ORIGINAL ARTICLE

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 (T_H) 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: T_H2 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, $P = .016$ for IL-4 and 321.1 vs 0.0 pg/mL, $P = .001$ for IL-10). However, there were no significant differences in T_H1 cytokines between the 2 groups (median, 116.5 vs 104.5 pg/mL, $P = .22$ for IL-2 and 50.5 vs 42.3 pg/mL, $P = .32$ for IFN-γ).
Conclusions: Increased levels of IL-4 and IL-10 could indicate high activation of T_H2 lymphocytes in this group of patients and consequently supports the concept of a bias towards T_H2-type responses.

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

Resumen

Objetivos: Este estudio se realizó para investigar los niveles de citocinas Th1 y 2 en pacientes con IDCV.
Material y métodos: Se han estudiado 24 casos de IDCV. Se midieron los niveles de las citocinas interleucina (il) 2, il-4, il-10 e interferón gamma en el suero de los pacientes comparados con controles sanos.
Resultados: Los niveles de citocinas Th2 (il-4, il-10) estaban significativamente aumentados en el grupo de pacientes, comparado con los controles (media: 64.5 vs 0.0 pg/mL, $P = .016$ para IL-4 y 321.1 vs 0.0 pg/mL, $P = .001$ para IL-10). Sin embargo, no existían diferencias significativas en las citocinas Th 1 entre los dos grupos (media, 116.5 vs 104.5 pg/mL, $P = .22$ para IL-2 y 50.5 vs 42.3 pg/mL, $P = .32$ para IFN-γ).
Conclusion: Los niveles aumentados de il-4 e il-10 podrían indicar un elevado grado de activación de los linfocitos Th2 en este grupo de pacientes y consecuentemente, este hallazgo apoya el concepto de un desplazamiento hacia una respuesta Th2.

Palabras clave: Immunodeficiencia común variable. Citocina. Interleucina 4. Interleucina 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 (T_H1) and T-helper 2 (T_H2) cells. T_H1 cells, which synthesize cytokines such as IL-2 and interferon (IFN)-γ, have a role in macrophage activation, whereas T_H2 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 T_H1 and T_H2 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_H2 Cytokine Production

The cytokine assay revealed a trend towards higher production of T_H2 cytokines (IL-4 and IL-10) in the patient group (Figure 1). Median IL-10 levels in patients and controls

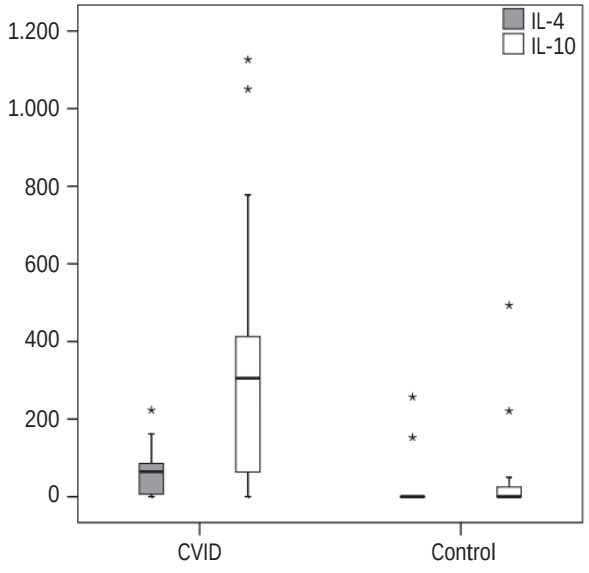

Figure 1. T-helper 2 serum cytokine levels (pg/mL) in CVID patients (n = 24). Boxes represent values between the 25th percentiles. The horizontal lines correspond to the median, minimum, and maximum.

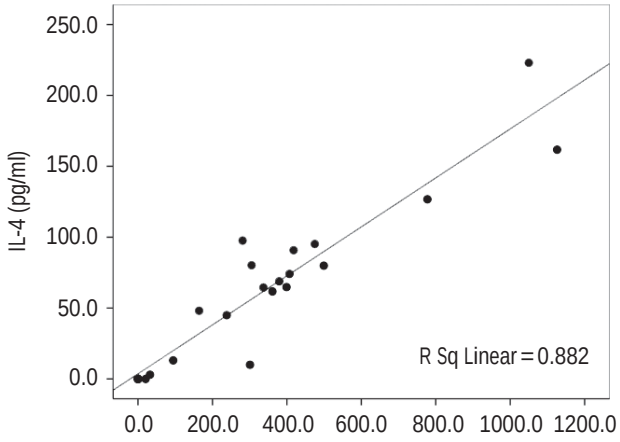

Figure 2. Association between serum levels of IL-4 and IL-10 in the patient group.

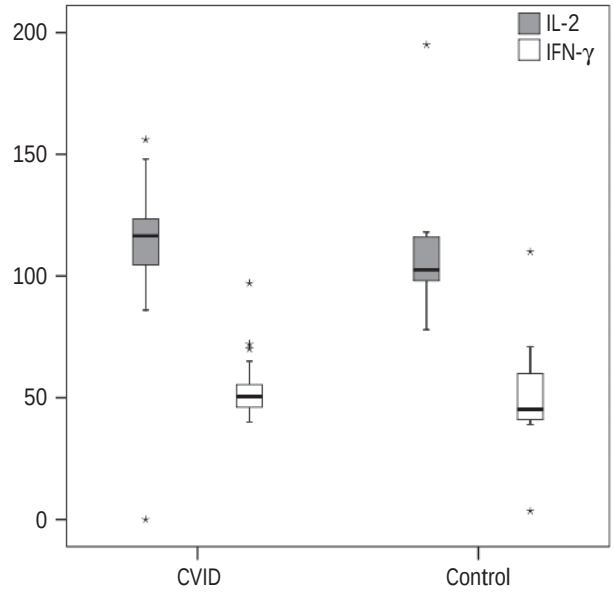

Figure 3. T-helper 1 serum cytokine levels (pg/mL) in CVID patients (n = 24). Boxes represent values between the 25th and 75th percentiles. The horizontal lines correspond to the median, minimum, and maximum.

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_H1 Cytokine Production

There were no significant differences in T_H1 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-γ 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-γ (T_H1 cytokines) and IL-4 and IL-10 (T_H2 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_H2 lymphocytes in this group and, consequently, supports the concept of a bias toward a T_H2-type response. Recently, there have been reports of increased serum levels of soluble CD30, an indicator of T_H2 cytokine production, thus indicating the predominance of T_H2 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_H1 and T_H2 cytokines have been contradictory.

Based on their finding of significantly increased levels of IFN-γ production in T-cells, North et al [30,31] suggest a T_H1 response in CVID, with normal cellular levels of IL-2 production [30]. Defects in IL-2 and IFN-γ 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-γ after stimulation with anti-CD3 than controls is insignificant. We find neither decreased nor increased secretion of IL-2 or IFN-γ in our patient group, and this result is similar to the results of Inoue et al [34]. This could suggest that the T_H1 function is normal in affected patients [34]. In addition, formation of granuloma could be associated with a T_H1 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_H2-type responses in CVID.

While the serum cytokine assay in our study revealed a predominance of T_H2, 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.

Acknowledgments

This work was supported by a grant from the Tehran University of Medical Sciences and Health Services. The authors are very grateful to all our colleagues in the Immunology, Asthma and Allergy Research Institute for their help and advice in the laboratory and to the patients and their families for their collaboration in this study.

References

1. Aghamohammadi A, Farhoudi A, Moin M, Rezaei N, Kouhi A, Pourpak Z, Yaseri N, Movahedi M, Gharagozlou M, Zandieh F, Yazadni F, Arshi S, Mohammadzadeh I, Ghazi BM, Mahmoudi M, Tahaei S, Isaeian A. Clinical and immunological features of 65 Iranian patients with common variable immunodeficiency. Clin Diagn Lab Immunol. 2005;12:825-32.
2. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92:34-48.
3. Hammarstrom L, Vorechovsky I, Webster D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). Clin Exp Immunol. 2000;120:225-31.
4. Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, Atarod L, Ghazi BM, Isaeian A, Mahmoudi M, Abolmaali K, Mansouri D, Arshi S, Tarash NJ, Sherkat R, Akbari H, Amin R, Alborzi A, Kashef S, Farid R, Mohammadzadeh I, Shabestari MS, Nabavi M, Farhoudi A. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian primary immunodeficiency registry. J Clin Immunol. 2006;26:519-32.
5. Spickett GP. Current perspectives on common variable immunodeficiency (CVID). Clin Exp Allergy. 2001;31:536-42.
6. Webster ADB. Clinical and immunological spectrum of common variable immunodeficiency (CVID). Iran J Allergy Asthma Immunol. 2004;3:103-13.
7. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, Hammarstrom L, Nonoyama S, Ochs HD, Puck JM, Roifman C, Seger R, Wedgwood J. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol. 2007;120:776-94.
8. Rezaei N, Aghamohammadi A, Siadat SD, Nejati M, Ahmadi H, Moin M, Pourpak Z, Kamali S, Norouzian D, Tabaraei B, Read RC. Serum bactericidal antibody response to serogroup C polysaccharide meningococcal vaccination in children with primary antibody deficiencies. Vaccine. 2007;25:5308-14.
9. Rezaei N, Aghamohammadi A, Siadat SD, Moin M, Pourpak Z, Nejati M, Ahmadi H, Kamali S, Norouzian D, Tabaraei B, Read RC. Serum bactericidal antibody responses to meningococcal polysaccharide vaccination as a basis for clinical classification of common variable immunodeficiency. Clin Vaccine Immunol. 2008;15:607-11.
10. Carsetti R, Rosado MM, Donnanno S, Guazzi V, Soresina A, Meini A, Plebani A, Aiuti F, Quinti I. The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. J Allergy Clin Immunol. 2005;115:412-7.
11. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, Eibel H, Schlesier M, Peter HH. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002;99:1544-51.
12. Vodjgani M, Aghamohammadi A, Samadi M, Moin M, Hadjati J, Mirahmadian M, Parvaneh N, Salavati A, Abdollahzade S, Rezaei N, Sarrafnejad A. Analysis of class-switched memory B cells in patients with common variable immunodeficiency and its clinical implications. J Investig Allergol Clin Immunol. 2007;17:321-8.
13. Ko J, Radigan L, Cunningham-Rundles C. Immune competence and switched memory B cells in common variable immunodeficiency. Clin Immunol. 2005;116:37-41.
14. Bayry J, Hermine O, Webster DA, Levy Y, Kaveri SV. Common variable immunodeficiency: the immune system in chaos. Trends Mol Med. 2005;11:370-6.
15. Di Renzo M, Zhou Z, George I, Becker K, Cunningham-Rundles C. Enhanced apoptosis of T cells in common variable immunodeficiency (CVID): role of defective CD28 co-stimulation. Clin Exp Immunol. 2000;120:503-11.
16. Isgro A, Marziali M, Mezzaroma I, Luzi G, Mazzone AM, Guazzi V, Andolfi G, Cassani B, Aiuti A, Aiuti F. Bone marrow clonogenic capability, cytokine production, and thymic output in patients with common variable immunodeficiency. J Immunol. 2005;174:5074-81.
17. Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Galicier L, Lepelletier Y, Webster D, Levy Y, Eibl MM, Oksenhendler E, Hermine O, Kaveri SV. Common variable immunodeficiency is associated with defective functions of dendritic cells. Blood. 2004;104:2441-3.
18. Scott-Taylor TH, Green MR, Eren E, Webster AD. Monocyte derived dendritic cell responses in common variable immunodeficiency. Clin Exp Immunol. 2004;138:484-90.
19. Nourizadeh M, Aghamohammadi A, Moazzeni SM, Mahdavi M, Rezaei N, Hadjati J. High production of IL-18 by dendritic cells induced by sera from patients with primary antibody deficiency. Iran J Allergy Asthma Immunol. 2007;6:59-65.
20. Rogge L. A genomic view of helper T cell subsets. Ann N Y Acad Sci. 2002;975:57-67.
21. Paganelli R, Capobianchi MR, Ensoli B, D'Offizi GP, Facchini J, Dianzani F, Aiuti F. Evidence that defective gamma interferon production in patients with primary immunodeficiencies is due to intrinsic incompetence of lymphocytes. Clin Exp Immunol. 1988;72:124-9.
22. Pons J, Ferrer JM, Martinez-Pomar N, Iglesias-Alzueta J, Matamoros N. Costimulatory molecules and cytokine production by T lymphocytes in common variable immunodeficiency disease. Scand J Immunol. 2006;63:383-9.
23. Holm AM, Aukrust P, Aandahl EM, Muller F, Tasken K, Froland SS. Impaired secretion of IL-10 by T cells from patients with common variable immunodeficiency--involvement of protein kinase A type I. J Immunol. 2003;170:5772-7.
24. Ferrer JM, Iglesias J, Hernandez M, Matamoros N. Alterations in interleukin secretion (IL-2 and IL-4) by CD4 and CD4 CD45RO cells from common variable immunodeficiency (CVI) patients. Clin Exp Immunol. 1995;102:286-9.
25. Zhou Z, Huang R, Danon M, Mayer L, Cunningham-Rundles C. IL-10 production in common variable immunodeficiency. Clin Immunol Immunopathol. 1998;86:298-304.

26. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol. 1999;93:190-7.
27. Cunningham-Rundles C. Common variable immunodeficiency. Curr Allergy Asthma Rep. 2001;1:421-9.
28. Di Renzo M, Pasqui AL, Auteri A. Common variable immunodeficiency: a review. Clin Exp Med. 2004;3:211-7.
29. Rezaei N, Haji-Molla-Hoseini M, Aghamohammadi A, Pourfathollah AA, Moghtadaie M, Pourpak Z. Increased serum levels of soluble CD30 in patients with common variable immunodeficiency and its clinical implications. J Clin Immunol. 2008;28-78-84.
30. North ME, Ivory K, Funauchi M, Webster AD, Lane AC, Farrant J. Intracellular cytokine production by human CD4+ and CD8+ T cells from normal and immunodeficient donors using directly conjugated anti-cytokine antibodies and three-colour flow cytometry. Clin Exp Immunol. 1996;105:517-22.
31. North ME, Webster AD, Farrant J. Primary defect in CD8+ lymphocytes in the antibody deficiency disease (common variable immunodeficiency): abnormalities in intracellular production of interferon-gamma (IFN-gamma) in CD28+ ('cytotoxic') and CD28- ('suppressor') CD8+ subsets. Clin Exp Immunol. 1998;111:70-5.
32. Fischer MB, Hauber I, Vogel E, Wolf HM, Mannhalter JW, Eibl MM. Defective interleukin-2 and interferon-gamma gene expression in response to antigen in a subgroup of patients with common variable immunodeficiency. J Allergy Clin Immunol. 1993;92:340-52.
33. Hauber I, Fischer MB, Eibl MM. Patients with common variable immunodeficiency (CVID) display aberrant IL-2 and IFN-gamma mRNA levels. Immunodeficiency. 1993;4:25-9.
34. Inoue Y, Kondo N, Motoyoshi F, Inoue R, Orii T. Interleukin-2 and interferon-gamma production by peripheral blood lymphocytes of patients with common variable immunodeficiency. J Investig Allergol Clin Immunol. 1994;4:122-5.
35. Aukrust P, Muller F, Froland SS. Elevated serum levels of interleukin-4 and interleukin-6 in patients with common variable immunodeficiency (CVI) are associated with chronic immune activation and low numbers of CD4+ lymphocytes. Clin Immunol Immunopathol. 1994;70:217-24.

Manuscript received February 23, 2008; accepted for publication April 30, 2008.

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