

Correspondence

Invariant natural killer T cells in patients with common variable immunodeficiency

To the Editor:

We have read with great interest the recent study by Gao et al¹ reporting invariant natural killer T (iNKT) cell frequency, phenotype, and function in a cohort of 36 patients with CVID. They confirm the findings of previous reports that patients with CVID have fewer iNKT cells than control subjects,^{2,3} with 15 patients having undetectable levels of these cells. Furthermore, they observed lower CD161 expression measured as mean fluorescence intensity on the residual iNKT cells and also observed that iNKT cells from most of the patients with CVID do not expand during a 7-day *in vitro* culture after stimulation with α -GalCer. Intriguingly, in the few samples from patients with CVID in which iNKT cells expanded during this culture, the authors observed increased IFN- γ but reduced IL-17 secretion compared with that seen in healthy control subjects.

Recently, we reported reduced frequencies of iNKT cells in 12 treatment-naïve patients with CVID and observed no significant increase as patients started IgG replacement therapy for 6 to 12 months.⁴ However, in contrast to the study by Gao et al,¹ all patients enrolled in our study had detectable iNKT cells (range, 0.01% to 0.3% of T cells). Gao et al¹ do not specify any details regarding the intravenous immunoglobulin (IVIg) treatment of the patients included in this study. IVIg treatment has many effects on the immune systems of patients with CVID,^{4,5} including effects on iNKT cells. Recently, we found CD161 mean fluorescence intensity levels to be increased on iNKT cells from treatment-naïve patients with CVID and found that these levels normalized as the patients started IVIg.⁴ This highlights the importance of distinguishing between the perturbations caused by CVID and the secondary effects of IVIg. Studying the same cohort of treatment-naïve patients with CVID, our new intracellular flow cytometric data based on unmanipulated PBMCs indicate a trend toward decreased IFN- γ production by iNKT cells in response to α -GalCer (Fig 1).

We are concerned that there is a risk that the methods used by Gao et al¹ to evaluate expression of cytokines in iNKT cells might skew the results. First, the 1-week expansion culture with IL-2 might change the functional phenotype of the iNKT cell population. Furthermore, the ELISPOT assay does not allow discrimination of which cells in the mixed-cell culture are producing the

cytokines. Thus an unknown proportion of the IFN- γ -producing cells could be non-iNKT cells, such as natural killer cells, that are activated in a bystander manner after iNKT cell activation. Finally, because cytokine production was analyzed only for 3 patients with higher iNKT cell frequencies, it is possible that the results are not representative of the majority of patients with CVID. Our approach to investigate iNKT cell function directly *ex vivo* in treatment-naïve patients has the advantage that cells are minimally manipulated *in vitro* before IFN- γ production is assessed and that the measurements take place before the influence of IgG replacement therapy on the disease phenotype. More longitudinal studies of patients with CVID before and after IVIg treatment are needed to better differentiate the disease phenotype from treatment effects.

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REFERENCES

- Gao Y, Workman S, Gadola S, Elliott T, Grimbacher B, Williams AP. Common variable immunodeficiency is associated with a functional deficiency of invariant natural killer T cells. *J Allergy Clin Immunol* 2014;133:1420-8.e1.
- Fulcher DA, Avery DT, Fewings NL, Berglund LJ, Wong S, Riminton DS, et al. Invariant natural killer (iNKT) T cell deficiency in patients with common variable immunodeficiency. *Clin Exp Immunol* 2009;157:365-9.
- Carvalho KI, Melo KM, Bruno FR, Snyder-Cappione JE, Nixon DF, Costa-Carvalho BT, et al. Skewed distribution of circulating activated natural killer

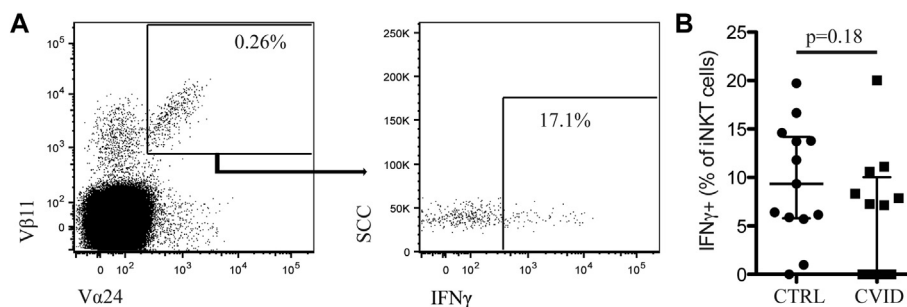


FIG 1. A, Gating strategy for IFN- γ production by iNKT cells. **B,** IFN- γ production by iNKT cells from healthy control subjects (CTRL) and treatment-naïve patients with CVID after α -GalCer stimulation for 6 hours.

- T (NKT) cells in patients with common variable immunodeficiency disorders (CVID). *PLoS One* 2010;5(9).
4. Paquin-Proulx D, Santos BA, Carvalho KI, Toledo-Barros M, Barreto de Oliveira AK, Kokron CM, et al. IVIg immune reconstitution treatment alleviates the state of persistent immune activation and suppressed CD4 T cell counts in CVID. *PLoS One* 2013;8:e75199.
 5. Paquin-Proulx D, Santos BA, Carvalho KI, Toledo-Barros M, Oliveira AK, Kokron CM, et al. Dysregulated CD1 profile in myeloid dendritic cells in CVID is normalized by IVIg treatment. *Blood* 2013;121:4963-4.
- <http://dx.doi.org/10.1016/j.jaci.2014.06.039>