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Lymphocyte characteristics in children with common variable immunodeficiency

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Abstract The diagnosis of common variable immunodeficiency (CVID) is reserved for patients who suffer from undefined B cell dysfunction. Division of the CVID population into subgroups enables research for underlying disease causes. We studied clinical features and lymphocyte characteristics in 38 children with CVID and compared them to 30 children with less severe antibody deficiencies (e.g. specific antibody deficiency combined with IgG subclass deficiency) and with 65 pediatric controls. Most pediatric immune phenotypes were comparable to adult CVID phenotypes, including a selective increase in newly formed B cells and a decrease in memory B cells and $CD4^+$ T cells. Eighteen percent of pediatric patients had a mutation in the TNFRSF13B gene, which requires further investigation. Finally, pediatric patients with decreased class-switched memory B cells had significantly more complications.
A pediatric classification for CVID may enable prediction and early diagnosis of disease related complications and provide a framework for further etiologic research.

Introduction

Common variable immunodeficiency (CVID) is a primary immunodeficiency characterized by B cell dysfunction [1,2]. The diagnosis is based on decreased serum immunoglobulins and a failure to produce antigen-specific antibodies in response to vaccinations or infections [3]. Besides recurrent infections, CVID patients have an increased tendency to develop autoimmunity, lymphoproliferative disease and malignancies [4]. Early prediction of complications is important as these disease complications cause severe morbidity, are associated with decreased survival and may require medical intervention. In addition to CVID, there is a spectrum of CVID-related antibody deficiencies that share clinical and immunological features. Patients diagnosed with selective antibody deficiency in combination with IgG subclass deficiency or symptomatic IgA deficiency may have as much recurrent infections as CVID patients and may thus require immunoglobulin substitution therapy as well. Moreover, those diseases occasionally progress to CVID [5,6].

The etiology of CVID is unknown for most patients. Mutations in the genes encoding the transmembrane

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activator and calcium-modulating cyclophilin ligand (CAML) interactor TACI [7,8], inducible costimulator (ICOS) [9], CD19 [10], B cell activating factor receptor (BAFFR) [11] and CD81 [12] have been described. Several biallelic and monoallelic variants of the TNFRSF13B gene encoding TACI have been described in CVID. Recently, however, heterozygous variants were found in healthy individuals [13], indicating that these variants are merely associated with increased susceptibility for CVID [14,15].

Classifications divide the heterogeneous CVID population into more similar subgroups to enable further etiological research and eventually give rise to personalized treatment options. Classifications of adult CVID patients have been ongoing: patients were classified based on flow cytometric markers of B cells [16–18], T cells [19] or non-infectious complications [20].

Immunological findings in pediatric CVID may exhibit important differences compared to CVID presenting in adults. Maturation of the immune system may influence the impact of gene mutations on clinical manifestations of CVID. Moreover, values for normality in this immature pediatric immune system are inherently different from those in adults [21]. For these reasons, there is need for evaluation of existing classifications in CVID. Here, we provide an overview of immunological characteristics of 68 children with CVID and related diseases. These data provide a framework for developing a pediatric CVID classification system.

Methods

Patients and controls

We retrospectively analyzed data obtained between July 1995 and July 2008. Thirty-eight pediatric CVID patients of the Wilhelmina Children's Hospital in Utrecht, The Netherlands, were included. Diagnoses were made consistent with the European Society for Immunodeficiencies (ESID) criteria [22]. Additionally, a group of 30 children with CVID-related diseases was investigated (selective antibody deficiency in combination with symptomatic IgA deficiency and/or IgG subclass deficiency, or 'probable CVID'). All CVID and CVID-like patients suffered from recurrent airway infections and showed significant clinical improvement after initiation of immunoglobulin replacement therapy.

Our diagnostic protocol for PID consists of assessment of serum immunoglobulin titers, vaccination responses, T and B cell phenotyping and *in vitro* mitogenic and antigenic T cell proliferation responses. B cell proliferation assays were performed on a subset of patients. The most recent data were used in case of repetitive phenotyping. Clinical data were extracted from the medical files. We classified the children according to the classifications reported by EUROClass [18], Giovannetti et al. [19] and Chapel et al. [20]. For comparison of phenotypical analyses, a control group of 65 healthy children was included. This cohort consisted of children without immune diseases who underwent elective surgery. [23].

Flow cytometry analysis

The T and B cell compartments were analyzed with four-color flow cytometry using whole blood and antibodies to CD3, CD45, CD27, CD4, CD8, HLA-DR, CD38, CD45RA and CD19, CD27, CD38, CD10, IgM, IgG, IgA, IgD, respectively, as described previously [23]. All monoclonal antibodies were derived from Becton Dickinson and the polyclonal goat $F(ab')_2$ anti-human immunoglobulin antibodies from Southern Biotechnology Associates, Birmingham, AL. After erythrocyte lysis, cells were analyzed on a FACSCalibur using CellQuest Pro data analysis software (both Becton Dickinson).

T and B lymphocyte functional assays

Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Paque density gradient centrifugation and 1.8×10^5/mL PBMC were cultured for 3 or 6 days in RH10 (RPMI1640 supplemented with L-glutamate and 25 mM HEPES (Gibco), containing 10% human AB serum (Sanquin), penicillin/streptomycin (100 U/mL) (Invitrogen) and 6.0×10^{-5}M β-mercaptoethanol (v/v) (Calbiochem)) and the following stimuli: phytohemagglutinin (5 μg/mL; Wellcome), Concanavalin A (10 μg/mL; Calbiochem), tetanus toxoid (70 Lf/mL; RIVM, The Netherlands), purified protein derivative (PPD) (13 μg/mL; Staten Serum Institute, Denmark), *Candida albicans* (7 μg/mL; Hal) and diphtheria toxin (70 Lf/mL; RIVM). ^{3}H-Thymidine (1 μCi/96 wells) was added 16 h prior to harvesting. A stimulation index >3 was considered positive. Assay conditions were verified by a control sample run in parallel. The percentage of response was defined by the number of positive responses to a stimulus divided by the total number of tests.

For B cell differentiation assays, 4×10^5 PBMC were cultured with either pokeweed mitogen (PWM, 3.5 μg/ml, Sigma) or *Staphylococcus aureus* antigen (STA, 40 U/mL) and IL-2 (10 U/mL, Sanquin). After 7 days, cells were harvested, cytospins (10^5/sample) were prepared, air-dried, fixed with 95% EtOH/5% HAc and stained with FITC-conjugated anti-Ig, IgM, IgA or IgG (SBA). Ten fields of 50 cells were analyzed by fluorescence microscopy and the fraction of plasmablasts was calculated.

Molecular analysis of the TNFRSF13B gene

Genomic DNA was extracted from peripheral blood granulocytes with an autopure kit (Qiagen). Up to 300-μmol DNA was used in a TaqMan 5′ nuclease assay. Primers and probes specific for the c.310T>C (p.C104R) variant, the c.542C>A (p.A181E) variant and their wild-type variants were designed with Primer Express software (Applied Biosystems), as shown in Appendix 1. Probes were labeled at the 5′ end with either FAM or VIC as reporter dyes and at the 3′ end with a minor groove binding (MGB) molecule (Applied Biosystems). The detection run consisted of a hot start at 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. All assays were performed as 20-μL reactions using PCR Mastermix 2× (Applied Biosystems) in 96-well plates using an ABI Prism 7900HT instrument (Applied Biosystems). We confirmed positive results by sequencing of the TNFRSF13B gene.

Statistical analysis

We corrected flow cytometry data for age and analyzed them with a Kruskal–Wallis one-way analysis of variance test. A p-value <0.05 was considered significant and post-hoc analyses were performed by Mann–Whitney U tests