CASE REPORT

Genetic polymorphism study of regulatory B cell molecules and cellular immunity function in an adult patient with Common Variable Immunodeficiency

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Abstract

A 43 year old female patient presented for recurrent bacterial lower respiratory infections. A research for immunodeficiency status revealed total hypogammaglobulinemia, reduced IgG1, IgG2, IgG3 subclass levels, and low number of B lymphocytes (CD19+). Common Variable Immunodeficiency (CVID) 11.2 category was diagnosed according to recent criteria of primary immunodeficiencies (PID). Further immunological study consisting of genetic polymorphism of genes relating to differentiation, activation and function of B cells (ICOS, BAFF receptor BCMA and TACI) was performed, which did not reveal any related mutations. T cell parameters and Th1/Th2 cytokine network did not show any disturbances. It is postulated that probable endstage B cell differentiation defects should be investigated. The patient receives IVIGs replacement thereafter and the rate and severity of infections have significantly improved. Hippokratia 2008; 12 (3): 188-190

Key words: common variable immunodeficiency, gene polymorphism

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Primary immunodeficiencies (PID) are inherited disorders of the immune system function that predispose affected individuals to increased rate and severity of infection, autoimmunity and malignancy. More than 120 related distinct genetic disorders have been identified to date, while prevalence is estimated to range between 1:500 to 1:10000 live births. In the majority of cases, PID are manifested in the first years of life, even though some forms may present in adults. The introduction of proteomic and genomic analysis in basic immunology, led to better understanding of the pathophysiology and more accurate diagnosis of these disorders. This allowed a more functional re-classification of PID, as new genes have emerged and clarified immune cellular interactions. Among PID, common variable immunodeficiency (CVID) is prevalent in approximately 1 in 25000 Caucasians and is the second most frequent PID after selective IgA deficiency. In this report, the diagnostic approach to an adult patient with CVID is presented.

Case Presentation

A 43-year old female patient was referred to the Clinical Immunology Unit of the 2nd Department of Internal Medicine, for the investigation of long term recurrent bacterial infections of the lower respiratory tract. Infections had led to bronchiectasis of the lower lung lobes and were hardly responsive to usual antibiotic regimens. During the last year, frequency and severity of these infections were increased, and the patient manifested splenomegaly and peripheral lemphadenopathy. Patient history challenged a research for a causative factor related to immune deficiency states. Causes of secondary immunodeficiency were carefully excluded.

In parallel, the quantitative evaluation of serum immunoglobulins showed total hypo-gammaglobulinemia with reduced levels of IgG in the serum and undetectable levels of IgM and IgA. Analysis of IgG subtypes revealed significantly low levels of IgG1, IgG2 and IgG3, while IgG4 levels were normal (Table 1). Peripheral blood im-

Table 1: Immunoglobulin classes and subclass levels on peripheral blood of the patient before treatment (concentrations in g/l)

<table>
<thead>
<tr>
<th>Ig’s</th>
<th>Measured Values (g/l)</th>
<th>Normal Values (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>2.97</td>
<td>7-16</td>
</tr>
<tr>
<td>IgM</td>
<td>Nd</td>
<td>0.6-3.5</td>
</tr>
<tr>
<td>IgA</td>
<td>Nd</td>
<td>0.7-3.1</td>
</tr>
<tr>
<td>IgG1</td>
<td>1.53</td>
<td>Low</td>
</tr>
<tr>
<td>IgG2</td>
<td>1.06</td>
<td>Low</td>
</tr>
<tr>
<td>IgG3</td>
<td>0.0363</td>
<td>Low</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.0762</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Nd: non detectable.
munophenotype revealed reduced percentage of helper T cells, increased percentage of cytotoxic T cells (reverse CD4+/CD8+ ratio) and an increased HLA-DR expression on T lymphocytes. Additionally, B lymphocytes (CD19+) were particularly low (Table 2).

These findings allowed the classification of the disease as Common Variable Immunodeficiency. The case was classified in II.2 category, according to the new classification of PID. CVID diagnosis was based on the basic immunologic profile (total hypo-gammaglobulinemia, low B-cell numbers), according to this classification.

Lymphocyte function was evaluated with peripheral blood cultures using phytohemagglutinin (PHA) for T cells and pokeweed (PKW) for B cells.

Cytokine levels (Th1 and Th2 type) were evaluated in culture supernatants. As displayed in Table 1, all cytokines studied (IFN-γ, IL-2, IL-10, IL-4, IL-5, IL-1 and TNFα) were detected in PKW culture supernatants; IFN-γ, IL-10, IL-4, IL-5, IL-1 and TNFα concentrations varied in a dose-dependent manner (Table 3). All cytokine studied were detected in PHA cultures as well.

New genes of the immune response have been described, which are related to the differentiation and activation of B cells, as well as to the ability of isotype switching. Mutations referring to the genes coding for inducible co-stimulatory molecule (ICOS), B-cell activating factor receptor (BAFF receptor), B-cell maturation antigen (BCMA), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), as well as other genes involved in the cross-talk of B and T lymphocytes have been discovered.

According to this rational, genomic analysis of the above mentioned genes and possible mutations concerning differentiation, activation and function of B lymphocytes was performed. The analysis was conducted with polymerase chain reaction methodology (PCR) in which the studied gene is overexpressed. The product of this reaction is analyzed for its primary structure (sequencing) where new single nucleotide polymorphisms (SNPs) and mutations are detected.

Genetic analysis (sequencing) of the BAFF-R, BCMA, ICOS and TACI genes did not reveal any related mutation.

The patient was administered polyclonal human immunoglobulin (replacement therapy) at 21 day intervals with immediate improvement on the frequency and severity of respiratory infections.

**Discussion**

Increased frequency and severity of various infections are usually the cause for initiating a diagnostic workup for PID, even in adults. It seems that 41% of CVID patients are diagnosed after the age of 18.

In this patient, CVID diagnosis was based on the basic immunologic profile (total hypo-gammaglobulinemia, low B-cell numbers), according to recent classification. This gave the opportunity to further investigate genetic disorders regarding B cell differentiation, activation and isotype switching (B-T cell interaction genes).

Genes of this cascade that so far have been described are:

**Inducible co-stimulator (ICOS)**, expressed in the membrane of activated T cells cross-reacting with its receptor (ICOS receptor) on the surface of B cells, leading to the secretion of IL-4, IL-5, IL-6 and IL-10. Lack of ICOS results in B lymphopenia, particularly decreased CD27+ memory B lymphocytes, total hypo-gammaglobulinemia and defective germinal center formation. Clinical symptoms are not apparent until the end of childhood or in the adult life; Nine cases of mutated ICOS have been described so far.

**TACI** interacts with **BAFF** and **APRIL** and is a proliferation inducing ligand. It promotes isotype switching in naive B cells in a T-cell independent manner. BAFF and APRIL are mainly expressed on dendritic cells; The most frequent identified mutation is C104R, although marked heterogeneity was observed regarding clinical manifestations in patients.

Other genes encoding for co-stimulatory molecules are believed to play a major role in CVID pathogenesis.

**Table 3**: Th-1 and Th-2 cytokines in peripheral blood culture supernatants using PHA, PKW 1μg and PKW 2μg (concentrations in pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ</th>
<th>IL-2</th>
<th>IL-10</th>
<th>IL-8</th>
<th>IL-6</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>IL-12p70</th>
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<tbody>
<tr>
<td>PHA</td>
<td>1960</td>
<td>4138</td>
<td>2923</td>
<td>788</td>
<td>13781</td>
<td>30</td>
<td>75</td>
<td>1279</td>
<td>1932</td>
<td>65</td>
</tr>
<tr>
<td>PKW 1μg</td>
<td>2222</td>
<td>116</td>
<td>22</td>
<td>4403</td>
<td>6633</td>
<td>68</td>
<td>28</td>
<td>129</td>
<td>198</td>
<td>40</td>
</tr>
<tr>
<td>PKW 2μg</td>
<td>3483</td>
<td>90</td>
<td>65</td>
<td>3084</td>
<td>12520</td>
<td>85</td>
<td>61</td>
<td>249</td>
<td>212</td>
<td>66</td>
</tr>
</tbody>
</table>
did not reveal any mutations, leading to further research regarding B cell maturation and/or antibody producing capability\(^6\). The findings of peripheral blood immunophenotype are consistent with CVID\(^6\)\(^\text{-}^\text{10}\). These patients tend to have low numbers of circulating CD4\(^+\) T cells, thus resulting in a reversed CD4\(^+\)/CD8\(^+\) ratio\(^11\). The expansion of CD8\(^+\) T cells, in association with high expression of activation markers (HLA-DR\(^+\)) may reflect the “effort” of the immune system, to overcome the defects of humoral immunity. Additionally, CD8\(^+\) T cell expansion may represent the result of the chronic immune stimulation by recurrent infections.

Furthermore, the cytokine network responsible for T cell – B cell interaction was found to be intact, and adequately responsive to mitogens as indicated on the cell cultures. B-cells, found to be relatively low, (Figure 1), correlated to low levels of serum immunoglobulins and became the target of further study of genetic polymorphisms described in B-cell activation, maturation and omeostasis. Since, no mutations on the so far co-involved genes were found, it is suggested, that the defect in this patient lies at the end stages of B cell differentiation, where intense antigenic pressure leads through somatic mutation to the isotype switch and the production of high affinity antibodies.

Finally, it should be stressed that gene mutations in CVID, coding for costimulatory molecules on one hand and memory B cells (CD27\(^+\)) lineage on the other hand, could comprise a cornerstone of disease pathophysiology although up to date, only 10-15% of CVID is attributed to gene mutation. Further understanding of the physiology of immune system will lead to a better understanding of cytoplasmic and cell membrane function concerning primary immunodeficiency diseases. This would facilitate diagnosis and management of CVID patients.

References