Altered dendritic cell function in response to sera of common variable immunodeficiency patients

M. Nourizadeh, A. Aghamohammadi, S. M. Moazzen, M. Mahdavi, A. Jalili, N. Rezaei, J. Hadjati

1 Immunology, Asthma and Allergy Research Institute, Medical Sciences University of Tehran, Tehran, Iran
2 Department of Immunology, Faculty of Medicine, Tarbiat Modarres University, Tehran, Iran
3 Division of Immunology, Allergy and Infectious Diseases (DIAID), Department of Dermatology, Medical University of Vienna, Allgemeines Krankenhaus, Vienna, Austria
4 Department of Immunology, Faculty of Medicine, Medical Sciences University of Tehran, Tehran, Iran, Fax: ++98 21 6641 9536, e-mail: hajatij@sina.tums.ac.ir or mnourizadeh@razi.tums.ac.ir

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Introduction

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders, characterized by defective antibody production and increased susceptibility to recurrent pyogenic infections as well as autoimmune and neoplastic diseases [1–5]. In CVID patients serum levels of at least 2 immunoglobulin isotypes are lower than normal [1, 3–6]. The number of circulating B cells is reduced or normal, but these cells can proliferate and produce immunoglobulins in vitro when stimulated with anti-CD40 and cytokines such as IL-4 and IL-10 [7].

Although CVID is a largely unknown disorder, several causes leading to alteration of immunoglobulin concentrations in the blood have already been identified. These include: T-cell abnormalities [8], accelerated T cell apoptosis [9], impaired cytokine production [10–12] and reduced generation of antigen-specific memory T cells [13]. Initially it had been believed CVID to be a defect in B cell maturation and function, but recently it has been shown that CVID-derived B cells are able to secrete IgM and IgG upon in vitro stimulation with B cell polyclonal activators despite these patients being hypogammaglobulinemic [7, 14]. Therefore, it is speculated that B cells may not receive appropriate signals from T helper lymphocytes or dendritic cells (DCs) and perturbed interaction in secondary lymphoid organs may be involved in the pathogenesis of the disease [15]. In addition to T cell stimulation, DCs regulate B-cell growth and immunoglobulin secretion/class switching and differentiation toward plasma cells [16, 17]. Although malfunctioning of DCs appears to be one of the prominent features of CVID patients [17], it is unclear whether the malfunction is associated to the effect of CVID patient’s microenvironmental factors such as blood cytokines or the DCs are inherently impaired.

The aim of the preset study was to study the effects of CVID patients’ sera on development, maturation, cytokine production and induction of adaptive immune response by monocyte-derived dendritic cells (MDCs) in vitro.
Materials and Methods

Patients and volunteers

Following informed consent and approval of the local Ethics Committee, 10 CVID patients, who had been referred to the Children Medical Center of Tehran University of Medical Sciences, were investigated. The diagnosis of CVID was based on standard criteria, which has been introduced by the Expert Committee of International Union of Immunological Societies (IUIS) on Primary Immunodeficiency [4]. To exclude other causes of hypogammaglobulinemia, patients with B cells number of less than 1% were subjected to BTK (Bruton’s Tyrosin kinase) [18] mutation analysis and the patients with B cells number of more than 1% were analyzed for AID (activation-induced cytidine deaminase) [19] (males and females), SH2D1A [20] and CD40L [21] (just in males) defects. Eight age and sex matched healthy volunteers served as control group.

Serum preparation

Serum samples were collected from patients before immunoglobulin replacement therapy (or four weeks after the last immunoglobulin replacement therapy) and controls. Briefly, 5 ml of whole blood obtained in order to get 2 ml of serum. Blood was allowed to clot for 30 minutes at room temperature, subsequently rinsed with a Pasteur pipette and after centrifugation at 3000 rpm for 10 min/37 °C, serum was separated and heat-inactivated, (56 °C for 30 min) then stored at –70 °C.

Generation of monocyte-derived dendritic cells (MDCs)

MDCs were generated as described elsewhere [22]. Briefly, heparinized peripheral blood was obtained from healthy volunteers (O blood group) different from control serum donors, mixed with an equal volume of PBS. PBMCs were collected over half volume of lymphoprep 1.077 ± 0.001 g/ml (Axis-Shield, Oslo, Norway) after 20 min centrifugation at 2000 rpm/37 °C and washed twice with RPMI-1640 (Invitrogen, Gibco, United Kingdom) to reduce platelets. MDCs were generated from PBMCs adherent to plastic flasks (Nunc, Roskilde, Denmark) in RPMI-1640 + 10% heat-inactivated human AB serum (Central Blood Bank, Tehran, Iran) after 2 h incubation at 37 °C. and after 5 day culture of adherent monocytes in 500 IU/ml rhIL-4, 1000 IU/ml rhGM-CSF (both from Bender MedSystems, Vienna, Austria) and 30% MCM (see Monocyte Conditioned Medium (MCM) section under Materials and Methods) [23] for 48 hrs. Yields of DCs averaged around 8 × 10⁶ per 10⁷ PBMCs.

Monocyte Conditioned Medium (MCM)

MCM was prepared as previously described with some modifications [24]. Briefly, Ig coated bacteriologic plates were prepared immediately before use by the addition of 10 ml of 5 mg/ml human gamma globulin (Baxter Healthcare, Deerfield, IL) for 5 min. The plates were washed three times with sterile PBS before use. PBMCs (10⁷) isolated as above were layered onto the Ig-coated bacteriologic plates in 10 ml complete medium with 20% human AB serum. After 2 hours nonadherent cells were washed away and discarded. Ig-adherent cells were incubated in fresh complete medium with 10% human AB serum at 37 °C for 24 hrs. The medium was collected, centrifuged at 3000 rpm for 10 min and the cell-free supernatant was passed through a 0.22µm filter and frozen at −20 °C until use.

Flow cytometry

The following monoclonal antibodies were used in flow cytometry: FITC–conjugated mouse anti-human CD14 (Clone TUK4), CD86 (Clone BU63), CD11c (Clone KB90) and PE-conjugated mouse anti-human HLA-DR (Clone AB3). These and the corresponding isotype control mAbs were purchased from DakoCytomation (Hamburg, Germany). FITC-conjugated mononuclear anti-human CD83 (Clone HB15e) and corresponding isotype control were obtained from Ancell (Bayport, MN). Stainings were performed according to manufacturer’s protocols. Briefly, 10⁵ MDCs were washed twice with PBS/1 % FCS (staining buffer) and incubated for 30 min with anti-CD14, CD1a (Clone NA1/34), CD86, CD11c, and HLA-DR and for 45 min with anti-CD83 at 4 °C. Cells were subsequently washed with staining buffer and analyzed by FACS using a FACS Caliber and CELLQuest software (Becton & Dickinson, San Joes, CA).

Allogeneic Mixed Lymphocyte Reaction Assay (MLR)

Allogeneic capacity of CVID and control sera treated MDCs was evaluated by co-culturing of different numbers (5000–40000) of irradiated (30 Gy) mature MDCs (effector) with 1 × 10⁵ allogeneic PBMCs/well/200 µl (responder) in RPMI 1640 medium supplemented with 10% human AB serum in 96-well plates (Greiner Bio-one, CELL STAR. Frickenhausen, Germany) [10, 25]. After 5 days of culture, proliferation of effector cells was measured by incorporation of 1 µCi/well [³H]-thymidine (Amersham, Aylesbury, UK) which was added during the final 18 h of the culture. Cells were then harvested onto glass-fiber filter papers (Titertek, Lier, Norway) by a mini-cell harvester (NUNC, Roskilde, Denmark) and incorporation of [³H]-thymidine was determined by liquid scintillation beta counter (LKB, NY). Thymidine incorporation was measured by standard liquid scintillation counting, and results were expressed as counts per minute (mean ± SD of triplicate values) [26]. Control wells contained only T cells, DCs, or T cells stimulated with 1% phytohemagglutinin (Invitrogen, Gibco, UK).

Cytokine production assays

A major trigger for cytokine secretion especially IL-12 in vivo is based on the T cell CD40L interaction with CD40 on DCs. The soluble CD40L molecule closely mimics the signals delivered to DC by CD40L expressed on the CD4+ T cells [26, 38].

To investigate the effect of CVID and controls sera on cytokine production by MDCs, these cells were stimulated with 1 µg/ml CD40L (Alexis, Lausen, Switzerland) in 24-well plates (Greiner Bio-one, CELL STAR, Frickenhausen, Germany) for 24 hrs and cell free supernatant were collected and used for cytokine assays (IL-10, IL-12 and IL-18) using Sandwich ELISA kits obtained from BMS (Bender MedSystems GmbH, Vienna, Austria).

Cytokine production by MDC-stimulated T cells was measured in supernatants collected 48 hr after co-culturing of MDCs and allogeneic PBMCs. Cytokine co-cetration (IFN-γ and IL-4) were measured in the supernatants using Sandwich ELISA kits obtained from BMS (Bender MedSystems GmbH, Vienna, Austria).

Statistical analysis

One-Sample Kolmogorov-Smirnov Test was performed in order to test normal distribution of quantitative variables in subgroups of qualitative variables. Parametric test were used for variables with normal distribution. Statistical analysis was performed by unpaired t or one-way ANOVA tests with Welch’s or Tukey corrections, respectively using GraphPad-Instat™ software. p values less than 0.05 were considered significant. Results were expressed as mean ± standard deviation (SD).