch there is evidence for the existence of multiple effector T_{FH} subsets (depicted in Figure 2). $CXCR5^+$ T_H1 cells that produce primarily IFN-γ would promote IgG2a production in the progeny of contacted antigen-specific B cells. We could consider this follicular homing subset, $T_{FH}1$ cells. In the same manner, $T_{FH}2$ would produce primarily IL-4 and promote switch to IgG1 and IgE in the clonal progeny of the primed B cells (Snapper and Paul, 1987). Antigen-specific inducible T_{reg} producing TGF-β and IL-10 could direct IgA switch as $T_{FH}10$ cells (Cazac and Roes, 2000;Defrance et al., 1992;Dullaers et al., 2009). There has also been a recent report of a TGF-β producing T_{FH} cell compartment that promotes not only IgA switch but also plasma cells migration to mucosal tissue by up-regulating CCR10 expression (Dullaers et al., 2009). IL-21 is another important secreted factor in the regulation and/or enhancement of antibody isotype switch (Bryant et al., 2007;Spolski and Leonard, 2008). The absence of IL-21 alone reduced IgG1 production with elevated IgE, while IL-4 and IL-21 DKO mice display decreases in all antibodies in the serum (Ozaki et al., 2002). Hence, pMHCII-specific effector T_{FH} cells may direct antibody isotype commitment through cognate and non-cognate contact modified by the cytokine combinations they deliver.

Bcl-6 and Blimp-1

Identifying antagonizing transcription factors allowed the more detailed molecular analysis of T_H lineage commitment. In the T_{FH} arena, the transcriptional repressor Bcl-6 has been used to distinguish the T_{FH} compartment from other T_H cell subsets as discussed above (Chtanova et al., 2004; Fazilleau et al., 2009; Nurieva et al., 2008; Vinuesa et al., 2005b; Vogelzang et al., 2008). This transcriptional repressor is highly expressed in GC B cells (Cattoretti et al., 1995; Onizuka et al., 1995) and has been demonstrated in a fraction of GC T_{FH} cells (Ree et al., 1999). Bcl-6 deficient mice have defective B cell responses with an absence of GC and memory development (Dent et al., 1997; Shaffer et al., 2000; Ye et al., 1997). In our recent analysis of T_{FH} cell development to protein vaccination, we reported the expression of Blimp-1 in the $CCR7^{hi}CXCR5^{lo}$ T zone resident effector T_H cell compartment (Fazilleau et al., 2009). Blimp-1 was first described as a master regulator for plasma cells (Turner et al., 1994) but has now found roles in natural T_{reg} development (Kallies et al., 2006; Kaplan et al., 1996a; Martins et al., 2006), the differentiation of T_H1 cells (Cimmino et al., 2008). Interestingly, these Bcl-6 and Blimp-1 are known to cross block (Martins and Calame, 2008) providing a means to extinguish the alternate cell fate in a feedback regulatory manner. This pair of transcription factors may represent antagonizing control of LN effector functions and used to balance the T zone versus B zone effector T_H cells that emerge in response to antigenic assault.

GERMINAL CENTER T_{FH} CELLS

It must be emphasized that defects in either the priming of naive T_H cells and B cells or the ineffective cognate interactions at the first contact between these two facets of the adaptive immune system will result in GC defects. Hence, the lack of GC formation is an unreliable phenotype for unraveling the impact of GC interactions themselves. Nevertheless, the GC functions of T_{FH} cells remain of intense interest and central importance to the understanding of high-affinity antigen-specific memory B cell generation (depicted in Figure 3).

The T cells within GC are predominantly $CD4^+$ and reside mainly in the light zones (Bowen et al., 1991; Fuller et al., 1993; MacLennan, 1994). Antigen-specific T_H cells have been shown to enter the GC in adoptive transfer models (Garside et al., 1998) and during the course of an immune response in polyclonal systems (Gulbranson-Judge and MacLennan, 1996; Zheng et al., 1996). Our early studies in protein vaccination noted the dynamics of movement between pre-GC follicular regions at day 7 to predominantly GC by day 9 after priming (McHeyzer-Williams et al., 1999). This sequential positioning of antigen-specific T_H cells was also found within the spleen of the polyclonal responders (Gulbranson-Judge and MacLennan, 1996). However, not all antigen-specific T_H cells entered the GC with evidence for particular sets of the responsive TCR repertoire not assorting into the GC (Mikszta et al., 1999). Importantly, these studies also monitored memory development for additional non-GC clonotypes to demonstrate that GC entry was not required for memory T_H cell development. Static analysis of GC T_H cell in situ suggested that GCs were open environments for T_H cells with evidence from micro-dissection studies for movement between different GC (Zheng et al., 1996).

The function of GC T_{FH} cells has been more difficult to ascertain. Interfering with CD28-CD86 or CD40-CD40L interactions leads to dissolution of established GC (Han et al., 1995). Using high frequencies of high affinity BCR transgenic B cells and multivalent forms of antigen can promote GC formation without T_H cells and in the absence of CD40 or CD28 signals (de Vinuesa et al., 2000). T_H cell independent GCs are histologically similar to typical T_H cell regulated GCs, containing immune complexes, FDC and B cells with a GC phenotype (Gaspal et al., 2006) but dissimilar global gene expression patterns (Yu et al., 2008). Importantly, TI GCs collapse early after priming with no evidence for somatic diversification of the BCR (de Vinuesa et al., 2000; Gaspal et al., 2006; Toellner et al., 2002). Thus, the presence of GC T_{FH} cells does not appear to be necessary for the generation of GC microenvironments but they appear important for the later events of GC B cell selection and the production of high-affinity B cell memory.

GC cellular dynamics

Recent dynamic imaging of the GC reaction in situ has provided outstanding clarity to the kinetics of GC B cell (Hauser et al., 2007a; Schwickert et al., 2007) and GC T_{FH} cell movements and interactions (Allen et al., 2007b). There was evidence for inter-zonal movement of GC B cells indicating bi-directional migration between light and dark zones (Allen et al., 2007b; Hauser et al., 2007a; Hauser et al., 2007b; Schwickert et al., 2007) with one study emphasizing the majority of movement to be intra-zonal (Hauser et al., 2007a; Hauser et al., 2007b). Surprisingly, cell division appeared in both zones of the GC in contrast to classical models (Allen et al., 2007b; Hauser et al., 2007a; Schwickert et al., 2007). There was also evidence for the ability of naive B cells to traverse the GC environment with evidence that high-affinity B cells could also enter and dominate existing GCs (Schwickert et al., 2007). All studies identified highly motile GC B cell movements with evidence for continuous uninterrupted movement over FDC processes. This movement contrasted with the capacity of GC B cells to form frequent short-term contacts but infrequent stable contacts with GC T_{FH} cells (Allen et al., 2007b). The latter group suggested that these infrequent cognate contacts play a dominant role in the selection of high-affinity BCR expressing GC B cells (Allen et al., 2007a). Hence, a new model for affinity-based selection emerges with uninterrupted access of GC B cells to antigen-coated FDC followed by the ability of “re-primed” B cells to express pMHCII complexes to levels that gain competitive access to pMHCII-specific GC T_{FH} cells.

It is important to emphasize that the functions of the pMHCII-specific GC T_{FH} cells appear broadly distinct from the effector T_{FH} counterparts. First, entry of T_{FH} into the GC environment appears dependent on prior interactions with pre-GC B cells (Qi et al., 2008). Entry of effector T_{FH} into the GC may not be the only pathway of GC entry, but appears to be one method of

gaining access into this microenvironment. Extensive clonal expansion ensues for the B cells within the GC environment (MacLennan, 1994) and therefore precludes continued contact with the effector T_{FH} cells at the time-point of entry. Furthermore, it is unclear whether there are developmental changes that occur within the effector T_{FH} cells between the point of entry and the capacity to form stable contacts with GC B cells after BCR diversification. Controlling GC B cell exit and the development of high affinity B cell memory is also correlated with the presence of GC T_{FH} cells (Toellner et al., 2002). It is possible that the GC T_{FH} cells promote and support antibody isotype switch within the GC. However, it is also likely that commitment to isotype switch has already occurred and does not require these secondary contact events as discussed above. Alternatively, there may exist subsets of GC T_{FH} cells specialized to select and support GC B cells of particular isotypes. Within this context, the major differential outcome of the GC interaction would be a major division represented by entry into the "reactive" memory B cell compartment or entry into long-lived plasma cell pathway (depicted in Figure 3). Both pathways contribute to long-term immune protection and represent two broad layers of antigen-specific B cell memory.

MEMORY T_{FH} CELLS

The original $CXCR5^+$ T_{FH} cells were identified in human peripheral blood as circulating sub-populations of resting T_H cells with a memory phenotype (Forster et al., 1994). Functional analysis of these circulating T_{FH} cells distinguishes their activity from tonsillar T_{FH} cells with reduced capacity for promoting antibody production by B cells (Kim et al., 2001). Nevertheless, the circulating T_{FH} compartment may play a differential role in the regulation of memory B cells compared with other the T_H cell memory compartment (Sallusto et al., 2004). The organization of antigen-specific memory T_{FH} cells and the regulation of memory B cell responses is an important but poorly studied facet of this research area.

In the polyclonal model, we recently characterized the presence of a $CXCR5^+$ T_{FH} compartment that remained resident within the LNs, had decreased levels of ICOS and cytokine mRNA in vivo, but rapid and differential induction of IL-4, IL-10 and IL-21 upon re-stimulation with antigen in vitro (Fazilleau et al., 2007b). We proposed that these cells were the memory counterpart of the effector T_{FH} population that emerged after initial priming. We provided evidence that local memory B cell responses arose more rapidly in their presence than elsewhere after antigen re-challenge. The continued expression of CD69 on the memory T_{FH} compartment suggested recent TCR triggering with pMHCII complexes. Adoptive transfer of naive TCR transgenic T_H cells located pMHCII depots in the draining LNs and not in the non-draining LNs or the spleen of previously immunized mice (Fazilleau et al., 2007b). These data suggested the presence of a resident B zone localized memory T_{FH} compartment able to rapidly regulate pMHCII-expressing memory B cells upon re-challenge with antigen. We predict that cognate interactions that define the regulation of memory B cells (Checkpoint IV) would be qualitatively and quantitatively distinct from the interactions that define cell fate after initial priming. Understanding and then controlling these molecular interactions will provide new impetus and novel targets for the basic strategies used in typical prime-boost vaccines regimes.

CONCLUSIONS

T_{FH} cells are a class of regulatory T_H cells that specialize in the cognate control of antigen-specific B cell immunity. Upon antigen-specific activation, the emergence and movement of $CXCR5^+CCR7^-$ pMHCII-specific T_{FH} cells into the follicular regions is the major attribute of T_{FH} cells. These T_{FH} cells express CD40L, ICOS and SAP to form stable contacts with antigen-primed B cells with evidence for IL-4, IFN-γ, IL-10 and IL-21 as soluble factors to modify B cell fate. Distinct control of IL-21 production and the expression of Bcl-6 help to

distinguish T_{FH} cells from other polarized T_H cell subsets and may define a separable T_H cell lineage. However, we favor a model in which all known T_H lineages can be deployed to the follicular regions to regulate a spectrum of outcomes in B cell immunity. In this model, both lineage and location would define the regulatory function of pMHCII-specific T_{FH} cells.

There are three major junctures in the delivery of cognate T_{FH} function in vivo. After T_H clonal selection and differentiation of effector function, first contact with antigen-primed B cells occurs pre-GC in the follicular regions. We propose that these effector T_{FH} cells control 1) commitment to antibody isotype and 2) entry into short-lived PC versus GC pathway to memory. Entry into the GC defines a distinct compartment of GC T_{FH} cells that control 1) GC B cell selection and survival and 2) entry into the long-lived memory B cell and PC compartments. Long-term persistence in LNs defines a separate resting memory T_{FH} compartment that control 1) memory B cell expansion and 2) rapid PC differentiation upon antigen re-challenge in vivo. Understanding the cellular development and molecular control of antigen-specific B cell immunity will provide new targets for the immunotherapeutic management of many clinical diseases with the potential to change the basic design of future vaccine strategies.

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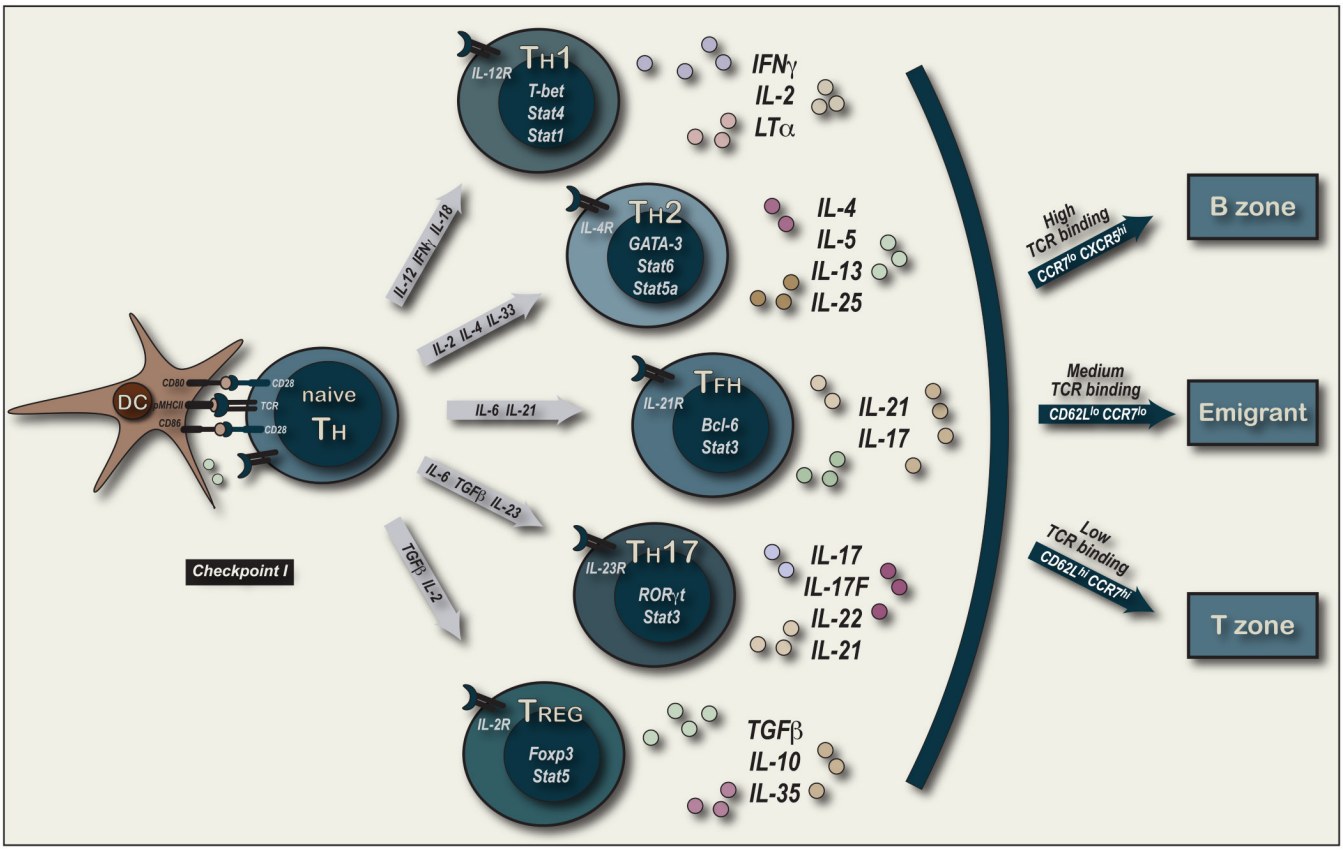

Figure 1. T_H Lineage Commitment and Micro-environmental Location

This figure depicted the first contact between pMHCII$^+$ DC and naive pMHCII-specific T_H cells (referred to as Checkpoint 1A). Intercellular cognate contact involves TCR-pMHCII interactions in the context of a range of co-stimulatory that include CD80/86 and CD28 interactions. The extent and quality of co-stimulation will be controlled by the inflammatory context of antigen exposure for the DC at immunization. The cytokines produced by the activated DC as well as the initial cytokines produced by the activated T_H cells will regulate the T_H cell lineage that develops after clonal selection, expansion and differentiation in effector function. Some of the major known influences to the developmental of separate T_H cell lineages are depicted to contrast to what is currently understood about effector T_{FH} development. The arc on the right depicts a time-point after clonal expansion and effector T_H cell differentiation when T_H cells from all lineages either 1) remain in the T cell areas of LNs or 2) migrate to the B cell areas or 3) leave the LNs to distal tissues and not return during the first week after priming. The migratory division and cell surface phenotype are predictions based on our recent study using protein vaccination in an adjuvant containing a TLR-4 agonist and the use of an FTY720 analog to block LN emigration after priming (Fazilleau et al., 2009).

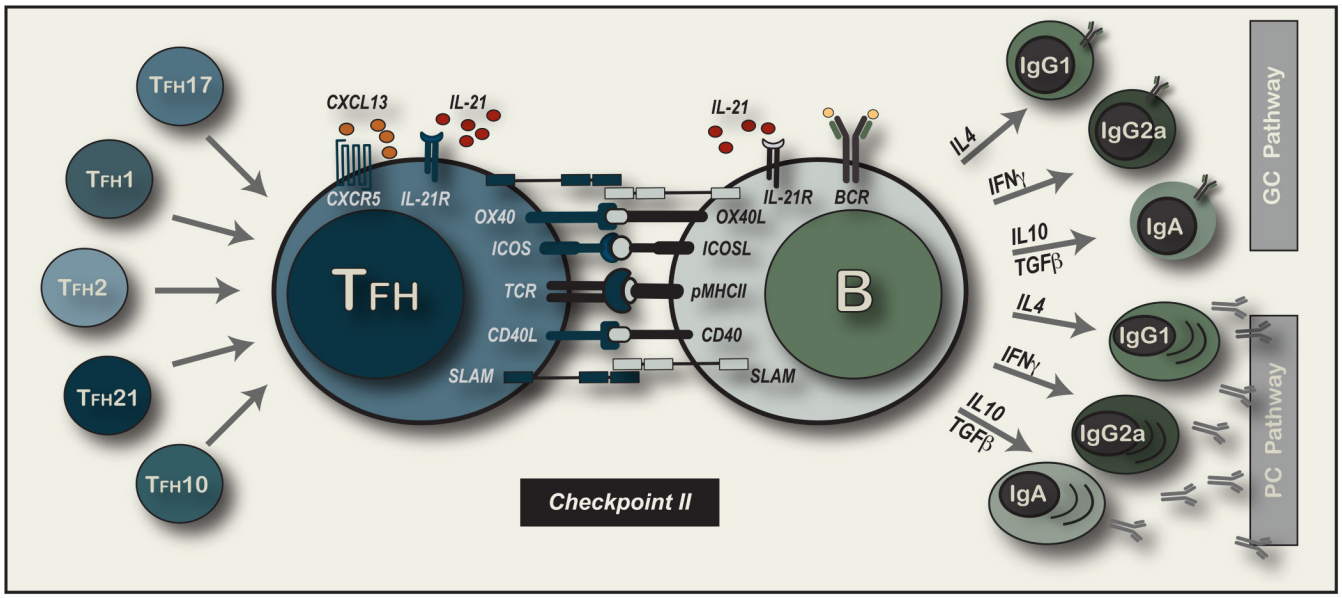

Figure 2. Delivery of Cognate Effector T_{FH} function to pMHCII-expressing B cells

The main focus of this display are the molecular interactions known to be involved at the cellular interface upon first contact of an effector T_{FH} cell and an antigen-primed B cells. Cell surface molecules are emphasized with the presence of IL-21 depicted as one of many cytokine exchanges that may ensue. On the left hand side of the figure, we depict multiple T_{FH} cell subsets that would arise as T_H cells of known lineages that adopt the developmental program of follicular placement. This program is largely defined as the loss of CCR7 and expression of CXCR5 but relies on appropriate positioning in the follicular regions and subsequent contact with an antigen-primed B cell. ON the riight hand side of the figure, we depict the two major outcomes for B cells after receipt of antigen-specific T cell help: 1) Entry into the extra-follicular short-lived PC pathway to terminal differentiation or 2) movement into the follicular areas to rapidly expand and form the GC in the pathway to memory B cell development. Isotype switch proceeds in both pathways controlled by the cytokine mixture and cell contact received at this critical second developmental checkpoint.

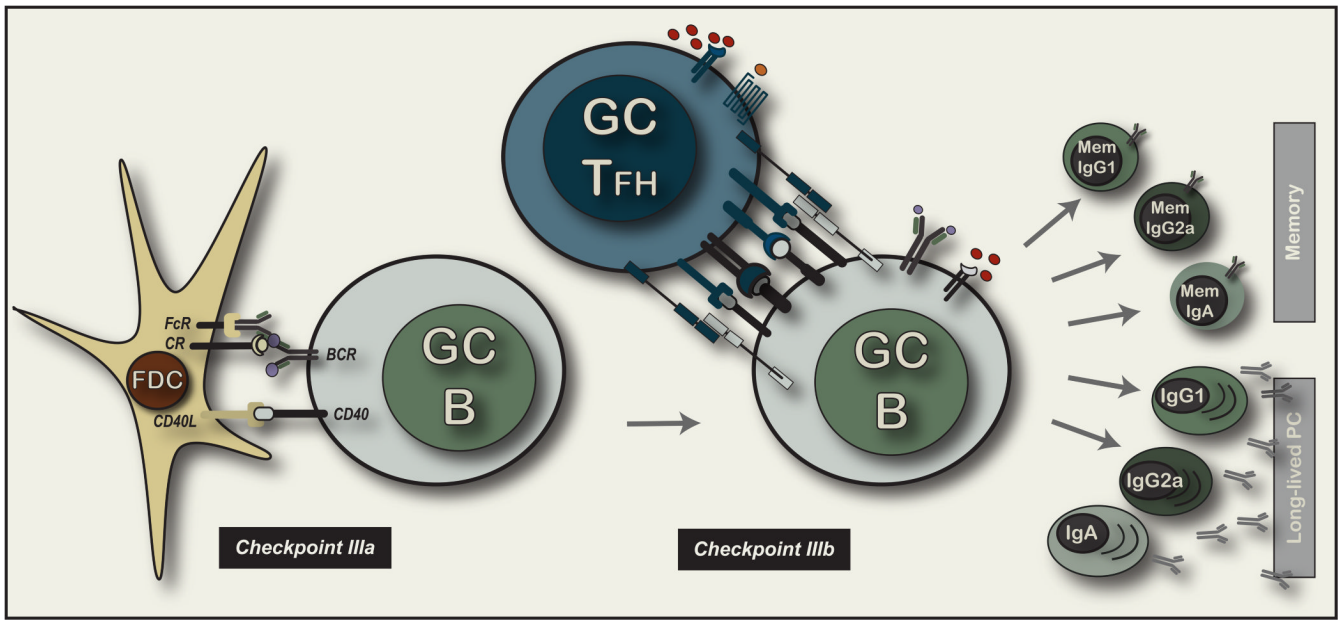

Figure 3. Selection of GC B cells and the cognate control of GC B cell

The left side of this figure depicts the molecular interactions associated with continuous movement of variant GC B cells along the stromal network of mature FDC that have coated immune complexes on their dense light zone processes through FcR and complement receptors. The right side of the figure depicts the intercellular engagement of a GC T_{FH} cell and a GC B cell. We have not labeled the molecules as there is still very little information on the molecular nature of this interaction regarding cell surface molecules or secreted cytokines. The phenotype that has been reported is largely similar between effector T_{FH} cells and their GC T_{FH} counterparts. The far right of figure depicts the predicted cellular outcomes of Checkpoint IIIA and IIIB interactions based on the known output of the GC reaction. There are broadly two classes of memory B cell that exits the GC 1) high affinity precursors for the memory response to antigen re-challenge that exist in a variety of physiological states and 2) long-lived PCs that contribute high affinity antibodies to the circulation of the animal largely for life. The latter compratment is not considered able to react to antigen re-challenge but is nevertheless considered a component of long-term B immunity. Finally, each post-GC pathway potentially comprises of all antibody isotypes that we propose may be considered to be separable lineages of memory B cells. For example, it is likely that IgG1 memory B cells have diverged in development from IgA memory B cells and not only received distinct inducing signals to develop in the first place (at Checkpoint II), but reside in separable microenvironmental niches around the body with distinguishable migratory phenotype, but also require unique activation requirements upon antigen re-challenge.