Common variable immunodeficiency is associated with a functional deficiency of invariant natural killer T cells

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Background: Common variable immunodeficiency (CVID) is the commonest symptomatic primary antibody disorder, with monogenic causes identified in less than 10% of all cases. X-linked proliferative disease is a monogenic disorder that is associated with hypogammaglobulinemia and characterized by a deficiency of invariant NKT (iNKT) cells. We sought to evaluate whether a defect in iNKT cell number or function was associated with CVID.

Objective: An evaluation of the function and number of iNKT cells in CVID.

Methods: Six-color flow cytometry enumerated iNKT cells in 36 patients with CVID and 50 healthy controls. Their proliferative capacity and cytokine production (IFN-γ, IL-13, IL-17) was then investigated following activation with CD1d ligand alpha-galactosylceramide.

Results: A reduction in the number of iNKT cells (31 iNKT cells/10^5 T cells) in patients with CVID compared with healthy controls (100 iNKT cells/10^5 T cells) was observed ($P < .0001$). Two cohorts could be discerned within the CVID group: group 1 with an abnormal number of iNKT cells (n = 28) and group 2 with a normal number of iNKT cells (n = 8). This segregation coassociated with the proliferative capacity of iNKT cells between the 2 groups. However, differences in the function of iNKT cells were noted in group 2, in which an increase in IFN-γ ($P = .0016$) and a decrease in IL-17 ($P = .0002$) production was observed between patients with CVID and controls. Finally, a significant association was seen between the number of iNKT cells and the percentage of class-switched memory B cells and propensity to lymphoproliferation ($P = .002$) in patients with CVID.

Conclusion: iNKT cells are deficient and/or functionally impaired in most of the patients with CVID. (J Allergy Clin Immunol 2014;133:1420-8.)

***Key words:** iNKT, CVID, XLA, cytokine, proliferation*

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Invariant natural killer T (iNKT) cells are a subset of thymic-derived innate T cells that are reactive to CD1d.[1-3] In human peripheral blood, approximately 0.01% to 1% of the T-cell pool can be defined as iNKT cells, characterized by their hallmark T-cell receptor (TCR)-invariant chain Vα24–Jα18 and Vβ11.[4] These cells are polyfunctional, producing multiple cytokines including IFN-γ, IL-13, and IL-17.[5] This diverse cytokine repertoire has contributed to their proposed immunoregulatory role in many autoimmune and infectious diseases and cancer. Recently, defective iNKT cells were identified in several immunodeficiency syndromes including X-linked lymphoproliferative (XLP) disease[6,7] and Omenn's syndrome.[8] The former showed a complete absence of iNKT cells due to a mutation in the SH2D1A gene,[9,10] causing a deficiency in the signaling lymphocytic activation molecules-associated protein (SAP), leading to defective SAP signaling for thymic selection, while the latter was also found to have deficient iNKT cells in the setting of aberrant DNA recombination, caused by mutations in the RAG2 gene.[11]

Common variable immunodeficiency (CVID) is the commonest symptomatic primary antibody deficiency, representing a heterogeneous group of primary defects.[12] Most patients with CVID have a B-cell defect characterized by a reduction in the number of memory B cells and failure of antibody production. Patients generally have a marked reduction in levels of serum IgG and IgA, often accompanied with a reduction in levels of IgM.[13] Patients typically present with recurrent sinopulmonary infections and impaired antibody responses to vaccination.[13] A bimodal distribution for the age of presentation for CVID has been described, with some presenting symptomatically in childhood and the majority being diagnosed as adults. The onset of CVID in men is relatively earlier (mean age of onset, 23 years) than in women (mean age of onset, 28 years).[14] Four major genetic defects have been identified in CVID following the first description in 2000. These are inducible T-cell costimulator,[15,16] transmembrane activator and calcium-modulating cyclophylin ligand interactor,[17-19] B-cell activating factor of the tumor necrosis factor family receptor,[20] and CD19.[21]

Abbreviations used
α-GalCer: Alpha-galactosylceramide
CVID: Common variable immunodeficiency
FITC: Fluorescein isothiocyanate
INKT: Invariant natural killer T
PE: Phycoerythrin
SAP: Signaling lymphocytic activation molecules-associated protein
TCR: T-cell receptor
XLA: X-linked agammaglobulinemia
XLP: X-linked proliferative

Recently, 2 groups have reviewed iNKT cell enumeration in patients with CVID and suggested that this subset is reduced or skewed compared with controls.[22,23] We have investigated a large cohort of patients with CVID for deficiencies of both iNKT cell function and distribution and compared these with healthy and disease controls. We show that 2 groups of patients with CVID can be identified on the basis of their number and function of iNKT cells. In contrast, the disorder X-linked agammaglobulinemia (XLA), a primary genetic defect of B cells, shows no defective iNKT cell function. Interestingly, the same 2 CVID groups can be identified on the basis of their percentage of peripheral switch memory B cells, which correlates with the presence or absence of iNKT cells.

METHODS

Cell culture

PBMCs were isolated by density gradient centrifugation using Ficoll-Plaque (GE-Health, Buckinghamshire, United Kingdom) from fresh buffy coat preparations of healthy blood donors (National Blood Services, Southampton, United Kingdom) and from adult patients with CVID and XLA. Cells were subject to 7 days culture with 100 IU of IL-2 (Peprotech, London, United Kingdom) in the presence of alpha-galactosylceramide (α-GalCer; School of Chemistry, University of Southampton, Southampton, United Kingdom). Informed consent was obtained from all contributing individuals according to the declaration of Helsinki.

Flow cytometry

iNKT cells were identified by using a TCR antibody panel and a CD1D tetramer approach. The tetramer approach used a human CD1d phycoerythrin (PE) tetramer loaded with α-GalCer (ProImmune, Oxford, United Kingdom) in combination with CD19 fluorescein isothiocyanate (FITC) (BD Biosciences, Oxford, United Kingdom) and CD3 PerCP (BD Biosciences).

iNKT cells were routinely identified by using a combination of anti-human Vα24 FITC (Beckman Coulter, Pasadena, Calif), anti-human Vβ11 PE (Beckman Coulter), anti-human CD3 PerCP (BD Biosciences), anti-human CD161 APC (BD Biosciences), anti-human CD4 PE-Cy7 (BD Biosciences), and anti-human CD8 APC-Cy7 (BD Biosciences) and analyzed on a fluorescence-activated cell sorting Canto.

B cells were identified by using a combination of anti–CD27 FITC (Dako, Glostrup, Denmark), anti–CD38 FITC (BD Biosciences), anti–CD21 PE (BD Biosciences), anti–IgD PE (Southern Biotechnology Associates, Birmingham, Ala), CD19 PC7 (Beckman Coulter), and anti–IgM Cy5 (Dianova, Hamburg, Germany).

Proliferation assay for iNKT cells

PBMCs were subjected to 7 days culture with 100 ng/mL of α-GalCer and IL-2. A proliferation index was calculated by using the number of iNKT cells per 10^5 T cells preculture compared with a similar quantitation postculture. The iNKT cells were identified by using the flow cytometry panel described previously.

Cytokine analysis for iNKT cells

PBMCs (4×10^5 per well) were pulsed with 100 ng/mL of α-GalCer on day 0 and again on day 7 for 18 hours. An ELISPOT assessment was undertaken for IFN-γ, IL-13, and IL-17 by using the human IFN-γ, IL-13, and IL-17 kits according to the manufacturer's instructions (Ready-SET-Go, eBiosciences, Hatfield, United Kingdom).

Statistical analysis

Paired *t* tests and correlation coefficients were undertaken on Prism Graphpad 4.0.

RESULTS

Investigating iNKT cells quantitatively and qualitatively in patients with CVID

To investigate the number of iNKT cells in patients with CVID, we used a 6-color fluorescence-activated cell sorting flow cytometry panel to identify this rare subset in whole blood using CD161, CD4, and CD8 to further characterize the Vα24:Vβ11 subset. To accurately quantitate the number of iNKT cells, a minimum of 10^5 T cells were collected and sequentially gated to examine the phenotype of those cells, that is, maturation and activation status.[24] A formal comparison of this TCR-based strategy for the detection of iNKT cells with the use of the CD1d tetramer showed equivalence of enumeration ($r^2 = 0.86$) (see Fig E1 in this article's Online Repository at www.jacionline.org). Representative phenotypes of iNKT cells identified in patients with CVID and controls show a clear difference in the populations of Vα24 T cells, as seen in Fig 1, *A*. While controls show a typical Vα24^{high} CD161-positive population of iNKT cells, patients with CVID show only conventional Vα24-positive T cells. The range of iNKT cells identified in the 36 patients with CVID and 50 controls is shown in Fig 1, *B*. Clinical phenotypes of these patients are shown in Table I. Unlike controls, 15 patients with CVID (42%) had no detectable iNKT cells, while all controls had detectable iNKT cells. Furthermore, a marked reduction in the number of iNKT cells in the CVID patient population was observed, with a mean value of 31 (0.031% of T cells) compared with 100 (0.1% of T cells) iNKT cells per 10^5 T cells in controls ($P < .0001$) (Fig 1, *B*). To ascertain whether these iNKT cell numbers were stable in patients with CVID, we followed up 4 of the 36 patients for up to 6 months (Fig 1, *C*). The results suggest that iNKT cells do not fluctuate greatly over time, consistent with our observations in healthy controls (data not shown). Furthermore, there was no association between the time from diagnosis and the number of iNKT cells observed in patients with CVID, consistent with the iNKT cell phenotype being a stable phenotype (data not shown).

Two phenotypic properties of iNKT cells, the ratio of CD4:double negative and the CD161 expression, were analyzed in the CVID and control cohorts (Fig 2). This analysis was restricted to the 12 of the 13 patients with CVID in whom the number of iNKT cells was more than 10 (0.01% of T cells) cells per 10^5 T cells. The intensity of CD161 expression was significantly reduced in patients with CVID than in controls ($P = .0061$). This observation is consistent with either an increased activation state or a relative immaturity of the iNKT population (Fig 2, *A*).

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