T-Helper 1 and 2 Cytokine Assay in Patients With Common Variable Immunodeficiency

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Abstract

Background: Common variable immunodeficiency (CVID) is a heterogeneous group of disorders, characterized by decreased immunoglobulin levels in serum and increased susceptibility to recurrent infections, autoimmunity, and malignancy. The pathogenesis of CVID is still unknown.

Objectives: This study was performed to investigate T-helper (Th) 1 and 2 cytokine levels in patients with CVID.

Material and Methods: Twenty-four cases of CVID were studied. Cytokine levels of interleukin (IL)-2, IL-4, IL-10, and interferon gamma were measured in the serum of the patients and compared with those of healthy controls.

Results: Th2 cytokine levels (IL-4 and IL-10) were significantly higher in the patient group than in the controls (median: 64.5 vs 0.0 pg/mL, \(P = 0.016\) for IL-4 and 321.1 vs 0.0 pg/mL, \(P = 0.001\) for IL-10). However, there were no significant differences in Th1 cytokines between the 2 groups (median, 116.5 vs 104.5 pg/mL, \(P = 0.22\) for IL-2 and 50.5 vs 42.3 pg/mL, \(P = 0.32\) for IFN-\(\gamma\)).

Conclusions: Increased levels of IL-4 and IL-10 could indicate high activation of Th2 lymphocytes in this group of patients and consequently supports the concept of a bias towards Th2-type responses.

Key words: Common variable immunodeficiency, Cytokine, Interleukin 4, Interleukin 10.

Introduction

Common variable immunodeficiency (CVID) is an antibody deficiency disease characterized by increased susceptibility to recurrent pyogenic infections, autoimmune diseases, and cancer. It is the most common symptomatic antibody deficiency disease, and is defined by decreased levels in serum of at least 2 immunoglobulins (Ig) [1-5].

The term CVID covers a heterogeneous group of disorders whose pathogenesis is not fully understood [6,7]. However, several general defects leading to alteration of immunoglobulin concentrations in the blood have been identified. Patients with CVID have a B-cell differentiation defect leading to impaired secretion of immunoglobulin. Some CVID patients cannot produce specific antibodies to protein or polysaccharide antigens, while others can [8,9]. It has also been shown that...
some patients with CVID have low populations of IgM memory B cells [10-13]. Additionally, several T-cell abnormalities have been reported [5,6,14], and include accelerated T-cell apoptosis [15] and reduced generation of antigen-specific memory T cells [16]. Abnormalities in the innate immune system including dendritic cells have also been reported in some patients with CVID [17-19].

Cytokines play an essential role in antibody synthesis. The patterns of cytokine production by subsets of CD4+ T cells are characteristic for T-helper 1 (Th1) and T-helper 2 (Th2) cells. Th1 cells, which synthesize cytokines such as IL-2 and interferon (IFN)-γ, have a role in macrophage activation, whereas Th2 cells, which produce cytokines such as IL-4 and IL-10, have a role in the regulation of the humoral immune response [20]. Although cytokine assay by CVID T cells has been performed in several studies [21-25], the results are contradictory. The present study was performed to investigate Th1 and Th2 serum cytokine levels in CVID patients. As little is known about the pathophysiology of this condition, our results could contribute to current knowledge of the underlying defects in CVID.

Materials and Methods

Participants

The study population was composed of 24 patients with CVID who were referred to the Division of Allergy and Clinical Immunology of the Children’s Medical Center Hospital, and 20 age- and sex-matched controls recruited from the medical personnel of this center and their families. CVID was diagnosed according to international criteria [7,26], including reduction of at least 2 serum immunoglobulin levels (serum IgG, IgA, and IgM) by 2 SD from the normal mean values for age, and genetic exclusion of other antibody deficiencies associated with well-defined single gene defects. Patients under 2 years of age were excluded from the study because of possible transient hypogammaglobulinemia.

Cytokine Assay

Once informed consent was obtained, blood samples were collected from the patients (and controls) 3 to 4 weeks after intravenous immunoglobulin therapy, and before the next scheduled infusion of immunoglobulin. Serum was separated and the cytokine assay for IL-2, IL-4, IL-10, and IFN-γ was performed using the enzyme-linked immunosorbent assay (ELISA) with commercial assay kits (R&D Systems, Inc. UK). The absorbance of each well was read at 492 nm. Cytokine concentrations in the samples were calculated with a standard curve generated from recombinant cytokines. Cytokine values were expressed as pg/mL.

Statistical Analysis

Data were analyzed using SPSS (version 15.0). Variables that were not normally distributed were presented as the median and interquartile range. The quantitative variables were correlated using the Spearman rank correlation. The medians of quantitative variables were analyzed using the Mann-Whitney U test. A P value of less than .05 was considered significant.

Results

Patient Characteristics

Twenty-four patients with CVID (17 males) and a median age of 16.2 (10.5-21.3) years, were analyzed in this study. All the patients presented recurrent infections, particularly in the respiratory tract and gastrointestinal system (83.3%). The most common manifestations were pneumonia, diarrhea, sinusitis, otitis media, eczema, conjunctivitis, septic arthritis, and superficial abscesses. In 7 patients, the course of the disease was complicated by bronchiectasis. Nine patients had splenomegaly. Autoimmune diseases and malignancies were detected in 6 and 3 cases, respectively.

Immunological Assay

All the patients had hypogammaglobulinemia with decreased serum levels in at least 2 of IgG, IgA, and IgM. The median serum levels of IgG, IgM, and IgA were 100 (50-209) mg/dL, 10 (2.5-20) mg/dL, and 5 (0-9) mg/dL, respectively. The patients’ T-cell counts were within the normal range, and a T-cell subset analysis indicated the reversal of the CD4+/CD8+ ratio in 11 cases (45.8%).

T<sub>h</sub>2 Cytokine Production

The cytokine assay revealed a trend towards higher production of T<sub>h</sub>2 cytokines (IL-4 and IL-10) in the patient group (Figure 1). Median IL-10 levels in patients and controls

![Figure 1. T-helper 2 serum cytokine levels (pg/mL) in CVID patients (n = 24). Boxes represent values between the 25th percentiles. The horizontal lines correspond to the median, minimum, and maximum.](image)

Cytokines Assay in CVID

were 321.1 (47.7-460.8) pg/mL vs 0.0 (0.0-37.5) pg/mL, respectively ($P = .001$). The median IL-4 level in the CVID patients was 64.5 (3.0-90.8) pg/mL, which was significantly higher than among the controls whose median IL-4 level was 0.0 pg/mL ($P = .016$). Statistical analysis of these data revealed a direct association between IL-4 and IL-10 levels in the patient group ($R = 0.939, R^2 = 0.882, F = 156.6, P < .001$) (Figure 2).

T\textsubscript{H}1 Cytokine Production

There were no significant differences in T\textsubscript{H}1 cytokine titers between the 2 groups (Figure 3). Median IL-2 levels in patients and controls were 116.5 (103.3-127.8) pg/mL vs 104.5 (98.5-117.5) pg/mL, respectively ($P = .22$), whereas median levels of IFN-\gamma in the patients and controls were 50.5 (45.5-56.3) pg/mL vs 42.3 (40.5-62.8) pg/mL, respectively ($P = .32$).

Discussion

CVID is a heterogeneous group of disorders, with unknown genetic defects. Different abnormalities in the number and function of immune components have been reported [1-3,27,28]. In this study, IL-2 and IFN-\gamma (T\textsubscript{H}1 cytokines) and IL-4 and IL-10 (T\textsubscript{H}2 cytokines) were evaluated in a group of CVID patients and compared with the same parameters in healthy controls.

This study revealed a trend towards higher production of IL-4 and IL-10 in the patient group. It indicated high activation of T\textsubscript{H}2 lymphocytes in this group and, consequently, supports the concept of a bias toward a T\textsubscript{H}2-type response. Recently, there have been reports of increased serum levels of soluble CD30, an indicator of T\textsubscript{H}2 cytokine production, thus indicating the predominance of T\textsubscript{H}2 in this disease [29].

During the last 20 years, the cytokine assay has been performed in several studies on patients with CVID, although results on the status of T\textsubscript{H}1 and T\textsubscript{H}2 cytokines have been contradictory.

Based on their finding of significantly increased levels of IFN-\gamma production in T-cells, North et al [30,31] suggest a T\textsubscript{H}1 response in CVID, with normal cellular levels of IL-2 production [30]. Defects in IL-2 and IFN-\gamma gene expression after T-cell antigenic stimulation had previously been reported [32,33], while the recent study by Pons et al [22] indicated that the role played by T cells in patients with CVID in the greater production of IL-2 and IFN-\gamma after stimulation with anti-CD3 than controls is insignificant. We find neither decreased nor increased secretion of IL-2 or IFN-\gamma in our patient group, and this result is similar to the results of Inoue et al [34]. This could suggest that the T\textsubscript{H}1 function is normal in affected patients [34]. In addition, formation of granuloma could be associated with a T\textsubscript{H}1 response, although this was not detected in our patients.

Our patients had increased levels of IL-4 and IL-10. Although other authors report a decreased level of IL-10 in CVID [23,25], recent studies have not confirmed this [22]. Moreover, several studies have revealed a higher production of IL-4 in CVID patients [22,24,35]. The trend towards a higher secretion of IL-4 and IL-10 could support the concept of responses that are biased towards T\textsubscript{H}2-type responses in CVID.

While the serum cytokine assay in our study revealed a predominance of T\textsubscript{H}2, T-cell proliferation and a cytokine assay after T-cell stimulation could provide valuable information that would improve our understanding of the pathophysiology of the disease. Further studies with larger groups of patients are recommended, in order to show cytokine patterns and their association with clinical disease in patients with CVID.
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