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Rapid Communication

Correlation of bone marrow abnormalities, peripheral lymphocyte subsets and clinical features in uncomplicated common variable immunodeficiency (CVID) patients

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ABSTRACT

B cell developmental defects in CVID were recently described in a limited number of cases. To date, a detailed correlation between this maturational defect and the clinical presentation of affected patients has not been reported. In this study, we correlated bone marrow B cell evaluation, peripheral B and T lymphocyte subsets and clinical findings in 15 CVID patients. Early B cell developmental defects were observed in one third of patients. Combined bone marrow and peripheral lymphocytes evaluation allowed to further subdivide CVID patients in three groups with shared clinical features at diagnosis and during follow-up. These data broaden the number of CVID patients with early B cell developmental defects and, together with the peripheral lymphocytes evaluation, offer insight into the related clinical features in affected patients.

1. Introduction

Common variable immunodeficiency (CVID) has been classically characterized to be associated with a peripheral B cell maturational defect, with associated T cell numerical or functional defects in a small percentage of cases [1,2,3]. To date, data on bone marrow B cell development are available for 37 patients [4,5]: while 12/37 reported patients were not complicated CVID cases [4], the remaining 25/37 underwent bone marrow analysis due to disease associated complications such as lymphoproliferative disease, lymphoma exclusion and others [3].

Here, we studied bone marrow B cell development in 15 uncomplicated CVID patients and correlated these findings with peripheral lymphocyte evaluation and clinical features at onset and during follow-up. Our data show that early B cell development may be perturbed in a fraction of CVID patients and that peripheral B cell lymphopenia may be present in CVID patients without an early bone marrow maturational impairment. Combining bone marrow B cell development with peripheral B and T cell evaluation as well as clinical features at onset and during follow-up, we show that CVID patients may be subdivided in 3 distinct groups with evident clinical implications.

Abbreviations: CVID, common variable immunodeficiency; RTE, recent thymic emigrants.

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2. Materials and methods

2.1. Patients

Fifteen patients (CVID1–CVID15) regularly followed at the Pediatrics Clinic, University of Brescia, Italy, were enrolled in this study. All patients were in good health, without any complications such as suspicion of lymphoproliferative disease, autoimmune cytopenias, or other confounding features at the time of evaluation. CVID diagnosis was made according to the ESID/PAGID criteria. The male: female ratio was 3:2. Mean age at diagnosis was 15 years, while mean age at the time the patients were evaluated for this study was 27.3 years. Immunoglobulin serum levels and peripheral B cells at diagnosis and at bone marrow evaluation are summarized in Supplementary Table 1. All patients were under regular immunoglobulin replacement treatment. All patients (or their parents in case of pediatric patients) signed an informed consent form according to local ethical committee indications.

2.2. Flow cytometry

Bone marrow aspirates and peripheral blood samples were obtained and processed from all patients on the same day. Samples from seven age-matched healthy donors were also examined using the same criteria. Surface staining was performed by using mixes of the following antibodies: CD34, CD10, CD19, CD20, CD22, CD45 for bone marrow B cell evaluation; CD19, CD20, CD10, CD21, CD27, IgD, CD38 for peripheral

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B cell evaluation; CD3, CD4, CD8, CD45RA, CCR7, CD31 for peripheral T cell evaluation. Samples were acquired on a Canto II flow cytometer (Becton Dickinson) and analyzed using the FlowJo software version 8.8.6 (TreeStar).

2.3. Statistical analysis

Statistical significance was analyzed using the unpaired Student's t-test by using GraphPad Prism Version 8.0 (GraphPad Software, San Diego, CA).

3. Results and discussion

3.1. Bone marrow evaluation of B cell development in 15 uncomplicated CVID patients

Patients' bone marrow aspirate was evaluated by means of six color flow cytometry and was compared to healthy donors'. A patient affected with Igβ deficiency was included as indicative for early B cell developmental arrest [6]. Five out of fifteen patients (33,3%) showed a distribution of B cell precursors similar to the Igβ deficient patient (group I) (Fig. 1A). Specifically, the proportion of pluripotent stem cells (CD34+, CD22−, CD19−, CD10−, CD45lo) was particularly abundant in these patients when compared to the other ten CVID patients (group II) ($p = 0{,}0007$) and seven healthy donors (HDs) ($p = 0{,}0025$) (Fig. 1B). The same trend was observed with pro-B cells (CD34+, CD22+, CD19−, CD10−, CD45lo) where patients from group I significantly exceeded the proportion of this cell subset when compared to patients from group II ($p = 0{,}0007$) and HDs ($p = 0{,}0025$) (Fig. 1C). Conversely, pre-B-I cells (CD34+, CD22+, CD19+, CD10hi, CD45lo) showed heterogeneous data, varying from 37.1% for CVID1 to 4.3% for CVID4, compatible with different maturation abnormalities (Fig. 1D).

From the pre-B-IIa stage, patients from group I showed a trend inverted from the one displayed at the level of stem cells and pro-B, suggestive of an early B cell developmental arrest. In fact, there was a

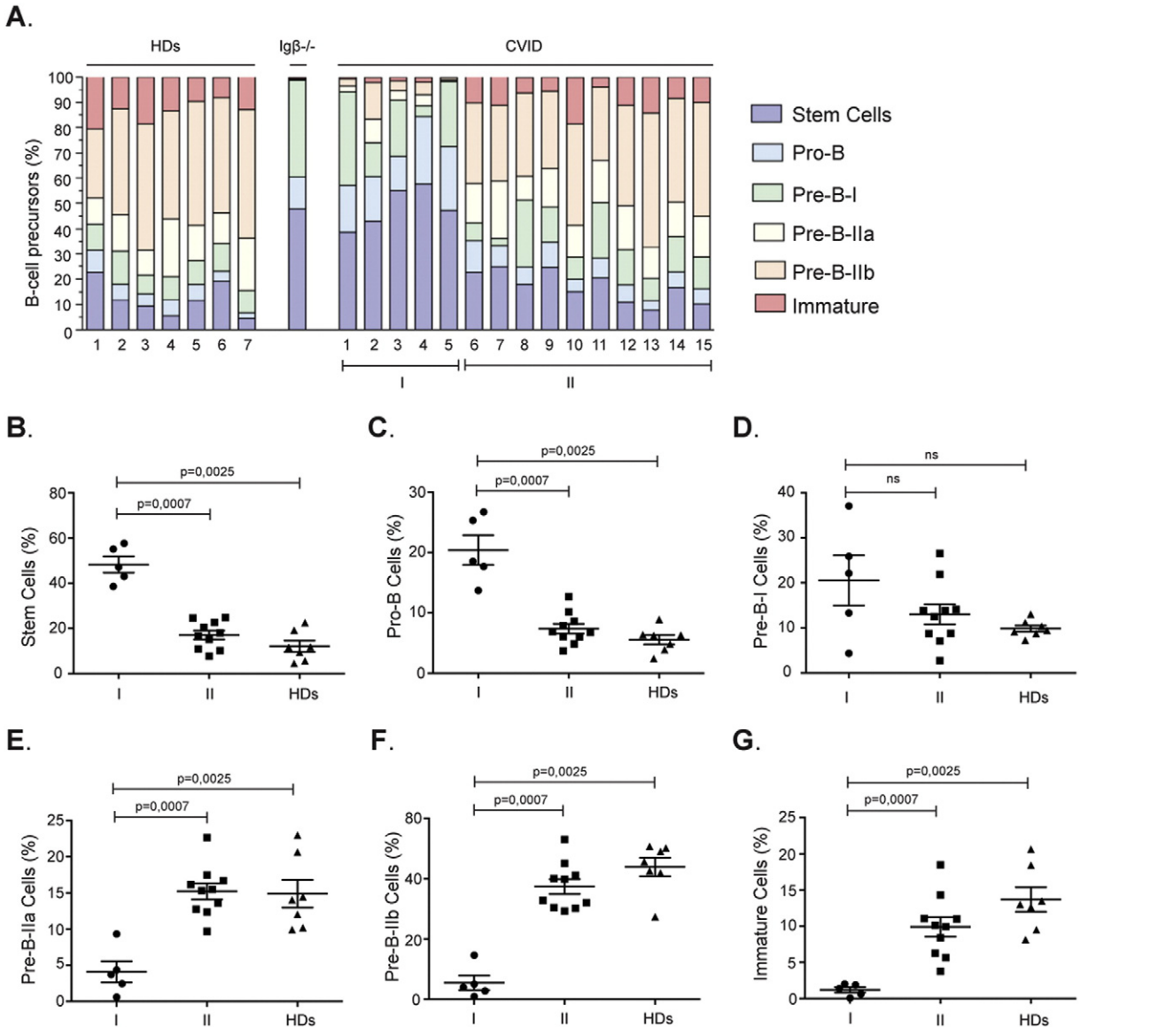

Fig. 1. Evaluation of bone marrow B cell development in 15 CVID patients. A. Summary of B cell developmental evaluation in 15 CVID patients and 7 healthy donors (HDs). Bone marrow evaluation of a patients affected with Igβ deficiency is also shown. B. Comparison of pluripotent stem cells (CD34+, CD22−, CD19−, CD10−, CD45lo) between CVID patients and HDs. C. Comparison of pro-B cells (CD34+, CD22+, CD19−, CD10−, CD45lo) between CVID patients and HDs. D. Comparison of pre-B I cells (CD34+, CD22+, CD19+, CD10hi, CD45lo) between CVID patients and HDs. E. Comparison of pre-BIIa cells (CD34−, CD22+, CD19+, CD10+, CD45+, CD20−/+, large cells) between CVID patients and HDs. F. Comparison of pre-BIIb (CD34−, CD22lo, CD19+, CD10+, CD45+, CD20+, small cells) stem cells between CVID patients and HDs. G. Comparison of immature B cells (CD22lo, CD19+, CD10lo, CD45hi, CD20+) between CVID patients and HDs. Statistical analysis was performed using the GraphPad Prism 4.0 software.