ORIGINAL ARTICLE

Cytokine Production by Activated T Cells in Common Variable Immunodeficiency

N Rezaei,[1,2] A Aghamohammadi,[1] M Nourizadeh,[3] GA Kardar,[3] Z Pourpak,[3] A Zare,[3] RC Read[2]

[1]Research Group for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Infection and Immunity, School of Medicine and Biomedical Sciences, The University of Sheffield, Sheffield, United Kingdom
[3]Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

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=.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.

Key words: Common variable immunodeficiency. Cytokine. Proliferation. T cell.

Resumen

Antecedentes: La inmunodeficiencia común variable (IDCV) es la deficiencia de anticuerpos sintomática más frecuente y se caracteriza por hipogammaglobulinemia, aumento de la susceptibilidad a infecciones recurrentes, autoinmunidad y neoplasias malignas.
Objetivos: Determinar si los pacientes con IDCV presentan deficiencias en la producción de citocinas tras la activación de los linfocitos T y evaluar si estos se correlacionan con marcadores de enfermedad grave.
Métodos: Se estudió a 27 pacientes con IDCV y a 17 voluntarios sanos. Se realizaron cultivos de células mononucleares de sangre periférica en condiciones normales, en presencia y ausencia de fitohemaglutinina. Se midieron la proliferación celular y la liberación de citocinas posteriores y se compararon los resultados entre células estimuladas y células no estimuladas.
Resultados: Se observó un aumento general de la producción de citocinas, tanto en los pacientes con IDCV como en los controles, tras la estimulación. Sin embargo, se detectó una menor producción de interferón-γ en los pacientes con IDCV que en los controles (p=0,026). Se apreció una deficiencia de producción de al menos 1 citocina en 12 pacientes. Diez de ellos no fueron capaces de generar títulos protectores en respuesta a la vacuna de polisacáridos, y la frecuencia de bronquiectasia en este grupo de pacientes fue del 91,7%. La liberación de citocinas se correlacionó claramente con la proliferación celular.
Conclusiones: Este estudio indica que algunos pacientes con IDCV presentan proliferación de linfocitos T y deficiencias de secreción que podrían estar asociadas con manifestaciones graves de enfermedad. La detección de estas deficiencias permitiría instaurar un seguimiento y estrategias terapéuticas más eficaces para los pacientes con IDCV.

Palabras clave: Inmunodeficiencia común variable. Citocina. Proliferación. Linfocito T.

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 (37ºC, 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 GmhH, 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,

4-56 years). The first manifestation of CVID occurred at a median age of 9 months (IQR, 1 month-50 years). The median age of patients at the time of diagnosis was 97 months (IQR, 2-54 years), with a median diagnosis delay of 50 months (IQR, 3-477 months).

Clinical Features of CVID Patients

All patients had experienced recurrent upper or lower respiratory tract infections, and 85.2% had had gastrointestinal manifestations. The most common pathologies were pneumonia (23 patients), diarrhea (n=23), sinusitis (n=20), otitis media (n=17), cutaneous infections (n=9), conjunctivitis (n=8), septic arthritis (n=7), and superficial abscesses (n=4). Failure to thrive was also a feature of 12 patients. Splenomegaly and radiologically confirmed bronchiectasis were each seen in 13 patients.

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 median 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/mm^3 and the mean absolute number of $CD3^+$ T cells was 1815.37 (737.58) cells/mm^3. The corresponding figures for $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were 821.41 (494.66) cells/mm^3 and 919.90 (418.18) cells/mm^3, 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

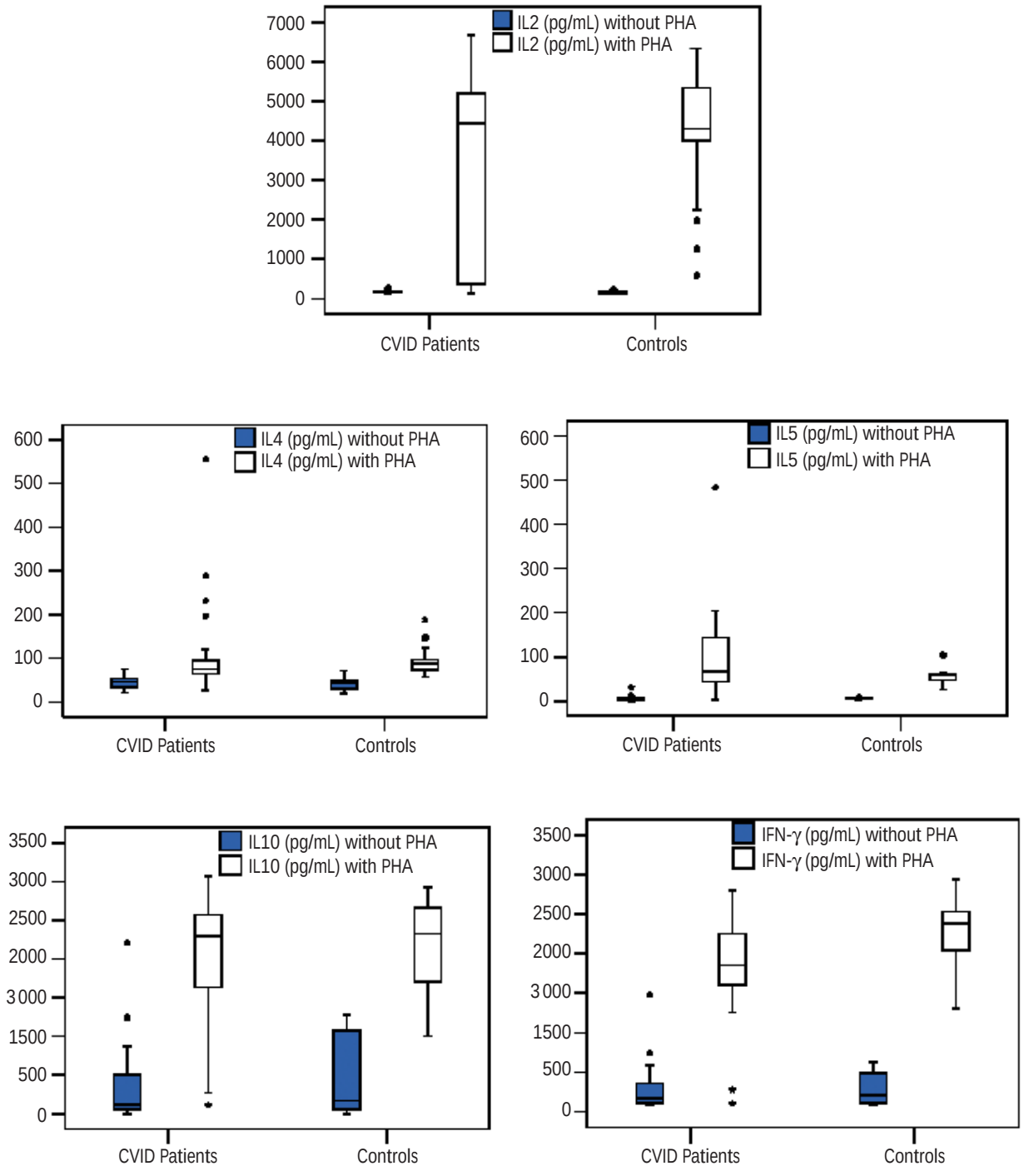

Figure 1. Comparison of cytokine production in unstimulated cells and cells stimulated with phytohemagglutinin (PHA) between patients with common variable immunodeficiency (CVID) and controls.

Table 1. Proliferative Response to Phytohemagglutinin in CVID Patients (n=27) Compared to Sex- and Age-Matched Controls

Patients			Controls			
OD of Unstimulated Cells	OD of Stimulated Cells	Stimulation Index[a]	OD of Unstimulated Cells	OD of Stimulated Cells	Stimulation Index	Cytokine Production Defect
0.083	0.531	6.40	0.087	0.460	5.29	–
0.069	0.463	6.71	0.087	0.460	5.29	–
0.120	0.570	4.75	0.086	0.525	6.10	–
0.100	0.508	5.08	0.086	0.525	6.10	IL10
0.114	0.381	3.34	0.170	0.555	3.26	IL2
0.154	0.489	3.18	0.170	0.555	3.26	IL4
0.152	0.424	2.79[b]	0.078	0.441	5.65	–
0.118	0.333	2.82[bc]	0.168	1.143	6.80	IL2,IL4,IL5,IL10
0.139	0.490	3.53	0.118	0.524	4.44	IL4
0.164	0.411	2.51[b]	0.118	0.524	4.44	–
0.142	0.472	3.32	0.118	0.524	4.44	IL4
0.193	0.437	2.26[b]	0.100	0.401	4.01	IL2
0.085	0.442	5.20	0.100	0.401	4.01	–
0.113	0.473	4.19	0.151	0.473	3.13	–
0.092	0.493	5.36	0.108	0.415	3.84	–
0.091	0.408	4.48	0.180	0.592	3.29	IL4
0.106	0.297	2.80[b]	0.180	0.592	3.29	IL2
0.152	1.251	8.23	0.143	1.210	8.46	–
0.053	0.250	4.72	0.117	0.951	8.13	IL2,IL5
0.085	0.894	10.52	0.117	0.951	8.13	–
0.044	0.058	1.32[bc]	0.060	0.565	9.42	IL2,IL4,IL5,IL10,IFN-γ
0.095	1.148	12.08	0.166	1.265	7.62	–
0.099	1.412	14.26	0.101	1.180	11.68	–
0.145	0.973	6.71	0.091	1.029	11.31	–
0.088	0.214	2.43[bc]	0.091	1.029	11.31	IL2,IL4,IL5,IFN-γ
0.088	1.023	11.63	0.112	0.906	8.09	–
0.058	0.823	14.19	0.112	0.906	8.09	–

Abbreviations: CVID, common variable immunodeficiency; IFN, interferon; IL, interleukin; OD, optical density.
[a]Calculated as the mean ratio of OD of cells stimulated with phytohemagglutinin to that of unstimulated cells.
[b]Less than cutoff of 2.98.
[c]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 (T_H2) T cell cytokine levels (IL-4 and IL-10) were significantly increased in CVID patients, but no significant differences were found for T_H1 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 T_H2 response in such patients has been suggested [21,22]. However, there is some evidence supporting dominance of T_H1 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].

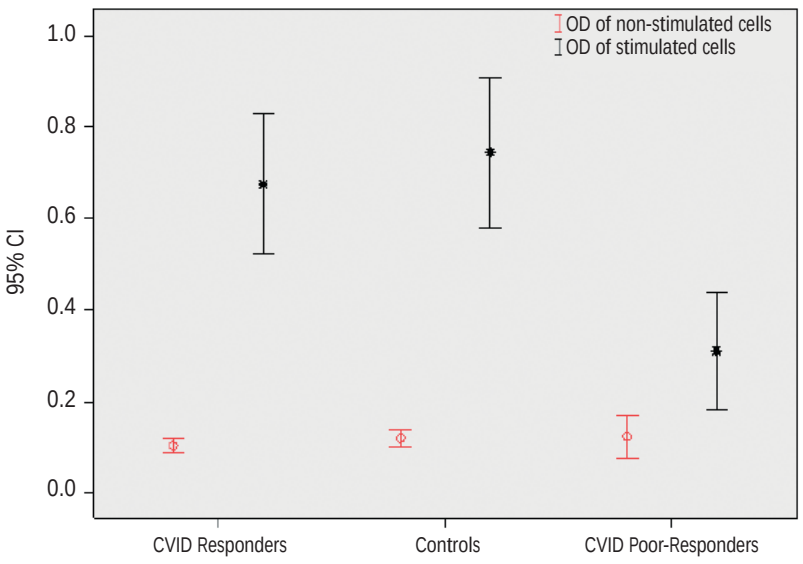

Figure 2. Error bar graph based on optical density (OD) of stimulated and unstimulated cells in patients with common variable immunodeficiency (CVID responders and poor responders to mitogen) and controls. CI indicates confidence interval.

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.

Acknowledgments

This study was supported by grant from Tehran University of Medical Sciences and Health Services (Grant 1452). The authors are

Table 2. Cytokine Production in Unstimulated Cells and Stimulated Cells in Patients with Common Variable Immunodeficiency and Controls[a]

Cytokine	Cells	Cytokine Production in Patients, pg/mL	Cytokine Production in Controls, pg/mL	*P* Value[b]
IL-2	Unstimulated	169.0 (152.0-183.0)	167.0 (150.0-193.5)	.809
	Stimulated	4436.0 (237.0-5231.0)	4299.0 (3127.0-5389.0)	.571
	P value[c]	<.001[d]	<.001[d]	
IL-4	Unstimulated	46.9 (30.6-56.0)	42.6 (29.8-49.2)	.283
	Stimulated	75.1 (61.8-96.6)	88.8 (70.7-100.8)	.306
	P value[c]	<.001[d]	<.001[d]	
IL-5	Unstimulated	3.7 (1.9-5.7)	5.1 (4.0-5.7)	.165
	Stimulated	66.9 (40.6-147.2)	58 (43.9-62.0)	.192
	P value[c]	<.001[d]	0.001[d]	
IL-10	Unstimulated	123.2 (57.1-516.3)	170.9 (54.9-1082.3)	.725
	Stimulated	2289.0 (1595.0-2671.0)	2323.5 (1626.3-2680.5)	.706
	P value[c]	<.001[d]	<.001[d]	
IFN-γ	Unstimulated	167.9 (111.0-364.5)	205.4 (108.5-493.6)	.700
	Stimulated	1855.0 (1582.3-2286.3)	2377.0 (1902.0-2646.5)	.029[d]
	P value[c]	<.001[d]	<.001[d]	

Abbreviations: IFN, interferon; IL, interleukin.
[a]Results are presented as median and interquartile range.
[b]*P* value between patients and controls, calculated using the Mann-Whitney U test.
[c]*P* value between unstimulated and stimulated cells, calculated using the Wilcoxon signed ranks test.
[d]Statistically significant *P* values.

very grateful to all the colleagues in the Department of Allergy and Clinical Immunology at the Children's Medical Center and the Immunology, Asthma and Allergy Research Institute for their kind help and laboratory assistance and all the patients and their families for agreeing to participate in this study. We would also like to thank S. Faridani, M. Siavashi, and R. Khalili for their help in collecting samples.

References

1. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92:34-48.
2. 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.
3. Aghamohammadi A, Parvaneh N, Tirgari F, Mahjoob F, Movahedi M, Gharagozlou M, Mansouri M, Kouhi A, Rezaei N, Webster D. Lymphoma of mucosa-associated lymphoid tissue in common variable immunodeficiency. Leuk Lymphoma. 2006;47:343-6.
4. Ramyar A, Aghamohammadi A, K. M, Rezaei N, Yeganeh M, Cheraghi T, Pouladi N, Heydari G, Moin M. Presence of idiopathic thrombocytopenia purpura and autoimmune hemolytic anemia in the patients with common variable immunodeficiency. Iran J Allergy Asthma Immunol. 2008;7:169-75.
5. 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.
6. Aghamohammadi A, Lougaris V, Plebani A, Miyawaki T, Durandy A, Hammarström L. Predominantly antibody deficiencies. In: Rezaei N, Aghamohammadi A, Notarangelo LD, editors. Primary immunodeficiency diseases: definition, diagnosis and management. Berlin Heidelberg: Springer-Verlag; 2008. p. 97-130.
7. Webster ADB. Clinical and immunological spectrum of common variable immunodeficiency (CVID). Iran J Allergy Asthma Immunol. 2004;3:103-13.
8. 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.
9. Vodjgani M, Aghamohammadi A, Samadi M, Moin M, Hadjati J, Mirahmadian M, Parvaneh N, Salavati A, Abdollahzade S, Rezaei N, Sarrafnejad A. The study of switched memory B cells in patients with common variable immunodeficiency and its clinical implications. J Investig Allergol Clin Immunol. 2007;17:321-8.
10. 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.
11. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, Vlkova M, Hernandez M, Detkova D, Bos PR, Poerksen G, von Bernuth H, Baumann U, Goldacker S, Gutenberger S, Schlesier M, Bergeron-van der Cruyssen F, Le Garff M, Debre P, Jacobs R, Jones J, Bateman E, Litzman J, van Hagen PM, Plebani A, Schmidt RE, Thon V, Quinti I, Espanol T, Webster AD, Chapel H, Vihinen M, Oksenhendler E, Peter HH, Warnatz K. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111:77-85.
12. 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.
13. 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.
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. Nourizadeh M, Aghamohammadi A, Moazzeni SM, Mahdavi M, Jalili A, Rezaei N, Hadjati J. Altered dendritic cell function in response to sera of common variable immunodeficiency patients. Inflamm Res. 2007;56:527-32.
16. 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.
17. 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.
18. Giovannetti A, Pierdominici M, Mazzetta F, Marziali M, Renzi C, Mileo AM, De Felice M, Mora B, Esposito A, Carello R, Pizzuti A, Paggi MG, Paganelli R, Malorni W, Aiuti F. Unravelling the complexity of T cell abnormalities in common variable immunodeficiency. J Immunol. 2007;178:3932-43.
19. Rezaei N, Wing JB, Aghamohammadi A, Carlring J, Lees A, Asgarian-Omran H, Pourpak Z, Sarrafnejad A, Kardar GA, Shahrestani T, Masoumi F, Zare A, Saghafi S, Sarrafzadeh S, Foster RA, Heath AW, Read RC. B-cell-T-cell activation and interaction in common variable immunodeficiency. Hum Immunol. 2010;71:355-62.
20. 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.
21. 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.
22. Rezaei N, Aghamohammadi A, Kardar GA, Nourizadeh M, Pourpak Z. T- helper 1 and 2 cytokines assay in patients with

common variable immunodeficiency. J Investig Allergol Clin Immunol. 2008;18:449-53.
23. 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.
24. 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.
25. Aghamohammadi A, Fiorini M, Moin M, Parvaneh N, Teimourian S, Yeganeh M, Goffi F, Kanegane H, Amirzargar AA, Pourpak Z, Rezaei N, Salavati A, Pouladi N, Abdollahzade S, Notarangelo LD, Miyawaki T, Plebani A. Clinical, immunological and molecular characteristics of 37 Iranian patients with X-linked agammaglobulinemia. Int Arch Allergy Immunol. 2006;141:408-14.
26. Rezaei N, Siadat SD, Aghamohammadi A, Moin M, Pourpak Z, Norouzian D, Mobarakeh JI, Aghasadeghi MR, Nejati M, Read RC. Serum bactericidal antibody response 1 year after meningococcal polysaccharide vaccination of patients with common variable immunodeficiency. Clin Vaccine Immunol. 2010;17:524-8.
27. Aghamohammadi A, Parvaneh N, Rezaei N. Common variable immunodeficiency: a heterogeneous group needs further subclassification. Expert Rev Clin Immunol. 2009;5:629-31.
28. Webster ADB. Common variable immunodeficiency. In: Roifman C, editor. Humoral deficiencies. Philadelphia, USA: Immunology and Allergy Clinics of North America; 2001. p. 1-22.
29. Funauchi M, Farrant J, Moreno C, Webster AD. Defects in antigen-driven lymphocyte responses in common variable immunodeficiency (CVID) are due to a reduction in the number of antigen-specific $CD4^+$ T cells. Clin Exp Immunol. 1995;101:82-8.
30. 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.
31. 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.
32. 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.
33. 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.
34. 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.
35. 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.
36. 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.
37. Rezaei N, Amirzargar AA, Shakiba Y, Mahmoudi M, Moradi B, Aghamohammadi A. Proinflammatory cytokine gene single nucleotide polymorphisms in common variable immunodeficiency. Clin Exp Immunol. 2009;155:21-7.
38. Rezaei N, Aghamohammadi A, Shakiba Y, Mahmoudi M, Jalali A, Moradi B, Amirzargar AA. Cytokine gene polymorphisms in common variable immunodeficiency. Int Arch Allergy Immunol. 2009;150:1-7.
39. Rezaei N, Aghamohammadi A, Mahmoudi M, Shakiba Y, Kardar GA, Mahmoudi M, Moradi B, Amirzargar AA. Association of IL-4 and IL-10 gene promoter polymorphisms with common variable immunodeficiency. Immunobiology. 2010;215:81-7.
40. 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.
41. 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.

Manuscript received May 22, 2009; accepted for publication August 12, 2009.

Nima Rezaei

Children's Medical Center Hospital
Tehran University of Medical Sciencies
Dr Qarib St., Keshavarz Bld.
Tehran 14194, Iran
E-mail: nima-rezaei@farabi.tums.ac.ir