

Letter to the Editor

Impaired T_H2 response in patients with Vav1-deficient common variable immunodeficiency with T-cell defects

To the Editor:

Common variable immunodeficiency (CVID) is a primary immune disorder including a heterogeneous group of diseases with the unifying feature of hypogammaglobulinemia.¹ Defects not only in B cells, which are directly responsible for antibody production, but also in other immune cells involved in the generation of an effective humoral response, including antigen-presenting cells and T_H cells, represent the molecular basis of CVID.²

T_H cells orchestrate the host immune response and can be divided into different subsets based on their pattern of cytokine secretion. T_H1 cells produce IFN- γ and TNF- β , which elicit macrophage activation and cell-mediated immunity, whereas T_H2 cells produce IL-4 and IL-13, which promote B-cell maturation and antibody production. The differentiation of T_H1 and T_H2 subsets is finely regulated by specific transcription factors, cytokines, and costimulatory signals. Among the lineage-specific transcription factors, T-box transcription factor (T-bet) plays a central role

in T_H1 cell development, whereas GATA-3 and c-Maf drive T_H2 cell differentiation.³⁻⁵

We have previously identified a subset of patients with CVID with impaired T-cell function characterized by defective activation of the protein tyrosine kinase cascade triggered by T-cell antigen receptor engagement.⁶ In these patients T cells harbor a profound defect in the Vav/Rac pathway, with consequent impaired F-actin reorganization.⁶ Interestingly, impaired IL-4 and c-Maf expression, together with enhanced T_H1 responses, which could be reversed by Vav1 reconstitution, have been observed in T cells from Vav1^{-/-} mice.⁷ Furthermore, germinal center formation and antibody class switching in response to immunization with a T-dependent antigen was found to be impaired in Vav1^{-/-} mice, implicating Vav1 in the differentiation of T_H2 cells and in the development of antibody responses in the mouse.^{7,8}

Based on these background studies, here we investigated the effect of the defect in Vav1 expression observed in 4 of 32 patients with CVID on T_H1/T_H2 differentiation. We measured IL-4 and IFN- γ T-cell production, as well as expression of GATA-3, c-Maf, and T-bet, in 4 patients with CVID with defective Vav1 expression (T-CVID1, T-CVID2, T-CVID3, and T-CVID4)⁶ and 5 healthy control subjects. Cytokines were also measured

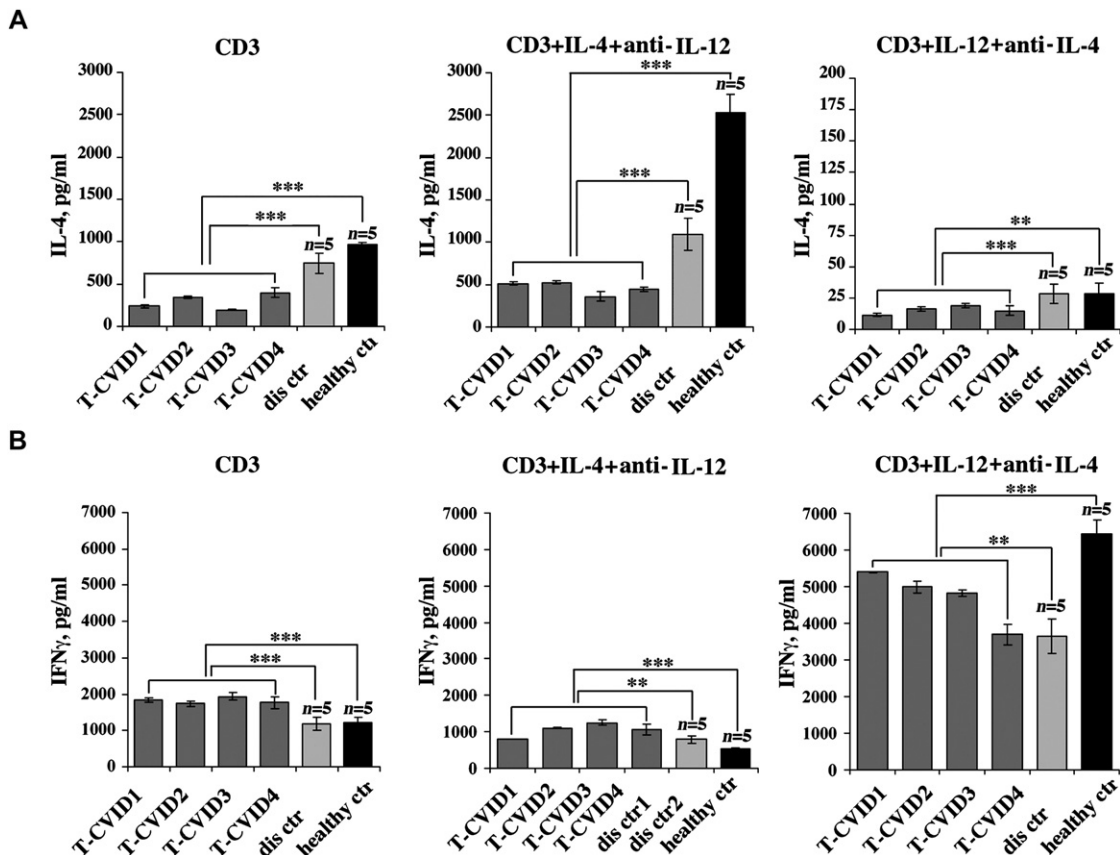


FIG 1. IL-4 (A) and IFN- γ (B) production of T_H cells from the 4 patients with T-CVID, the 5 patients without T-CVID (disease control subjects), and the 5 healthy donors differentiated in neutral, T_H2-polarizing, or T_H1-polarizing conditions. Data are representative of 3 independent experiments, each in duplicate. The combined data obtained on the patients with T-CVID (mean $\pm$ SD) and healthy donors were subjected to statistical analysis. *** $P \leq .001$. ** $P \leq .01$. Error bars = SD.

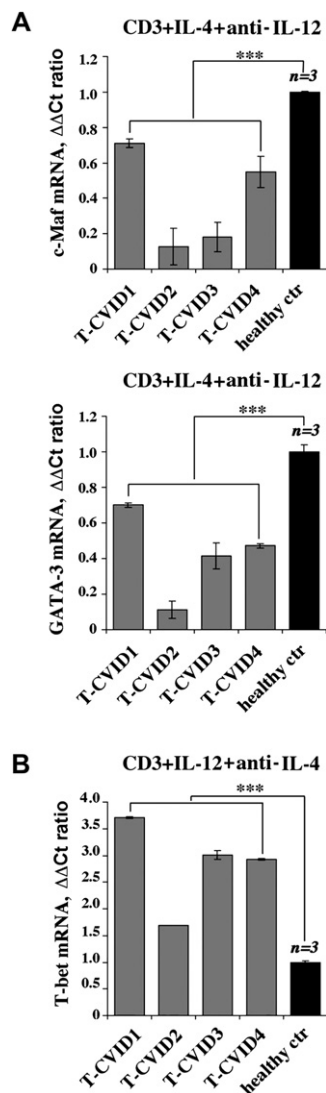


FIG 2. Levels of mRNA encoding c-Maf, GATA-3 (**A**), and T-bet (**B**) quantified by means of real-time RT-PCR in T_H cells and differentiated in T_H2 - or T_H1 -polarizing conditions of patients with T-CVID and healthy donors. Data are representative of 3 independent experiments, each in duplicate. The combined data obtained on the patients with T-CVID (mean $\pm$ SD) and healthy donors were subjected to statistical analysis. *** $P \leq .001$. Error bars = SD.

in 5 patients without T-CVID (disease control subjects). Enriched $CD4^+$ T cells purified by means of MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) from patients with CVID and control subjects were activated by anti-CD3 mAb in the absence or presence of recombinant human IL-12 (Sigma-Aldrich, Milan, Italy) and neutralizing anti-IL-4 antibodies (BioLegend, San Diego, Calif) to promote T_H1 differentiation or recombinant human IL-4 (Sigma-Aldrich) and neutralizing anti-IL-12 (Serotec, Oxford, United Kingdom) to promote T_H2 differentiation. After 10 days and after anti-CD3 stimulation, cytokine levels were measured by means of ELISA (Biosource International, Camarillo, Calif). $CD4^+$ T cells from healthy control subjects and T_H2 clones were investigated for intracellular IL-4 and IFN- γ production by means of FACS analysis after pretreatment with 6-thio-GTP (Jena Bioscience, Jena, Germany), as previously described,⁹

and subsequent stimulation for 6 hours with anti-CD3 mAb. Moreover, T cells from T-CVID1, T-CVID2, T-CVID3, and T-CVID4 and 5 healthy control subjects were nucleofected with either empty vector (pEF-BOS) or the same vector encoding human Vav16 by using Amaxa technology. Vav1 reconstitution was checked by means of immunoblotting (data not shown). The ability of nucleofected T cells to provide B-cell help for antibody production was investigated, as previously described, by using purified autologous B cells.^{10,11} Real-time quantitative PCR for c-Maf, GATA-3, T-bet, and glyceraldehyde-3-phosphate dehydrogenase was performed with the SYBR Green I SensiMix dT Kit (Quantace, Watford, United Kingdom) with appropriate primers according to the manufacturer's instructions. Sequence analysis of the Vav1 gene promoter, as well as of all Vav1 exons and the respective flanking regions, carried out on PCR fragments amplified from genomic DNA from all patients with T-CVID did not reveal any point mutation (data not shown).

T_H cells from Vav1-deficient patients with CVID were significantly impaired in their ability to produce IL-4 (mean $\pm$ SD, 307 ± 105 pg/mL) compared with healthy donors and disease control subjects (Fig 1, A, left). On the other hand, the amount of IFN- γ (mean $\pm$ SD, $1,850 \pm 141$ pg/mL) produced by cells from Vav1-deficient patients with CVID was significantly higher than that found in healthy control subjects (Fig 1, B, left).

Priming T_H cells in the presence of exogenous IL-4 and neutralizing anti-IL-12 antibodies (T_H2 -inducing conditions) further revealed the extent of the impaired T_H2 response in Vav1-deficient patients with CVID. Although the amount of secreted IL-4 increased from 307 ± 105 to 460 ± 79 pg/mL (mean $\pm$ SD) under T_H2 -inducing conditions (Fig 1, A, central panel), it remained lower than that generated by control T_H cells cultured under neutral conditions (Fig 1, A, left). Moreover, under T_H2 -inducing conditions, T_H cells from Vav1-deficient patients with CVID still produced significantly more IFN- γ than those from healthy control subjects and disease control subjects (Fig 1, B, central panel). Thus all 4 Vav1-deficient patients with CVID but neither the healthy control subjects nor the disease control subjects displayed a defective T_H2 cell polarization and an alteration in the T_H1/T_H2 balance, as assessed by the consistent significant reduction in IL-4 and the overall enhancement in IFN- γ production.

To address at a molecular level the mechanisms that account for the impaired IL-4 expression and enhanced IFN- γ production by T_H cells from Vav1-deficient patients with CVID, we used real-time RT-PCR to quantify the expression of T-bet,³ c-Maf, and GATA-3^{4,5} crucially required for T_H cell differentiation to T_H1 or T_H2 effectors, respectively. T_H cells from all patients with T-CVID showed a severe impairment of c-Maf expression (Fig 2, A). GATA-3 expression was also markedly reduced, especially in patient 2 (Fig 2, A). Conversely, T-bet expression was enhanced in a fashion that reflected, to a large extent in each patient, the respective T_H1 cytokine production (Fig 2, B).

To investigate a potential direct role of Vav-1 in IL-4 production downstream of the T-cell receptor, we treated peripheral blood-derived $CD4^+$ T cells from healthy control subjects, as well as T_H2 clones, with 6-thio-GTP, a cell-permeable Vav1 inhibitor,⁹ before and during priming. On 6-thio-GTP treatment, the intracellular expression of IL-4 was significantly reduced, with the IL-4 impairment being more prominent in $CD4^+$ T cells than in stabilized T_H2 clones. The *in vitro* treatment with 6-thio-GTP resulted in a significant reduction of IL-4-producing cells in

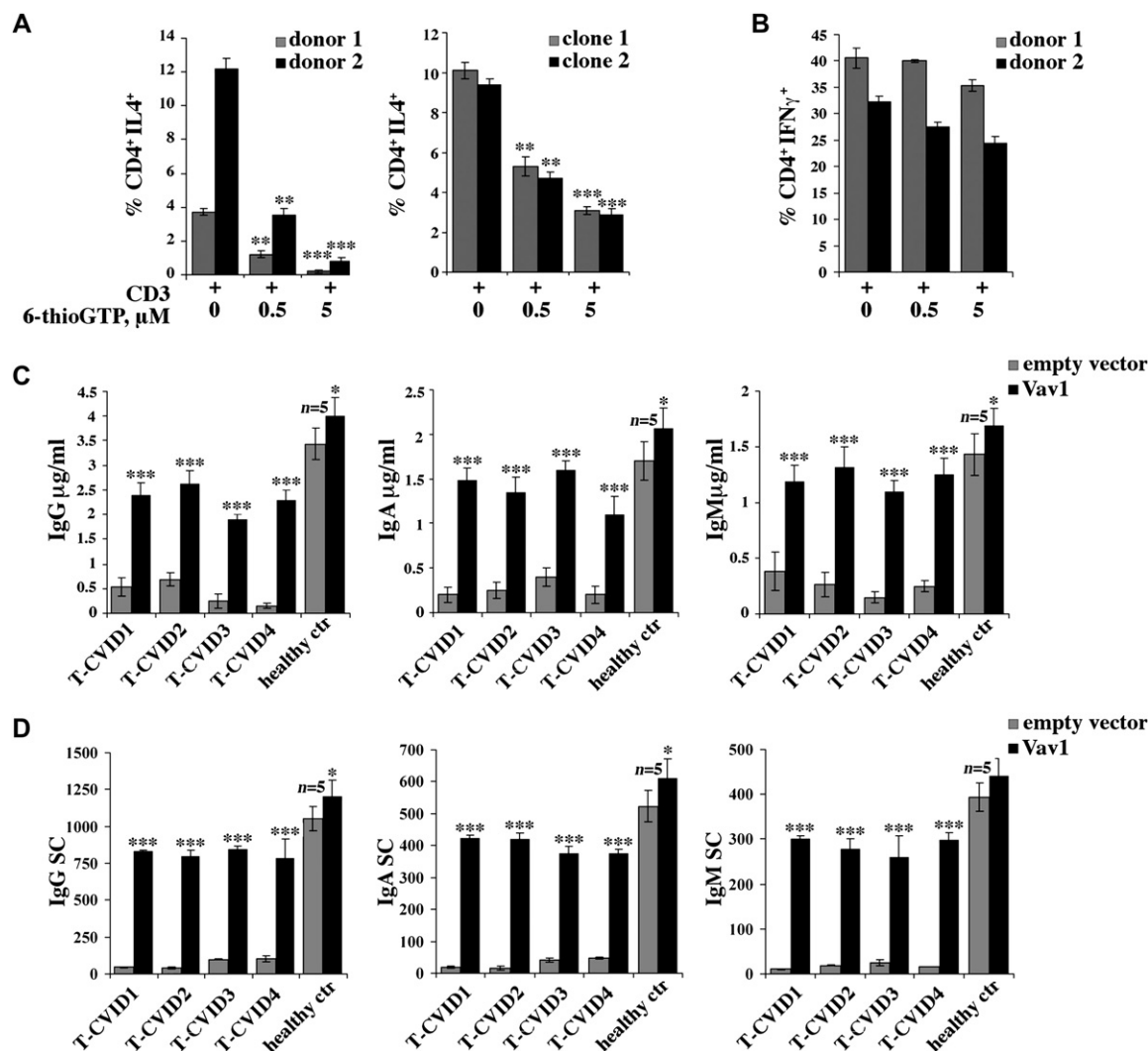


FIG 3. Intracellular IL-4 (A) or IFN- γ (B) production by T_H cells derived from peripheral blood from 2 healthy donors (Fig 3, A, left; and Fig 3, B) and by 2 IL-4-producing T_H clones (Fig 3, A, right) stimulated with anti-CD3 and treated or not *in vitro* with 6-thio-GTP. The histograms summarize the results (mean $\pm$ SD) of 2 independent experiments, each in duplicate. *** $P \leq .001$ compared with baseline (6-thioGTP, 0 mmol/L). ** $P \leq .01$ compared with baseline (6-thioGTP, 0 mmol/L) for each donor. C and D, *In vitro* production of IgG, IgA, and IgM induced in purified autologous B cells by activated T cells from 4 patients with T-CVID and 5 healthy control subjects nucleofected with either empty vector or a Vav1-encoding construct. The results show the immunoglobulin levels (Fig 3, C) and the immunoglobulin spot-forming cells (SC; Fig 3, D) induced by T cells over the spontaneous immunoglobulin production in cultures of B cells alone. For each patient, the P value (*** $P \leq .001$) refers to the comparison of the Vav1-transfected samples versus the samples transfected with empty vector.

a dose-dependent fashion (Fig 3, A and B), indicating an impairment in IL-4 production related to defective Vav1 function similar to that reported in the Vav1-deficient patients with CVID, whereas it does not substantially affect IFN- γ production (Fig 3, B). Of note, reconstitution of Vav1 expression in T cells from patients with T-CVID resulted in their functional rescue, measured as the capacity to provide B-cell help. Indeed, taking into account the transfection efficiency (30% to 40%), autologous B cells secreted levels of IgG, IgA, and IgM comparable with those seen in B cells from healthy donors when cocultured with Vav1-reconstituted T cells but not when cocultured with T cells transfected with empty vector (Fig 3, C and D). No significant effect of exogenous Vav1 expression was observed on the helper function of T cells from healthy control subjects (Fig 3, C and D).

The present study indicates that Vav1 deficiency and impaired expression of the T_H2 lineage-specific transcription factors c-Maf and GATA-3 underlie the T-cell defects harbored by a proportion of patients with CVID and suggests a potential future therapeutic intervention strategy based on restoring normal Vav1 levels in the defective patient's T cells.

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