HUMAN LEUKOCYTE CLASS I AND II ANTIGENS IN IRANIAN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

A. Aghamohammadi[*1], A. Farhoudi[1], H. Hosseini Nik[1], Sh. Khazali[1], Z. Pourpak[1], F. Khosravi[2], A. Sarrafnezhad[2], AA. Amir-Zargar[2], N. Rezaei[1], K. Abolmaali[1], D. Mansouri[1] and B. Nikbin[2]

1) Department of Immunology and Allergy, Children Hospital Medical Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2) Department of Immunogenetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Common variable immunodeficiency (CVID) is a heterogeneous heritable disease characterized by arrest in B cell differentiation. An association between CVID and two HLA haplotypes, haplotype I (HLA-A1, HLA-B8, HLA-DR3) and haplotype II (HLA-A29, HLA-B44, HLA-DR7) has been previously documented. In the present study, we have attempted to find an association between susceptibility to CVID and HLA class I and II antigens in Iranian population. Seventeen Iranian patients with CVID (mean age 17, range 3-28 years; 12 male and 5 female), including two couples of brothers and 100 healthy controls were studied. All subjects were typed for HLA class 1, and 12 patients and all controls were typed for HLA class II, using microdroplet lymphocytotoxicity technique. Out of 12 CVID patients typed for HLA-DR and DQ specificities, five patients presented DR-1, which showed an increased frequency in patient (41.6% vs. 12% in controls), and 3 presented DQ-2, which also showed an increased frequency (25% vs. 4% in controls), both of which reached statistical significance (P = 0.018 and P = 0.026, respectively). HLA-DR10 was present in 2 patients (16.6%), which was markedly more frequent compared to controls, but this difference was not significant statistically. Our results suggest that HLA-DR1 and DQ-2 may contribute to susceptibility to CVID. We did not find any significant association between HLA-A1, B8 and DR3 that has been previously reported to be associated with CVID.

Acta Medica Iranica, 42(4): 272-276; 2004

Keywords: Common Variable Immunodeficiency, human leukocyte antigen

INTRODUCTION

Common variable immunodeficiency (CVID) is a heterogeneous primary immunodeficiency disorder, characterized by hypogammaglobulinemia and increased susceptibility to recurrent bacterial infections (1). The familial predisposition to CVID suggests that genetic factors influence disease susceptibility. Friedman was the first to show that genetic factors might be important by finding an increased incidence of autoantibodies in the serum of first-degree relatives of CVID patients (2). Although the molecular defect in this disease is unknown, different prevalence in different ethnic groups and familial clustering of the disorder (3,4) suggest involvement of a thus far unidentified susceptibility gene(s) in arresting B cell differentiation pathways (5,6), impairing T cell-mediated cell signaling (7,8) and/or isotype class switching (7-9).

Evidence for linkage with the HLA region has been obtained in some (10,11) but not all studies (4).It has been previously documented that all or part of two HLA haplotypes which are referred to as haplotype I (HLA-DQBl 0201, HLA-DR3, C4B-Sf,

Received: 29 Jun. 2002, Revised: 28 Sep. 2003, Accepted: 19 Nov. 2003

*** Corresponding Author:**
A. Aghamohammadi, Department of Immunology and Allergy, Children Hospital Medical Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 8966246, 8951462, Fax: +98 21 8956476
E-mail: shahin_kh@hotmail.com

C4A-0, Gl-15, Bf-0.4, C2-A, HSP-7.5, TNF alpha-5, HLA-B8, HLA-Al) and haplotype II (HLA-DQBl 0201, HLA-DR7, C4B-S, C4A-L, Gll-4.5, Bf-0.6, C2-b, HSP-9, TNF alpha-9, HLA-B44, HLA-A29) are associated with the susceptibility to CVID (12,13). An association between HLA alleles DR3, B8, and A1 and familial CVID has also been documented (14). In contrast, in a study done by de Asis and his colleagues on two-generation family with three members affected by CVID, HLA typing did not reveal HLA-A1, B8, DR3 haplotype in this family (15).

These findings prompted us to attempt to investigate if similar HLA class I and class II antigens were to be found in our CVID patients and also to seek confirmation of previous findings in a well defined Iranian CVID patient group.

MATERIALS AND METHODS

Subjects

Tehran University of Medical Sciences research center approved the study protocol. The children's Medical center, immunology and allergy clinic serves as a tertiary referral center for inhabitants of the province of Tehran.

The clinical records of all immunodeficient patients referred to this clinic or of those who regularly take their IVIG in the hospital were reviewed. After careful exclusion of uncertain cases, 17 CVID patients (5 females and 12 males, mean age 17 years, ranging 3-28 years) who were available and accepted to participate were included in this study. All individuals participating in the study were Iranian. Diagnosis of patients was established in accordance with accepted recommendations (16-18). Patients' laboratory data are summarized in table 1.

One hundred randomly selected healthy Iranian were used as controls.

All subjects and their families gave us their informed consent before their inclusion in this study.

HLA Typing

Typing antisera obtained from commercial sources were utilized for HLA A, B, C, DR, and DQ typing by the microdroplet lymphocytotoxicity test.

Table 1. Demographic and immunologic specifications of 17 CVID patients

No	Sex	Age	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)
1	M	8	289	120	54
2	F	11	490	60	50
3	M	11	320	40	< 20
4	M	15	180	25	45
5	F	15	280	Abs	Abs
6	M	3	160	< 20	< 10
7	M	18	440	210	41
8	F	15	530	20	46
9	F	17	260	150	< 10
10	M	7	250	228	27
11	M	11	180	< 20	< 10
12	M	28	230	120	44
13	F	25	185	90	32
14	M	9	320	100	< 20
15	M	5	350	51	47
16	M	12	290	32	19
17	M	13	450	80	50

Abbreviation: CVID, common variable immunodeficiency.

Statistical Analysis

Differences in the frequency of study parameters between two populations were analyzed by the chi square test or, in case of small numbers, Fisher's exact test. *P* values were not corrected for multiple comparisons.

RESULTS

All 17 patients were typed for HLA class I, and 12 patients were typed for HLA class II. The HLA typing results are summarized in table 2.

A, B, and C antigens frequency

The frequency of A, B, and C antigens in our patients and controls are summarized in table 3. This shows an increased frequency of HLA-A23 (5.8% vs. 3%), A30 (5.8% vs. 1 %), B7 (17.6% vs. 6%), B8 (17.6% vs. 10%), B21 (23.5% vs. 16%), B38 (17.6% vs. 6%), CW4 (58.8% vs. 37%), and CW6 (23.5% vs. 13%). Among these antigens, odds ratio for A23 (2.02), A30 (6.19), B7 (3.36), B38 (3.36), and CW4 (2.43) were remarkable, but none of these increases in frequencies reached statistical significance. Frequency of none of the other class I antigens was significantly different in two groups.

Table 2. HLA class I and II results in 17 CVID patients

	HLA Class I						HLA Class II					
No	**A**		**B**		**C**		**DR**				**DQ**	
1	A3		B5				DR3	DR52			DQ2	
2	A24		B35	B38	CW4		DR1	DR11	DR52		DQ1	DQ3
3	A1	A3	B38									
4	A26	A30	B13	B38	CW6		DR52	DR53				
5	A2	A28	B5	B15	CW4		DR52					
6	A28		B18	B22			DR6	DR2	DR52		DQ1	
7	A2	A28	B5	B21	CW4		DR1	DR6	DR52		DQ1	DQ2
8	A2		B5	B21								
9	A2	A23	B7	B12	CW4							
10	A24		B35	B8	CW4		DR3	DR7	DR52	DR53	DQ3	
11	A1	A11	B35		CW4	CW6						
12	A3	A24	B53		CW1	CW6	DR1	DR4	DR53		DQ1	DQ3
13	A11	A24	B35		CW4		DR4	DR11	DR52	DR53	DQ1	DQ3
14	A1	A2	B8	B21	CW6		DR1	DR10	DR53		DQ1	
15	A1	A2	B8	B21	CW6		DR1	DR10			DQ1	
16	A11		B7	B35	CW4		DR2				DQ1	DQ2
17	A11		B7	B35	CW4							

Abbreviation: CVID, common variable immunodeficiency.

Table 3. HLA-A, B, and C antigens frequency in CVID patients and normal subjects

	Patients(n=17)		Controls (n= 100)	Odds Ratio
Antigen	**N**	**%**	**%**	
A1	4	23.5%	30%	0.72
A2	6	35.3%	35%	1/01
A3	3	17.6%	21%	0.81
A11	4	23.5%	32%	0.65
A23	1	5.9%	3%	2.02
A24	4	23.5%	21%	1.16
A26	1	5.9%	24%	0.2
A28	3	17.6%	17%	1.05
A30	1	5.9%	1%	6.19
B5	4	23.5%	41%	0.44
B7	3	17.6%	6%	3.36
B8	3	17.6%	10%	1.93
B12	1	5.9%	11%	0.51
B13	1	5.9%	6%	0.98
B15	1	5.9%	15%	0.19
B18	1	5.9%	6%	0.98
B21	4	23.5%	16%	1.62
B22	1	5.9%	9%	0.63
B35	6	35.3%	35%	1.01
B38	3	17.6%	6%	3.36
B53	1	5.9%	18%	0.28
CW1	1	5.9%	7%	0.83
CW4	9	52.9%	37%	2.43
CW6	5	29.4%	13%	2.06

DR and DQ antigens frequency

The frequency of DR, and DQ antigens in our patients and controls are summarized in table 4. Five of the 12 CVID patients (41.6%) had HLA-DR1 antigen, whereas only 12 of the 100 healthy controls (12%) did so (odds ratio = 5.24). Also the result indicates that the frequency of HLA-DQ2 is remarkably higher than controls (25% vs. 4%; odds ratio, 8).

Table 4. HLA-DR and DQ antigens frequency in CVID patients and normal subjects

	Patients(n=12)		Controls (n= 100)	Odds Ratio
Antigen	**N**	**%**	**%**	
DR1	5	41.7%	12%	5.24
DR2	2	16.7%	37%	0.34
DR3	2	16.7%	24%	0.63
DR4	2	16.7%	37%	0.34
DR6	2	16.7%	4%	4.8
DR7	1	8.3%	19%	0.39
DR10	2	16.7%	3%	6.47
DR11	2	16.7%	17%	0.98
DR52	8	66.7%	66%	1.03
DR53	5	41.7%	55%	0.58
DQ1	8	66.7%	65%	1.08
DQ2	3	25.0%	4%	8
DQ3	4	33.3%	50%	0.5

P value for HLA-DR1 and HLA-DQ2 are 0.018 and 0.026 respectively. *P* values for other antigens are higher than 0.05.

The Fisher exact test confirmed a significant difference ($P = 0.018$ for HLA-DR1 and $P = 0.026$ for HLA- DQ2) between the frequencies of these two antigens in patients and controls. The results also showed an increased frequency of HLA-DR6 (16.67% vs. 4%; odds ratio, 4.8) and HLA-DR10 (16.67% vs. 3%; odds ratio, 6.47), but they were not statistically significant, although *P* value of 0.08 for HLA-DR10 was remarkable. Frequency of none of the other class II antigens was significantly different (Table 4).

DISCUSSION

Our results showed an increased frequency of some HLA class I antigens in patients comparing with controls, especially HLA-A30 with odds ratio of 6.19, but none of them reached statistical significance. While the association of HLA-A1 (12-14) and HLA-B8 (12-14,19) to susceptibility to familial CVID has been reported in previous studies, the frequency of HLA-Al in our patients was less than controls; and although HLA-B8 in the patients was more frequent than healthy controls, this was not significant statistically (Table 3). In our patients, just two brothers (cases no. 14 and 15 in table 2) possessed both these antigens (HLA-Al and B8). There was also a significantly increased frequency of HLA-B5 among patients with CVID in another Iranian study, which didn't occur in present study (20). Four out of 17 patients had HLA-A11, B35 (case 11, 13, 16, and 17) and another 4 patients had HLA-A2, B21 (case 7, 8, 14, and 15) (Table 2), but because we didn't type their families, we could not consider any of them as haplotypes to compare their frequencies in patients with general population.

The results of HLA class II typing showed an increased frequency of HLA-DRl and HLA-DQ2 antigens compared to normal controls (41.67% vs. 12% and 25% vs. 4%, respectively); these differences reached statistical significance ($P = 0.018$ and $P = 0.026$, respectively). The increased frequency of HLA-DR10 in our patients was remarkable (16.67% vs. 3%) but was not significant statistically ($P = 0.088$). While the association of HLA-DR3 (12, 13, 19), and in some studies, HLA-DR7 (12, 13) to susceptibility to familial CVID has been reported in previous studies, the frequency of them in our patients was less than controls.

Our results suggest that some HLA class II antigens (mainly HLA-DR1 and HLA-DQ2) confer an increased susceptibility to develop CVID in our patients; and like a few other studies (15,20), the reported association of HLA Al, B8, DR3 haplotype with familial CVID was not revealed in our study. Because of the strong linkage disequilibrium between the different MHC loci, it is extremely difficult to differentiate whether the primary association is with the gene(s) coding one or both of these two antigens, or another gene somewhere else within the MHC. Our different results from previous studies may be explained by the heterogeneous nature of CVID.

REFERENCES

1. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: Clinical and immunological features of 248 patients. Clin Immunol. 1999; 92(1): 34-48.

2. Schaffer FM, Palermos J, Zhu ZB, Barger BO, Cooper MD, Volanakis JE. Individuals with IgA deficiency and common variable immunodeficiency share polymorphisms of major histocompatibility complex class III genes. Proc Natl Acad Sci USA. 1989; 86(20): 8015-8019.

3. Koistinen J. Familial clustering of selective IgA deficiency. Vox Sang. 1976; 30(3):181-90.

4. Oen K, Petty RE, Schroeder ML. Immunoglobulin A deficiency: genetic studies. Tissue Antigens. 1982; 19(3): 174-182.

5. Conley ME, Cooper MD. Immature IgA B cells in IgA-deficient patients. N Engl J Med. 1981; 305(9):495-497.

6. Cooper MD, Lawton AR, Bockman DE. Agammaglobulinaemia with B Iymphocytes. Specific defect of plasma-cell differentiation. Lancet. 1971; 2(7728):791-794.

7. Eisenstein EM, Chua K, Strober W. B cell differentiation defects in common variable immunodeficiency are ameliorated after stimulation with anti-CD40 antibody and IL-10. J Immunol. 1994; 152(12):5957-5968.

8. 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 USA. 1994; 91(3): 1099-1103.

9. Islam KB, Baskin B, Nilsson L, Hammarstrom L, Sideras P, Smith CI. Molecular analysis of IgA deficiency. Evidence for impaired switching to IgA. J Immunol. 1994; 152(3):1442-1452.

10. Lakhanpal S, O'Duffy JD, Humburger HA, Moore SB. Evidence for linkage of IgA deficiency with the major histocompatibility complex. Mayo Clin Proc. 1988; 63(5): 461-465.

11. Wilton AN, Cobain TJ, Dawkins RL. Family studies of IgA deficiency. Immunogenetics. 1985; 21(4) :333-342.

12. Ashman RF, Schaffer FM, Kemp JD, Yokoyama WM, Zhu ZB, Cooper MD, Volanakis JE. Genetic and immunologic analysis of a family containing five patients with common-variable immune deficiency or selective IgA deficiency. J Clin Immunol. 1992; 12(6):406-414.

13. Johnson ML, Keeton LG, Zhu ZB, Volanakis JE, Cooper MD, Schroeder HW Jr. Age related changes in serum immunoglobulins in patients with familial IgA deficiency and common variable immunodeficiency (CVID). Clin Exp Immunol. 1997; 108(3): 477-483.

14. Schroeder HW Jr, Zhu ZB, March RE, Campbell RD, Berney SM, Nedospasov SA, Turetskaya RL, Atkinson TP, Go RC, Cooper MD, Volanakis JE. Susceptibility locus for IgA deficiency and common variable immunodeficiency in the HLA-DR3, -B8, -A1 haplotypes. Mol Med. 1998; 4(2): 72-86.

15. de Asis ML, Iqbal S, Sicklick M. Analysis of a family containing three members with common variable immunodeficiency. Ann Allergy Asthma Immunol. 1996; 76(6): 527-529

16. [No authors listed]. Primary Immunodeficiency diseases. Report of an IUIS committee. International Union of Immunological Societies. Clin Exp Immunol. 1999; 118(Suppl 1): 1-28.

17. Spickett GP, Misbah SA, Chapel HM. Primary antibody deficiency in adults. Lancet. 1991; 337(8736): 281-284.

18. Webster AD. Common variable immunodeficiency. Immunol Allergy Clin North Am. 2001; 21 (1): 1-22.

19. Alper CA, Marcus-Bagley D, Awdeh Z, Kruskall MS, Eisenbarth GS, Brink SJ, Katz AJ, Stein R, Bing DH, Yunis EJ, Schur PH. Prospective analysis suggests susceptibility genes for deficiencies of IgA and several other immunoglobulins on the [HLA-B8, SC01, HLA-DR3] conserved extended haplotype. Tissue Antigens. 2000; 56(3): 207-216.

20. Farhoudi A, Hashemian H. HLA typing in some immunodeficient patients. Thesis for PhD in laboratory science; Tehran University of Medical Sciences. 1995.