ORIGINAL ARTICLE

Naïve CD4+ T cells and Recent Thymic Emigrants in Common Variable Immunodeficiency

M Oraei,[1] A Aghamohammadi,[2] N Rezaei,[1,2,3] K Bidad,[1] Z Gheflati,[1] A Amirkhani,[4] H Abolhassani,[2] A Massoud[1]

[1]Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[3]Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Epidemiology, Pasteur Institute of Iran, Tehran, Iran

■ **Abstract**

Background: Common variable immunodeficiency (CVID) comprises a heterogeneous group of disorders classified as predominantly antibody deficiencies. The aim of this study was to analyze levels of naïve CD4+ T cells and recent thymic emigrant (RTE) cells in CVID patients and healthy controls.

Methods: Twenty patients with CVID and 20 age- and sex-matched healthy controls were studied. CD4+ T cells were negatively isolated from peripheral blood mononuclear cells by magnetic beads, and cell surface markers (CD45RA, CD62L and CD31) were assessed by flow cytometry. The normal range of naïve CD4+ T cells detected in the control group (33%-63%) was used to classify the CVID patients into 2 groups (≤33% and >33% naïve CD4+ T cells).

Results: Naïve CD4+ T cells (CD45RA+ CD62L+) and RTE cells (CD45RA+CD62L+CD31+) were significantly lower in male CVID patients compared to both female patients and healthy male controls. There were also more male patients in the group with naïve CD4+ T-cell levels of 33% or less. Autoimmunity was only observed in this group.

Conclusions: Lower numbers of naïve CD4+ T cells and RTE cells in male CVID patients might be due to lower thymic output in these patients. The classification of patients based on naïve CD4+ T cell levels seems to be consistent with clinical features.

Key words: CVID. Naïve CD4+ T cells. RTE. Sex.

■ **Resumen**

Antecedentes: La inmunodeficiencia común variable (IDCV) incluye un grupo heterogéneo de trastornos clasificados principalmente como deficiencias de anticuerpos. El objetivo de este estudio fue analizar los niveles de linfocitos T CD4+ indiferenciados y de linfocitos emigrantes tímicos recientes (ETR) en pacientes con IDCV y en controles sanos.

Métodos: Se estudió a 20 pacientes con IDCV y 20 controles sanos emparejados por edad y sexo. Los linfocitos T CD4+ se aislaron negativamente de las células mononucleares de sangre periférica mediante perlas magnéticas, y los marcadores de la superficie celular (CD45RA, CD62L y CD31) fueron evaluados mediante citometría de flujo. El intervalo normal de linfocitos T CD4+ indiferenciados detectado en el grupo de control (33-63%) se utilizó para clasificar a los pacientes con IDCV en 2 grupos (linfocitos T CD4+ indiferenciados ≤ 33% y > 33%).

Resultados: El número de linfocitos T CD4+ indiferenciados (CD45RA+ CD62L+) y linfocitos ETR (CD45RA+CD62L+CD31+) fue significativamente inferior en varones con IDCV frente a mujeres y controles varones sanos. Asimismo, hubo más pacientes varones en el grupo con niveles de linfocitos T CD4+ indiferenciados del 33% o menos. Solo se observó autoinmunidad en este grupo.

Conclusiones: El recuento menor de linfocitos T CD4+ indiferenciados y de linfocitos ETR en varones con IDCV puede deberse a una menor producción tímica en estos pacientes. La clasificación de los pacientes basada en los niveles de linfocitos T CD4+ indiferenciados parece corresponderse con las características clínicas.

Palabras clave: IDCV. Linfocitos T CD4+ indiferenciados. ERT. Sexo.

Introduction

Common variable immunodeficiency (CVID) is a primary immunodeficiency disease consisting of a group of heterogeneous disorders of unknown etiology and characterized by low serum immunoglobulin levels and recurrent bacterial infections [1-3]. Patients have a high risk of autoimmunity, cancer, and splenomegaly [4-7].

CVID can occur at any age, but its incidence peaks in the first and third decades of life. It affects males and females similarly, with prevalence ranging from 1 case in 15 000 to 1 in 200 000, depending on ethnicity. The molecular mechanism of CVID is not clear and diagnosis is based mainly on the exclusion of other known single gene defects associated with hypogammaglobulinemias [8].

Because of the clinical and immunologic heterogeneity of CVID, one of the main goals in this disease is to design accurate classification systems, and to do this, it is necessary to describe homogeneous subgroups of patients [8,9]. There have been several attempts to classify CVID based on clinical and immunological phenotypes [10-14]. The first accepted classifications were based on immunoglobulin synthesis and markers of B-cell maturation [9,10,15]. T-cell defects, such as decreased lymphocytic proliferation in response to mitogens and antigens [16,17], increased apoptosis in T cells [18], cytokine production impairment [19-22], reduced CD40L expression on activated T cells [23,24] and impaired antigen-primed T-cell generation after prophylactic vaccination [25,26] have also been reported in CVID. Thanks to these advancements, Giovanetti et al [27] recently classified CVID according to number of naïve CD4+ T cells and proposed a classification system based on the association between these cells and clinical symptoms.

The thymus is the primary location of thymopoiesis in fetal life and the infantile period. It is developed by the time of birth, but undergoes gradual involution by aging. The main cells produced in the thymus are T cells. After TCR rearrangement in these cells, extrachromosomal circles, known as T-cell receptor rearrangement excision circles (TRECs), are released into the cytoplasm. TRECs were the first biomarkers used to evaluate thymus output. Later, it was shown that naïve CD31+ T cells have high TREC levels in comparison to naïve CD31- T cells. CD31+ naïve CD4+ T cells are in fact recent thymic emigrant (RTE) cells, while CD31- naïve CD4+ T cells are cells that have proliferated in the periphery [28]. In CVID patients, thymic output appears to be decreased in comparison with healthy individuals [1,27,29].

The aims of this study were to evaluate thymic output in Iranian CVID patients and healthy controls by measuring CD31+ naïve CD4 T cells and to assess the association between cell numbers and clinical symptoms and signs.

Patients and Methods

Participants

The study population consisted of 20 well-documented CVID patients (age range, 15-50 years) and 20 age- and sex-matched healthy controls. The patients were selected from among patients undergoing regular follow-up at the Children's Medical Center, the Pediatrics Center of Excellence in Tehran, Iran. Diagnosis of CVID was based on international criteria, which includes a reduction in at least 2 serum immunoglobulin (Ig) levels (IgG, IgA, and IgM) by 2 SDs from the normal mean values for age, and genetic exclusion of other causes of hypogammaglobulinemia. Clinical data, including disease-related symptoms, signs, and complications, were compiled from the patients' medical records. All the patients were evaluated for autoimmune disorders common in CVID such as idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA), pernicious anemia, autoimmune thyroiditis, rheumatoid arthritis, celiac disease, and vitiligo.

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

Heparinized peripheral blood was obtained from all the participants in the study. In the case of the patients, sampling was performed 3 to 4 weeks after intravenous immunoglobulin administration, before the next infusion.

Separation of CD4+ T cells

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll/Hypaque (Histoprep) and washed twice with phosphate buffered saline (pH, 7.2).

CD4+ T cells were negatively isolated from PBMCs by magnetic beads according to the manufacturer's instruction (Dynabeads Untouched Human CD4 T Cells; Invitrogen Dynal AS). The purity of separated CD4+ T cells was over 95%, as determined by flow cytometry.

Flow Cytometry Analysis

For immunophenotypic analysis, the CD4+ T cells were stained with 3 fluorescent conjugated monoclonal antibodies specific for cell surface markers. The following conjugated monoclonal antibodies along with their isotype controls were purchased from BD PharMingen: Anti-CD62L-FITC, anti-CD45RA-PE-CY5, and anti-CD31-PE. Analyses were performed using a FACSCalibur flow cytometer and CellQuest software (BD FlowCytometry Systems). CD4+ T cells positive for both CD45RA and CD62L were gated as the naïve population and analyzed for the percentage of CD31+ cells. The RTE cell population was defined as the percentage of CD31+ CD62L+CD45RA+ cells in CD4+ T cells (Figure 1).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 16.0). P values of less than .05 were considered significant.

Results

Characteristics of Participants

We included 20 CVID patients and 20 age- and sex-matched controls in this cross-sectional study. The mean (SD) age of
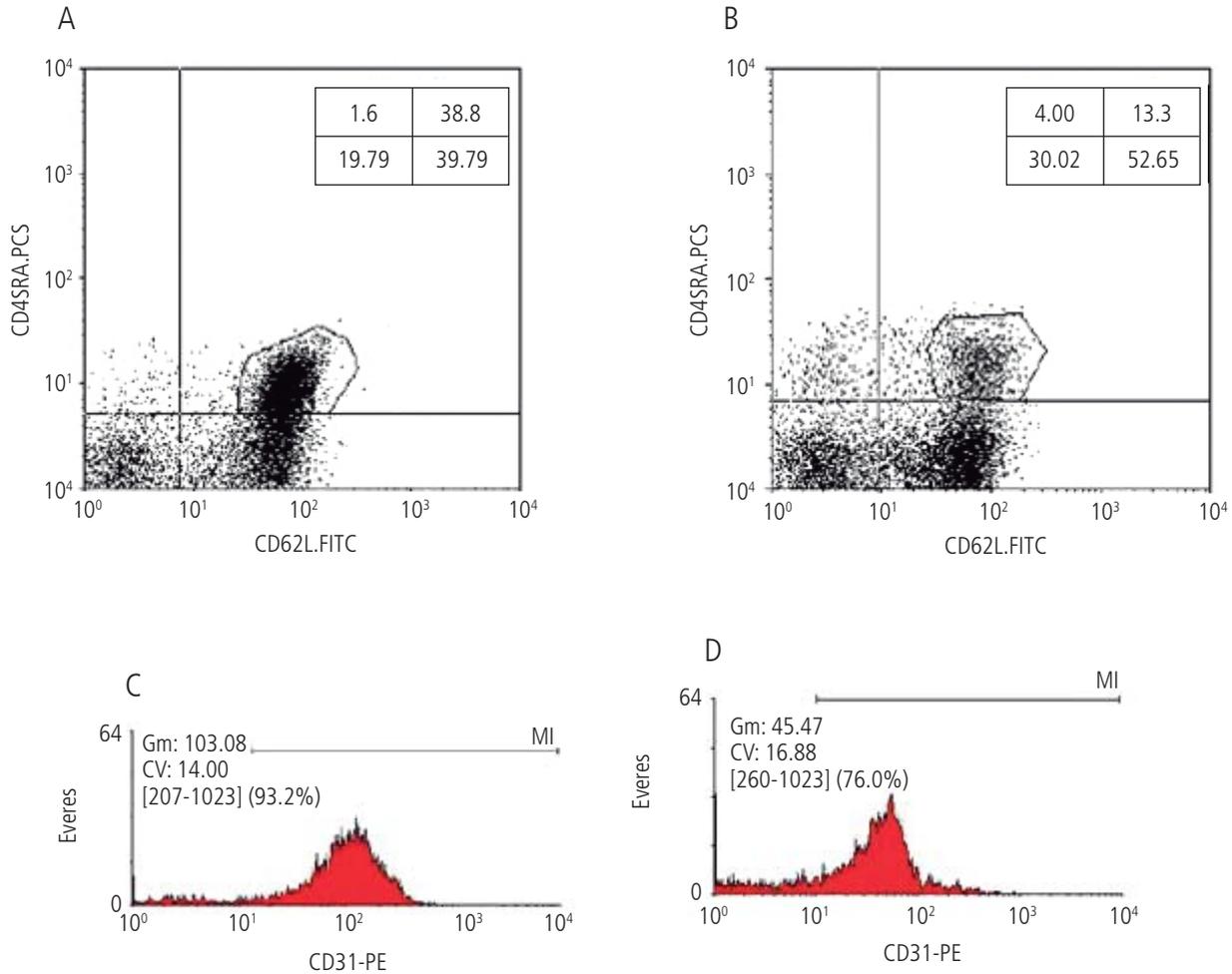
Figure 1. Representative sample of flow cytometry analysis for naïve CD4+ T cells and recent thymic emigrant cells in a healthy donor (A and C) and a patient with common variable immunodeficiency (CVID) (B and D). Dot blots represent the flow cytometry analysis of naïve CD4+ T cells (CD62L+CD45RA+) based on CD62L and CD45RA expression in a healthy control (A) and a CVID patient (B). Histograms for CD31 expression in gated naïve population in a CVID patient (C) and a healthy control (D). CV indicates coefficient of variance; GM: geometric mean.

participants was 23.20 (7.3) years. The female to male ratio was 16:24. Table 1 shows the demographic characteristics of the case and control groups as well as the mean percentage of naïve CD4+ T cells and RTE cells. Table 2 shows the demographic, laboratory, and clinical characteristics of each of the patients individually.

Three patients were found to have an autoimmune disorder: ITP/AIHA in 1 case, autoimmune thyroiditis in another, and celiac disease in the third.

Table 1. Demographic Characteristics and Percentage of Naïve CD4+ T Cells and RTE Cells in Patients With Common Variable Immunodeficiency (CVID) and Healthy Controls[a]

	CVID Patients (n=20)	Healthy Controls (n=26)	Total (n=46)	P Value
Age, y	22.95 (7.74)	23.45 (7.09)	23.20 (7.30)	NS
Female to male ratio	8:12	8:12	16:24	NS
CD45RA+ CD62L+ CD4+ T cells (naïve cells)	32.12 (15.18)	44.30 (9.44)	38.21 (13.92)	.004
CD31+CD45RA+CD62L+ CD4+ T cells (RTE cells)	29.37 (14.34)	40.16 (9.46)	34.76 (18.18)	.008

Abbreviations: NS, not significant; RTE, recent thymic emigrant.
[a]Data are expressed as mean (SD) unless otherwise indicated.

Naïve CD4⁺ T Cells and RTE Cells in Male and Female Cases and Controls

Naïve CD4$^+$ T cells (CD45RA$^+$ CD62L$^+$ CD4$^+$ T cells) were significantly lower in male patients compared to female patients (26.24 [14.64] vs 40.94 [11.87], P=.03) and compared to male healthy controls (44.66 [10.00], P=.002). RTE cells (CD31$^+$ CD45RA$^+$ CD62L$^+$ CD4$^+$ T Cells) were also significantly lower in male patients compared to both female patients (23.52 [13.47] vs 38.14 [11.26], P=.02) and male healthy controls (40.08 [10.68], P=.003) (Figure 2). However, there were no significant differences between female and male controls for either of the cells. In the patient group, there was a significant negative correlation between the percentage of naïve cells in isolated CD4$^+$ T cells and that of CD8$^+$ T cells in PBMCs (Pearson correlation coefficient=-0.48, P=.04).

Subgroups of Patients Based on Percentage of CD4⁺ T cells

Patients were divided into 2 subgroups based on the normal range of naïve CD4+ T cell levels detected in the control group (33%-63%). Patients with levels lower than or equal to 33% were classified as group 1 while those with levels higher than 33% were classified as group 2. Table 3 illustrates the characteristics of the patients in these groups. The percentage of males in group 1 and 2, respectively, was 82% and 33%. The difference in the sex composition of the 2 groups was statistically significant (P=.02). Autoimmunity was only observed in the first group. Splenomegaly, bronchiectasis, and lymphadenopathy were more common in group 1, but

the differences with group 2 were not statistically significant. IgG and IgA levels were also lower in group 1 but again, the differences were not significant.

Naïve CD4⁺ T cells and RTE Cells in Relation to Clinical Manifestations

The mean percentage of naïve CD4$^+$ T cells and RTE cells was compared between patients with different clinical manifestations. Both types of cells were significantly reduced in patients with autoimmunity compared to those without (Table 4). The probability of autoimmunity (celiac disease, thyroiditis, AIHA, and ITP) was significantly higher in patients in group 1 (≤33% naïve CD4$^+$ T cells) than in group 2 (>33%). However, the differences were nonsignificant for other manifestations, namely splenomegaly, bronchiectasis, gastrointestinal complications, conjunctivitis, and lymphadenopathy (Table 3).

The number of white blood cells (WBCs) and the percentage of CD19$^+$ cells were significantly lower in patients with autoimmunity than in those without (WBCs: 5350 [353] vs 8167 [347], P=.01; CD19$^+$ cells: 5.79% [2.29] vs 13.31% [10.45], P=.02).

Discussion

CVID, as the name implies, consists of a heterogeneous group of disorders of unknown etiology. B-cell defects are a well-established possible cause, but T-cell defects have recently begun to attract a lot of attention.

Table 2. Demographic, Laboratory, and Clinical Characteristics of Patients With Common Variable Immunodeficiency

Patients	Age, y	Sex	%CD4	%CD8	CD4:CD8	Naïve CD4⁺ T Cells	RTE cells, %	Auto-immunity	Spleno-megaly	Bronchiec-tasis	Lympha-denopathy
P1	25	F	68.37	19.73	3.47	59.30	55	No	Yes	No	Yes
P2	35	F	44.14	31.98	1.38	31.87	29	No	No	No	No
P3	18	F	34.19	32.58	1.05	38.00	36	No	No	No	No
P4	20	M	21.00	18.00	1.17	17.35	16	No	Yes	No	Yes
P5	17	M	31.85	55.48	0.57	13.33	12	Yes	Yes	Yes	Yes
P6	19	M	31.70	34.20	0.93	20.74	19	No	Yes	No	No
P7	24	M	NA	NA	NA	17.14	15	No	No	No	No
P8	23	M	28.90	41.90	0.69	37.00	34	No	Yes	Yes	Yes
P9	21	F	38.33	35.08	1.09	45.48	43	No	Yes	No	Yes
P10	15	F	46.07	25.52	1.81	53.09	50	No	Yes	No	No
P11	23	F	23.42	49.45	0.47	22.00	20	Yes	No	Yes	No
P12	19	M	20.85	43.85	0.48	25.81	24	No	Yes	Yes	Yes
P13	16	F	35.23	31.29	1.13	42.38	39	No	No	No	No
P14	49	M	49.40	NA	NA	14.90	10	Yes	Yes	No	Yes
P15	16	M	29.10	38.99	0.75	28.00	27	No	Yes	Yes	Yes
P16	29	M	57.00	52.00	1.10	17.91	16	No	No	No	No
P17	22	M	14.00	65.00	0.22	20.39	18	No	Yes	Yes	Yes
P18	24	M	20.00	66.00	0.30	37.00	33	No	No	No	No
P19	20	M	26.00	24.00	1.08	65.41	59	No	No	No	No
P20	24	F	27.00	66.00	0.41	35.40	33	No	No	Yes	No

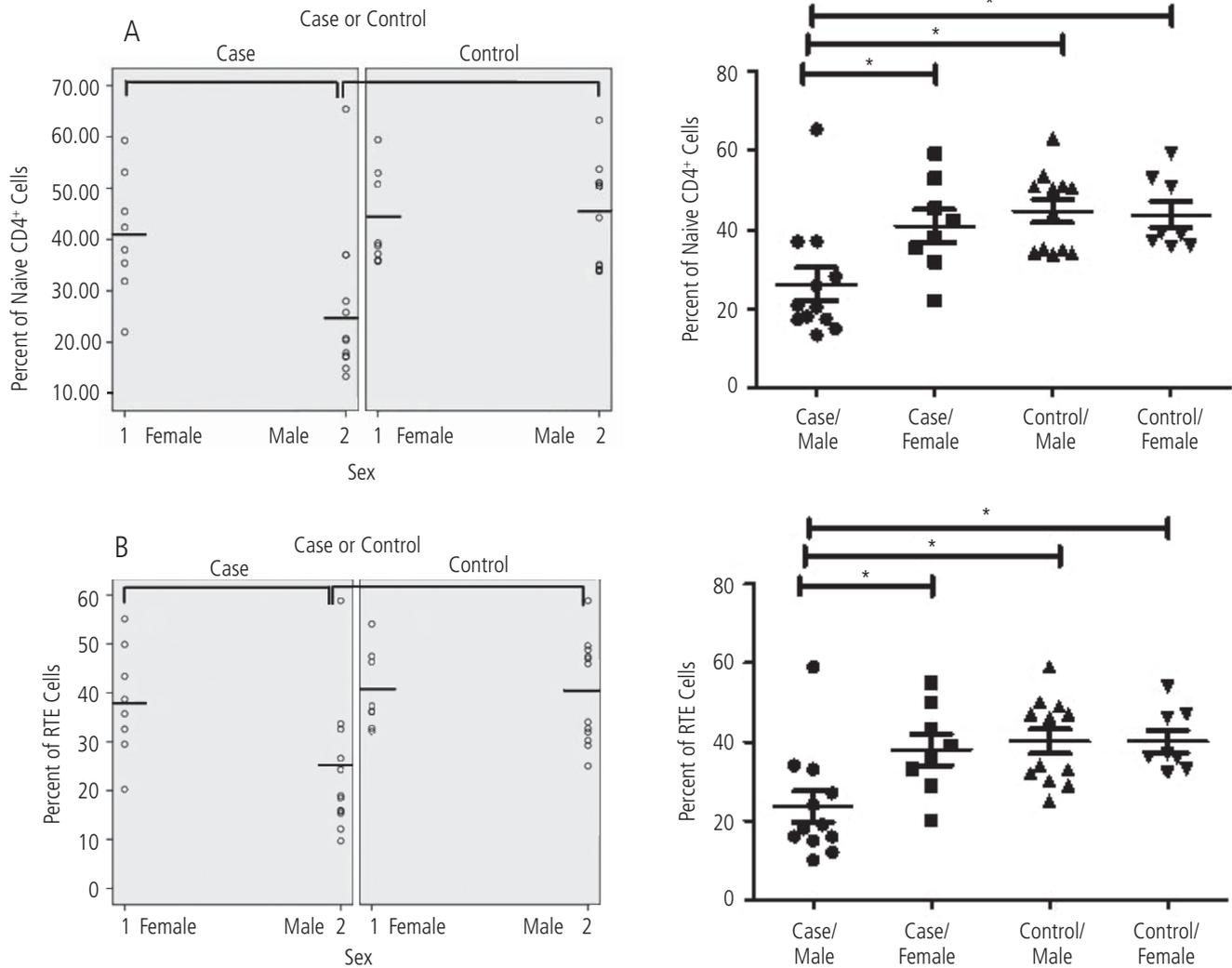Abbreviations: F, female; M, male; NA, not available.

Figure 2. Percentage and mean (SD) numbers of naïve CD4+ T cells (A) and recent thymic emigrant (RTE) cells (B) in patients with common variable immunodeficiency and healthy controls according to sex. An asterisk indicates a *P* value of less than .05.

Table 3. Characteristics of Patients by Percentage of Naïve CD4+ T Cells

	Group 1[a]		Group 2[b]		RR	95% CI
	No.	%	No.	%		
Sex						
Male	9	75	3	25	2.45	0.93-6.44
Female	2	25	6	75	0.11	0.01-0.87
Autoimmunity	3	100	0	0	--	---
Splenomegaly	7	63.6	4	36.4	1.43	0.60 -3.37
Bronchiectasis	5	71.4	2	28.6	2.04	0.51-8.15
Gastrointestinal complications	9	64.3	5	35.7	1.47	0.77-2.81
Conjunctivitis	5	62.5	3	37.9	1.36	0.44-4.21
Lymphadenopathy	6	66.7	3	33.3	1.63	0.56-4.77
Cancer	1	50	1	50	0.81	0.05-11.33

Abbreviation: RR, relative risk.
[a]≤33% naïve CD4+ T Cells.
[b]>33% naïve CD4+ T Cells.

In our study, we compared naïve CD4+ T cells in CVID patients and healthy controls, and like Giovanetti et al [27], Guazzi et al [29] and Isgro et al [1], found that these were significantly lower in patients than in controls. In the absence of age- and stress-related changes, naïve CD4+ T cell levels generally remain constant in healthy adults, indicating strict homeostatic control of these cells. Two pathways are responsible for T-cell renewal: thymic T-cell production and peripheral T-cell expansion [30]. T-cell defects may therefore be attributable to thymopoietic defects in the thymus. The thymus is the primary site of

T-cell generation and has been shown to remain functional during adult life, despite thymic involution [31]. Thymic output in CVID patients was assessed for the first time in 2002 by measurement of TRECs in naïve CD4+ T cells [29]. It was subsequently evaluated, also in CVID patients, by measurement of both TREC levels and the percentage of naïve CD4+ T cells coexpressing CD31 (RTE population) [1,27]. A good correlation was found between these 2 methods. In our study, we evaluated thymic output by measuring the percentage of cells coexpressing CD45RA, CD62L, and CD31 in isolated CD4+ T cells. Our results, consistent with those of previous studies [1,27], showed a decline in thymic output in CVID patients compared to healthy controls.

Table 4. Mean Percentage of Naïve CD4+ T cells and Recent Thymic Emigrant (RTE) Cells in Patients With Common Variable Immunodeficiency by Clinical Manifestation[a]

Clinical Manifestation		No. of Patients	Naïve CD4+ T Cells, %	P	RTE Cells, %	P
Autoimmunity	Yes	3	16.74 (4.6)	0.05	14.02 (5.5)	.04
	No	17	34.83 (14.8)		30.40 (12.2)	
Splenomegaly	Yes	11	30.49 (15.9)	0.6	27.99 (15.5)	.6
	No	9	34.12 (14.9)		27.57 (9.1)	
Bronchiectasis	Yes	7	25.99 (8.4)	0.1	23.98 (7.7)	.1
	No	13	34.83 (14.8)		30.05 (14.9)	
Gastrointestinal complications	Yes	14	31.47 (17.2)	0.7	26.28 (14.3)	.7
	No	6	33.63 (9.5)		31.13 (9.23)	
Conjunctivitis	Yes	8	27.84 (15.6)	0.4	25.87 (15.1)	.3
	No	12	34.37 (15.1)		29.23 (11.5)	
Lymphadenopathy	Yes	9	29.06 (15.4)	0.4	26.58 (15.1)	.4
	No	11	34.63 (15.2)		28.94 (11.2)	

[a]Data are expressed as mean (SD) unless otherwise indicated.

When levels of naïve CD4+ T cells and RTE cells were analyzed according to sex, it was observed that the lower levels detected in the CVID group were due to lower percentages of these cells in male patients. To the best of our knowledge, our study is the first to report significantly lower naïve CD4+ T cell levels and RTE cell levels in male CVID patients compared with female patients.

It has been previously reported that thymic output is different in males and females. In a study on B-cell and T-cell hematopoietic malignancies in childhood, TREC levels were found to be higher in girls than in boys, in both healthy individuals and patients with T-cell malignancies [32]. In 2 other studies on peripheral blood T cells, TRECs were also reported to be higher in females than in males, although T-cell numbers were similar in both groups [33,34]. These studies suggest better thymic function in females and might contribute to explaining why women have a longer life expectancy than men.

Sex-related differences in thymic output could be due to variations in sexual hormones. Male sexual hormones have been shown to accelerate thymic involution more profoundly than female hormones, with reports of improved thymic function in male gonadectomized mice [35]. Epidemiological differences between the sexes (such as weight, lifestyle, smoking, and medication) could also account for differences observed.

Studies of T cells in CVID patients have attempted to design new classification systems for CVID. The first systems were based on functional and phenotypic B-cell disorders [9,10,15], and these were followed by attempts to organize CVID patients on the basis of T-cell disorders. The most acceptable classification based on T-cell abnormalities is that suggested by Giovanneti et al in 2007 [27]. They classified CVID patients into 3 groups based on naïve CD4+ T cell levels that were more consistent with clinical manifestations than markers used in other recommended classifications. The 3 homogeneous groups in the Giovannetti subclassification were identified according to tertiles of naïve CD4+ T-cell levels

in PBMCs (<15%, 15%-29%, and ≥30%). In our study, we divided our patients on the basis of normal ranges of naïve cells (CD45RA+CD62L+) in the CD4+ T-cell pool isolated in the control group. Patients fell into 2 groups: group 1 consisting of 11 patients (≤33% naïve CD4+ T cells), and group 2 consisting of 9 patients (>33%). Male patients were again significantly more present in the first group, characterized by lower naïve CD4+ T-cell levels, more complications (eg, autoimmunity), and greater disease severity.

In the classification by Giovannetti et al [27], naïve CD4+ T-cell levels were related to overall clinical severity and splenomegaly. In our study, splenomegaly, while not significant, was more common in the first group. Autoimmunity was also present only in this group.

Previous studies have reported B-cell defects in CVID patients with autoimmunity, and decreased thymic output has been documented in studies investigating diverse autoimmune diseases [36,37]. To the best of our knowledge, our study is the first to report significantly decreased levels of naïve CD4+ T cells and RTE cells in CVID patients with autoimmunity compared to those without. This suggests that autoimmunity might be related to lower thymic output as a cause or a consequence. It should, however, also be borne in mind that this is a preliminary finding that needs to be investigated further with larger numbers of patients.

In conclusion, our study shows T-cell defects in male CVID patients and reports a good correlation between classification based on naïve CD4+ T cell levels and clinical manifestations. However, studies with larger numbers of patients, a greater age range, and a focus on sex differences are recommended.

Acknowledgments

References

1. 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.

2. Livaditi O, Giamarellos-Bourboulis EJ, Kakkas I, Kapsimali V, Lymberi P, Papastariades C, Douzinas EE. Grouping of patients with common variable immunodeficiency based on immunoglobulin biosynthesis: comparison with a classification system on CD4-naive cells. Immunol Lett. 2007;114:103-9.

3. 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.

4. Aghamohammadi A, Rezaei N, Gharagozlou M, Ramyar A, Mahjoub F, Rezaei-Kalantari K, Moin M. Hodgkin lymphoma in two siblings with common variable immunodeficiency. Pediatr Hematol Oncol. 2007;24:337-42.

5. Aghamohammadi A, Pouladi N, Parvaneh N, Yeganeh M, Movahedi M, Gharagolou M, Pourpak Z, Rezaei N, Salavati A, Abdollahzade S, Moin M. Mortality and morbidity in common variable immunodeficiency. J Trop Pediatr. 2007;53:32-8.

6. 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.

7. Ramyar A, Aghamohammadi A, Moazzami K, Rezaei N, Yeganeh M, Cheraghi T, Pouladi N, Heydari G, Abolhassani H, Amirzargar AA, Parvaneh N, Moin M. Presence of Idiopathic Thrombocytopenic Purpura and autoimmune hemolytic anemia in the patients with common variable immunodeficiency. Iran J Allergy Asthma Immunol. 2008;7:169-75.

8. Aghamohammadi A, Parvaneh N, Rezaei N. Common variable immunodeficiency: a heterogeneous group needs further subclassification. Expert Rev Clin Immunol. 2009;5:629-31.

9. Bryant A, Calver NC, Toubi E, Webster AD, Farrant J. Classification of patients with common variable immunodeficiency by B cell secretion of IgM and IgG in response to anti-IgM and interleukin-2. Clin Immunol Immunopathol. 1990;56:239-48.

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. Chapel H, Cunningham-Rundles C. Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions. Br J Haematol. 2009;145:709-27.

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, 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.

14. 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.

15. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, Debre P, Schmitt C, Oksenhendler E. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003;23:385-400.

16. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92:34-48.

17. 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.

18. Di Renzo M, Serrano D, Zhou Z, George I, Becker K, Cunningham-Rundles C. Enhanced T cell apoptosis in common variable immunodeficiency: negative role of the fas/fasligand system and of the Bcl-2 family proteins and possible role of TNF-RS. Clin Exp Immunol. 2001;125:117-22.

19. Sneller MC, Strober W. Abnormalities of lymphokine gene expression in patients with common variable immunodeficiency. J Immunol. 1990;144:3762-9.

20. 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.

21. Rezaei N, Aghamohammadi A, Kardar GA, Nourizadeh M, Pourpak Z. T- helper 1 and 2 cytokine assay in patients with common variable immunodeficiency. J Investig Allergol Clin Immunol. 2008;18:449-53.

22. 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.

23. Farrington M, Grosmaire LS, Nonoyama S, Fischer SH, Hollenbaugh D, Ledbetter JA, Noelle RJ, Aruffo A, Ochs HD. CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. Proc Natl Acad Sci U S A. 1994;91:1099-103.

24. Oliva A, Scala E, Quinti I, Paganelli R, Ansotegui IJ, Giovannetti A, Pierdominici M, Aiuti F, Pandolfi F. IL-10 production and CD40L expression in patients with common variable immunodeficiency. Scand J Immunol. 1997;46:86-90.

25. Stagg AJ, Funauchi M, Knight SC, Webster AD, Farrant J. Failure in antigen responses by T cells from patients with common variable immunodeficiency (CVID). Clin Exp Immunol. 1994;96:48-53.

26. Kondratenko I, Amlot PL, Webster AD, Farrant J. Lack of specific

antibody response in common variable immunodeficiency (CVID) associated with failure in production of antigen-specific memory T cells. MRC Immunodeficiency Group. Clin Exp Immunol. 1997;108:9-13.

27. 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.

28. Kimmig S, Przybylski GK, Schmidt CA, Laurisch K, Mowes B, Radbruch A, Thiel A. Two subsets of naive T helper cells with distinct T cell receptor excision circle content in human adult peripheral blood. J Exp Med. 2002;195:789-94.

29. Guazzi V, Aiuti F, Mezzaroma I, Mazzetta F, Andolfi G, Mortellaro A, Pierdominici M, Fantini R, Marziali M, Aiuti A. Assessment of thymic output in common variable immunodeficiency patients by evaluation of T cell receptor excision circles. Clin Exp Immunol. 2002;129:346-53.

30. Nickel P, Kreutzer S, Bold G, Friebe A, Schmolke K, Meisel C, Jurgensen JS, Thiel A, Wernecke KD, Reinke P, Volk HD. CD31+ naive Th cells are stable during six months following kidney transplantation: implications for post-transplant thymic function. Am J Transplant. 2005;5:1764-71.

31. Zhang L, Lewin SR, Markowitz M, Lin HH, Skulsky E, Karanicolas R, He Y, Jin X, Tuttleton S, Vesanen M, Spiegel H, Kost R, van Lunzen J, Stellbrink HJ, Wolinsky S, Borkowsky W, Palumbo P, Kostrikis LG, Ho DD. Measuring recent thymic emigrants in blood of normal and HIV-1-infected individuals before and after effective therapy. J Exp Med. 1999;190:725-32.

32. Petridou E, Klimentopoulou AE, Moustaki M, Kostrikis LG, Hatzakis A, Trichopoulos D. Recent thymic emigrants and prognosis in T- and B-cell childhood hematopoietic malignancies. Int J Cancer. 2002;101:74-7.

33. Pido-Lopez J, Imami N, Aspinall R. Both age and gender affect thymic output: more recent thymic migrants in females than males as they age. Clin Exp Immunol. 2001;125:409-13.

34. Li Y. Recent thymic output function in patients with hematological malignancy. Hematology. 2005;10:297-305.

35. Hince M, Sakkal S, Vlahos K, Dudakov J, Boyd R, Chidgey A. The role of sex steroids and gonadectomy in the control of thymic involution. Cell Immunol. 2008;252:122-38.

36. Thewissen M, Somers V, Venken K, Linsen L, van Paassen P, Geusens P, Damoiseaux J, Stinissen P. Analyses of immunosenescent markers in patients with autoimmune disease. Clin Immunol. 2007;123:209-18.

37. Duszczyszyn DA, Beck JD, Antel J, Bar-Or A, Lapierre Y, Gadag V, Haegert DG. Altered naive CD4 and CD8 T cell homeostasis in patients with relapsing-remitting multiple sclerosis: thymic versus peripheral (non-thymic) mechanisms. Clin Exp Immunol. 2006;143:305-13.

▌ **Ahmad Massoud and Asghar Aghamohammadi**

Department of Immunology
2nd Floor, 7th Building
Tehran University of Medical Sciences
Ghods St, Enghelab Ave
Tehran 1417613151, Iran
E-mails: massoud.ahmad@yahoo.com,
aghamohammadi@tums.ac.ir