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FOLLICULAR HELPER T CELLS AS COGNATE REGULATORS OF B CELL IMMUNITY

Louise J. McHeyzer-Williams, **Nadege Pelletier**, **Linda Mark**, **Nicolas Fazilleau**, and **Michael G. McHeyzer-Williams**
Department of Immunology and Microbial Sciences, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla CA 92037

SUMMARY

Follicular helper T (T_{FH}) cells are a class of helper T cells specialized in the cognate control of antigen-specific B cell immunity. Upon first contact with antigen-primed B cells, pre-germinal center effector T_{FH} cells promote B cell clonal expansion, antibody isotype switch, plasma cell differentiation and the induction of germinal centers. In contrast, within germinal centers, T_{FH} cells regulate the fate of antigen-specific GC B cells expressing high-affinity variant B cell receptors to promote memory B cell and long-lived plasma cell development. Recent studies unravel multiple signals controlling T_{FH} development and functional sub-types of antigen-specific T_{FH} cells, including memory T_{FH} cells that accelerate memory B cell responses to antigen re-challenge in vivo.

INTRODUCTION

Helper T cell regulated B cell immunity is considered the basis of long-term immune protection provided by most vaccines in use today. There are spatial and temporal constraints on cognate programming events following antigen-specific priming that serve as developmental checkpoints in clonal selection and the commitment to adaptive immune function [1,2]. The rules that govern information exchange at each developmental checkpoint define the molecular mechanisms of antigen-specific immune protection in this pathway and are the focus of the current review.

Follicular helper T cells are now recognized as the class of helper T cells that regulate the multiple stages of B cell immunity (Figure 1) [3–6]. After initial contact with antigen-experienced DC (Checkpoint I), antigen-specific effector T_{FH} cells emerge as $CXCR5^{+}CCR7^{-}$ T_H cells that migrate to the follicular regions of lymphoid organs to form stable contacts with antigen-primed B cells (Checkpoint II). Subsequent to cognate B cell contact, a cohort of effector T_{FH} cells migrate to germinal centers, form stable contacts with variant GC B cells (Checkpoint III) to regulate the development of antigen-specific memory B cell compartment in ways that remain poorly understood. Finally, memory T_{FH} cells persist within the priming environment to regulate the antigen-specific memory B cell response to re-challenge (Checkpoint IV). We propose that the strength of antigen receptor binding, the

Corresponding Author: Michael McHeyzer-Williams (mcheyzer@scripps.edu).

duration of cellular contact and the molecular context of cognate interactions are the defining attributes of each developmental checkpoint in vivo.

INITIATING ADAPTIVE IMMUNITY: CHECKPOINT I

Vaccines provide foreign antigen within an inflammatory context to initiate dendritic cell (DC) maturation. Antigen-experienced DC will express peptide-MHC class II (pMHCII) complexes and a spectrum of secreted and surface-expressed molecules to recruit naive pMHCII-specific T_H cells (Checkpoint Ia), promote T_H clonal expansion and effector T_H cell differentiation. The strength of TCR-pMHCII interactions and the extended molecular context of these cognate events impact antigen-specific T_H cell fate and the acquisition of effector T_H cell function. Our recent findings indicated the requirement of a threshold TCR affinity to reach maximal local clonal accumulation [7*]. Surprisingly, antigen dose did not alter the clonal selection threshold but changing the vaccine adjuvant altered clonal composition and pMHCII binding profiles of responder T_H cells. More recently, we provided evidence for a casual link between TCR binding strength and the differentiation of effector T_H cells [8**]. In this protein vaccination model, we identified three separable sub-types of antigen-specific effector T_H cells expressing a hierarchy of TCR binding strength. T-zone localized effector T_H cells expressed the lowest binding, emigrant effector T_H cells an intermediate binding and the effector T_{FH} cell compartment the highest binding to pMHCII complexes. Hence, adjuvant controls the threshold for clonal selection and strength of TCR-pMHCII binding regulates the deployment of effector T_H cell function.

Naive B cells that can recognize soluble or cell-associated antigen with sufficient binding strength (Checkpoint 1b) will internalize antigen, process and present pMHCII complexes. Vaccine adjuvants can influence these early events in B cell priming through the engagement of innate receptors [9,10], however their mechanism of action and developmental consequence in vivo remains poorly resolved. Specific recognition by BCR will lead to increased co-stimulatory molecule expression and movement towards the T cell zones of secondary lymphoid tissue [11]. Here, the antigen-primed pMHCII-expressing B cells receive cognate help as a prerequisite for secondary developmental programming events. Without cognate help, protein antigen-primed B cells will largely die without expansion or plasma cell differentiation. Strength of germ-line encoded BCR binding, even at this earliest developmental checkpoint, may impact levels of pMHCII or co-stimulatory molecule expression by antigen-primed B cells, thereby, indirectly influencing subsequent B cell fate and function.

PRE-GC EFFECTOR T_{FH} CELLS: CHECKPOINT II

The cardinal feature of antigen-specific $CXCR5^+$ T_{FH} cells is migration towards the B cell zones of secondary lymphoid tissue [12,13] and then placement within the GCs of an ongoing immune response [14,15]. Functional analysis in vitro [15] and then in vivo upon adoptive transfer [16] established the propensity of the $CXCR5^+$ T_H compartment to support antibody production by B cells. The presence of antigen-specific T_H cells within the GC has been described in human tonsils [17,18] and across multiple murine models [19–22]. In this review, we will refer to the pre-GC compartment involved in checkpoint II interactions as “effector T_{FH} cells” to distinguish them from the “GC T_{FH} cells” associated with checkpoint III interactions (Figure 1). While it remains unclear whether functional and developmental differences exist between these two T_{FH} cell types, it is important to approach current analysis with this possibility in mind.

First contact between pMHCII-specific effector T_{FH} cells and antigen-primed $pMHCII^+$ B cells occurs at the T-B borders [2,19,23]. Cyster and colleagues [24*] first captured dynamic ‘monogamous’ cognate interactions between individual T_H cells and B cells demonstrating highly motile conjugates with the B cell leading movement. It was known that in the absence

of SAP, an adaptor protein for signaling through the SLAM family of receptors [25], T_{FH} cells were still able to secrete cytokines [26] but unable to promote a GC reaction [27]. Germain and colleagues [28**] recently connected these observations by interrogating the control, stability and consequence of effector T_{FH}-B cell early events using dynamic imaging in vivo. This elegant study demonstrated the requirement for SAP in the formation of stable pre-GC T_{FH}-B interactions. There was no role for SAP in the initial contact with $pMHCII^+$ DC allowing all other known features of effector T_{FH} cells to develop (expression of CXCR5, CD40L, ICOS and OX40). Importantly, the antigen-primed B cells had to express the pMHCII specificity of the T_{FH} cells to form the stable contacts [28**]. Finally, the long duration interactions were required for subsequent entry of effector T_{FH} cells into the GC reaction. Hence, it is likely that effector T_{FH} cells produce the GC T_{FH} cell compartment and may require signals from the antigen-primed B cells at this earlier developmental juncture.

EFFECTOR T_{FH} FUNCTION: LINEAGE AND LOCATION

The spectrum of helper T cell functions with regulatory impact on different cellular targets is extensive and expanding [29*]. The programming and organization of these functions across different subtypes of effector T_H cells is still an area of intense research. In this context, the assignment of cytokine production by effector T_{FH} cell functions remains controversial. Early assessments of cytokine production by in vitro re-stimulated $CXCR5^+$ T_{FH} cells indicated IL-2, IFN-γ and IL-10 from human peripheral blood [15] with evidence for IL-4 and IFN-γ from TCR transgenic mouse T_{FH} cells [16]. Molecules important in the development of normal B cell immunity were implicated in early studies. CD40L, ICOS and OX40 expression were candidates for the delivery of effector T_{FH} cell function and shown to be expressed on antigen-specific T_{FH} cells [30*]. Early microarray analyses suggested separable gene expression programs for T_{FH} cells and other known T_H cell subsets. CXCL13 was highlighted early [31] with evidence for ICOS, IL-21 [32,33], IL-21R [34] and the differential expression of Bcl-6 [32] being used as the most reliable attributes of T_{FH} function in vivo. Thus, the acquisition of special effector T_{FH} cell functions may be associated with the programming of a separate T_H cell lineage.

IL-21 has been highlighted as an important cytokine in T_{FH} development [5,35**,36**]. IL-21 and IL21R deficiency has a detrimental impact antibody isotype switch and GC formation [37]. Either deficiency also blocks the development of T_H17 cells in studies that indicate IL-21 acts in an autocrine manner to amplify the T_H17 cell subset [38,39]. Recent studies have highlighted the role of IL-21 in the generation of T_{FH} cells as an autocrine factor [35**, 36**] with further capacity to control B cell immunity as an effector molecule delivered to B cells. King and colleagues [36**] demonstrated that IL21R expression on T_H cells partially rescued the B cell defect in IL-21R deficient animals. These studies indicated IL-21 enhanced co-stimulatory stimuli to developing T_H cells that enhanced T_{FH} induction. Dong and colleagues [35**] preferentially induced CXCR5+ $Bcl\text{-}6^+$ T_{FH} cells in vitro using IL-21 and antibodies to IL-4, IFN-γ and TGF-β blocking the development of T_H1, T_H2 and T_H17 development. Importantly, both groups demonstrated the requirement of ICOS-L on B cells for induction of complete T_{FH} cell development in vivo. These studies argue for a separate T_{FH} lineage with IL-21 as the primary effector function and Bcl-6 as an important regulator of this program.

Antigen-specific B cell development proceeds in at least two separate pathways following checkpoint II interactions (Figure 2). A major developmental decision involves commitment to plasma cell differentiation without BCR diversification or entry into the germinal center pathway to memory B cell development. Both pathways support antibody class switch recombination that can vary depending on the cytokine milieu upon cognate T_H cell contact. Our recent analysis of cytokine expression in vivo [8**], demonstrated the presence of IL-2,

IFN-γ, IL-10 as well as IL-4 and IL-21 in the antigen-specific effector T_{FH} compartment. Elegant studies by Locksley and colleagues using IL-4 and IFN-γ reporter mice [40**], demonstrated the production of IL-4 and IFN-γ by T_{FH} cells at the T-B borders and within GCs after antigen-specific priming. Two further studies by Mohrs and colleagues [41*] and Pearce and colleagues [42*] using reporter mice provided further evidence for a connection between T_H2 cells and T_{FH} cell development. Together, these trends suggest that multiple T_H cell functional subsets may express a secondary migration program to deploy them to the appropriate tissue location to deliver their local regulatory T_{FH} functions.

GERMINAL CENTERS: CHECKPOINT III

The GC cycle of activity begins with recruitment and massive expansion of antigen-specific B cells in the B cell zones of secondary lymphoid tissue. There is evidence for BCR-affinity based selection for entry into the GC cycle [43]. It is curious to speculate that the strength of pMHCII-TCR contact at checkpoint II influences this developmental progression. In this model, the initial strength of BCR binding to antigen may be indirectly responsible for the duration of effector T_{FH} cell $pMHCII^+$ B cell contact at checkpoint II. Nevertheless, stable contact at checkpoint II impacts migration of effector T_{FH} cells in the GC environment. Diversification of the BCR by somatic hypermutation accompanies expression of the variant BCR and then by some means that remains unclear, high affinity GC B cells are selected to enter the long-lived memory compartment. Selection coincides with developmental decisions to become memory response precursors or the long-lived plasma cells to provide long-term antigen-specific immune protection [44]. Antibody isotype also divides across these two major developmental pathways providing multiple layers of memory B cell function in vivo.

Three separate dynamic imaging studies provided breakthrough insights into our current appreciation of GC cellular dynamics in vivo [45–47**]. All three studies highlighted the zonal organization of the GC and documented movement of GC B cells between these areas in both directions. All groups reported the continuous movement of GC B cells along the antigen-laden processes of the follicular dendritic cells (FDC), the stromal element of the GC [48*]. Importantly, the GC B cells did not pause in a synapse-like manner on these FDC with constant velocities at checkpoint IIIa interaction. Nussenzweig and colleagues [47**] reported the sporadic appearance of naïve B cells traversing the GC structures and the capacity of high affinity antigen-specific B cells to join formed GC indicating a more open selection process than previously considered for B cells. Cyster and colleagues [45**,49], reported the transient and then stable contacts between GC B cells and GC T_{FH} cells suggesting that the immune synapse at checkpoint IIIb might be the rate-limiting step in GC B cell selection. This fascinating proposal suggests that the TCR-pMHCII interactions or some other controller of checkpoint IIIb stability could indirectly discriminate the affinity of the GC B cell's BCR for antigen.

GC T_{FH} FUNCTION: SEPARATE B CELL LINEAGES

In their recent analysis of T_{FH}-GC B cell doublets, Locksey and colleagues [40**] provided another surprising and potentially important insight into our understanding of GC cognate interactions. These studies isolated doublets from day 21 of an immune response and demonstrated the phenotype of T_{FH} cells and phenotype/genotype of antigen-specific GC B cells. Most remarkably, IL-4 producing T_{FH} cells paired with IgG1 expressing GC B cells and IFN-γ producing T_{FH} cells paired with IgG2a expressing GC B cells. The authors suggested a continued requirement for these particular growth factors well beyond the initiation of antibody isotype switch. These data also provide evidence for separable cytokine-producing T_{FH} cell lineages dedicated to the regulation of isotype-switched B cells (Figure 2). Thus, antibody isotype would also define separable functional lineages across the antigen-experienced B cell

compartment. In this model, T_{FH} cognate contact may control functional commitment in GC B cells and/or deployment of the memory compartment to the different locations in vivo.

Many different varieties of GC T_{FH} have been reported. Kuchroo and colleagues [50*] recently demonstrate that T_{FH} cells can produce IL-17, while Mountz and colleagues [51*] indicate IL-17 producing GC T_{FH} cells within the GCs of animals that are prone to develop autoimmunity. Using FoxP3 reporter mice, Fagarasan and colleagues [52**] demonstrate that FoxP3 expressing T_H cells were precursors of $FoxP3^{neg}$ T_{FH} cells within the GCs of peyers patches (PP). Importantly, this T_{FH} lineage was restricted to the PP with no capacity to form LN or splenic GC T_{FH} cells. Hence, local environmental cues were highlighted as an important discriminating feature of the functional program in vivo. GC T_{FH} populations have been demonstrated to produce TGF-β1 and IL-21 in ways that synergize to over-ride IgG switch and promote IgA and migration of plasma cells towards mucosal tissues [53]. There have also been recent reports of $ICOS^+$ extra-follicular T_H cells that do not express CXCR5 but may be required for the development/maintenance of the short-lived plasma cell pathway [54*]. Thus, heterogeneity of GC T_{FH} function suggests that the priming context across different types of immune responses impacts the composition of T_{FH} function.

Where there is somatic BCR diversification there must be mechanisms for selecting against self-reactivity. Recent clear evidence for this process came when Goodnow and colleagues [33] demonstrated the induction of autoimmunity in animals with dysregulated ICOS expression on T_H cells. There were substantially higher numbers of T_{FH} cells in these animals with exaggerated spontaneous GC formation that correlated with the induction of autoantibody and pathology. Recently, Vinuesa and colleagues [55*] demonstrated rescue of this model by altering T_{FH} generation GC formation and abrogating disease. These data further support the role of GC T_{FH} cells in the selection of the memory B cell compartment and highlight the possibility that too many GC T_{FH} cells may cause problems in GC B cell selection and the maintenance of peripheral immune tolerance.

MEMORY B CELL RESPONSE: CHECKPOINT IV

$CXCR5^+$ T_H cells were first reported in the peripheral blood of humans [56] but were subsequently demonstrated to express reduced capacity to promote antibody production in vitro [31]. How these circulating $CXCR5^+$ cells relate to the resident T_{FH} compartment in peripheral LNs [4,30] has not been resolved. Our recent demonstration of a memory counterpart to the antigen-specific effector T_{FH} population indicates a cohort of $CXCR5^+$ T_{FH} cells that persists locally to regulate accelerated memory B cell responses. These memory T_{FH} cells re-capitulate the cytokine expression pattern of the primary response effector T_{FH} compartment. Importantly, we provided clear evidence for persistent cell associated pMHCII complexes that may play a role in the local retention mechanism confirm earlier reports in the response to influenza virus infection [57]. Overall, the systemic organization of memory T_{FH} cells suggests a substantial regional component for the cognate regulators of antigen-specific memory B cell responses. This critical arena of research has received very little attention in recent years but underlies the future rational design of vaccine boost strategies.

CONCLUSIONS

Follicular helper T cells define a class of regulatory T_H cells specialized for the cognate control of all stages of antigen-specific B cell development. Recent advances in this field indicate that initial contact with pMHCII-expressing DC induces multiple subtypes of $CXCR5^+$ effector T_{FH} cells producing different cytokines involved in the programming of B cell immunity. Antibody isotype switch is one major facet of the B cell response regulated by cognate control that may define antigen-specific B lineage commitment throughout plasma cell and memory

B cell differentiation. The mechanisms used by pMHCII-specific GC T_{FH} cells to regulate GC B cells control the fate and function of the antigen-specific memory B cell compartment are now experimentally accessible but remain poorly resolved. Finally, pMHCII-specific memory T_{FH} cells persist in the local priming environment for the cognate control of accelerated memory B cell responses to antigen re-challenge.

Understanding how to regulate these cognate regulators is the central design challenge for the next generation of protein vaccines. It is now feasible to select vaccine adjuvants and antigen delivery strategies to optimize different T_{FH} functions and subsequent B cell fates. Valuable recent advances emphasize experimental models that are accessible to quantitative cellular and detailed molecular analysis in physiologically relevant settings. These studies allow us to devise complex but layered models for the regulation of antigen-specific B cell immunity that are experimentally tractable and promise mechanistic insight to the future vaccine effort.

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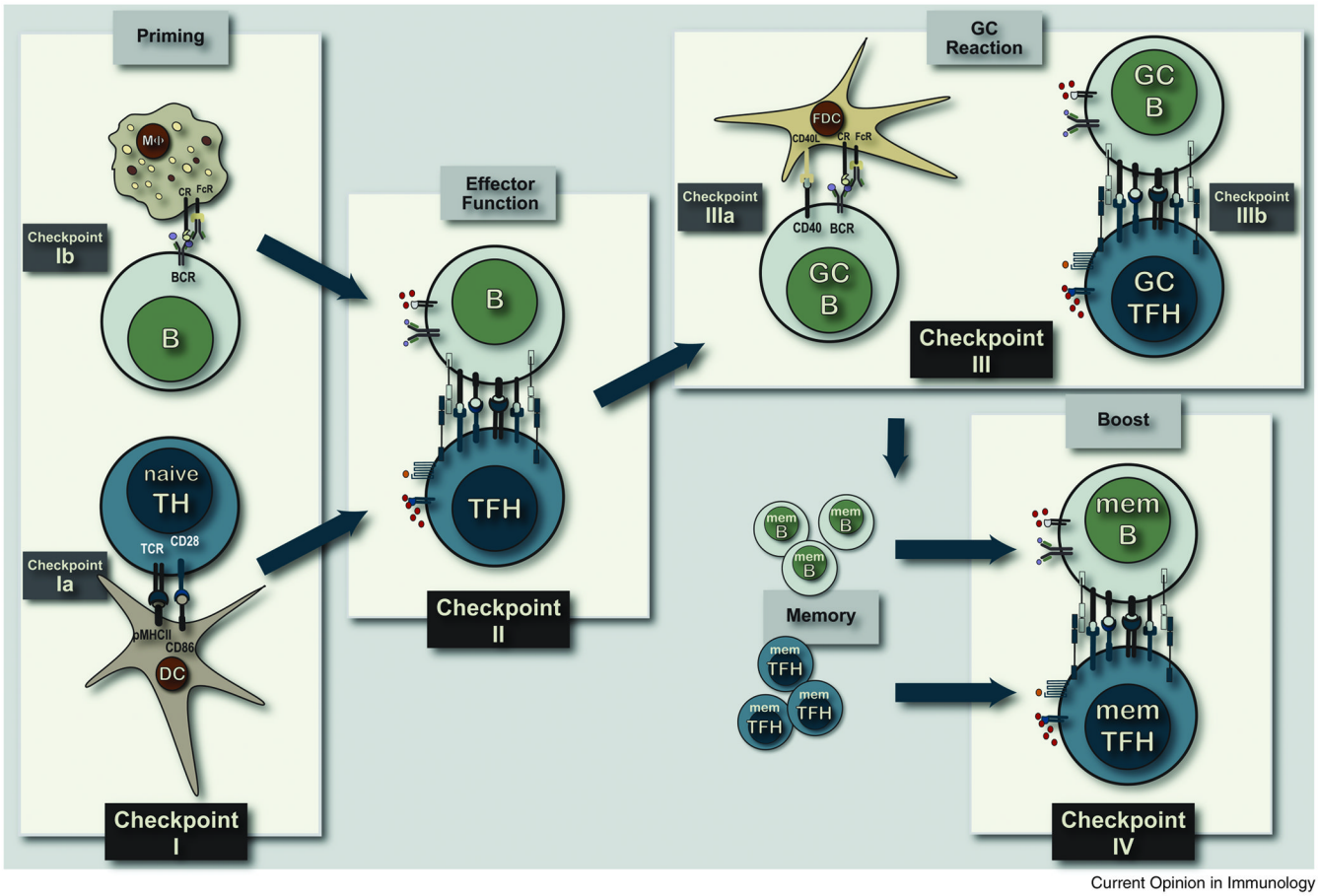

FIGURE 1. COGNATE TH CELL REGULATION OF B CELL IMMUNITY

Following local protein vaccination, mature antigen-experienced dendritic cells from the site of injection traffic to the draining lymphoid tissue to prime pMHCII-specific naive T_H cells at Checkpoint Ia. Antigen can also be transported to the subcapsullar sinus by macrophage to present native cell associate antigen to B cells at Checkpoint Ib. Antigen-specific B cells will take up protein antigen, process and present pMHCII complexes and move towards the T-B borders to interact with pMHCII-specific effector T_{FH} cells at Checkpoint II. Following stable cognate contact a cohort of antigen-specific B cells will move into the follicular regions, massively expand to form secondary follicles, somatically diversify their BCR, express the variant BCR and then traverse FDC networks in the light zone of germinal center at continuous Checkpoint IIIa interactions. GC B cells expressing high affinity variant BCR form stable contacts with GC T_{FH} cells at Checkpoint IIIb prior to GC exit and entry into the memory B cell compartment as either memory response precursors or long-lived plasma cells. Antigen-specific memory T_{FH} cells and memory B cells persist in the priming lymphoid tissue to interact upon secondary challenge with antigen at Checkpoint IV a requisite regulatory interaction for expansion of memory B cells and formation of memory response plasma cells.

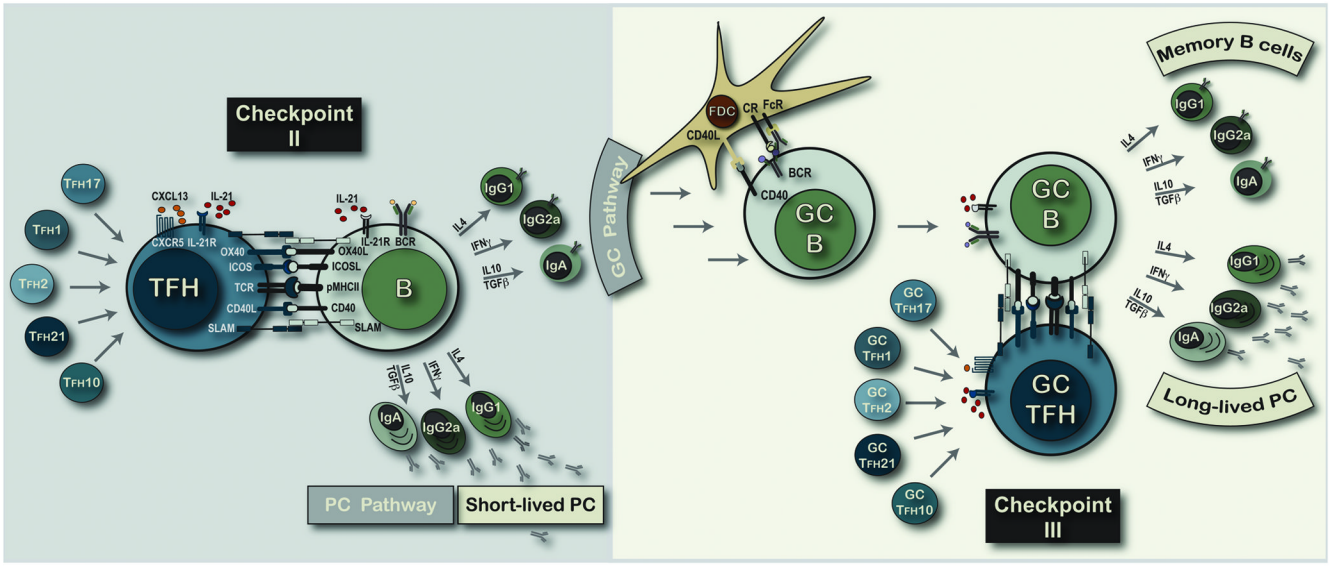

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FIGURE 2. SEPARABLE ANTIGEN-SPECIFIC T_{FH} AND B CELL LINEAGES

We depict critical sets of molecular interactions at Checkpoint II that appear common to all antigen-specific effector T_{FH} interactions with antigen-primed pMHCII-expressing B cells. We propose that all sub-types of functionally distinct T_H cell lineages can also program the migration to the T-B borders and B cell zones to become T_{FH} cells and regulate B cell fate and function. Further, these separate effector T_{FH} lineages, depicted according to their original T_H cell program, will promote distinct B cell lineage decisions at Checkpoint II. We propose that antibody isotype is a major 'lineage' decision for antigen-specific B cells and that within each isotype-specific lineage the secondary outcomes of plasma cell versus GC B cell fate may be controlled by the duration of contact at Checkpoint II. Entry into the GC pathway involves clonal expansion, somatic BCR diversification and affinity-based selection for all isotype-specific GC B cells at Checkpoint IIIa. GC B cells with a positive selection 'imprint' at Checkpoint IIIa will form stable cognate interactions with pMHCII-specific GC T_{FH} cells at Checkpoint IIIb. We propose that there is some lineage maintenance between the effector T_{FH} and the GC T_{FH} creating distinct T_{FH} lineages dedicated to the regulation of separate isotype-specific B cells. In this model, IL-4 expressing GC T_{FH} will re-connect with $IgG1^+$ GC B cells and IFN-γ+ T_{FH} will re-connect with $IgG2a^+$ GC B cells as demonstrated by Locksley and colleagues [40**]. It is plausible that the duration of intercellular contact regulates memory B cell versus long-lived plasma cell fate and function at this point of memory B lineage development.