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ORIGINAL RESEARCH

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Phenotypically defined subpopulations of circulating follicular helper T cells in common variable immunodeficiency

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Abstract

Background: Common variable immunodeficiency (CVID) is characterized by low immunoglobulin G and IgA/IgM, decreased switched memory B cells, impaired response to vaccine, and an increased susceptibility to infections and autoimmunity. T_{FH} cells play an important role in germinal center reaction where it supports isotype switching, somatic hypermutation, generation of memory B cells, and differentiation of B cells to plasma cells. The objective was to study the distribution of three subsets of T_{FH} cells and their relationship with autoimmune diseases associated with CVID.

Methods: T_{FH} cells have been divided into $T_{FH}1$ (interleukin 21 [IL-21] and interferon γ), $T_{FH}2$ (IL-21 and IL-4), and $T_{FH}17$ (IL-21 and IL-17) cells. Mononuclear cells from 25 patients with CVID and age and gender-matched controls were stained with various monoclonal antibodies (anti-CD4 APC, anti-CXCR5 FITC, anti-CCR6 PerCP, and anti-CXCR3 PE) and isotype controls and analyzed for $T_{FH}1$ ($CD4^+CXCR5^+CXCR3^+CCR6^-$), $T_{FH}2$ ($CD4^+CXCR5^+CXCR3^-CCR6^-$), and $T_{FH}17$ ($CD4^+CXCR5^+CXCR3^-CCR6^+$) cells by multi-color flow cytometry. Twenty thousand cells were acquired and analyzed by FlowJo software. Statistical analysis of comparison of patients and healthy controls was performed by paired *t* test using PRISM 7 software.

Results: $T_{FH}2$ and $T_{FH}17$ cells subpopulations of T_{FH} cells were significantly decreased ($P < .003$ and $P < .006$, respectively) in CVID as compared with controls. No significant difference was observed in any of T_{FH} cell subpopulations between CVID with and those without autoimmunity group.

Conclusion: Alterations in T_{FH} cell subpopulation may play a role in defects in B cell compartment in CVID.

KEYWORDS

autoimmunity, CVID, follicular helper T cells

1 | INTRODUCTION

Common variable immunodeficiency (CVID) is heterogeneous and most common primary immunodeficiency disease in adults characterized by low serum immunoglobulins immunoglobulin G (IgG), IgA, and/or IgM, impaired specific antibody response to vaccines, and increased susceptibility to recurrent infections.[1-5] In addition, patients with CVID have increased prevalence of allergic, autoimmune, and granulomatous disorders, and malignancy, the majority being lymphoreticular malignancy.[5-10]

A number of gene mutations have been reported in CVID; however, they account for less than 20% of CVID patients.[11-13] Therefore, in majority of patients with CVID cause(s) is unknown. The predominant defects appear to be in the B cell compartment including impaired immunoglobulin isotype switching and differentiation of B cells into plasma cells despite normal number of B cells; postgerminal center B cells are defective and switched memory B cells are reduced.[14-16]

The follicular helper (T_{FH}) cells are major $CD4^+$ T helper subset that are essential for B cell differentiation into immunoglobulin producing plasma cells, and for the generation of memory B cells in the germinal center (GC).[17,18] GCs are primary sites for class-switched recombination and affinity maturation. T_{FH} cells regulate GC formation, and selection of high-affinity antibody-producing B cells and support isotype class switching.[19,20] An increased cT_{FH} cells response in the GC is associated with the expansion of low affinity and autoreactive B cells.[21,22]

T_{FH} cells are characterized by the expression of CXCR5 and transcription factor B cell lymphoma 6 (Bcl6), and production of their signature cytokine, the interleukin 21 (IL-21).[23-25] CXCR5 plays and important role in the migration of B cells to germinal follicles to support immunoglobulin production.[26] Although T_{FH} cells are predominantly found in lymph nodes and spleen, a small proportion of these cells are also found in the circulation. Vella et al[27] compared T_{FH} cells from lymph nodes, thoracic duct lymph, and blood and showed that they share TCR clonotype, phenotype, and transcriptional signature, and therefore cT_{FH} represents T_{FH} cells in GC.

Morita et al[28] also reported that blood $CXCR5^+$ $CD4^+$ T cells induce naive B cells differentiation and class switching more than $CXCR5^-$ $CD4^+$ T cells. According to the expression of CXCR3 and CCR6 on CD4 + CXCR5, they identified three different subsets of T_{FH} cells with different functions. In addition to IL-21, these different cT_{FH} subsets can also produce, albeit in lower amounts, IL-4, interferon γ (IFN-γ), and IL-17. $cT_{FH}1$ ($CXCR5^+CXCR3^+CCR6^-$) produce IFN-γ, $cT_{FH}2$ ($CXCR5^+CXCR3^-CCR6^-$) produce IL-21 and IL-4, and $cT_{FH}17$ ($CXCR5^+CXCR3^-CCR6^+$) produce IL-21 and IL-71A; all of them are able to efficiently induce antibody response by memory B cells.

A role of T_{FH} cells in antibody-mediated autoimmune disease has been established in both mice and humans.[21,22] Because T_{FH} cells play a role in class switching and autoimmunity, and an observed deficiency of switched memory B cells and increased autoimmunity in CVID, we evaluated $cT_{FH}1$, $cT_{FH}2$, and $cT_{FH}17$ cells in CVID patients and examined their relationship with autoimmune diseases associated with CVID.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 25 patients (seven men and 18 women, aged 15-82 years) with CVID and 25 healthy controls (13 men and 12 women, aged, 20-67 years) were enrolled in the study. Pan American and ESID Criteria were used to diagnose CVID patients.[1] Clinical and immunological features of these patients have been published.[29] All patients were receiving immunoglobulin replacement treatment. Blood samples were drawn at trough level. The Institutional Review Board committee (human research), University of California at Irvine approved this study protocol. Written and signed informed consent was obtained from all subjects.

2.2 | Antibodies

Anti-CD4 APC, anti-CXCR5 (CD185) FITC (clone-2G8), anti-CCR6 (CD196) PerCP (clone-11A9), anti-CXCR3 (CD183) PE (clone-1C6/CXCR3) monoclonal antibodies, and isotype control antibodies were purchased from Pharmingen BD Sciences, San Jose, CA.

2.3 | Immunophenotyping

Ten ml of heparinized blood was diluted with Hank's buffered salt solution (HBSS). Mononuclear cells (MNC) were separated by Ficoll-Hypaque density gradient using lymphocyte separation medium. Cells were suspended in HBSS and used for immunophenotyping. Cells were incubated with different monoclonal antibodies and isotype controls (below) for 30 minutes on ice in the dark. Cells were washed and $cT_{FH}1$, $cT_{FH}2$, and $cT_{FH}17$ analyses were performed by multicolor flow cytometry

(FACSCelesta; Becton-Dickinson, San Jose, CA). Twenty thousand cells were acquired and analyzed by FlowJo software (Treestar Inc., Ashland, OR).

For cT_{FH} cells: anti-CD4 APC, anti-CXCR5 FITC, anti-CCR6 PerCP, anti-CXCR3 PE; three subsets of cT_{FH} cells were identified as: $cT_{FH}1$ ($CD4^+CXCR3^+CCR6^-$), $cT_{FH}2$ ($CD4^+CXCR3^-CCR6^-$), and $cT_{FH}17$ ($CD4^+CXCR3^-CCR6^+$).

Statistical analysis of comparison of patients and healthy controls was performed by paired *t* test for equality of means using PRISM 7 software.

3 | RESULTS

3.1 | cT_{FH} subpopulations in CVID

CXCR5 + CD4 cT_{FH} are further subdivided by the expression of CXCR3 and CCR6 and cytokines they produce into $T_{FH}1$, $T_{FH}2$, and $T_{FH}17$ cells.[28] MNC were incubated with panel of monoclonal antibodies defining $T_{FH}1$, $T_{FH}2$, and $T_{FH}17$ cells and isotype controls and analyzed using multicolor flow cytometry. Cumulative data from 25 patients with CVID and healthy controls are shown in Figure 1. $cT_{FH}2$ and $cT_{FH}17$ cells were significantly decreased in CVID patients when compared to controls ($P < .003$, $P < .006$, respectively). $cT_{FH}1$ cells were comparable between two groups ($P < .802$).

controls to defined $T_{FH}1$, $T_{FH}2$, and $T_{FH}17$ subsets of follicular helper T cells and analyzed with multicolor flow cytometry using FACSCelesta. Data are expressed as mean ± SD. Statistical analysis was performed with GraphPad Prism version 8.4.3 for Windows (GraphPad Software, San Diego, CA).

3.2 | cT_{FH} subpopulations in CVID with and without autoimmunity

cT_{FH} cells play a role in autoimmunity and autoimmune diseases.[21,22,30] Therefore, we analyzed our data for the presence and absence of autoimmunity in CVID. Data are shown in Figure 2. $cT_{FH}17$ cells tended to be higher in CVID patients with autoimmunity as compared with those without autoimmunity. However, we observed no significant difference in $cT_{FH}1$, $cT_{FH}2$, and $cT_{FH}17$ cells between CVID patients with or without autoimmune disease ($P > .754$, $P > .177$, $P > .230$, respectively). There were only seven of 25 CVID patients with autoimmune disease.

4 | DISCUSSION

Patients with CVID display increased susceptibility to recurrent infections, and increased incidence of autoimmune and inflammatory disorders, and malignancy.[2-10]

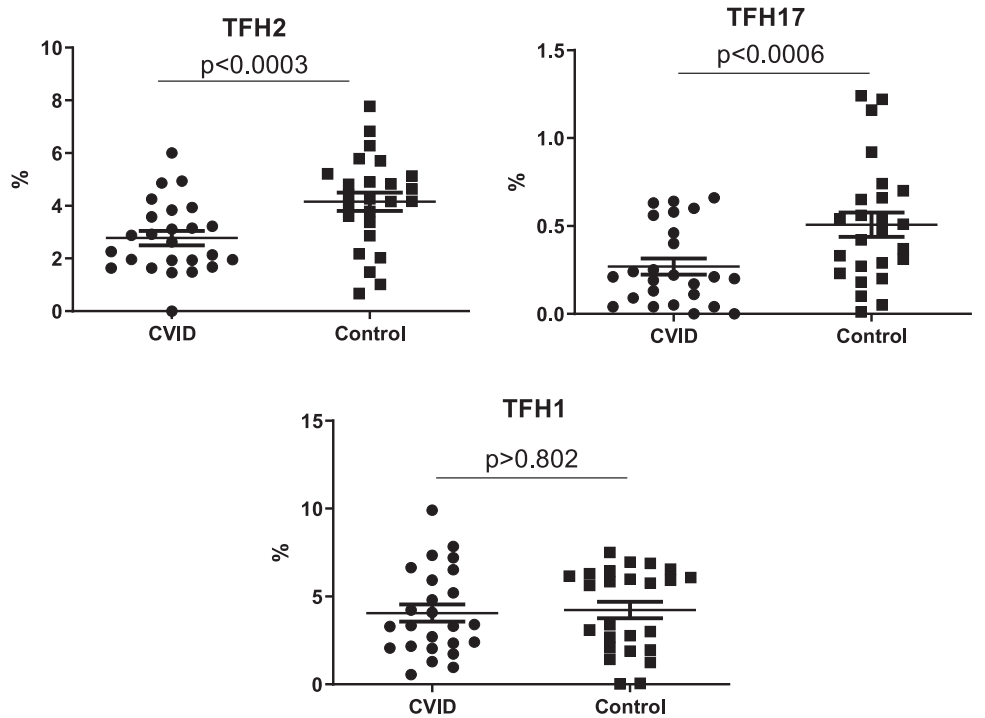

FIGURE 1 T_{FH} cell subsets in CVID and controls. Mononuclear cells from 25 CVID patients and healthy controls were stained with monoclonal antibodies and isotype. CVID, common variable immunodeficiency

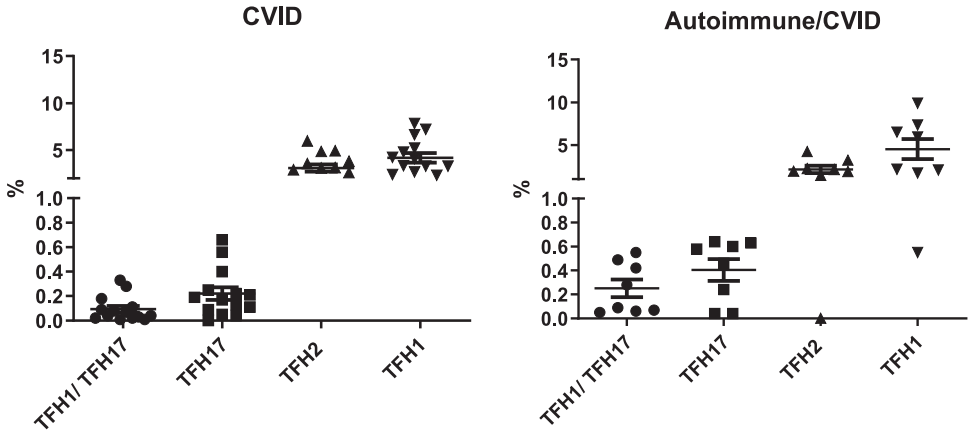

FIGURE 2 T_{FH} cell subsets relations to autoimmune diseases in CVID. $T_{FH}1$, $T_{FH}2$, and $T_{FH}17$ subsets and $T_{FH}1/T_{FH}17$ ratio were compared for CVID patients with autoimmune diseases ($n = 7$) and without autoimmune diseases ($n = 18$).
CVID, common variable immunodeficiency

The hallmark of defect In CVID is an impaired specific antibody response to vaccine, decreased switched memory B cells, and impaired differentiation of B cells to plasma cells that takes place in GCs of follicles.[14-16]

T_{FH} cells are specialized helper T cells that provide help to B cells and are essential for the formation of GC B cells, affinity maturation, and generation of high-affinity antibodies and memory B cells. T_{FH} cells.[17-28,31] T_{FH} cells are characterized by high expression of the transcription factor Bcl6, CXCR5, and IL-21 production.[24,26] The GC is also regulated by T follicular regulatory cells.[17]

CVID is the most common and genetically heterogeneous antibody deficiency disorder in adults. However, with the use of genome-wide association studies and next-generation sequencing have delineated several gene mutations in CVID including *CD19*, *CD20*, *CD21*, *CD81*, *TACI (TNFRSF13B)*, *BAFF (TNFRSF13C)*, *PTEN*, *PI3KD*, *PIK3R1*, *TWEAK*, *TRNT1*, *TTC37*, *NFKB1*, *NFKB2*, *IKZF1*, *IRF2BP*, *ATP6AP1*, *ITPKB*, *PRKCD*, *LRBA*, *and ICOS*.[13,32,33] However, these genetic mutations contribute to less than 20% of CVID patients. Therefore, in majority of patients with CVID genetic basis and pathogenesis remain unclear.

Bossaller et al[34] and Grimbacher et al[35] reported decreased proportions of $CXCR5^+CD4^+$ cT_{FH} cells in CVID patients with inducible T cell costimulator (ICOS) deficiency. Cunill et al[14] observed increased $CD4^+CXCR5^+cT_{FH}$ cells in CVID as compared with controls; however, these differences were observed only between CVID with low-switched B cells (smB^-) vs normal controls. Coraglia et al[36] reported no difference in $CD4^+CXCR5^+$ cT_{FH} cells that expressed IL-10, IL-21, or IL-4 between CVID with and without autoimmune diseases as compared with controls. However, they observed increased proportions of $PD1^+CCR7^+$ T_{FH} in CVID with autoimmune diseases as compared with CVID without autoimmune diseases and controls. Cunill et al[14] when used expression of CXCR3 and CCR6 to define $cT_{FH}1$, cT_{FH} 2, and $cT_{FH}17$, observed increased $cT_{FH}1$ cells, and decreased $T_{FH}17$ cells in CVID with low-switched memory B cells as compared with CVID with normal switched memory B cells and healthy controls. No difference was observed in $T_{FH}2$ cells. Unger et al[37] also observed increased $T_{FH}1$ and decreased $T_{FH}17$ cells in CVID patient. Increased $T_{FH}1$ cells were observed in patients with autoimmune manifestations and strongest shift in $T_{FH}1$ cells was observed in CVID with increased $CD21^{low}$ B cells. Turpin et al[38] reported higher proportions of $cT_{FH}1$, $cT_{FH}17$ and low cT_{FH} 2 in CVID patients than control subjects. Increased IFN-γ-producing $T_{FH}1$ cells in CVID were observed in CVID with noninfectious manifestations. However, Le Coz et al[39] did not observed increased IFNγ producing T_{FH} cells in CVID. They observed increased IL-21 producing T_{FH} cells and imbalance in $T_{FH}1$ /$T_{FH}2$ to $T_{FH}17$. We observed significantly decreased cT_{FH} 2 in CVID that is in agreement with report by Turpin et al.[39] Our observations of decreased $T_{FH}17$ cells in CVID are in agreement with reports of Cunill et al[14] and Unger et al.[37] However, similar to Le Coz et al,[39] we did not observed any significant difference in $T_{FH}1$ cells in CVID. Our results are different from those of increased $T_{FH}1$ cells reported by Cunill et al[14] and Unger et al.[35] However, we did not analyze our data in relation to switched B cells. The role of $T_{FH}1$ cells in the pathogenesis of CVID is questionable. Desjardins et al[40] demonstrated that an addition of exogenous IFNγ to cultures of B cells had no effect on B cells from CVID patients. We did not observed significant difference in any of subsets of cT_{FH} cells between CVID patients with and without autoimmune disease. In various autoimmune diseases including SLE, IgG4-related diseases, Sjogren's syndrome, rheumatoid arthritis, myasthenia

gravis, autoimmune thyroid disease, different patterns in cT_{FH} cell subsets have been reported (reviewed in Ueno[31]]. Therefore, type of autoimmune diseases associated with CVID as well difference in characterization of CVID may explain discrepancy among various studies. Furthermore, we need to consider a role of regulatory lymphocytes in autoimmunity associated with CVID. We have reported decreased proportion of $CD4^+$ Treg, $CD8^+$ Treg, and Breg cells in CVID patients.[29] More recently, cT_{FR} has been shown to regulate GC reaction at multiple levels.[41-43] cT_{FR} regulate proliferation and cytokine production, as well as B cell proliferation and immunoglobulin production.[43-45] Cunill et al[14] reported decreased cT_{FR} cells in patients with CVID with low proportions of switched memory B cells. Borte et al[46] did not observe any defect in IL-21 or IL-21R expression or mutations in *IL-21* gene in CVID. However, they demonstrated that a combination of IL-21, IL-4, and anti-CD40 induced class-witched recombination and differentiation of B cells to immunoglobulin secreting cells in CVID. IL-21R/IL-4 double deficient mice exhibit a CVID phenotype with low IgG and IgA and normal IgM, suggesting a critical role of IL-21, that is produced by cT_{FH} cells, in regulating immunoglobulin isotype switch.[47]

In summary, a decreased in T_{FH} cell subsets may play a role in poor GC reactions including decreased isotype switching, impaired affinity maturation, generation of memory B cells, and B cell differentiation to plasma cells that are characteristics of CVID. To understand the pathogenesis of defects in B cell compartment and autoimmune and inflammatory manifestations, further comprehensive studies of all phenotypic and functionally defined subsets cT_{FH} cells, including cT_{FR} in homogenously subclassified groups of CVID patients are needed.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

YS performed the experiments, collected and analyzed the data, and wrote preliminary draft. SG conceived the idea, supervised YS, and edited the manuscript.

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