Cytokine Production by Activated T Cells in Common Variable Immunodeficiency

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Abstract

Background: Common variable immunodeficiency (CVID) is the most common symptomatic antibody deficiency. It is characterized by hypogammaglobulinemia, increased susceptibility to recurrent infections, autoimmunity, and malignancies.

Objectives: To determine whether patients with CVID have cytokine production defects after T-cell activation and to assess whether or not these are correlated with markers of severe disease.

Methods: Twenty-seven patients with CVID and 17 healthy volunteers were investigated. Peripheral blood mononuclear cells were cultured under standard conditions in the presence and absence of phytohemagglutinin. Subsequent cell proliferation and cytokine release were measured and compared between stimulated and unstimulated cells.

Results: A general enhancement in cytokine production was observed in both CVID patients and controls after stimulation. However, we detected a lower production of interferon-γ in CVID patients than in controls (p=0.026). A production defect for at least 1 cytokine was observed in 12 patients. Ten of these failed to generate protective titers in response to the polysaccharide vaccine, and the frequency of bronchiectasis in this group of patients was 91.7%. Cytokine release correlated strongly with cell proliferation.

Conclusions: This study indicates that some CVID patients have T-cell proliferation and secretory defects and that these may be associated with severe manifestations of disease. Screening for such defects could permit more effective monitoring and therapeutic strategies for CVID patients.

Keywords: Common variable immunodeficiency. Cytokine. Proliferation. T cell.
Introduction

Common variable immunodeficiency (CVID) is a heterogeneous group of primary immunodeficiency disorders, and is the most common symptomatic form of antibody deficiency. Patients with this condition usually develop recurrent bacterial infections, autoimmune disorders, and neoplastic diseases [1-6].

The underlying cause of CVID is unknown in most cases [7,8]. Diagnosis is based on hypogammaglobulinemia in the absence of recognized genetic abnormalities [8]. Discrete B cell–differentiation defects [7] and defective immunoglobulin (Ig) M memory B cells [9,10] have been demonstrated in some CVID patients. The presence of such defects has been used in the sub-classification of the disorder [11]. Antibody response to polysaccharide vaccines has also been posited as a method of clinical classification [12,13]. Dendritic cell abnormalities also occur in some patients with CVID [14-17], and T cell function defects [7,14,18,19] add further complexity. Increased T-cell activation, accelerated T-cell turnover and apoptosis, altered cytokine production, and reduced generation of antigen-specific memory T cells have all been reported in CVID [18,20-23].

This study was performed to evaluate T-cell proliferation and cytokine production in a cohort of Iranian patients with CVID.

Patients and Methods

Patients

Twenty-seven patients with CVID, each under regular follow-up and immunoglobulin replacement therapy, were enrolled. Diagnosis of CVID was based on international criteria [8,24], including a reduction in at least 2 serum Ig levels (IgG, IgA, or IgM) and genetic exclusion of other antibody deficiencies associated with well-defined single-gene defects. BTK mutation analysis, for example, was performed in patients with B-cell populations of less than 2% of the total lymphocyte count to exclude Btk deficiency [25]. Patients under 2 years of age were excluded from this study because of a possible diagnosis of transient hypogammaglobulinemia.

Seventeen sex- and age-matched healthy individuals recruited from the families of health-care workers at the hospital were used as controls. In some instances, 2 or 3 patients were matched to 1 sex- and age-matched control.

The study was approved by the ethics committee of the Tehran University of Medical Sciences, and written informed consent was obtained from all participants before sampling.

Cell Culture

Five mL of blood anticoagulated with heparin was drawn from the participants. Blood samples from CVID patients were taken at least 4 weeks after intravenous immunoglobulin therapy, just prior to the next scheduled intravenous immunoglobulin substitution. The fraction of peripheral blood mononuclear cells (PBMCs) was isolated by Ficoll–Hypaque density gradient centrifugation and washed twice with phosphate-buffered saline. PBMCs were cultured at a final concentration of 1×10^6 cells per mL in RPMI 1640 culture medium (Gibco, Invitrogen, Paisley, Scotland, UK) containing 10% heat-inactivated fetal bovine serum (Gibco, Invitrogen, Paisley, Scotland, UK) under standard conditions (37°C, 5% carbon dioxide) in the presence and absence of 10 µg/mL phytohemagglutinin (PHA) (Sigma, St Louis, Missouri, USA) for 3 days. After incubation, 10 µL of 5-bromo-2’-deoxyuridine (Brdu; Roche Diagnostics GmbH, Mannheim, Germany) was added to each well, and centrifuged after 18 hours’ incubation. The proliferation of cells was read at an optical density (OD) of 450 nm.

Cytokine Assay

The cytokines interleukin (IL) 2, IL-4, IL-5, IL-10, and interferon (IFN) -γ were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, UK). The absorbance of each well was read at 492 nm. Cytokine concentrations of the samples were calculated using a standard curve generated from recombinant cytokines and expressed as pg/mL.

Statistical Analysis

Data were analyzed using the SPSS statistical software package (version 15.0). Low production of a given cytokine was defined as a post-stimulation cytokine value of greater than 2 SDs below the mean level for the same cytokine in the control group. For proliferation, the stimulation index (SI) was calculated as the ratio of OD of cells stimulated with PHA to that of the OD of unstimulated cells. The SI for each patient was compared to that of the corresponding control in each experiment. A receiver operating characteristic (ROC) curve was used to identify the level of SI most sensitively and specifically associated with bronchiectasis. This cutoff was then used as a surrogate marker of severe symptomatic CVID and also used arbitrarily to identify poor-responding patients in the proliferation assay. Poor responses to polysaccharide vaccines and cytokine defects were also tested to identify the optimal cutoff. A P value of less than .05 was considered significant. Bonferroni corrections were used in multiple analyses. Cytokine production by stimulated and unstimulated cells was also compared in patient and control groups. Data that were not normally distributed were presented as medians and interquartile range (IQR) and results were compared using nonparametric tests. The Wilcoxon signed ranks test was used to compare cytokine results between stimulated and unstimulated cells. The Mann-Whitney U test was used to compare results between the patient and control groups.

Results

Characteristics of CVID Patients

Twenty-seven patients with CVID (19 male and 8 female) were investigated. The mean (SD) age of patients at the time of study was 19.04 (14.29) years (median age, 15 years; IQR,
were 821.41 (494.66) cells/mm³ and 919.90 (418.18) cells/mm³, respectively. IgA levels were 10 mg/dL (IQR, 0-140 mg/dL) and 5 mg/dL (IQR, 0-67 mg/dL), respectively. Subpopulations, and Antibody Responses

Serum Immunoglobulins, Lymphocyte Subpopulations, and Antibody Responses

Serum IgG, IgM, and IgA levels of all the patients enrolled in the study were more than 2 SDs below the normal mean values for age. The mean serum IgG level was 100 mg/dL (IQR, 0-770 mg/dL). The median serum IgM and IgA levels were 10 mg/dL (IQR, 0-140 mg/dL) and 5 mg/dL (IQR, 0-67 mg/dL), respectively.

The mean (SD) absolute number of CD19⁺ B cells was 310.75 (289.45) cells/mm³ and the mean absolute number of CD3⁺ T cells was 1815.37 (737.58) cells/mm³. The corresponding figures for CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells were 821.41 (494.66) cells/mm³ and 919.90 (418.18) cells/mm³, respectively. While the CD4/CD8 ratio was more than 1 in almost half of the patients (n=14), T cell–subset analysis showed a reversed CD4/CD8 ratio in the remaining 13 patients.

We have previously reported normal vaccination response in a group of CVID patients after immunization with polysaccharide vaccine [12]. A more recent analysis of the same group revealed that some of the patients failed to maintain protective titers in the long term [26]. Of the patients analyzed in the current study, 15 were nonresponders to polysaccharide vaccine in both the short term and long term after vaccination.

Cytokine Production

There were no significant differences in cytokine production by unstimulated cells between patients and controls. Following stimulation, however, we observed a significant increase in all cytokine levels in both groups (P<.001) (Table 2). Nonetheless, in CVID patients, there was a significant trend towards a lower production of IFN-γ by stimulated cells (Figure 1).

A production defect in at least 1 cytokine was detected in 12 patients (44.4%) after PHA stimulation (Table 1). (Seven patients, for example, had low IL-2 production after stimulation). In contrast, no production defects were detected in any of the controls (P=.001).

Proliferative Response to Mitogens

Overall, there was no significant difference in SI between CVID patients and controls. However, in vitro stimulation of cells with PHA resulted in a heterogeneous response profile amongst patients. While 20 patients had an SI that matched or even exceeded that of the control, 7 patients exhibited poor response, with an SI of lower than the cutoff of 2.98, and 3 of these had an SI of less than 0.5 times that of the matched control (Table 1). The cutoff of 2.98 was identified using a ROC curve based on bronchiectasis and associated with a sensitivity of 91.7% and a specificity of 50%. A ROC curve based on poor antibody responses to polysaccharide vaccines and cytokine defects produced the same cutoff with a sensitivity of over 90%.

The OD of cells after PHA stimulation in poor responders to mitogen was significantly lower than that of other CVID patients (P=.029) and controls (P=.009) (Figure 2).

Clinical Implications

Of the 12 patients with cytokine production defects, only 3 had multiple defects and they all had an SI of less than 0.5 times that of the matched controls (Table 1). These 3 patients were also nonresponders to the vaccine; 7 of the 9 patients with a single cytokine defect were also nonresponders. There were significantly more nonresponders in the cytokine defect group than in the rest of CVID patients (P=.027).

The mean SI of patients with cytokine defects was 3.27 (1.08), which was significantly lower than that of the other CVID patients (7.70 [3.92]), P=.001) and controls (6.50 [2.75], P=.016). Indeed 10 (83.3%) of the 12 patients with cytokine defects developed bronchiectasis, compared to just 3 (20%) of the 15 without these defects (P=.0039). A reversed CD4/CD8 ratio was also detected in 9 patients (75%) with cytokine defects. This reversal was detected in a significantly lower proportion of CVID patients without these defects (4 of 15 patients, 26.7%; P=.034).

Discussion

CVID is a heterogeneous group of disorders with a wide variety of clinical and immunological manifestations [1,2,6,8,27]. Although there have been several attempts to sub-classify CVID according to T-cell abnormalities in the past 3 decades, the results have been controversial [7].

It is estimated that 10% of patients with CVID have very low lymphocyte proliferation in vitro after stimulation with PHA [7]. According to other studies, however, subnormal lymphocyte proliferation responses (to 1 or more mitogens) could be as high as 40% [1]. In the present study, 26% of the patients were poor responders. Of these, only 3 (11%) had very low lymphocyte proliferation (SI of less than 0.5 of that of matched controls). Nevertheless, it should be noted that we only evaluated proliferation responses to PHA; other mitogens such as concanavalin A or pokeweed mitogen were not tested.

While patients with a T-cell proliferation defect have a reversed CD4/CD8 ratio (due to either a decreased number of CD4⁺T cells or an increased number of CD8⁺T cells), the poor proliferation response might be due to altered subpopulations rather than a fundamental T-cell defect or defects [7,28,29].

Cytokine production by T cells in CVID patients has
Figure 1. Comparison of cytokine production in unstimulated cells and cells stimulated with phytohemagglutinin (PHA) between patients with common variable immunodeficiency (CVID) and controls.
Table 1. Proliferative Response to Phytohemagglutinin in CVID Patients (n=27) Compared to Sex- and Age-Matched Controls

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
<th>Cytokine Production Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD of Unstimulated Cells</td>
<td>OD of Stimulated Cells</td>
<td>Stimulation Index</td>
</tr>
<tr>
<td>0.083</td>
<td>0.531</td>
<td>6.40</td>
</tr>
<tr>
<td>0.069</td>
<td>0.463</td>
<td>6.71</td>
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<tr>
<td>0.120</td>
<td>0.570</td>
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<td>0.100</td>
<td>0.508</td>
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<tr>
<td>0.114</td>
<td>0.381</td>
<td>3.34</td>
</tr>
<tr>
<td>0.154</td>
<td>0.489</td>
<td>3.18</td>
</tr>
<tr>
<td>0.152</td>
<td>0.424</td>
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<td>0.490</td>
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<td>0.411</td>
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<tr>
<td>0.142</td>
<td>0.472</td>
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<tr>
<td>0.193</td>
<td>0.437</td>
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</tr>
<tr>
<td>0.085</td>
<td>0.442</td>
<td>5.20</td>
</tr>
<tr>
<td>0.113</td>
<td>0.473</td>
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<td>10.52</td>
</tr>
<tr>
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</tr>
<tr>
<td>0.095</td>
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<tr>
<td>0.099</td>
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<td>0.145</td>
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<tr>
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</tr>
<tr>
<td>0.058</td>
<td>0.823</td>
<td>14.19</td>
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</table>

Abbreviations: CVID, common variable immunodeficiency; IFN, interferon; IL, interleukin; OD, optical density.

*Calculated as the mean ratio of OD of cells stimulated with phytohemagglutinin to that of unstimulated cells.

Less than cutoff of 2.98.

Less than 0.5 times the stimulated index of matched control.

been evaluated in a number of studies, but it is still a subject of active debate [22,30-34]. Our preliminary report on serum cytokine levels revealed that type 2 helper (Th2) T cell cytokine levels (IL-4 and IL-10) were significantly increased in CVID patients, but no significant differences were found for Th1 serum cytokine levels (IL-2 and IFN-γ) [22]. On the basis of these findings and the increased serum levels of soluble CD30 in patients with CVID [21], a bias toward a Th2 response in such patients has been suggested [21,22]. However, there is some evidence supporting dominance of Th1 response in this disease [14,35,36].

While the production of some cytokines might be affected by certain genetic polymorphisms [37-39], a comparison of cytokine levels between unstimulated cells and stimulated cells shows the ability of T cells to produce cytokines.

In the current study, we detected a significant trend towards a lower production of IFN-γ in CVID patients. Evaluation of cytokine production in individual patients revealed at least 1 defect in 12 patients. A significantly lower level of IL-2 production was also detected in this group. Although insignificantly higher production of IL-2 and IFN-γ has been reported following the stimulation of T cells with anti-CD3 [31], there have also been reports of impaired IL-2 and IFN-γ gene expression and IL-2 release after antigenic stimulation [40,41]. The reason for low IL-2 production in CVID is still unclear. While a defect in T-cell activation by the T-cell receptor has been hypothesized [40], a reversed CD4/CD8 ratio could be another reason [7]. In the present study, an altered circulating subpopulation of T cells was significantly more common in patients with cytokine defects. However, it is not clear whether this phenomenon was a fundamental T-cell defect or the result of chronic infection [7,12].
A proliferative response to mitogens and cytokine evaluation could have clinical benefits. Poor T-cell responses to PHA have been previously associated with mortality in CVID [1]. In the present study, a number of patients with cytokine defects were poor responders to PHA, nonresponders to the polysaccharide vaccine, and significantly prone to bronchiectasis complications. While development of bronchiectasis in this group may reflect the severity of the immune defect, these patients need more effective monitoring and therapeutic strategies [12]. Further studies on B-cell and T-cell interactions in CVID patients should be performed to gain greater insight into the pathophysiology of the disease.

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References


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