Members of the Regulatory Lymphocyte Club in Common Variable Immunodeficiency

Sudhir Gupta*, Yesim Demirdag and Ankmalika Abha Gupta

Basic and Clinical Immunology, Change affiliation of Ankmalika Abha Gupta to Division of Basic and Clinical Immunology, Irvine, CA, United States

The role of CD4 T regulatory cells is well established in peripheral tolerance and the pathogenesis of the murine model and human autoimmune diseases. CD4 T regulatory cells (CD4 Tregs) have been investigated in common variable immunodeficiency (CVID).

Recently, additional members have been added to the club of regulatory lymphocytes. These include CD8 T regulatory (CD8 Tregs), B regulatory (Bregs), and T follicular helper regulatory (TFR) cells. There are accumulating data to suggest their roles in both human and experimental models of autoimmune disease. Their phenotypic characterization and mechanisms of immunoregulation are evolving. Patients with CVID may present or are associated with an increased frequency of autoimmunity and autoimmune diseases. In this review, we have primarily focused on the characteristics of CD4 Tregs and new players of the regulatory club and their changes in patients with CVID in relation to autoimmunity and emphasized the complexity of interplay among various regulatory lymphocytes. We suggest future careful investigations of phenotypic and functional regulatory lymphocytes in a large cohort of phenotypically and genotypically defined CVID patients to define their role in the pathogenesis of CVID and autoimmunity associated with CVID.

Keywords: Treg, CD8 Treg, Breg, T follicular regulatory cells, CVID, autoimmunity, germinal center

INTRODUCTION

The major function of the immune system is to protect from foreign pathogens, allergens, and intrinsic aberrant malignant cells and maintain tolerance to self-antigens (1). Immune tolerance is maintained by central and peripheral tolerance (2, 3). Central tolerance occurs in the primary lymphoid organs (thymus and bone marrow), where T- or B-cell clones that recognize autoantigens with high affinity are deleted predominantly by apoptosis and by receptor editing in B cells. Peripheral tolerance that occurs in the secondary lymphoid organs (spleen, lymph nodes) involved suppression of effector functions of autoantigen-recognizing T or B cells that have escaped central tolerance, and it is mediated by inducing anergy, deletion by apoptosis, or regulatory cells of self-recognizing T and B cells. Genetic and epigenetic factors disturb immune tolerance (4). Loss of immune tolerance to self-antigens results in the development of autoimmunity and autoimmune diseases (5–8).
Though paradoxical, immunodeficiency and autoimmunity may occur simultaneously. Recent studies of two rare monogenic inborn errors of immunity (IEI) associated with immunodeficiency and autoimmunity—autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and immunedysregulation-polyendocrinopathy-enteropathy-X-linked (IPEX)—have established the critical role of transcription regulators [autoimmune regulator (AIRE)] that regulate the transcription of numerous self-antigens in central tolerance and of Forkhead Box P3 (FOXp3), which is expressed in CD4 Treg, CD8 Treg, and T follicular regulatory cells (TFH), in suppressing autoreactive T cells in the periphery, which is called peripheral tolerance (9, 10).

Recently, plasma cells with regulatory properties have been reported in experimental models of autoimmune and infectious diseases (11–13). Shen et al. (12) demonstrated that CD138(+) plasma cells produce both IL-35 and IL-10. IL-35 limits experimental autoimmune encephalitis via inhibition of pathogenic TH1 and TH17 cells, and in the Samonella infection model, IL-10 inhibits anti-Salmonella immunity. These regulatory plasma cells express surface IgM, CD80, CD86, CD40, CD69, CD44, TACI, CXCR4, MHC II, Tim1, and Blimp1. Lino et al., in a murine model of Salmonella typhimurium infection, reported that IL-10-producing CD138(+) plasma cells express LAG-3, PD-L1, PD-L2, CD200, and BLIMP1 (13). The role of regulatory plasma cells in humans has not been explored. Furthermore, plasma cells are reduced in common variable immunodeficiency (CVID); therefore, it is unlikely that they play a significant role in the pathogenesis of CVID or autoimmune and autoimmunene associated with CVID.

Autoimmunity and autoimmune diseases are observed with increased frequency in several IEI (14, 15). Autoimmunity and autoimmune diseases are common complications in CVID, affecting at least 25% of patients and may be the first presenting non-infectious manifestations (16–22). Both organ- and tissue-specific systemic autoimmune diseases are associated with CVID, with autoimmune cytopenia (e.g., immune thrombocytopenia, autoimmune hemolytic anemia) being the most frequent autoimmune manifestations. Several mechanisms have been reported to explain autoimmunity associated with CVID. These include increased T helper type 1 (TH1), TFF cells, and CD21(+) B cells and decreased CD4(+)CD25(+)FoxP3(+) regulatory cells [reviewed in (23)].

Several investigators have studied CD4 Tregs in CVID (24–33); however, only limited data are available for other members of the regulatory lymphocyte club (33, 34). Here, we review them in-depth and their possible role in the pathogenesis of CVID and autoimmunity associated with CVID.

GERMINAL CENTER REACTION AND ITS REGULATION

The two important events in effective immune response, the class-switched recombination (CSR) and somatic hypermutation (SHM) or affinity maturation, resulting in generating high-affinity protective antibodies, occur in the dark zone of germinal centers (35, 36). However, clones of self-reactive B cells that are not eliminated can initiate autoantibody production in germinal centers (GCs) (35). There is evidence to support that self-reactive B cells are generated by SHM in GCs (35). The survival of these self-reactive B-cell clones depends upon the location and concentrations of autoantigens in GCs. SHM-mediated alteration of the antigen specificity of GC B cells can also play an important role in preventing autoantibody production in peripheral lymphoid tissues (36).

The regulation of the GC occurs at multiple levels and by multiple mechanisms. Several mechanisms have been proposed to explain B-cell autoimmunity, including chronic infection, molecular mimicry, excess production of memory B cells with a CD21(+) phenotype, IL-21 produced by T follicular helper cells (TFH), and regulatory lymphocyte dysfunctions. In this article, we also reviewed the role of CD4 Treg, CD8 Treg, and TFH regulatory cells in GC reaction.

**TFH Cells and GC Reaction**

CD4(+) T cells that express high levels of the chemokine receptor CXCR5 migrate to GCs and regulate GC formation, selection of high-affinity antibody-producing B cells, isotype class switching, and generation of long-lived memory B cells and plasmablasts (37–40). In addition to CXCR5 expression, TFH cells also express transcription factor B-cell lymphoma-6 (Bcl-6), programmed cell death receptor-1 (PD-1), inducible T-cell co-stimulator (ICOS), and CD40 ligand (CD40L/CD154) (41, 42). IL-21, the signature cytokine of TFH cells, signaling the JAK and STAT pathway, supports the proliferation, survival and SHM, and differentiation of B cells to antibody-producing cells and long-lived memory B cells. Martin and colleagues (43), based upon the expression of CXCR3 and CCR6 markers, have divided TFH cells into TFH1 (CXCR5(+)CXCR3(+)CCR6(-)), TFH2 (CXCR5(+)CXCR3(-)CCR6(+) or TFH17 (CXCR5(+)CXCR3(+)CCR6(-)) cells. TFH2 and TFH17 cells are able to help naive B cells to differentiate to produce antibodies; however, all subsets of TFH cells can induce differentiation of memory B cells to antibody-producing cells.

An increased TFH cell response in the GC is associated with the expansion of low affinity and autoreactive B cells, and overactive TFH cells are observed in a variety of systemic autoimmune diseases (44–48). Therefore, balanced responses of TFH and B cells are required to eliminate pathogens and simultaneously prevent autoimmune disease.

**CD4 Treg Cells and T Follicular Regulatory Cells in GC Reaction**

CD4 T cells with regulatory activity were originally described in 1982 by Damle and Gupta (49), who demonstrated that CD4(+) T cells upon activation in-vitro suppressed proliferative responses of T cells to phytohemagglutinin and alloantigens in mixed lymphocyte culture reaction. In 1995, Sakaguchi and colleagues further defined CD25(+) subsets of CD4 T cells with regulatory activity and termed them as Treg cells (50). In 2003, Tregs were further defined by the presence of transcription factor FoxP3 (51). The significance of the FoxP3 transcription factor in immune tolerance was reported in IPEX in which mutation of FoxP3 resulted in the development of autoimmunity.
The role of CD4 Tregs in the suppression of T cells and antibody responses is well established. Sakaguchi and colleagues (50) reported that depletion of CD4+CD25+ T cells leads to induction of antiparietal cell antibodies by gastric epithelia and of antithyroglobulin antibodies by thyroid follicular cells. Leonardo and colleagues (52) demonstrated the role of CD4 Tregs on germinal center formation and antibody response in a mouse model in which CD4 Tregs express the primate diphtheria toxin receptors. In these mice, depletion of specific CD4 Tregs resulted in enhanced GC formation, TFR cell expansion, and autoantibody responses. Strongly enhanced GC/TFH responses are also observed in patients with IPEX (53). Lim et al. (54, 55) reported that Foxp3+ Tregs can also directly suppress B-cell response without the need to first suppress T helper cells. Following activation, a subset of CD4 Tregs (CD4+CD25+CD69+) acquires CXCR5 expression while losing CCR7, allowing them to migrate to the B-cell follicle and suppress B-cell responses including B-cell survival, expression of activation-induced cytosine deaminase, and immunoglobulin production (56). Therefore, a subset of CD4 Tregs (CD4+CD25+CD69+) appears to transition to TFR cells, and this subset of CD4 Tregs regulates antibody responses in GC by suppressing T FH cells. T FR cells were not normally defined until 2011, when three groups simultaneously defined T FR cells as CXCR5+PD-1+BCL6+Foxp3+ cells (57–59). TFR cells appear to have critical roles in controlling both foreign antigen-specific and self-reactive B cells. T FR cell differentiation and maturation are facilitated by DCs and B cells (57). TFR Cells prevent T FH cell-induced activation of autoreactive B cells. T FR cells modify GC reaction by controlling the size of GCs and the selection of antigen-specific T FR cells and B-cell clones and by regulating immunoglobulin isotype switch and affinity maturation of antibodies (60). The precise molecules that are responsible for such effects are unknown; cognitive interactions via CTLA-4 appear to mediate suppression (61, 62).

Fu and colleagues (63) studied the role of T FR cells in autoimmunity in Bcl6 fl Foxp3 Cre KO mice. These mice, as they age, develop spontaneous autoimmune diseases, associated with increased number of T FH cells, production of autoantibodies, and IgG deposition in the kidney, supporting the role of T FR cells in germinal center formation and control of autoimmunity. T FR cells have been studied in a variety of autoimmune diseases (64–67). Increased T FR cells are associated with decreased autoantibodies and stable disease in rheumatoid arthritis (66). An imbalance between T FR/T FH cells correlates with disease activity in a number of autoimmune diseases (67–71). Several subsets of CD8+ Treg cells have been described in mice and humans [reviewed in (84–86)]. Shi et al. (87) reported that human central memory CD183+CD8+ T cells contain regulatory activity against T-cell responses mediated by IL-10. We further characterized CD8 Tregs both phenotypically and functionally (88–90). We examined the effect of CD183+CD45RA−CCR7+CD8+ T cells on various subpopulations of cells and observed that CD183+CD45RA−CCR7+CD8+ T cells suppress plasmablasts only. Furthermore, they did not have any significant effect on BAFF-R expression, suggesting that CD8 Tregs do not regulate B-cell survival (88). We further examined the direct effect of CD8 Tregs on GC cells and demonstrated that CD8 Tregs as defined by CD183+CD25+CD278+CD8+ have greater inhibitory activity against B-cell proliferation and immunoglobulin production than CD183+CD45RA−CCR7+CD8+ Tregs (89).

Breg Cells and GC Reaction

Regulatory B cells are immunosuppressive cells that downregulate immune responses and maintain immunological tolerance (91, 92). In 1974, Katz and colleagues (93) reported B cells suppressing the delayed type of hypersensitivity. However, it is in the last decade that Bregs have been investigated for their role in immune homeostasis and tolerance. Following exposure to the autoantigen, B cells mature into Breg cells that can express PD-1 and PD-L1, and suppress inflammation in autoimmune diseases via PD-1–PD-L1 interactions. In mice, B cells regulate immune responses through the release of IL-10, TGFβ, and IL-35 (91). In mice, IL-35 produced by plasma cells plays an important role in the negative regulation of immunity during autoimmune and infectious diseases (12). The role of IL-35 in B-cell-mediated negative regulation of immunity in humans has not been studied in detail. In a single report, Ye et al. (94) reported decreased plasma IL-35 and IL-35+ plasma cells in early-onset SLE patients. Bregs downregulate T- and B-cell immune responses via IL-10. In addition, Bregs promote the generation of CD4 Tregs and induce suppressive natural killer T cells [reviewed in (95)].

In humans, B cells regulate immune responses by secreting IL-10 and TGFβ (96, 97). Distinct subsets of B cells, namely, CD24hiCD38hi (similar to transitional B cells) and CD24+CD27− (memory B cell, B10 cells), and CD25+CD171+CD73+ display regulatory activities. Although CD19+CD24+CD38hi Bregs are enriched in IL-10+ B-cell fraction in peripheral blood (95, 96), CD24hiCD27+Bregs (B10) are relatively more suppressive for T-cell proliferation and IL-17/IFNγ expression. Both subsets produce IL-10; however, CD24hiCD27+Bregs are enriched in TGFβ and granzyme B (96). Therefore, these two phenotypically distinct Bregs mediate immunosuppression via distinct mechanisms. Achour et al. (98) also reported that human Bregs inhibit T FH cell differentiation and maturation and...
GERMINAL CENTER REACTION IN CVID

CVID is characterized by severely reduced numbers of circulating class-switched memory B cells and reduced levels of SHM resulting in impaired pathogen-protective high-affinity antibody response (102–108). Therefore, GCs as the primary site for both CSR and SHM may be disturbed in CVID patients.

Unger and colleagues (109) studied lymph node biopsies from CVID patients with lymphadenopathy. In the majority of cases, varying degrees of ill-defined GC hyperplasia were observed that correlated with the increased percentage of circulating CD21low B cells. Class-switched plasma cells were severely reduced. Therefore, large GCs and the reduction of circulating memory B cells and class-switched plasma cells suggest a failure of GC output rather than GC formation in CVID patients with lymphadenopathy.

van Schouwenburg and colleagues (110) studied naive and the antigen-selected BCR repertoire in CVID patients and were able to identify the GC reaction as the process most often deregulated in CVID patients. They also observed that some patients have possible defects in early B-cell development or selection against autoimmune features. Their study indicated that in the majority of CVID patients, repertoire formation is intact, while repertoire specification is often impaired. Therefore, CVID patients, in addition to having a quantitative defect in B-cell development, also had impaired quality of B cells.

T_{FH} Cells in CVID

As discussed above, T_{FH} cells play an important role in GC reaction. CVID patients with ICOS deficiency show severely impaired GC formation in lymphoid tissues and severely decreased blood memory T_{FH} cells, accompanied by a severe deficiency of memory B cells. Bossaller et al. (111) and Grimbacher et al. (112) reported decreased proportions of CXCR5+CD4+ T_{FH} cells in CVID patients with ICOS deficiency. Cunill et al. (113) analyzed T_{FH} cells in CVID patients. Patients were divided into smB− (<2% switched memory B cells) and smB+ (switched B cells >2%). They observed an increased percentage of CD4+CXCR5+ T_{FH} cells in CVID as compared with controls; however, these differences were observed only between smB− CVID patients. These T_{FH} cells have increased PD-1 expression.

Coraglia et al. (114) studied T_{FH} cells in 21 CVID patients divided into group I with autoimmune/ granulomatous (AI/GD) diseases and group II without AI/GD. They observed increased CD4+CXCR5+ T_{FH} cells in group I as compared with group II and healthy controls. Group II was not different from healthy controls. When data were analyzed for CCR7 and PD-1 expression, CD4+CXCR5+CCR7loPD-1hi cells were universally present in group I but not in group II. Kasahara et al. (115) reported decreased T_{FH} cells expressing PD-1 and ICOS-1 and reduced IL-21 secretion but a normal function of T_{FH} cells in CVID patients suggesting intrinsic B-cell defect. Borte et al. (116) reported that exogenous IL-21 restored immunoglobulin production in CVID. They reported decreased IL-21 mRNA in T cells; however, they did not find any mutation in IL-21. They did not examine IL-21 secretion.

Several investigators have reported increased levels of the T_{FH}1 subset in CVID patients with splenomegaly and/or AI/GD, when compared with CVID patients without AI/GD or splenomegaly and healthy controls. Cunill et al. (113), Unger et al. (117), and Kasahara et al. (115) have observed increased cT_{FH}1 cells in CVID. Cunill et al. (113) observed a significant increase in T_{FH}1 cells in smB− CVID patients. Unger et al. (117) observed increased T_{FH}1 cells in patients with autoimmune manifestations, and the strongest shift in T_{FH}1 cells was observed in CVID with increased CD21low B cells.

Cuhill et al. (113) and Kasahara et al. (115) did not observe any significant difference in T_{FH}2 cells between CVID and controls. Several investigators reported decreased T_{FH}17 cells in CVID patients (113, 115–118). Reduced production of IL-17 by CD4+ T cells has been associated with the reduced number of CD27+ IgD− B cells in CVID patients and healthy subjects (119, 120). Cunill et al. (113) observed reduced T_{FH}17 cells and increased T_{FH}1/T_{FH}17 ratio in smB− CVID patients. Berrón-Ruiz and colleagues (119) observed decreased IL-17A production in CVID. Barbosa et al. (120) also observed decreased IL-17 in CVID that correlated with increased CD21low; however, they did not observe any correlation in CVID with autoimmunity and autoimmune disease.

T_{FR} Cells in CVID

T_{FR} cells appear to have critical roles in controlling both foreign antigen-specific and autoreactive B cells. T_{FR} cells suppress antibody responses by suppressing T_{FH} cells (57–61). Fu et al. (6) reported an association between T_{FR} cell deficiency and the development of autoimmunity in mice. Cunill et al. (113) reported a reduction in CXCR5+CD25hiCD127low T_{FR} cells in CVID patients as compared with controls. Furthermore, a significant reduction was observed in smB− CVID but not in smB+ CVID patients. They demonstrated that the sorted CXCR5+CD25hiCD127low T_{FR} cells from smB-I CVID had decreased regulatory activity. Kasahara et al. (115) investigated T_{FR} cells (CD4+CD45RA− CXCR5+CD25+FoxP3+) in CVID patients with (n = 12) and without (n = 20) autoimmune diseases. They observed a decreased percentage of T_{FR} cells in CVID patients; however, no significant difference was observed between the autoimmune and non-autoimmunity groups. They also observed an increase in the T_{FH}/T_{FR} Ratio in CVID patients with autoimmune diseases as compared with controls but not between CVID patients without autoimmune diseases and controls. Coraglia et al. (114) reported similar proportions of
T<sub>FR</sub> cells (CD4<sup>+</sup>CXCR5<sup>+</sup>FoxP3<sup>+</sup>) in CVID patients and healthy controls. Furthermore, no difference in T<sub>FR</sub> cells was observed between CVID patients with or without AI/GD.

**CD4 Treg Cells in CVID**

A number of investigators have reported decreased CD4 Tregs in freshly isolated mononuclear cells (nTregs) in patients with CVID (24, 26–34, 119, 120). However, Kutukculer et al. (25) in 20 pediatric CVID patients reported no change in CD4 Tregs regardless of the severity of disease, and the presence of autoimmunity was not associated with decreased CD4 Tregs. Melo et al. (30), using the CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>FoxP3<sup>+</sup> phenotype as criteria for CD4 Tregs, reported decreased CD4 Tregs in CVID; however, they observed no difference between those with and without autoimmunity. Romberg et al. (34) also reported reduced frequency of Tregs in CVID patients especially with autoimmune cytopenia. Furthermore, they reported that CD4 Tregs from CVID with autoimmune cytopenia were impaired in suppressing allogeneic T cells of healthy controls. There was an inverse relationship between the expansion of T<sub>FH</sub> and CD4 Tregs and CVID patients. Furthermore, they did not analyze their data in relation to autoimmunity. Louis et al. (24) reported decreased CD4 Tregs in CVID patients with mutation of the inositol trisphosphate kinase beta (ITPKb) gene. Horn et al. (31), using two different phenotypic markers (CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>FoxP3 and CD4<sup>+</sup>CD25<sup>high</sup>FoxP3<sup>+</sup>CTLA-4<sup>+</sup>), reported decreased CD4 Treg cells in CVID patients with granulomatous disease and immune cytopenia. Several other investigators have reported an association between reduced CD4 Tregs and CVID with autoimmune diseases (29, 31, 32). Kofod-Olsen et al. (32) reported an association between decreased CD4<sup>+</sup> Tregs and increased pro-B10 Breg cells and CD4<sup>+</sup>CD25<sup>high</sup>FoxP3<sup>+</sup>CTLA-4<sup>+</sup> phenotype, reported decreased CD4 Tregs (without in-vitro activation) and iCD4 Tregs (ex vivo activated with anti-CD3/CD28) in 25 subjects with CVID. The proportions of iCD8 Tregs were significantly reduced in CVID; however, no significant difference was observed in nCD8 Tregs between CVID and controls. Furthermore, they did not observe any difference in the proportions of iCD8 Tregs between CVID with or without autoimmunity. Therefore, CD8 Tregs need to be studied both phenotypically and functionally in a large cohort of CVID patients with and without autoimmunity to delineate their role in autoimmunity-associated CVID.

**CD8 Treg Cells in CVID**

CD8 Tregs suppress the differentiation of T<sub>FR</sub> cells from naive CD4 T cells and are shown to regulate both T- and B-cell responses and GC reaction. CD8 Tregs regulate immune responses and development of several experimental models of autoimmune diseases and in human autoimmune diseases (77, 124–126); however, CD8 Tregs have not been studied in detail in patients with IEL. In humans, Shi and colleagues (87) have shown that CD8 Tregs (CD8<sup>+</sup>CD183<sup>+</sup>CXCR3<sup>+</sup>) regulate T-cell proliferation and effector functions. CD8 Tregs as defined by CD8<sup>+</sup>CD25<sup>high</sup>FoxP3<sup>+</sup> play an important role in the maintenance of self-tolerance (125). Churlaud et al. (83), Wang et al. (127), and Lu et al. (124) have demonstrated that CD8<sup>+</sup>CD25<sup>high</sup>FoxP3<sup>+</sup> Treg cells that have been expanded in vitro inhibit the proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. We have reported alterations in iCD8 Tregs in patients with selective IgM deficiency (128), Good syndrome (129), syndrome of selective IgM deficiency, severe T-cell deficiency and Mycobacterium avium complex infection (130), and hypogammaglobulinemia associated with CMV colitis and deficiency of CMV-specific CD8 T cells (131). Yesililk et al. (33) recently analyzed both nCD8 Tregs (without in-vitro activation) and iCD8 Tregs (ex vivo activated with anti-CD3/CD28) in 25 subjects with CVID. The proportions of iCD8 Tregs were significantly reduced in CVID; however, no significant difference was observed in nCD8 Tregs between CVID and controls. Furthermore, they did not observe any difference in the proportions of iCD8 Tregs between CVID with or without autoimmunity. Therefore, CD8 Tregs need to be studied both phenotypically and functionally in a large cohort of CVID patients with and without autoimmunity to delineate their role in autoimmunity-associated CVID.

**Breg Cells in CVID**

Breg cells regulate T- and B-cell responses including the maintenance of CD4 Tregs (88, 89, 95). B regulatory cell frequency and functions are decreased in a number of systemic autoimmune diseases (92, 93, 95, 96). Barsotti et al. (132) studied both phenotypically and functionally in a large cohort of CVID patients with and without autoimmunity to delineate their role in autoimmunity-associated CVID.

autoimmunity. In none of the published reports have investigators examined ex-vivo activated CD4<sup>+</sup> Tregs (iCD4 Tregs). Yesililk et al. (33), using the CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>FoxP3<sup>+</sup> phenotype to define CD4<sup>+</sup> Tregs, observed decreased proportion of both nCD4<sup>+</sup> Tregs and iCD4<sup>+</sup> Tregs (ex vivo activation of T cells with anti-CD3/CD28 in CVID; however, they did not observe any significant difference between the autoimmune and non-autoimmune disease groups. This could be due in part to the small sample size of patients with autoimmunity.
CD24hiCD38hi was significantly decreased in the cytopenia group as compared with the gastrointestinal autoimmunity group and healthy controls. Therefore, Bregs may have a differential effect on autoimmune manifestations associated with CVID. Furthermore, they did not observe any correlation between the frequency of Breg and CD4 Treg cells. We also observed decreased proportions of CD19+CD24hiCD38hi regulatory B cells in CVID patients as compared with controls; however, we did not observe any correlation with autoimmunity in CVID (30). Vlkova et al. (133) investigated Breg cells by stimulating peripheral blood B cells via stimulation of T cells by a plastic-coated anti-CD3 monoclonal antibody for 72 h and adding PMA and ionomycin for the last 4 h. They observed no difference in the frequency of CD19+CD24hiCD38hi Breg cells. However, they did observe a significantly reduced frequency of CD19+CD24hiCD38hiIL-10+ Bregs in CVID as compared with controls. No relationship was observed with the EUROclass categories of CVID. Furthermore, they observed an impaired Breg function in CVID as demonstrated by failure to suppress IFNγ and TNFα production by CD4+ T cells and an increased number of CD4+IFNγ+TNFα+ cells. In contrast, Arumugakani et al. (29) used CpG + rhCD40L to stimulate B cells for 43 h and analyzed Breg cells, which they termed pro-B10 cells, as CD19+IL-10+. They observed an increased frequency of pro-B10 Breg cells in total CVID patients as compared with controls. Furthermore, they observed an even more significant increase in pro-B10 cells in the CVID with autoimmune group as compared with controls, whereas the frequency of pro-B10 cells in the non-autoimmune group was similar in total controls. In addition, they observed a correlation with EUROclass categories. They also did not observe any correlation with decreased CD4 Tregs. Different experimental conditions and different phenotypic criteria to define Breg cells may account for the discrepancies among these studies. An increase in Breg cells has also been reported in patients with primary selective IgM deficiency (126).

SUMMARY AND CONCLUDING REMARKS

B-cell clones expressing self-reacting BCRs in GCs can initiate autoantibody production. Peripheral tolerance is induced by

CD4 Treg, CD8 Treg, TFR, and Breg cells that regulate GC reaction by multiple mechanisms, including anergy, apoptosis, and suppression of effector functions of self-reacting T and B cells. Furthermore, these regulatory lymphocytes regulate themselves (regulators of regulatory lymphocytes). In the majority of CVID studies, regulatory lymphocytes have been phenotypically examined, and their functions have been examined in very few studies. There is a general consensus with regard to decreased CD4 Tregs in CVID; however, there are conflicting data regarding their relationship with autoimmunity. A subset of CD4 Tregs (CD4+CD25+CD69+), migrating to lymphoid organs and transitioning into TFR cells, suppresses antibody response. Data on TFR cells are conflicting. CD8 Tregs regulate directly both TFR and B-cell responses. There are very little data about CVID. Similarly, Breg cells have not been studied in detail. The role of regulatory lymphocytes in the pathogenesis of low immunoglobulins in CVID remains to be explored. Since regulatory lymphocytes regulate each other, this poses another challenge to sort out the role of individual regulatory lymphocytes in the pathogenesis of CVID. There appears to be a phenotypic heterogeneity in subsets of CD8 Treg, Breg, and TFR cells. Perhaps a multicenter comprehensive study of both the phenotypic and functional analyses of regulatory lymphocytes in a well-categorized large cohort of CVID patients is needed to delineate their role in the pathogenesis of CVID and associated autoimmunity and autoimmune diseases. Furthermore, additional studies are needed to examine the effect of biologics on regulatory lymphocytes in CVID patients with autoimmune diseases.

AUTHOR CONTRIBUTIONS

SG conceptualized, formatted, wrote, and edited the manuscript. YD and AG wrote part of the paper and edited the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors extend their thanks to Houfen Su for technical support.
Monogenic Disorders in Mice and Men. *Carr Opin Immunol* (2008) 20:646–56. doi: 10.1016/j.coi.2008.10.004

11. Pillarreui S. Regulatory Plasma Cells. *Carr Opin Pharmacol* (2015) 23:1–5. doi: 10.1016/j.coph.2015.04.006

12. Shen P, Roch T, Lampropoulou V, O’Connor RA, Stervbo U, Hilgenberg E, et al. IL-35 Producing B Cells Are Critical Regulators of Immunity During Autoimmunity and Infectious Diseases. *Nature* (2014) 507(7492):366–70. doi: 10.1038/nature12979

13. Lino AC, Dang VD, Lampropoulou V, Welle A, Joedicke J, Pohar J, et al. LAG-3 Inhibitory Receptor Expression Identifies Immunosuppressive Natural Regulatory Plasma Cells. *Immunity* (2018) 49(1):120–135.e9. doi: 10.1016/j.immunity.2018.06.007

14. Fischer A, Provot J, Jais J, Alcais A, Malaoui N, Members of the CEREDHI French PID Study Group. Autoimmune and Inflammatory Manifestations Occur Frequent in Patients With Primary Immunodeficiencies. *J Allergy Clin Immunol* (2017) 140:1388–93.e8. doi: 10.1016/j.jaci.2016.12.978

15. Schmidt RE, Grimbacher B, Witte T. Autoimmunity and Primary Immunodeficiency: Two Sides of the Same Coin? *Nat Rev Rheumatol* (2018) 14:7–18. doi: 10.1038/nrrheum.2017.199

16. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Decrease in Phenotypic Regulatory T Cells Function in Patients With Common Variable Immunodeficiency. *Clin Immunol* (2019) 10:2753. doi: 10.1016/j.clinim.2019.03.002

17. Boileau J, Mouillot G, Gerard L, Carmagnat M, Rabian C, Oksenhendler E, et al. Autoimmunity in Common Variable Immunodeficiency: Correlation With Lymphocyte Phenotype in the French DEFI Study. *J Autoimmun* (2011) 36 (1):25–32. doi: 10.1016/j.jaut.2010.10.002

18. Arandi N, Mirshafey A, Jeddi-Tehrani M, Abolhasani H, Sadeghi B, Mirinbachi B, et al. Evaluation of CD4+CD25+FOXP3+ Regulatory T Cells Function in Patients With Common Variable Immunodeficiency. *Cell Immunol* (2013) 281:129–33. doi: 10.1016/j.cellimm.2013.03.003

19. Genre J, Errante PR, Krockon CM, Toledo-Barros M, Camara NO, Ruzzo LV. Reduced Frequency of CD4+CD25 (High) FOXP3+ Cells and Diminished FoxP3 Expression in Patients With Common Variable Immunodeficiency. A Link to Autoimmunity? *Clin Immunol* (2009) 132:215–21. doi: 10.1016/j.clim.2009.03.519

20. Carter CR, Aravind G, Smalle NL, Cole JY, Savic S, Wood PM. CVID Patients With Autoimmunity Have Elevated T Cell Expression of Granzyme B and HLA-DR and Reduced Levels of Treg Cells. *J Clin Pathol* (2013) 66:146–50. doi: 10.1136/jclinpath-2012-201046

21. Aranoguzakis G, Wood PM, Carter CR. Frequency of Treg Cells is Reduced in CVID Patients With Autoimmunity and Splenomegaly and is Associated With Expandedcd211o B Lymphocytes. *J Clin Immunol* (2010) 30:292–300, 2010. doi: 10.1007/s10875-009-9351-3

22. Patuzzo G, Barbieri A, Tinazzi E, Veneri D, Argentino G, Moretta F, et al. Altered Fraction of Regulatory B and T Cells Is Correlated With Autoimmune Phenomena and Splenomegaly in Patients With CVID. *Clin Immunol* (2016) 162:49–57. doi: 10.1016/j.clim.2015.11.003

23. Yeilik S, Agrawal S, Gollapudi SV, Gupta S. Phenotypic Analysis of CD4+ Treg, CD8+ Treg, and Breg Cells in Adult Common Variable Immunodeficiency Patients. *Int Arch Allergy Immunol* (2019) 180(2):150–8. doi: 10.1159/000501457

24. Ronborg N, Le Coz C, Glazzy S, Schickel JN, Trofa M, Nolan BE, et al. Patients With Common Variable Immunodeficiency With Autoimmune Cytopenias Exhibit Hyperplastic Yet Inefficient Germline Center Responses. *J Allergy Clin Immunol* (2019) 143(1):258–65. doi: 10.1016/j.jaci.2018.06.012

25. Brink R. The Imperfect Control of Self-Reactive Germinal Center B Cells. *Carr Opin Immunol* (2014) 28:97–101. doi: 10.1016/j.coi.2014.03.001

26. Brink R, Phan TG. Self-Reactive B Cells in the Germinal Center Reaction. *Ann Rev Immunol* (2018) 36:339–57. doi: 10.1146/annurev-immunol-051116-052510

27. Cyster JG. B Cell Follicles and Antigen Encounters of the Third Kind. *Nat Immunol* (2010) 11:989–996. doi: 10.1038/ni.1946

28. De Silva NS, Klein U. Dynamics of B Cells in Germinal Centres. *Nat Rev Immunol* (2015) 15:137–148. doi: 10.1038/nri3804

29. Rodda LB, Banard O, Ludewig B, Nagasawa T, Cyster JG. Phenotypic and Morphological Properties of Germinal Center Dark Zone Cxcl12-Expressing Reticular Cells. *Immunity* (2015) 45:145–91. doi: 10.4049/immunity.1501191

30. Gatto D, Brink R. The Germinal Center Reaction. *J Allergy Clin Immunol* (2012) 128:998–907. doi: 10.1016/j.jaci.2010.09.007

31. Schmitt N, Benteblief S-E, Umed H. Phenotype and Function of Memory Tfh Cells in Human Blood. *Trend Immunol* (2014) 35:434–42. doi: 10.1016/j.it.2014.06.002

32. Schaefer P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC Chemokine Receptor 5 Expression Defines Follicular Homing T Cells With B Cell Helper Function. *J Exp Med* (2000) 192:1533–6. doi: 10.1084/jem.192.11.1533

33. Morita R, Schmitt N, Benteblief SE, Ranganathan R, Bourdier L, Zurawski G, et al. Human Blood CCR5+CXCR5+CD4+ T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets That Differentially Support Antibody Secretion. *Immunity* (2011) 34(1):108–21. doi: 10.1016/j.immuni.2010.12.012

34. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, et al. Expansion of Circulating T Cells Resembling Follicular Helper T Cells Is A Fixed Phenotype That Identifies a Subset of Severe Systemic Lupus Erythematosus. *Arthritis Rheumatol* (2010) 62:234–44. doi: 10.1002/art.25032

35. Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL, et al. Follicular Helper T Cells Are Required for Systemic Autoimmunity. *J Exp Med* (2009) 206:561–76. doi: 10.1084/jem.20081886

36. Ueno H, Banchereau J, Vinuesa CG. Pathophysiology of T Follicular Helper Cells in Humans and Mice. *Nat Immunol* (2015) 16:142. doi: 10.1038/ni.3054

37. Vinuesa CG, Sanz I, Cook MC. Dysregulation of Germinal Centers in Autoimmune Disease. *Rev Immunol* (2009) 9:945–57. doi: 10.1002/imm.20637

38. Domeier PP, Schell SL, Rahman ZSM. Spontaneous Germinal Centers and Autoimmunity. *Autoimmun* (2017) 50(1):4–18. doi: 10.1080/08919343.2017.1280671
Hori S, Nomura T, Sakaguchi S. Control of Regulatory T Cell Development

51. Hori S, Nomura T, Sakaguchi S. Control of Regulatory T Cell Development
52. Leonardo SM, De Santis JL, Gehrand A, Malherbe LP, Gauld SB. Expansion
53. Lim HW, Hillsamer P, Kim CH. Regulatory T Cells Can Migrate to Follicles
54. Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting Edge: Direct Suppression of B Cells by CD4+CD25+ Regulatory T Cells. *J Immunol* (2005) 175:4180–3. doi: 10.4049/jimmunol.175.7.4180
55. Wing JB, Tekgöz M, Sakaguchi S. Control of Germinal Center Responses by T-Follicular Regulatory Cells. *Front Immunol* (2018) 9:1910. doi: 10.3389/fimmu.2018.01910
56. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3+ Follicular Regulatory T Cells Control the Germinal Center Response. *Nat Med* (2011) 17:795–82. doi: 10.1038/nm.2245
57. Wollenberg I, Gea-Duarte A, Hernandez A, Almeida C, Oliveira VG, Faro J, et al. Regulation of the Germinal Center Reaction by Foxp3+ Follicular Regulatory T Cells. *J Immunol* (2011) 187:4553–60. doi: 10.4049/jimmunol.1101328
58. Chung Y, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, et al. Follicular T Cells Expressing Foxp3 and Bcl-6 Suppress Germinal Center Reactions. *Nat Med* (2011) 17:983–8. doi: 10.1038/nm.2426
59. Stebegg M, Kumar SD, Silva-Cayetano A, Fonseca VR, Linterman MA, Graca L. Regulation of the Germinal Center Response. *Front Immunol* (2018) 9:2469. doi: 10.3389/fimmu.2018.02469
60. Sage PT, Paterson AM, Lovitch SB, Sharpe AH. The Coinhibitory Receptor CTLA-4 Controls B Cell Responses by Modulating T Follicular Helper, T Follicular Regulatory, and T Regulatory Cells. *Nat Immunol* (2001) 27(1):20–1. doi: 10.1038/33713
61. Bennett CL, Christie J, Ramsdell F, Brunenkow ME, Ferguson PJ, Whitesell L, et al. The Immune Dysfunction, Polyendocrinopathy, Enteropathy, X-Linked Syndrome (IPEX) Is Caused by Mutations of FOXP3. *Nat Genet* (2001) 27(1):20–1. doi: 10.1038/33713
62. Shi Z, Okuno Y, Suzuki H. Essential Role of CD8+CD25+ Regulatory T Cells in the Recovery From Experimental Autoimmune Encephalomyelitis. *J Immunol* (2008) 180:825–32. doi: 10.4049/jimmunol.180.2.825
63. Fu W, Liu X, Lin X, Feng H, Sun L, Li S, et al. Deletion Of B-Cell Anergy. *J Immunol* (2004) 173(11):6640–9. doi: 10.4049/jimmunol.173.11.6640
64. Hao H, Nakayamada S, Tanaka Y. Differentiation, Functions, and Roles of T Follicular Regulatory Cells in Autoimmune Diseases. *Eur J Immunol* (2005) 35(11):2931–8. doi: 10.1002/eji.200526216
65. Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T Cells Control Antigen-Specific Expansion of Th Cell Number and Humoral Immune Responses via the Coreceptor CTLA-4. *Immunity* (2014) 41:1013–25. doi: 10.1016/j.immuni.2014.12.006
66. Fu W, Liu X, Lin X, Feng H, Sun L, Li S, et al. Deficiency in T Follicular Regulatory Cells Promotes Autoimmunity. *J Exp Med* (2018) 215(3):815–25. doi: 10.1084/jem.20170901
67. Hao H, Nakayamada S, Tanaka Y. Differentiation, Functions, and Roles of T Follicular Regulatory Cells in Autoimmune Diseases. *Inflamm Regen* (2021) 41(1):14. doi: 10.1186/s41322-021-00164-9
68. Jacqueminin C, Augusto JF, Scherling M, Gensous N, Forcade E, Douchet L et al. Ox40L/Ox40 Axis Impairs Follicular and Natural T Reg Function in Human SLE. *JCI Insight* (2018) 3(24):122167. doi: 10.1172/jci.insight.122167
69. Liu C, Wang D, Lu S, Xu Q, Zhao I, Zhao J, et al. Increased Circulating Follicular Treg Cells Are Associated With Lower Levels of Autoantibodies in Patients With Rheumatoid Arthritis in Stable Remission. *Arthritis Rheumatol* (2018) 70(5):711–21. doi: 10.1002/art.40430
70. Wang X, Yang C, Xu F, QI L, Wang J, Yang P. Imbalance of Circulating Tfh/Tfh Ratio in Patients With Rheumatoid Arthritis. *Clin Exp Med* (2019) 19(1):55–64. doi: 10.1007/s10288-018-0530-5
71. Niu Q, Huang ZC, Wu XJ, Jin XY, An YF, Li YM, et al. Enhanced IL-6/Phosphorylated STAT3 Signaling Is Related to the Imbalance of Circulating T Follicular Helper/T Follicular Regulatory Cells in Patients With Rheumatoid Arthritis. *Arthritis Res Ther* (2018) 20(1):200. doi: 10.1186/s13075-018-1690-0
72. Cao Z, Fang P, Cui Z, Yue X, Chi S, Ma A, et al. An Imbalance Between Blood CD4+CXCR5+Foxp3+ Tfh Cells and CD4+CXCR5+Th Cells May Contribute to the Immunopathogenesis of Rheumatoid Arthritis. *Mol Immunol* (2020) 125:1–8. doi: 10.1016/j.molimm.2020.06.003
73. Liang M, Liwen Z, Juan D, Yun Z, Yanbo D, Jianping C. Dysregulated TFR and TFF Cells Correlate With B-Cell Differentiation and Antibody Production in Autoimmune Hepatitis. *J Cell Mol Med* (2020) 24(7):3948–57. doi: 10.1111/jcmm.14997
74. Long Y, Xia C, Xu L, Liu C, Fan C, Bao H, et al. The Imbalance of Circulating Follicular Helper T Cells and Follicular Regulatory T Cells Is Associated With Disease Activity in Patients With Ulcerative Colitis. *Front Immunol* (2020) 11:104. doi: 10.3389/fimmu.2020.00104
75. Reinhirz EL, Kung PC, Goldstein G, Schlossman SF. A Monoclonal Antibody Reactive With the Human Cytotoxic/Suppressor T Cell Subset Previously Defined by a Heteroantiserum Termed TH2. *J Immunol* (1980) 124(3):1301–7
76. Endharty AT, Okuno Y, Shi Z, Misawa N, Toyokuni S, Ito M, et al. CD8+CD122+ Regulatory T Cells (Treg) and CD4 Tregs Cooperatively Prevent and Cure CD4+Cell-Induced Colitis. *J Immunol* (2011) 186:41–52. doi: 10.4049/jimmunol.1000880
77. Suzuki M, Konya C, Goronzy JJ, Weyand CM. Inhibitory CD8+Tcells in Rheumatoid Arthritis. *Eur J Immunol* (2018) 48(1):55–66. doi: 10.1002/eji.201748125
78. Vuddamalay Y, Attia M, Vicente R, Pomie J, Laplace S, et al. Unique Phenotype of Human Tonsillar and In Vivo-Induced FOXP3+CD4+ T Cells. *J Immunol* (2009) 182(4):2124–30. doi: 10.4049/jimmunol.0802271
79. Cosimi L, Porta I, Fabbri G, Lazzari F, Francalanci M, Angeli R, Mazzinghi B, et al. Human CD8+CXCR3+ T Cells Have the Same Function as Murine CD8+CD122+ Treg. *Eur J Immunol* (2009) 39:2106–19. doi: 10.1002/eji.200939314
80. Niederlova V, Tsyklauri O, Chadimova T, Stepanek O. CD8+ Tregs Regulate Autoimmunity and During Interleukin 2 Therapy. *Front Immunol* (2015) 6:102. doi: 10.3389/fimmu.2015.00171
81. Smith TRF, Kumar V. Revival of CD8 T Reg-Mediated Suppression. *Trend Immunol* (2008) 29:337–42. doi: 10.1016/j.it.2008.04.002
82. Mishra S, Srinivasan S, Ma C, Zhang N. CD8+ Regulatory T Cell - A Mystery to Be Revealed. *Front Immunol* (2021) 12:708874. doi: 10.3389/fimmu.2021.708874
83. Niederlova V, Tsyklauri O, Chadimova T, Stepanek O. CD8+ Tregs Revisited: A Heterogeneous Population With Different Phenotypes and Properties. *Eur J Immunol* (2021) 51(3):512–30. doi: 10.1002/eji.202048614
Primary Immunodeficiency Due to Damaging Mutations in NFKB2. *Front Immunol* (2019) 10:297. doi: 10.3389/fimmu.2019.00297

124. Lu L, Cantor H. Generation and Regulation of CD8 (+) Regulatory T Cells. *Cell Mol Immunol* (2008) 5:401–6. doi: 10.1038/cmi.2008.50

125. Bacchetta R, Gamberini E. Roncarolo MG Role of Regulatory T Cell and FOXP3 in Human Diseases. *J Allergy Clin Immunol* (2007) 120:227–35. doi: 10.1016/j.jaci.2007.06.023

126. Mohr A, Mallhotra R, Mayer G, Gorochov G, Miyara M. Human FoxP3+ T Regulatory Cell Heterogeneity. *Clin Trans Immunol* (2018) 7:e1005. doi: 10.1002/cti2.1005

127. Wang YM, Alexander SI. CD8 Regulatory T Cells: What’s Old is Now New. *Immunol Cell Biol* (2009) 87:192–3. doi: 10.1038/icb.2009.8

128. Louis AG, Agrawal S, Gupta S. Analysis of Subsets of B Cells, Breg, CD4Treg and CD8Treg Cells in Adult Patients With Primary Selective IgM Deficiency. *Am J Clin Exp Immunol* (2016) 5(1):21–32.

129. Caperton C, Agrawal S, Gupta S. Good Syndrome Presenting With CD8+ T Cell Large Granular Lymphocyte Leukemia. *Oncotarget* (2015) 6(34):36577–86. doi: 10.18632/oncotarget.5369

130. Gharib A, Louis AG, Agrawal S, Gupta S. Syndrome of Selective IgM Deficiency With Severe T Cell Deficiency Associated With Disseminated Cutaneous Mycobacterium Avium Intracellulare Infection. *Am J Clin Exp Immunol* (2015) 4(2):15–27.

131. Agrawal S, Khokar A, Gupta S. Cytomegalovirus Colitis in Primary Hypogammaglobulinemia With Normal CD4+ T Cells: Deficiency of CMV-Specific CD8+ T Cells. *Front Immunol* (2019) 10:399. doi: 10.3389/fimmu.2019.00399

132. Barsotti NS, Almeida RR, Costa PR, Barros MT, Kalil J, Kokron CM. IL-10-Producing Regulatory B Cells Are Decreased in Patients With Common Variable Immunodeficiency. *PloS One* (2016) 11:e0151761. doi: 10.1371/journal.pone.0151761

133. Vlkova M, Ticha O, Nechvatalova J, Kalina T, Mauri C, et al. Regulatory B Cells in CVID Patients Fail to Suppress Multifunctional IFN-γ+TNF-α+ CD4+ T Cells Differentiation. *Clin Immunol* (2015) 16:292–300. doi: 10.1016/j.clim.2015.06.013

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Gupta, Demirdag and Gupta. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.