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Research paper

Evaluation of class switch recombination in B lymphocytes of patients with common variable immunodeficiency

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ABSTRACT

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by hypogammaglobulinemia and recurrent bacterial infections. Impaired antibody production in these patients is due to defect in B-cell differentiation into specific plasma cells. Class switch recombination (CSR), which plays a critical role in the production of different immunoglobulin isotypes, may be defective in a group of CVID patients. The aim of this study was to investigate the CSR process in B cells of CVID patients, by evaluating the expression of IgE mRNA and production of its protein in an IgE inductive medium. Peripheral blood mononuclear cells (PBMCs) from 29 CVID patients and 21 healthy controls were isolated and cultured in the presence of rhIL-4 and CD40L. IgE mRNA and IgE protein were measured by RT-PCR and ELISA techniques, respectively. Normal production of IgE mRNA was recorded in 23 out of 29 patients (79.31%) as well as all controls; while the remaining 6 patients (20.69%) were unable to express IgE mRNA indicating a defect in CSR. PBMCs of 16 out of 29 patients (55.2%) could not produce normal amounts of IgE compared with controls, after being stimulated by IL-4 and CD40L. Post B cell stimulation IgE production was impaired in about half of studied CVID patients. Defects in processes occur following the CSR process such as IgE mRNA transcription, protein production, and secretion can be the causative mechanism of CVID in patients with normal mRNA expression of the immunoglobulin but impaired protein production. Determination of these defects can help to clarify the various underlying mechanisms responsible for the development of CVID.

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1. Introduction

Common variable immune deficiency (CVID) is a heterogeneous disorder characterized by impaired production of specific antibodies against special foreign antigens. Hypogammaglobulinemia, including reduced levels of Immunoglobulin (Ig) G, A and/or M, predisposes patients with CVID to recurrent infections (Aghamohammadi et al., 2005; Cunningham-Rundles and Bodian, 1999). Divergent clinical findings and immune dysregulation in CVID patients can reflect the heterogeneity of the mechanisms leading to this disorder (Di Renzo et al., 2004). Defective antibody production in CVID is result of a failure in differentiation of B cells into immunoglobulin-secreting cells (Nonoyama et al., 1993).

Class switch recombination (CSR) has a critical role in late stages of Ig production. It occurs in mature B cells after antigen engagement and requires co-stimulatory signals from CD4+ T cells such as CD40 ligand (CD40L) and various cytokines like IL-4 and IL-10 to produce different isotypes of antibody

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(Kracker and Durandy, 2011). CSR deficiencies lead to normal or increased IgM production, and reduced or absent Ig isotype switching to produce Ig G, Ig A, or Ig E (Durandy et al., 2007).

There are some reported defects in the function of B cells in CVID patients. They include incompetence in early and late B cell differentiation, impaired expression of CD27, CD86, and CD70, impaired antibody affinity maturation and somatic hyper-mutation, impaired signaling, and deficiency of memory B cells (Bayry et al., 2005; Bonhomme et al., 2000; Levy et al., 1998). So far various defects in the following genes such as *BAFF-R*, *CD19*, *TACI*, *ICOS*, and *MSH5* have been reported (Castigli et al., 2005; Salzer et al., 2005; Sekine et al., 2007; van Zelm et al., 2006). These known genetic defects involve only about 25% of CVID patients, while other mechanisms involved in the etiology in this disorder are not clear. Therefore a clarified causing mechanism is yet to be determined (Blanco-Quiros et al., 2006).

There are some investigations on the CSR process and Ig production in CVID patients after stimulation and activation by various required cytokines such as IL-2, IL-4, and IL-10 in combination with anti-CD40 (Eisenstein et al., 1994; Nonoyama et al., 1993; Pastorelli et al., 1990). However, different results on B cell response to stimulation, subsequent differentiation, and Ig production were reported. Combination of CD40-L and IL-4 directly stimulates the proliferation of B cells and IgE isotype switching (Shapira et al., 1992). Therefore this method of stimulation can exclusively evaluate the CSR function of B cells in CVID patients. The aim of this study is to investigate the CSR process in B cells of CVID patients by evaluating the production of IgE mRNA and its protein to improve our understanding of defective molecular and cellular mechanisms responsible for the etiology of CVID.

2. Materials and methods

2.1. Patients

Twenty-nine CVID patients (24 male and 5 female) and a control group of 21 age and sex matched healthy individuals were enrolled in this study. On some occasions, two patients were matched with one individual in the control group. The diagnosis of CVID was made in all of the patients by using standard criteria of the European Society for Immune Deficiency (ESID) and Pan American Group for Immunodeficiency (PAGID) (Chapel, 1994). According to these criteria, the diagnosis of CVID was confirmed when patient's age was higher than 4 years and serum levels of IgG and at least one of serum IgA or IgM were lower than 2 standard deviations from normal mean range for age and sex matched individuals. All patients also had evidence of defect in T-cell independent immune response (as measured by lacking isohemagglutinins) and/or defect of T-cell dependent immune response (as measured by lacking response to vaccination). The other causes of hypogammaglobulinemia also were excluded in these patients. All patients received monthly intravenous immunoglobulin (IVIG) and blood samples were prepared before taking their IVIG. None of the patients had active infection at the time of study. The process of this study was approved by the ethic committee of the Tehran University of Medical Sciences and informed consent was taken from all subjects.

2.2. Blood cell culture

A 5-ml blood sample was taken from each patient or healthy donor. Peripheral blood mononuclear cells (PBMCs) were isolated from the samples using Ficoll–Paque density gradient centrifugation. Separated PBMCs were then washed twice with Phosphate-Buffered Saline (PBS). Isolated and washed PBMCs (1×10^5 cells) were cultured in Iscove's Modified Dulbecco's Medium (IMDM) (Sigma Aldrich, Germany) containing 10% fetal bovine serum (FBS), 1% sodium pyruvate and 1% non-essential amino acids (all prepared from Invitrogen, Germany) at 37 °C and 5% CO_2.

To create the suitable environment for class switching of B lymphocyte to IgE, 400 ng/ml rh-IL4 and 200 IU/ml rh-CD40L (both prepared from Invitrogen, Germany) were added to culture mediums. Moreover, 100 U/ml penicillin and 100 μg/ml of streptomycin were also added to culture medium in order to prevent bacterial contamination (Armitage et al., 1993; Shapira et al., 1992).

After 5 and 12 days of culturing the expression of IgE mRNA and protein was determined in cultured cells and their supernatants respectively.

2.3. Evaluation of IgE mRNA expression

The mRNA of cultured PBMCs was extracted after 5 days by the use of 0.5 ml Guanidinium thiocyanate-phenol-chloroform (TRIzol, Gibco-BRL, USA) at 4 °C. The contaminated DNA was removed with DNase 1 (Gibco-BRL, USA). This temperature was reached by using crushed ice. For determination of mRNA purity the ratio of optical density (OD) at 260 nm to the OD at 280 nm was measured by BioPhotometer (Roche, Germany). This ratio was always more than 1.8. The expression of *Actin* gene mRNA was also determined as internal control.

Real time polymerase chain reaction (RT-PCR) was performed using cDNA Synthesis Kits (Fermentas, Canada). Simply, after mRNA extraction, cDNA (for *IgE* and *Actin* genes) were produced using Oligo (dT) primer and reverse transcriptase enzyme. Specific primers for each gene were designed using the information on GenBank nucleotide sequence database and their characteristics were evaluated using Oligo V6 software (Molecular Biology Insights, Inc., Cascade, CO). Oligonucleotide sequences of these primers are shown in Table 1. Finally, PCR method was performed on produced cDNAs using Master Mix Kit (Fermentas, Canada).

2.4. Evaluation of B cell proliferation

Isolated and washed PBMCs from patients and controls (1×10^6 cells) were suspended in Roswell Park Memorial Institute medium (RPMI 1640) (Gibco, Germany) supplemented with 10% fetal bovine serum (FBS), 1% sodium pyruvate and 1%

Table 1
Primer sequences for *Actin* and *IgE*.

Gene	Primer sequence
Actin	Forward: 5′-TACCACTGGCATCGTGATGGACT-3′
	Reverse: 5′-TCCTTCTGCATCCTGTCGGCAAT-3′
IgE	Forward: 5′-GACACGGCCGTGTATTACTG-3′
	Reverse: 5′ACGGAGGTGGCATTGGAGGGAATGT-3′

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