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Review

Regulation of Germinal Center Reactions by B and T Cells

Young Uk Kim [1], Xindong Liu [2], Shinya Tanaka [3], Dat Quoc Tran [4] and Yeonseok Chung [1,*]

[1] Center for Immunology and Autoimmune Diseases, Institute of Molecular Medicine, the University of Texas Medical School at Houston, Houston, TX 77030, USA; E-Mail: Young.Uk.Kim@uth.tmc.edu

[2] Department of Immunology, MD Anderson Cancer Center, Houston, TX 77030, USA; E-Mail: XLiu5@mdanderson.org

[3] Immunology Frontier Research Center, Osaka University, Osaka 565-0871, Japan; E-Mail: stanaka@ifrec.osaka-u.ac.jp

[4] Department of Pediatrics, the University of Texas Medical School at Houston, Houston, TX 77030, USA; E-Mail: Dat.Q.Tran@uth.tmc.edu

* Author to whom correspondence should be addressed; E-Mail: Yeonseok.Chung@uth.tmc.edu; Tel.: +1-713-500-3190; Fax: +1-713-500-2420.

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Abstract: Break of B cell tolerance to self-antigens results in the development of autoantibodies and, thus, leads to autoimmunity. How B cell tolerance is maintained during active germinal center (GC) reactions is yet to be fully understood. Recent advances revealed several subsets of T cells and B cells that can positively or negatively regulate GC B cell responses *in vivo*. IL-21-producing $CXCR5^+$ $CD4^+$ T cells comprise a distinct lineage of helper T cells—termed follicular helper T cells (T_{FH})—that can provide help for the development of GC reactions where somatic hypermutation and affinity maturation take place. Although the function of T_{FH} cells is beneficial in generating high affinity antibodies against infectious agents, aberrant activation of T_{FH} cell or B cell to self-antigens results in autoimmunity. At least three subsets of immune cells have been proposed as regulatory cells that can limit such antibody-mediated autoimmunity, including follicular regulatory T cells (T_{FR}), Qa-1 restricted $CD8^+$ regulatory T cells ($CD8^+T_{REG}$), and regulatory B cells (B_{REG}). In this review, we will discuss our current understanding of GC B cell regulation with specific emphasis on the newly identified immune cell subsets involved in this process.

Keywords: T_{FH}; T_{FR}; B_{REG}; Qa-1 restricted $CD8^+T_{REG}$; Germinal center; B cells; antibody

1. Introduction

Negative selection during the development of B cells in the bone marrow and T cells in the thymus leads to the deletion of self-reactive B and T cells. Although some of the self-reactive B cells can escape from negative selection in the bone marrow, they are seldom activated due to the lack of proper help from T cells, since most of self-reactive T cells in the periphery are in an anergic state. These processes—termed central and peripheral tolerance—represent a primary mechanism by which the immune system prevents the development of autoimmunity. During active immune responses in the periphery, antigen-specific T:B cell interaction induces somatic hypermutation of the B cells in the complementarity determining regions. Multiple rounds of somatic hypermutations not only enable the generation of high affinity antibodies, but also diversify the repertoire of B cell receptors. As a result, some of the newly generated B cells possibly acquire B cell receptors that recognize self-antigens. Hence, the somatic hypermutation process is a double-edged sword that may lead to the generation of high affinity antibodies, as well as auto-reactive B cells in the periphery. Importantly, this T: B interaction mainly occurs in a specialized region of B cell area, termed germinal center (GC). Thus, understanding the regulation of GC responses is crucial for vaccine development, as well as for the treatment of antibody-mediated autoimmune diseases.

Recent advances have clearly demonstrated the crucial role of $CXCR5^+$ follicular helper T cells (T_{FH}) in GC responses. T_{FH} cells promote GC responses by providing developmental and survival signals, as well as factors important for B cells, which eventually become memory B cells and long-lived antibody secreting plasma cells [1]. However, unnecessary activation of T_{FH} and B cells against self-antigens may induce autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren syndrome, and juvenile dermatomyositis [2].

The identity of cells that control T_{FH} and GC B cell responses are not fully understood. Recent studies have simultaneously discovered at least three specialized immune cells that specifically suppress GC responses, namely follicular regulatory T cells (T_{FR}), Qa-1 restricted CD8 regulatory T cells, and regulatory B cells (B_{REG}) [3]. Despite the differences in their lineages, all three types of regulatory cells express CXCR5, just like T_{FH} cells. Hence, the balance between T_{FH} cells and the regulatory cells likely determine the magnitude and duration of GC B cell responses. Understanding how T_{FH} cells and these regulatory cells control GC B cell responses will pave the way for the development of novel therapeutic targets in vaccine design, as well as for the treatment of antibody-mediated autoimmune diseases.

2. Follicular Helper T Cells

The help of $CD4^+$ T cells is required for GC formation, Ig class-switching, and antigen specific memory B cell and plasma cell production [4]. The concept of T_{FH} cells was first described in humans as $CD4^+$ T cells in secondary lymphoid tissues (tonsil) that express CXCR5 and localize in B cell follicles, especially in germinal centers (GCs) [5–7]. GCs are histologically specialized structures that

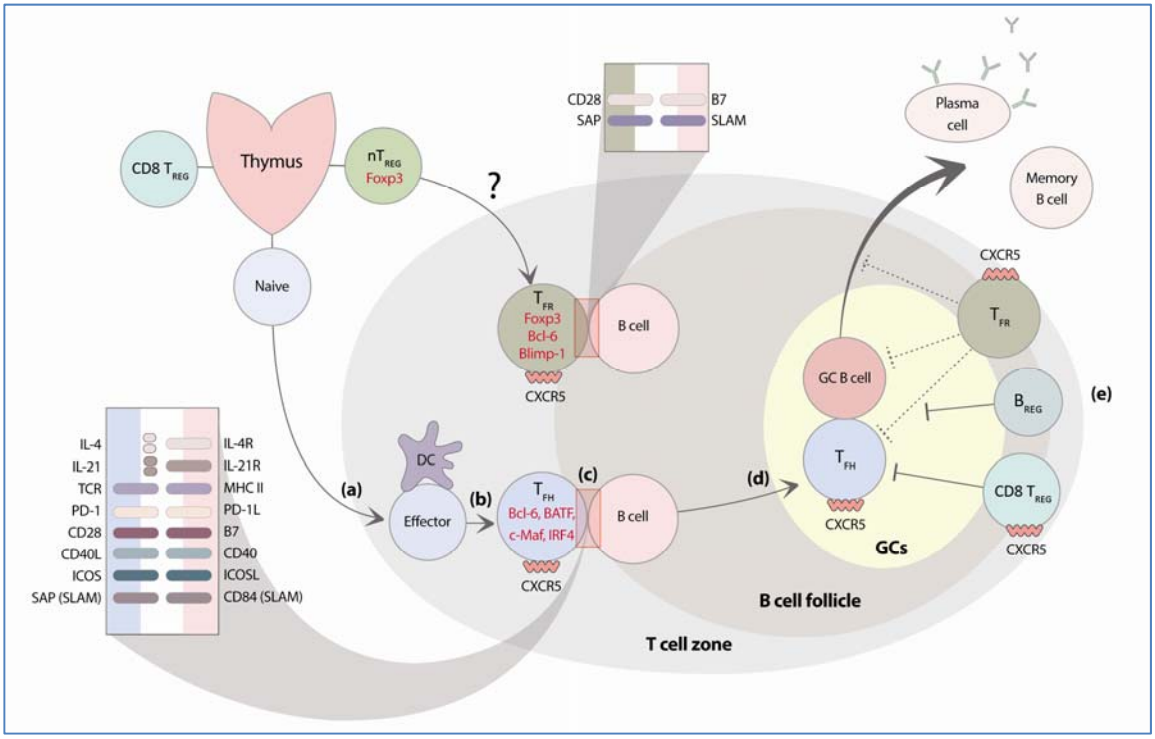
develop within B cell follicles of secondary lymphoid tissues where somatic hypermutation, selection of high affinity B cells, class switch recombination, as well as plasma cell and memory B cell differentiation mainly occur [1]. CXCR5 expression allows T_{FH} cells to migrate into B cell follicles in response to a CXCL13 gradient [7,8], and thus it serves as a surface marker of this T_H subset. In the past, $CXCR5^+$ T_H cells were thought to be a subpopulation of Th2 cells, since they were able to express IL-4 [9–11]. However, recent studies have shown that $CXCR5^+$ T_{FH} cell generation was intact in $STAT6^{-/-}$, as well as $IL\text{-}4^{-/-}$ mice [9,12]. Moreover, T_{FH} are normal in $STAT4^{-/-}$ and $Rorc^{-/-}$ mice. Hence, the differentiation of T_{FH} cells is independent of the Th1, Th2 and Th17 lineage programs. More recently, B cell lymphoma 6 (Bcl-6) has been reported as an essential transcriptional repressor for T_{FH} cell differentiation [13–15].

Differentiation of GC B cells and T_{FH} cells is likely initiated in the interfollicular (IF) zone, where initially activated $CD4^+$ T cells are further primed by CXCR5 expressing dendritic cells [16,17]. Interaction between antigen-specific T cells and B cells also occurs in the IF zone for the first two to three days after immunization, prior to their migration to the follicles to form GCs [16]. In the GCs, B cell division is restricted to the dark zone (DZ), while B–T interaction occurs in the light zone (LZ). B cell assistance from T_{FH} cells in the LZ facilitates B cell return to the DZ, which is accompanied by clonal expansion of B cells in the DZ [18]. Unlike GC B cells, T_{FH} cells have been shown to continually emigrate into follicles and neighboring GCs [19]. Moreover, newly differentiated T_{FH} cells can migrate into preexisting GCs and augment ongoing GC responses [19]. These polyclonal colonization and invasive properties of T_{FH} cells might be critical for prolonged GC B cell responses. T_{FH} cells express CXCR5, inducible co-stimulator (ICOS), programmed cell death protein 1 (PD-1), Bcl-6, basic leucin zipper transcription factor ATF-like (BATF), signal transducer and activator of transcription 3 (STAT3), c-Maf and interferon regulatory factor 4 (IRF4), as well as cytokines IL-21, IL-4, and IL-10 [1,20,21]. Other surface molecules, such as CD40 ligand (CD40L), BTLA, CD84, and cytoplasmic adaptor protein SLAM-associated protein (SAP), are also expressed in T_{FH} cells (Figure 1) [5,6,13,14,22–27].

2.1. Transcription Factors and Molecules Required for the Differentiation of T_{FH} Cells

In the last five years, a number of studies have identified multiple transcription factors that are required for T_{FH} differentiation. As mentioned above, Bcl-6 has been reported as a master transcriptional regulator for T_{FH} lineage commitment [13–15]. As a transcriptional repressor, expression of Bcl-6 can inhibit Th1, Th2, and Th17 differentiation. Interestingly, some of these transcription factors, such as STAT3, BATF, and IRF4 are also necessary for Th17 cell commitment. In addition, IL-21 is the most important effector cytokine for T_{FH} cells, and it is also expressed in Th17 cells. Therefore, T_{FH} and Th17 cells share many common features including developmental requirements and effector cytokines.

Figure 1. Schematic view of germinal center reactions. (**a**) Naïve $CD4^+$ T cells interact with antigen-presenting dendritic cells (DCs) in the T cell zones. (**b**) Activated T cells transiently express CXCR5 and migrate to the T-B border. (**c**) Interaction of $CXCR5^+$ T cells with Ag-specific B cells and follicular DCs further promote T_{FH} differentiation, as well as the formation of germinal center (GC). (**d**) In GC, T_{FH} cells induce clone expansion, somatic hypermutation, and class switching of B cells. (**e**) The interaction of T_{FH} and B cells leads to the generation of memory B cells and long-lived antibody-producing plasma cells. Transcription factors, Bcl-6, c-Maf, BATF and IRF4, in T_{FH} cells direct the expression of T_{FH} signature genes, including CXCR5, ICOS, IL21, and PD1. Interactions of CD28:CD86, CD40L:CD40, PD-1:PD-1L, SLAM:SAP and ICOS: ICOSL between T_{FH} and B cells are required for GC formation. (**f**) Follicular regulatory T cells (T_{FR}), Qa-1 restricted $CD8^+$ regulatory T cells, and IL-10 producing regulatory B (B_{REG}) cells are known to suppress GC responses.



2.1.1. Bcl-6

Bcl-6 was first described in a subset of $CD4^+$ T cells in GCs in human tonsils [28]. $CD4^+$ T cells from Bcl-6 deficient mice failed to differentiate into T_{FH} cells, while constitutive expression of Bcl-6 induced T_{FH} cell generation *in vivo* [13–15]. Bcl-6 drives T_{FH} cell differentiation by suppressing the differentiation of the other T_H subsets. For instance, Bcl-6 suppresses GATA3 expression [29], and can directly bind to *Tbx21* (encoding T-bet) and *Rorc* (encoding RORγt) promoter regions to suppress the transcription of these genes [15]. Moreover, Bcl-6 suppresses Blimp-1 and a cluster of microRNAs which suppress T_{FH} generation [15]. Blimp-1 (encoded by *Prdm1* gene) and its antagonist Bcl-6 are reciprocally expressed in T_{FH} cells and other subsets of T helper cells [13,30–32]. Enforced Blimp-1 expression in $CD4^+$ T cells suppresses Bcl-6 expression and generation of T_{FH} cells [13]. However,

over-expression of Bcl-6 is likely not sufficient to induce IL-21 and CXCR5 expression on T cells [1]. The mechanism of Bcl-6 induction during T_{FH} differentiation remains unclear.

2.1.2. c-Maf

c-Maf plays an important role in Th2 and Th17 development [33]. c-Maf is induced by IL-6 and IL-27 [34] and triggers IL-4 and IL-21 in activated $CD4^+$ T cells *in vitro* [27,34] and *in vivo* [35]. In addition, the expansion of T_{FH} cells induced by IL-27 is c-Maf-dependent [27,35,36]. c-Maf directly binds to the IL-21 promoter region [34,36,37], and constitutive c-Maf expression is known to be sufficient to enforce IL-21 expression in activated $CD4^+$ T cells [34]. With NFAT and JunB help, c-Maf induces IL-4 [38] in T cells in a GATA3-independent manner [10]. In naïve $CD4^+$ T cells, co-expression of c-Maf and Bcl-6 significantly increases the expression of CXCR5, as well as ICOS and PD-1 [27].

2.1.3. BATF

BATF belongs to the activator protein 1 (AP-1) superfamily and is originally known to be required for Th17 differentiation [39]. Of note, BATF-deficient mice also show defects in T_{FH} cells and GC responses [40,41]. During T_{FH} cell differentiation, BATF directly regulates Bcl-6 and c-Maf expression by binding to the promoter regions of these genes [41]. Defective generation of T_{FH} cells in BATF-deficient $CD4^+$ T cells can be restored by simultaneous expression of Bcl-6 and c-Maf [41].

2.1.4. STAT3

STAT3 is the main transducer of IL-6R and IL-21R signals in T and B cells [42,43]. These two signaling pathways are important for T_{FH} differentiation and affect Bcl-6 expression in stimulated CD4 T cells [12,14]. In T_{FH} cells, STAT3 can bind to the *Bcl-6* promoter and induce Bcl-6 expression [44–46]. On the other hand, STAT3 also induces Blimp-1, an antagonist of Bcl-6 in $CD4^+$ T cells [45]. STAT3-deficient $CD4^+$ T cells are shown to be defective in T_{FH} cell differentiation [12], while another study showed normal population of $CXCR5^+CD4^+$ T cells in the absence of STAT3 [47,48]. In human T cells, STAT3 does not seem to be important for T_{FH} cell differentiation and function [49,50]. Thus, it is likely that although STAT3 is generally required for optimal T_{FH} differentiation, T_{FH} cells can be generated in a STAT3-independent fashion.

2.1.5. IRF4

IRF4, like BATF and STAT3, is also known as a Th17 cell transcription factor [49,51,52]. IRF4 can bind to the *Il21* promoter and play a crucial role during T_{FH} cell development [45,53]. Defect in the T_{FH} population has been reported in IRF4-deficient mice, however, it is not clear whether this was T_{FH} cell specific, or due to a general defect in $CD4^+$ T cells [45]. In cooperation with BATF, IRF4 binds to the AP1-IRF composite element and stimulates IL-21 expression during Th17 differentiation [54,55]. However, whether the same complex mediates the expression of T_{FH} transcription factors, such as Bcl-6 and c-Maf, remains to be determined. A number of studies have also demonstrated that deregulation of IRF4 is associated with auto-reactive B cell responses. For instance, Pernis and colleagues have

identified the IRF4-binding protein (IBP) as a negative regulator of IRF4. Mice deficient in IBP develop spontaneous autoimmune phenotypes associated with enhanced responsiveness of T cells to low levels of stimulation [56,57]. Mechanistically, IBP inhibits the binding of IRF4 to the transcriptional regulatory regions of IL-17 and IL-21, and thus, IBP-deficient T cells produce enhanced amounts of IL-17 and IL-21 [57]. The increased production of IL-21 in IBP deficient mice results in the increased expression of the *Aicda* gene (encoding AID) in B cells [58]. These results strongly suggest a crucial role for IRF4 in T_{FH} cell differentiation and autoimmune B cell responses.

2.1.6. microRNAs

MicroRNAs of the miR-17~92 family have originally been reported as negative regulators of T_{FH} differentiation [15]. On the contrary, two recent studies have demonstrated that the miR-17~92 family is essential for T_{FH} differentiation. For instance, Kang *et al.* described that mice with T cell-specific deletion of miR-17~92 have a significantly diminished T_{FH} population, while mice with T cell-specific transgenic expression of the same microRNA family developed a fatal immunopathology with spontaneous T_{FH} responses [59]. Mechanistically, the miR-17~92 family has been shown to contribute to the migration of T_{FH} cells into B cell follicles by suppressing the expression of phosphatase PHLPP2 [59]. In addition, Baumjohann *et al.* have demonstrated that miRNA-17~92 suppresses the expression of *Rora*, thus inhibiting the induction of Th17-related genes during T_{FH} cell differentiation [60]. Collectively, these studies clearly demonstrate that the miR-17~92 cluster is necessary for T_{FH} lineage specification *in vivo*.

2.1.7. Cytokines

In addition to its indispensable role in Th17 cell differentiation [61], IL-6 has also been shown to play a role in T_{FH} cell differentiation through its capacity to induce IL-21 by T cells [12,47,62]. Both IL-6 and IL-21 signaling occurs through the activation of STAT3 and STAT3-deficient $CD4^+$ T cells show defects in T_{FH} differentiation [12]. An initial study showed that IL-6 induces the transcription of Bcl6 and CXCR5 [14], while other studies demonstrated normal development of T_{FH} cells in the absence of IL-6 signaling [47,48,63]. In addition, it has recently been described that the increase of T_{FH} cells and GC responses during the late stage of chronic LCMV infection requires IL-6, and that late blockade of IL-6 signaling delays viral clearance [64]. In the absence of IL-6 signaling, virus-specific $CD4^+$ T cells express significantly decreased Bcl-6, indicating a crucial contribution of this cytokine to T_{FH} lineage programming during viral infections [64]. Another study showed that the absence of either IL-6 or IL-21 alone does not limit the T_{FH} differentiation, while removing both signals substantially abrogates T_{FH} cells. These observations suggest a redundant function for these two cytokines in this process [48], which might be crucial for late maintenance of T_{FH} cells.

IL-2 has been reported to play a suppressive role in T_{FH} differentiation [65–67] by inducing STAT5 mediated Blimp-1 [65,67]. Moreover, in Th1 polarization conditions, a high concentration of IL-2 inhibits Bcl-6 expression by regulating STATs and Foxo binding to the *Bcl-6* promoter. In addition, T-bet interacts with Bcl-6 to form a Tbet-Bcl6 complex, resulting in the inhibition of Bcl-6 dependent gene repression [66]. *Toxoplasma gondii* and viral infections enhance humoral responses by increasing

T_{FH} cells in the absence of T-bet [68,69], indicating that the ratio of T-bet and Bcl-6 in T helper cells is a critical determinant of Th1 and T_{FH} cell lineages.

IL-12 is also induces IL-21 in both human and mouse $CD4^+$ T cells and these cells have T_{FH}-like cell phenotypes [30,48,50,69–71]. Interestingly, IL-21 induction by IL-12 depends on STAT3 signaling [69,70]. During *in vitro* Th1 differentiation, IL-12 induces both IL-21^+Bcl-6^+ and IFN-γ^+T-bet^+ $CD4^+$ T cell populations at an early stage [50,69,70]. However, Th1 cells became a major population under the Th1 polarizing conditions, whereas T_{FH} differentiation is blocked by persistent expression of T-bet [69] via its ability to suppress Bcl-6 [66].

2.2. Molecular Requirements for T_{FH} Cell Function

2.2.1. CXCR5

CXCR5 represents a reliable marker of T_{FH} cells and its expression helps them migrate into the B cell follicles in response to CXCL13 (Figure 1) [7,8]. During differentiation, T_{FH} cells down-regulate CCR7 to avoid their relocation to the T cell zone [1,25,72]. A recent study demonstrated that expression of CXCR5 on DCs was required for optimal T_{FH} and Th2 differentiation in response to *H. polygyrus* infection [17]. Deficiency of CXCR5 in T cells up-regulates Blimp-1 and decreases T_{FH} cell frequency [13,32,73,74]. CXCR5-deficient T cells fail to migrate into B cell follicles and thus fail to induce GC B cells [25,72,75]. Bcl6-deficient T cells do not express CXCR5 [14,15]. Kitano *et al.* demonstrated that up-regulation of Bcl-6 and CXCR5 on all T cells were initiated two days after immunization, and peaked at day 3 [76]. In contrast, Liu and colleagues reported that, while Bcl-6 expression was gradually increased from day 2 to day 7, the expression of CXCR5 was dramatically increased at day 2, and reached a plateau at day 3 and was maintained at a high level in activated T cells [77]. Notably, over-expression of Bcl-6 alone minimally increased the expression of CXCR5 on $CD4^+$ T cells [27,77]. Future studies will be needed to identify one or more transcription factors that directly trigger the expression of CXCR5.

2.2.2. ICOS

ICOS is up-regulated on $CD4^+$ T cells after CD28 co-stimulation [78,79]. ICOS provides an important signal for the survival of effector $CD4^+$ T cells, as well as for the differentiation of T_{FH} cells. ICOSL is constitutively expressed on most of DCs and B cells [78,80,81]. ICOS signaling recruits PI3K to its cytoplasmic tail, which in turn triggers the expression of IL-21, IL-4, c-Maf and CXCR5 [31,35,82–86]. ICOS-deficient mice have a reduced number of T_{FH} cells and form immature GCs after immunization [35,87,88]. ICOS has recently been shown to directly control follicular recruitment of activated T helper cells by follicular bystander B cells, rather than by antigen presenting DCs, or cognate B cells [89]. ICOS engagement induces coordinated pseudopod formation and increases persistent T cell migration at the border between the T-B zone *in vitro* and *in vivo* [89]. As a result, in the absence of ICOSL on follicular bystander B cells, activated T helper cells cannot develop into T_{FH} cells [89].

2.2.3. CD40L–CD40

CD40 is involved in multiple stages of B cell activation and differentiation. CD40L, which is the only ligand of CD40, is highly expressed in activated $CD4^+$ T cells [1]. Deficiency of CD40L or CD40, or patients with mutations in *CD40LG*, has a decreased number of T_{FH} cells and a failure of GC formation [84,90,91]. CD40 is critical for B cell activation, proliferation and survival, and CD40L–CD40 engagement is critical for the maintenance of GC B cells [1,92]. It has been reported that CD40L together with IL-21 or IL-4 is required for the maintenance of GC B cells. In the absence of CD40L, GC B cells differentiate into plasma cells [93–96]. Furthermore, the CD40–CD40L interaction is important for migration of T cells into follicles since T_{FH} cells from $CD40L^{-/-}$ mice failed to migrate into B cell follicles, which can be restored by anti-CD40 [97]. Therefore, the CD40–CD40L interaction is bidirectional and CD40L signaling to the $CD4^+$ T cells is essential for the migration, priming and maintenance of T_{FH} cell [97–99].

2.2.4. SAP:SLAM Family Receptor

SAP plays multiple roles in T_{FH} function. First, SAP is required for the formation of T–B conjugates, which is critical for T_{FH} differentiation [100,101]. SAP-deficient $CD4^+$ T cells are unable to form stable conjugates with cognate B cells and fail to differentiate into T_{FH} cells [10,26,100–103]. Importantly, patients with X-linked lympho-proliferative disease have mutations in *SH2D1A* (encoding SAP), and they exhibit impaired T_{FH} cell function and poor humoral immunity [104,105]. In addition, SAP is also required for the induction of IL-4 by T_{FH} cells, which is independent of Th2, but requires SLAM-SAP-PKCθ signaling [10,106].

Additional molecules in the T-B cell interaction include the SLAM family of receptors: SLAM (CD150), CD84 (SLAMF5), Ly108 (SLAMF6;NTB-A in humans), Ly9 (CD229, SLAMF3) and 2B4 (CD244, Natural Killer cell receptor) [107]. These SLAM family receptors can recruit SAP which is a cytoplasmic adaptor molecule and activate signal cascade through PKCθ, BCL-10, NF-κB and FYN [105]. CD84 is a SAP-binding SLAM family receptor that is up-regulated on both mouse and human T_{FH} cells and GC B cells [22,101]. After primary integrin interaction between B and T cells, CD84 stabilizes the B:T cell conjugates and helps T_{FH} function and GC formation *in vivo* [101]. Ly108 is a SAP-binding SLAM family member and has two distinct isoforms [1]. Ly108 has a role in B cell negative selection and T-B cells adhesion [101,108]. Deletion of Ly108 in $CD4^+$ T cells reversed the *Sh2d1a*$^{-/-}$ phenotype by eliminating the SAP requirement for GCs. Its inhibitory function is dependent on immunotyrosine switch motifs (ITSMs) and SHP-1 [109]. Recruitment of high levels of SHP-1 at the T:B synapse enables Ly108 to limit T:B cell adhesion [109]. Ly9 is constitutively expressed at a high level on $CD4^+$ T cells and there is no defect in T_{FH} differentiation, GCs, and antibody responses in Ly9-deficient mice [110]. SLAM is known to be important for Th2-independent IL-4 expression in GC T_{FH} cells, although IL-4 is likely not required for T_{FH} differentiation [10,111].

2.2.5. IL-21

IL-21 has been demonstrated to be essential for T_{FH} cell differentiation and function *in vitro* and *in vivo* [112,113]. IL-21 production in $CD4^+$ T cell is induced by IL-21 itself, or IL-6, IL-12 and

IL-27 [12,62,114,115]. IL-21 increases the expression Bcl6 and CXCR5 transcripts in $CD4^+$ T cells *in vitro* [12,14]. IL-21-deficient mice exhibit relatively normal frequency of T_{FH} cells upon immunization, however, the number of T_{FH} cells rapidly declines [112], indicating that IL-21 signaling is essential for the maintenance of differentiated T_{FH} cells. Importantly, IL-21 from T_{FH} cells drives the differentiation of plasma cells from activated B cells in GC [44,116–119], and it also promotes the proliferation of GC B cells [112,113]. IL-21 is also important for the affinity maturation of immunoglobulins, but it does not affect the formation of memory B cells [112,113]. Furthermore, IL-21 signaling triggers immunoglobulin isotype switching to produce IgG3, IgA and IgG1 in human B cells, or IgG1 in murine B cells [120–122].

2.2.6. IL-4

Th2 cytokine IL-4 has been thought as a B cell survival and differentiation factor [123]. A series of studies have demonstrated that T_{FH} cells, rather than Th2 cells, provide help for B cell differentiation and maturation [1]. By using IL-4 reporter mice, Reinhardt *et al.* showed that the majority of $CD4^+$ T cells secreting IL-4 after *Leishmania major* infection are T_{FH} cells and that IL-4 production is induced by SAP:SLAM interaction in a Th2-independent manner [10,11,106]. IL-4 triggers the Ig isotype switch to IgG1 and IgE [1,124,125]. IL-4 signaling in B cells induces Bcl-XL, an anti-apoptotic Bcl-2 family gene, and strongly enhances glucose uptake [125,126]. Therefore, IL-4 production by T_{FH} cells mediates the survival and proliferation of GC B cells.

2.3. T_{FH} Cells and Autoimmunity

While T_{FH} cells are necessary for humoral immune responses to infectious agents and cancerous cells, excessive T_{FH} responses may lead to the induction of self-reactive B cells [102,127]. Indeed, increased T_{FH} responses are tightly associated with systemic autoimmunity, such as SLE, in both humans and mice [127–129]. Spontaneous generation of GCs and expansion of T_{FH} cells are key features of SLE [2,23,102,130–133].

2.3.1. T_{FH} Cells in Animal Models of Antibody-Mediated Autoimmunity

Sanroque mice have a single recessive mutation in the *Roquin* gene that encodes a RING-type ubiquitin ligase protein that interrupts a repressor of ICOS expression [23]. The mutation of *sanroque* causes excessive numbers of T_{FH} cells with high expression of ICOS and IL-21in T cells that lead to the development of SLE-like pathologies [23]. Adoptive transfer of T_{FH} cells from *sanroque* mice into wild-type mice triggers spontaneous GC formation and production of autoantibodies [134]. SAP deficiency or Bcl-6 deletion in the *sanroque* mice results in the reduction of T_{FH} cells and IL-21 production, and thus alleviates lupus-like symptoms [102,135,136]. Of note, deletion of *Roquin* in hematopoietic cells results in deregulation of immune homeostasis, such as increased GC B cells and effector memory T cells in the secondary lymphoid organs, but does not lead to spontaneous autoimmunity [137]. Thus, the exact role of *Roquin* in the generation of self-reactive T_{FH} cells remains to be determined.

BXSB.*Yaa* mice also exhibit spontaneous GC formation and T_{FH} cells [44,136]. These SLE-like autoimmune phenotypes are associated with increased IL-21 production due to the duplicated *Tlr7* gene that mediates excessive signaling in response to self-RNA [135]. Genetic deletion of the IL-21 receptor in BXSB.*Yaa* mice significantly decreases the production of autoantibodies, T_{FH} cell numbers and disease severity [136]. This result supports the concept that IL-21 is required for auto-reactive B cell differentiation [112,113], and that blockade of this cytokine might be a promising therapeutic approach [138].

The MRL/MpJ-Fas$^{lpr/lpr}$/J (MRL^{lpr}) mouse strain is also a widely used as an experimental model of human lupus. Similar to *Sanroque* and BXSB.*Yaa* mice, MRL^{lpr} mice exhibit increased IL-21 production. Of note, the production of autoantibodies in these mice is from B cells in the extrafollicular foci, and this process is mediated by extrafollicular helper T (T_{eFH}) cells [139,140]. ICOS signaling is essential for the function of T_{eFH} cells, demonstrated by the fact that ICOS deficiency in MRL^{lpr} mice results in diminished production of autoantibodies [140–142]. Deficiency in IL-21R in MRL^{lpr} mice leads to a dramatic decrease of T_{FH}, T_{eFH}, GC B cells, plasma cells, and plasmablasts [134].

The NZB/W F1 model is another classical experimental autoimmune model that exhibits a spontaneous lupus-like disease [143]. NZB/W F1 mice also show increased T_{FH} and GC B cell responses that appear to be dependent on the ICOS/ICOSL pathway. Accordingly, treatment with anti-ICOSL ameliorates the disease severity by decreasing T_{FH} and GC B cell responses [144].

BXD2 mice also exhibit a lupus-like phenotype. This strain has been established by inbreeding the intercross progeny of C57BL/6K and DBA/2J mice for more than 20 generations [145]. BXD2 mice spontaneously produce pathogenic auto-antibodies and develop glomerulonephritis and erosive arthritis [146]. $CD4^+$ T cells in BXD2 mice express increased IL-17 levels and *Il17*-deficient BXD2 mice produce significantly reduced auto-antibodies [133]. Therefore, unlike the other animal model of lupus, the lupus-like phenotype of BXD2 mice is known to be IL-17-dependent. Mechanistically, B cells in BXD2 mice exhibit increased expression of IL-17RA compared to C57BL/6 mice and thus quickly activate the canonical NF-κB signaling pathway upon IL-17 [147]. Inhibition of NF-κB signaling diminishes IL-17 induced chemotactic arrest of B cells in response to CXCL12 [147]. Interestingly, the number of IL-17RA expressing $CXCR5^+ICOS^+$ T_{FH} cells is significantly increased in BXD2 mice, and blockade of IL-17 signaling reduces T_{FH}:B cell interaction and the generation of auto-antibody producing B cells in BXD2 mice [148].

2.3.2. T_{FH} Cells in Human Autoimmune Diseases

While the role of T_{FH} cells in mouse autoimmunity has been established, the role of T_{FH} cells in humans remains largely unexplored. High levels of class-switched auto-antibodies and abnormal GC B cell populations in patients with autoimmune diseases strongly suggest the involvement of T_{FH} cells in the pathogenesis of autoimmune diseases [139,149]. Increased frequencies of circulating T_{FH}-like cells ($CXCR5^{hi}PD\text{-}1^{hi}ICOS^{hi}CD4^+$) are found in patients with SLE, Sjögren's syndrome, RA, and juvenile dermatomyositis. In addition, the frequencies of T_{FH}-like cells are positively correlated with autoantibody titers, as well as disease symptoms, in SLE patients [150–153]. These circulating T_{FH}-like cells share phenotypic and functional properties with GC T_{FH} cells in follicles; but they

express low levels of BCL6 and IL-21 [150]. While Bcl-6 is gradually down-regulated as the GC response progresses in mouse T_{FH} cells [76], BCL-6 is rapidly re-expressed after TCR stimulation in circulating human $CXCR5^{hi}$ central memory $CD4^+$ T cells [83]. It is possible that the circulating T_{FH}-like cells may act as T_{FH} precursors with the potential for rapid CXCR5-mediated follicular access and B cell helper functions [83,151]. Further studies will be required to demonstrate the role of T_{FH} cells in the pathogenesis of antibody-mediated diseases in humans.

3. Regulatory Cells that Control GC B Cell Responses

While help from T_{FH} cells promotes the generation of high-affinity IgGs and memory B cells, excessive activation of T_{FH} and GC B cells can lead to autoimmunity as evident in a wide range of animal models of autoimmune disorders, as well as in humans. While the existence of suppressor T cells, including $Foxp3^+$ T cells, has been well established over the last two decades, the existence and function of regulatory cell subsets specialized for GC B cell responses and T_{FH} cells have only recently received attention. These include IL-10-producing regulatory B cells (B_{REG}), $CXCR5^+Bcl6^+$ follicular regulatory cells (T_{FR}), and Qa-1-restricted $CD8^+$ regulatory T cells (Figure 2). Although their role in autoimmunity and the mode of their suppressive activity are still under investigation, evidence from animal models strongly suggests that these cells are necessary for preventing excessive germinal center reactions *in vivo*.

3.1. Regulatory B Cells

Provision of ICOSL by B cells is required for the differentiation of T_{FH} cells [12]. Thus, B cells are generally considered as positive regulator of GC reactions. However, secreted antibodies are known to influence GC responses by limiting the acquisition of antigens by B cells [154]. In addition, recent studies have identified a few subsets of B cells, termed regulatory B cells (B_{REG}), which exhibit immunoregulatory functions [155].

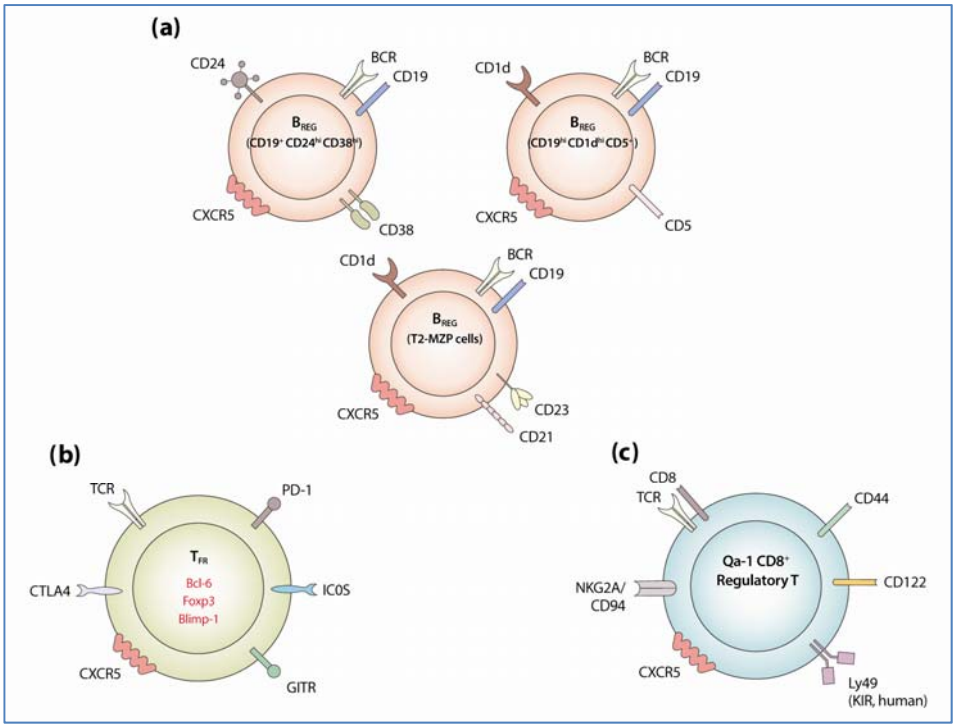
3.1.1. Identification and Development of Regulatory B Cells

Mice with a genetic deficiency of B cells are more susceptible to experimental autoimmune encephalomyelitis (EAE) [156]. Subsequent studies revealed that IL-10 production by B cells plays an immunoregulatory role in animal models of inflammatory diseases, including EAE, inflammatory bowel disease and rheumatoid arthritis [157–159]. This evidence indicates that B cells have regulatory properties. These IL-10 producing B_{REG} cells seem to be heterogeneous, as no appropriate surface marker(s) or master transcription factor(s) has been described to define these cellular subsets [155].

Marginal-zone (MZ) B cells are shown to produce IL-10 in response to CpG stimulation and ameliorate disease severity in a murine model of lupus [160]. Phenotypically, a subset of $CD1d^{hi}CD5^+$ B cells produces IL-10 and B-1a, MZ B and transitional 2-MZ precursor (T2-MZP) cells share this phenotype [161]. IL-10 secretion is largely restricted in $CD1d^{hi}CD5^+$ B cells which consist only 1~2% of splenocytes in wild type mice. $CD19^+CD23^+CD21^+CD1d^{hi}$ T2-MZP cells also express IL-10 [162]. Similarly, some of human B cells can produce IL-10 [163,164]. In particular, $CD19^+CD24^{hi}CD38^{hi}$ B cells contain the highest fraction of IL-10 producing B cells upon CD40 stimulation in human

peripheral blood from healthy individuals [165]. In addition, $CD24^{hi}CD27^{+}$ B cells are also known to produce IL-10 [166]. Interestingly, $CD19^{+}CD24^{hi}CD38^{hi}$ B cells are associated with immature B cells, while $CD24^{hi}CD27^{+}$ B cells are related to memory B cells [165,166]. Thus, it is likely that multiple subsets of B cells are able to produce IL-10 in mice, as well as in humans.

Figure 2. Diverse regulatory cells that control GC B cell responses. In order to maintain B cell tolerance to self-antigens and to control the size of GC reactions, the immune system establishes multiple subsets of regulatory cells, such as T_{FR} cells, Qa-1 restricted $CD8^{+}$ regulatory T cells, and regulatory B cells. (**a**) At least three subsets of B_{REG} cells are known to exert immunosuppressive activity: $CD1d^{hi}CD5^{+}$ B cells, $CD19^{+}CD24^{hi}CD38^{hi}$ B cells, and $CD19^{+}CD23^{+}CD21^{+}CD1d^{hi}$ T2-MZP B cells. They can be stimulated by TLR/CD40 signaling and secrete IL-10. (**b**) T_{FR} cells express Bcl-6 and Blimp-1 in addition to Foxp3. Phenotypically, they express T_{FH}-related molecules such as CXCR5, PD-1, ICOS, as well as the T_{REG}-associated molecules CTLA4, GITR and IL-10. (**c**) T cell receptor of Qa-1 restricted $CD8^{+}$ regulatory T cells recognize the MHC class Ib molecule, Qa-1, that is expressed exclusively on T_{FH} cells. This TCR/Qa-1-peptides interaction triggers the suppressive activity of $CD8^{+}$ regulatory T cells and limits GC reactions by inhibiting the function of T_{FH} cells.



B_{REG} cells are known to suppress inflammatory responses by producing IL-10, or by directly interacting with pathogenic T cells via a cell-to-cell contact dependent manner [167]. Activation of B cells with certain TLR agonists triggers the production of IL-10 and inhibits dendritic cell-mediated activation of T cells *in vitro* [168]. Interestingly, mice deficient in TLR2 and TLR4, or MyD88 in B cells, show a significantly delayed recovery from EAE, indicating that those TLR signals activate the regulatory function of B cells [168]. Human B cells express TLR9, and stimulation with CpG plus anti-Ig synergistically induces IL-10 production by human B cells [169].

CD40-CD40L interaction is required for B-cell mediated suppression. Agonistic anti-CD40 induces the differentiation of IL-10 producing B cells in splenocytes in an animal model of collagen-induced arthritis mice [159]. In humans, blood B cells treated with CD40L are shown to induce $Foxp3^+$ T_{REG} cells via an unknown mechanism [170]. In addition, CD40L stimulation increases the $CD19^+CD24^{hi}CD38^{hi}$ B cell population that can inhibit the differentiation of Th1 cells via IL-10 [165]. Notably, B cells from patients with SLE were insensitive to CD40L and produced limited amount of IL-10 [165]. Together, these findings strongly suggest that the CD40-CD40L interaction is critical for the activation of B_{REG} cells.

B cell activating factor (BAFF) is a TNF family protein and plays a key role in B cell maturation and survival [155]. A study with BAFF transgenic mice suggests that BAFF can induce $Foxp3^+$ T_{REG} cells to suppress T cell responses in a B cell dependent manner [171]. Moreover, *in vitro* cultures, with a low dosage of BAFF, can induce $CD1d^{hi}CD5^+$ B_{REG} cells, and *in vivo* treatment with BAFF also increases the number of B_{REG} cells in the marginal zone [172].

3.1.2. Regulatory Mechanism of B_{REG} Cells

The mechanisms by which B_{REG} cells employ their regulatory functions during the immune response have been explored. Two different B_{REG} subsets, $CD19^{hi}CD1d^{hi}CD5^+$ and $CD19^+CD23^+CD21^+CD1d^{hi}$ T2-MZP cells, are rare in normal conditions, however, they repress both the T cell proliferation and Th1 cytokines (IFN-γ and TNF-α) production through IL-10 [161,162,172]. B_{REG} cells can convert effector T cells into regulatory Tr1 cells *in vitro* in an IL-10 dependent manner [173,174].

B_{REG} cells are also known to control the balance between $Foxp3^+$ and IL-17 producing T cells [175,176]. For instance, B_{REG} cells induced by *Schistosoma mansoni* infection suppressed allergic airway inflammation by increasing pulmonary infiltration of $Foxp3^+$ T_{REG} cells in an IL-10 dependent manner [175,177]. Moreover, mice deficient in IL-10 in B cells develop severe arthritis and increased pro-inflammatory T cells (Th1/Th17) and decreased $Foxp3^+$ T_{REG} cells [176,178]. *In vitro* induced B_{REG} cells suppress the differentiation of Th17 from naïve T cells by down-regulating the phosphorylation of STAT3 [179].

B cells can express FasL and other death-inducing ligands to promote activation-induced cell death [167]. $CD5^+$ B cells highly express FasL and IL-10 and their regulatory functions come from their Fas-FasL mediated killing ability [167]. The expression of FasL on $CD5^+$ B cells is increased by stimulating them with the schistosome antigens IL-4 and IL-10 [180]. These $FasL^+CD5^+$ B cells are $IL\text{-}5R^{high}$ and can be expanded by CD40L and IL-5 stimulation without losing FasL-dependent killing capacity [181]. A recent study showed that the frequency of $FasL^+CD5^+$ B cells is inversely correlated with the severity of the disease in an animal model of collagen-induced arthritis [182], suggesting that $FasL^+$ B cells may have a role in the suppression of autoimmune diseases. Hence, although IL-10 serves as a key mechanism for the suppressive activity of B_{REG} cells, other mechanisms of suppression, including cell-to-cell contact dependent suppression, are also involved in their regulatory capacity.

3.1.3. B_{REG} Cells in Animal Models of Autoimmune Diseases

B_{REG} cells mediate immunosuppression in many types of autoimmunity [155]. Rheumatoid arthritis is associated with infiltration of activated T cells, B cells and macrophages that eventually trigger continuous destruction of cartilage and bone structure [183]. Collagen-induced arthritis (CIA) is triggered by $CD4^+$ T cells infiltrating into the synovial membrane and by B cells producing collagen-specific antibodies [184]. Depletion of B cells by CD20 monoclonal antibody treatment prior to collagen immunization delays the onset of arthritis. On the contrary, transfer of *in vitro* activated B cells significantly reduces the incidence and severity in a DBA mouse model of arthritis [159]. B cells from IL-10-deficient mice failed to protect recipient mice from arthritis [159]. Moreover, adoptive transfer of B_{REG} cells that were previously expanded *ex vivo* in the presence of BAFF suppresses the development of arthritis and relieves the disease severity in CIA mice [172,179]. These findings suggest that B_{REG} cells can ameliorate the severity of RA in experimental animal models.

Both T cells and B cells are involved in the pathogenesis of SLE [155]. Interestingly, it has been shown that B cell depletion in young NZB/W F1 (four weeks) mice promotes disease onset, while B cell depletion in older mice (12–28 weeks old) delays the disease progression [185]. IL-10-producing B_{REG} cells are known to be heavily expanded in young NZB/W F1 mice [186], an observation that might explain the different role of B cell depletion on disease severity in NZB/W F1 mice. $CD19^{-/-}$ NZB/W mice display delayed autoantibodies production; however, these mice show early nephritis development and poor survival rate due to the lack of B_{REG} cells [187]. Accordingly, transfer of splenic B_{REG} cells from NZB/W mice into $CD19^{-/-}$ NZB/W mice significantly increases survival rates. Similarly, transfer of anti-CD40 antibody induced B_{REG} cells into MPL^{lpr} mice ameliorates the disease severity and increases survival rate in an IL-10-dependent manner [174]. Therefore, B_{REG} cells can efficiently suppress different types of antibody-mediated experimental autoimmune diseases.

3.2. Follicular Regulatory T Cells

$Foxp3^+$ regulatory T cells (T_{REG}) are a subset of $CD4^+$ T cells and are necessary for immunological self-tolerance and homeostasis [188]. Previous studies demonstrated that T_{REG} cells are found in B cell follicles and GCs [189] and directly suppress B cell responses and auto-reactive B cells [190,191]. Over the last five years, a series of studies have unveiled that distinct subsets of T_{REG} cells selectively mediate the suppression of Th1, Th2, and Th17 responses in a CXCR3, IRF4, and STAT3-dependent mechanism, respectively [188,192]. More recently, three independent groups simultaneously discovered the existence of T_{FR} cells—a specialized subset of $Foxp3^+$ T_{REG} cells—that control GC reactions *in vivo* [193–195].

3.2.1. Identification and Development of T_{FR}

Foxp3-deficient scurfy mice exhibit a profound population of spontaneous GC B cells as early as four weeks of ages [193]. Similarly, the size of T_{FH} population in scurfy mice is significantly increased compared to wild-type mice. These observations clearly demonstrate that $Foxp3^+$ T_{REG} cells are essential for the maintenance of B cell tolerance to self-antigens in the periphery. Importantly, approximately 10%~15% of the $CXCR5^+CD4^+$ T cell population expresses Foxp3. $CXCR5^+Foxp3^+$ T

cells express Bcl-6 and Blimp-1, and they are absent in Bcl6-deficient, but not Blimp1-deficient mice [193,194]. Such $CXCR5^+Bcl6^+Foxp3^+$ T cells are termed 'follicular regulatory T cells' or 'T_{FR}'. Importantly, T_{FR} cells are present in all secondary lymphoid organs, but not in the thymus, and appear to express the transcription factor, Helios, that has been shown to be exclusively expressed by thymus-derived T_{REG} cells. Thus, it is likely that T_{FR} can be differentiated from thymus-derived T_{REG} cells in the periphery as a result of certain inflammatory signals. Although Bcl-6 is required in this process, the type(s) of inflammatory signals that are driving the differentiation of T_{FR} cells remains to be determined. The surface phenotype of T_{FR} cells resembles that of T_{FH}. T_{FR} cells express Bcl-6, CXCR5, PD-1, ICOS, and BTLA, but they lack CD40L, IL-4, and IL-21 [193,194]. Another difference between T_{FR} and T_{FH} cells is that the former express Blimp-1 together with Bcl-6, while the latter only express Bcl-6. As a subset of T_{REG}, T_{FR} cells express GITR, CTLA4, CD25 and KLRG1. However, T_{FR} cells do not express CXCR3, indicating that they are distinct from $CXCR3^+$ T_{REG} cells which are shown to be specialized for suppressing type I immune responses. Similar to that of T_{FH} cells, differentiation of T_{FR} cells depends on CD28, ICOS, and SAP [194]. Moreover, T_{FR} cells are absent in B cell-deficient mice, indicating that B cells provide crucial signals during T_{FR} differentiation *in vivo* [194]. Interestingly, Sage *et al.* recently unveiled a regulatory role for PD-1 during T_{FR} differentiation. They showed that PD-1-deficient mice harbor a significantly increased T_{FR} population, defined as $CXCR5^+ICOS^+Foxp3^+$ T cells [196]. Moreover, they showed that PD-1-deficient T_{FR} cells exhibit greater suppression of immunization-induced GC reactions *in vivo*. Hence, although T_{FR} cells express PD-1, their interaction with PDL1 seems to inhibit the expansion and suppressive activity of T_{FR} cells *in vivo*. It would be interesting to determine the involvement of the transcription factors, cytokines, and microRNA clusters that are known to mediate T_{FH} differentiation in the differentiation of T_{FR} cells.

3.2.2. Mechanism of T_{FR} Suppressive Activity

T_{REG} cells from *Cxcr5*$^{-/-}$, *Bcl6*$^{-/-}$, *Sh2d1a*$^{-/-}$ mice are significantly less efficient in suppressing T cell-dependent antibody production *in vivo*, compared to wild-type T_{REG} cells [193,194]. Therefore, T_{FR} cells play an essential role in controlling GC reactions [194–196]. Interestingly, a study by Linterman *et al.* showed that T_{FR} cells control GC B cell responses by suppressing the differentiation of T_{FH} cells [194], while our own study propose that T_{FR} might directly suppress B cells, rather than through T_{FH} cells [193]. These differences might be due to the difference in the experimental systems. The former study utilized mixed bone marrow chimeras with 1:1 ratio of *Sh2d1a*$^{-/-}$ and $Foxp3^{DTR}$ bone marrow to generate an *in vivo* system lacking T_{FR} cells [194]. In the latter study, T_{REG} cells from *Bcl-6*$^{-/-}$ or *Cxcr5*$^{-/-}$ mice and naïve $CD4^+$ T cells were co-transferred into *Tcrb*$^{-/-}$ mice to establish mice lacking T_{FR} cells [193]. Thus, it remains to be determined whether T_{FR} cells directly suppress T_{FH}, B cells, or both, during GC reactions *in vivo*.

The molecular mechanism by which T_{FR} cells regulate GC reactions remains unclear. Nevertheless, current studies give some evidence that high levels of CTLA4, GITR, and IL-10 expression in T_{FR} cells might be important for their regulatory function [193,194,197]. Cretney *et al.* showed that a certain population of T_{REG} cells residing in mucosal sites expresses Blimp-1, and that Blimp-1 induces the expression of IL-10 in T_{REG} cells [198]. Deletion of Blimp-1 caused impaired T_{REG} activation and homeostasis. Increased transcription levels of *Prdm1* and *Il10* in T_{FR} cells indicate that Blimp-1

expression mediates IL-10 expression in T_{FR} cells [194]. As described above, PD-1 is related to a suppressive function in T_{FR} cells. Although deletion of PD-1 does not affect T_{FR} cell migration into GCs, PD-1 deficient mice have higher numbers of T_{FR} cells in the lymph nodes and these cells exhibit increased suppressive ability [196]. Thus, PD-1 negatively regulates the suppressive function of T_{FR} cells, as well as the development of the cells. Further studies will be needed to define the molecular mechanism of T_{FR}-mediated suppression of GC reactions.

3.3. Qa-1 Restricted CD8$^+$ T Cells

In addition to Foxp3$^+$ T_{REG} cells, CD8$^+$ regulatory (CD8$^+$ T_{REG}) cells have been known to suppress B cell responses by suppressing Th2 immunity [199]. Subsequent studies demonstrated that Qa-1 restricted CD8$^+$ T_{REG} cells inhibit the immune responses mediated by Qa-1 expressing CD4$^+$ T cells. Accordingly, Qa-1-deficient mice appeared to be more susceptible to experimental autoimmune diseases, including EAE, RA, and lupus [200,201]. Strikingly, a recent study showed that Qa-1 is exclusively expressed on $T_{FH,}$ but not on the other subsets of CD4$^+$ T cells, and that CD8$^+$ T_{REG} cells specifically inhibit T_{FH} responses *in vivo* [202].

3.3.1. Identification of Qa-1 Restricted CD8$^+$ T_{REG} Cells

Early studies have provided fundamental evidence for the existence of CD8$^+$ T_{REG} cells that suppress T cell-dependent B cell responses in a Qa-1-dependent manner [199,203,204]. In addition, a series of animal studies with experimental autoimmune encephalomyelitis also revealed the immune-regulatory function of Qa-1-restricted CD8$^+$ T cells [205]. Qa-1 is the murine homolog of non-classical the MHC class 1b molecule, human leukocyte antigen-E (HLA-E) [205]. Engagement of the Qa-1/peptide complex by TCR activates and expands Ag-specific CD8$^+$ T cells, while its binding to the CD94/NKG2A receptor decreases the activities of CD8$^+$ T, NK, and NKT cells [205].

Importantly, the HLA-E restricted regulatory mechanism has also been reported in human autoimmune diseases. For instance, CD8$^+$ T cells from patients with recent-onset T1D exhibit defects in the suppression of auto-reactive CD4$^+$ T cells, a condition that can be restored by stimulation with DC primed with the HLA-E binding peptide [206]. Moreover, the frequency CD8$^+$ T cells that specifically recognize and lyse activated myelin-reactive CD4$^+$ T cells in an HLA-E restricted manner appeared to be largely reduced in the peripheral blood of multiple sclerosis patients during disease exacerbation, compared with patients in remission and healthy individuals [207]. These clinical observations strongly suggest a critical contribution of CD8$^+$ T_{REG} cells to the prevention of autoimmunity.

3.3.2. Mechanism of Qa-1 Restricted CD8$^+$ T_{REG}-Mediated Suppression on GC Reactions

Qa-1 can bind to two different receptors with opposing functions. Qa-1 can act as a MHC I molecule and bind to TCR and activate, as well as expand, Ag-specific CD8$^+$ T cells. On the other hand, when Qa-1-Qdm (Qa-1 determinant modifier) binds to the CD95/NKG2A receptor, expressed by CD8$^+$ T, NK, and NKT cells, it attenuates the activities of the cells [205]. A peptide from Heat Shock Protein 60 (Hsp60) is dominantly bound to Qa-1 and Qa-1-peptide/TCR signaling activates antigen-specific CD8$^+$ T cells [205]. Replacement of an amino acid at Qa-1 position 227 (D to K) disrupts

Qa-1 binding to the TCR/CD8 co-receptor, but has no effect on the inhibitory NKG2A receptor on CD8 and NK cells [202,208]. Accordingly, mice with this Qa-1 single mutation (D227K) do not have Qa-1 restricted $CD8^+$ T_{REG} cells. These mice exhibit significantly increased numbers of T_{FH} cells in the secondary lymphoid organs and develop SLE-like disease [202,205].

Qa-1 restricted $CD8^+$ T_{REG} cells express CXCR5 and ICOSL and migrate to B cell follicles, and attenuate the function of highly Qa-1 expressing T_{FH} cells [202]. Unlike conventional $CD4^+$ T_{REG} cells, these $CD8^+$ T_{REG} cells do not express Foxp3, and their suppressive function is mediated by perforin and IL-15 [202,205]. Treatment with anti-IL-15 inhibits the suppressive activity of $CD8^+$ T_{REG} cells [202]. Subsequent studies demonstrated the suppressive role of $CD8^+$ T_{REG} cells in a B6-*Yaa* mouse model of lupus and a mouse model of collagen induced arthritis [209,210]. Notably, *in vitro* expanded $CD8^+$ T_{REG} cells successfully inhibit CIA by reducing auto-reactive T_{FH} and Th17 cells via a perforin-dependent mechanism [210].

Killer cell immunoglobulin-like receptors (KIR) in humans, a functional counterpart of the Ly49 receptor in mice, is expressed in about 4%~5% of $CD8^+$ T cells in humans [211,212]. Similar to mouse $Ly49^+CD8^+$ T cells, human KIR^+CD8^+ T cells recognize HLA-E, and express perforin [211]. The role of this KIR^+CD8^+ T cell population remains to be determined.

4. Translational Potentials

Generation of high-affinity antibodies and strong memory B cells improve vaccine efficacy. On the other hand, suppression of auto-antibody production would ameliorate systemic autoimmune diseases. Thus, the immune cell subsets discussed in this review might provide a wide range of translational opportunities for the development of immunotherapy for human diseases.

4.1. Vaccine Design

Considering the crucial contributions of T_{FH} cells to GC B cell responses and memory B cells, enhancement of T_{FH} differentiation/function would improve the efficacy of vaccination [213]. Recent studies showed that certain adjuvants enhanced the generation of T_{FH} cells. For instance, vaccination with a nanoparticle (NP) delivery system promotes high-titer, high-avidity Ab responses to malaria antigens by enhancing the number of T_{FH} cells, compared to a US FDA-approved adjuvant monophosphoryl lipid A (MPLA) [214]. In addition, IFN-α has been shown to enhance the expansion of T_{FH} cells in an animal model of adenoviral-vector based vaccination [215].

Interestingly, Zheng *et al.* have recently shown that immunization with a DNA vaccine containing the *mGITRL* gene significantly enhanced the T_{FH} population and increased the levels of Ag-specific IgG to RagB of *Porphyromonas gingivalis* (*P. gingivalis*) [216]. GITR/GITRL signaling can act like co-stimulatory molecules in TCR signal mediated T cell activation and proliferation [217]. However, the role of the GITR signaling pathway in $T_{FH,}$ or GC B cell, responses is not clear. Modulating SAP and SLAM family receptor expression on T or B cells could be another target for optimizing vaccine efficacy since SAP:SLAM family receptor interaction is critical for long-term B:T_{FH} interaction during GC reactions [218]. Notably, over-expression of EAT2—a SLAM adapter protein—in DCs and macrophages enhances the induction of Ag-specific immune responses [219].

Similarly, blockade of inhibitory pathways during T_{FH} and GC B cell responses might also offer attractive targets, since inhibitory co-stimulators are involved in chronic infections and cancer as immunosuppressive mechanisms [220]. For instance, since PD-L1 is known to be highly expressed in various tumors and chronic infections [220], and since its ligand PD-1 is highly expressed on T_{FH} cells, blockade of PD-1/PD-L1 would improve the expansion and function of T_{FH} cells in tumor-bearing or infected hosts. In addition, it has been described that blocking B7-H1, but not B7-DC, increases the differentiation of T_{FH} cells and enhances antigen specific Ig responses [221]. Another study also demonstrated that blockade of PD-L1 and LAG3 signals in *Plasmodium yoelii* infected mice rapidly clears malaria by enhancing humoral immune responses with an increased number of T_{FH} and GC B cells [222].

Ex vivo expansion of tumor-infiltrating lymphocytes has been established in order to obtain a large number of tumor-specific T cells. Administration of tumor-specific antibodies is one of the most successful immunotherapeutic approaches for the eradication of tumor cells *in vivo*. As described above, the molecular and cellular factors required for T_{FH} cell generation are well documented. Thus, it seems possible to obtain a large number of tumor-specific T_{FH} cells. It would be interesting to investigate whether transfusion of tumor-specific T_{FH} cells would enhance anti-tumor immunity by inducing anti-tumor antibodies.

4.2. Autoimmune Diseases

As discussed above, in *Section 2.3*, exaggerated GC B cells and T_{FH} responses represent key pathophysiologic features of antibody-mediated autoimmune diseases in humans. Despite differences in the cellular origin and suppressive mechanism, the three types of suppressor cells discussed in this review share a common outcome when it comes to their activities: inhibition of GC reaction. It is not clear if defects in these suppressor cell populations lead to autoimmunity in humans. Nevertheless, it is plausible to surmise that *in vivo* expansion, or administration of the suppressor cells, would be beneficial to alleviate the problem of production of autoantibodies.

Importantly, transfer of circulating blood T_{FR} cells results in the suppression of Ag-specific IgG responses, and PD-1-deficient T_{FR} cells show stronger suppression [196]. Similarly, adoptive transfer of Qa-1 restricted $CD8^+$ T_{REG} cells and B_{REG} cells induce diminished antibody production [155,202,209]. As a next step, further studies will be needed to address if transfer of these GC suppressor cells can ameliorate the production of autoantibodies in animal models of lupus and other antibody-mediated disorders. The availability of only a limited number of suppressor cells is likely the main obstacle preventing their use in *in vivo* experimental studies. Given that T and B cells can be easily expanded, finding the right conditions for *ex vivo* expansion (or for differentiation) would enable us to overcome this obstacle. A series of future studies will be needed before GC suppressor cell-based immunotherapy can be developed as a therapy for personalized medicine.

5. Conclusions

The diverse types of immune cells involved in GC B cell responses reflect an orchestrated regulation of this process. In the past decade, there have been a number of milestone discoveries describing the regulation of GC reactions. Identification of the T_{FH} cell lineage and the discoveries of T_{FR} cells, $CD8^+$ T_{REG} cells, and B_{REG} cells have broadened our understanding of the complex balance of B cell immunity and tolerance. We expect that future studies will define the suppressive mechanisms by which each suppressor cell subset inhibits GC B cell responses. Various types of expanded or engineered immune cells are currently under pre-clinical and clinical investigations. As discussed above, the suppressor cells of the GC reaction offer great potential for translational research and may pave the way for the development of novel therapeutics for autoimmune diseases, as well as infections and cancers [212].

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Conflicts of Interest

The authors declare no conflict of interest.

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