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ORIGINAL ARTICLE

FREQUENCY ANALYSIS OF HLA ANTIGENS IN IRANIAN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

Amir Amanzadeh DVM*, Mohammad-Ali Shokrgozar PhD*, Zahra Samadi-Bahrami MSc*, Asghar Aghamohammadi MD**, Fazel Shokri PhD* ***•

**National Cell Bank, Pasteur Institute of Iran, **Immunology and Allergy Clinic, Tehran University of Medical Sciences, ***Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

Background – **The etiology of most primary immunodeficiency disorders is unknown, though a variety of genetic imbalances have been reported to be implicated. The histocompatibility locus antigen (HLA) genes and antigens play a decisive role in immune regulation. Therefore, lower or higher representation of some HLA alleles may contribute to the presentation of some immunodeficiency conditions. One important human immunodeficiency that has recently been shown to be associated with particular HLA antigens is common variable immunodeficiency (CVID). We investigated for the first time the association between HLA antigens and this condition in Iranian patients.**

Methods – **Epstein-Barr virus (EBV)-transformed B-cell lines established from 16 patients with CVID and 85 healthy controls were screened using the microlymphocytotoxicity method, with a panel of anti-HLA antisera. The statistical of differences was determined using Chi-squere test with Yate's correction.**

Results – **Expression of HLA-A2 ($p < 0.02$) and A33 ($p < 0.001$) was significantly increased in patients compared to controls. A significant negative association was also evident for DR2 ($p < 0.05$), DR7 ($p < 0.001$), DR52 ($p < 0.05$), and DQ2 ($p < 0.05$) alleles.**

Conclusion – **Our study demonstrated a significantly greater representation of HLA-A2 and A33 and lower frequencies of HLA-DR2, DR7, DR52, and DQ2 in patients compared to controls. This may suggest involvement of the HLA complex in the presentation of CVID in the Iranian population.**

Keywords CVID HLA antigens immunodeficiency

Introduction

Common variable immunodeficiency (CVID) is a primary immunodeficiency disorder with unknown etiology diagnosed by absence of or decreased serum immunoglobulins (Igs) and increased susceptibility to infections.[1–2] In principle, the disease may present during childhood, but most cases are diagnosed in the third decade of life. The prevalence of CVID varies among different ethnic populations from 1 per 50,000–100,000 to 1 per 200,000 – 500,000.[1]

Although panhypogammaglobulinemia and susceptibility to recurrent infections are seen in all patients, CVID is clinically heterogeneous, and probably represents a group of disorders.[1, 3]

The high incidence of immunodeficiency and autoimmunity in relatives of these patients suggests that genetic factors are important.[4, 5] Recent observations support the hypothesis that CVID and selective IgA deficiency syndrome may reflect a common underlying genetic defect.[6]

Differential expression of some histo-compatibility locus antigen (HLA) class-I and class-II alleles and antigens has been found in

•**Correspondence:** *F. Shokri PhD, Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. P.O. Box: 14155-6446, Fax: +98-21-6462267 , E-mail: fazshok@yahoo.com.*

CVID patients of different ethnic origin.[7–13] Relatively small numbers of patients and a lack of clinical and essential immunophenotypic data limit previous reported HLA studies in CVID.

In the present study, the frequency of HLA class-I and class-II antigens was investigated in 16 Iranian patients with CVID using the micro-lymphocytotoxicity assay. To enhance sensitivity and precision of the assay, we used B-cell lines established from patients by Epstein-Barr virus (EBV) transformation.

Materials and Methods

Clinical samples

Heparinized peripheral blood was collected from 16 Iranian CVID patients attending the Allergy and Immunology Clinic of Children's Medical Center, Tehran University of Medical Sciences, over 18 months from March 1999 to September 2000.

Of the 16 patients, six were female and 10 were male; ages ranged from 4 to 25 years, with a mean of 14.6 ± 5.4 years. The diagnosis of CVID was based on a reduction in or an absence of three major serum immunoglobulin classes (panhypo-gammaglobulinemia), recurrent infections and exclusion of known causes of humoral immune system defects. All patients were receiving replacement therapy with intravenous immuno-globulin (IVIG) preparations (Table 1). Immuno-globulin isotype levels were measured using nephelometry and concentrations of the leukocyte-associated antigens CD3 and CD19 were determined using flow cytometry with specific monoclonal anti-bodies (Dako, Denmark).

Establishment of B-lymphoblastoid cell lines

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density gradient centrifugation using Ficoll-Paque (Sigma, USA) and transformed with EBV as previously described.[14] Briefly, PBMC were resuspended in filtered supernatant collected from EBV-infected B95.8 Marmoset cell line (NCBI C110, National Cell Bank of Iran) and incubated for 60 minutes at 37ºC under 5% carbon dioxide. The cells were then washed with RPMI-1640 medium (Sigma, USA) and resuspended in culture medium containing 1 ìg/mL cyclosporine A (Sandoz, Switzerland), penicillin 100 IU/mL, and streptomycin 100 ìg/mL (Sigma, USA). Outgrowth of immortalized B-cells was visible within 10 – 14 days from the time of infection.

HLA typing

HLA typing was performed using a standard microlymphocytotoxicity technique, as described elsewhere.[15] Briefly, Terasaki microtiter plates (Nunc, Denmark) containing various anti-HLA class-I and class-II antisera (Blood Transfusion Center) were seeded with $3 – 4 \times 10^3$ immortalized B-cells. After incubation at room temperature and addition of rabbit complement, cell viability was determined using 5% eosin dye (Merck, Germany) under an inverted microscope. Normal AB blood group serum was used as a negative control and antilymphocyte globulin and anti-HLA DR (polyspecific) antibodies were used as positive controls for HLA class-I and class-II microplates, respectively. Results were compared with the control group, which consisted of 85 EBV-transformed B-cell lines established from healthy individuals.

Data analysis

Statistically significant differences in expression of HLA antigens between CVID patients and controls were determined using the Chi-squared test with Yate's correction. Woolf`s relative risk (RR), etiological fraction (EF), and preventive fraction (PF) were calculated.[16] EF and PF values greater than 0.15 were considered to reflect positive and negative association, respectively. *P* values of less than 0.05 were considered significant.

Results

The major clinical and hematological findings of our CVID patients are delineated in Table 1. HLA-typing results obtained for HLA class-I antigens are summarized in Table 2. The frequency of expression of HLA-A2 ($p < 0.02$) and A33 ($p < 0.001$) was significantly greater in CVID patients compared to controls. Increased expressions of HLA-B22 and A24(9) were also observed in patients, although the differences were not statistically significant. A number of HLA class-I antigens including A3, A25, A28, and Bw4 were expressed at lower frequencies in patients than controls, but the differences were not statistically significant.

Of the HLA class-II antigens, HLA-DR11 and DQ3 were expressed more frequently in CVID patients than controls, but the difference was not statistically significant (Table 3). There was a significant negative association for DR2 ($p < 0.05$), DR7 ($p < 0.001$), DR52 ($p < 0.05$) and DQ2 ($p <$

Table 1. Major clinical and hematological findings in Iranian CVID patients.

Patient	Sex	Age	CD19%	CD3%	IgG mg/dL	IgA mg/dL	IgM mg/dL	WBC /ìL	Lymph %	PMN %	Mono %	Eosin %	Clinical Conditions
CVID 1	M	13	10	89	290	ab	ab	8000	25	58	8.8	2.2	Aseptic arthritis, Diarrhea, Pneumonia
CVID 2	F	25	10.1	88	ab	ab	ab	9300	42	64	4.0	1.0	Pneumonia, Chronic sinusitis
CVID 3	F	23	11.1	79	440	ab	ab	7000	34	65	1.0	2.0	Resp. infection, Diarrhea, Sinusitis
CVID 4	M	18	10	55	420	105	210	2000	31	7.0	5.0	7.0	Mastoidis, Resp. infection
CVID 5	F	10	2.7	82	250	ab	483	13070	21	59	10.8	2.1	Pneumonia, Hemolytic anemia
CVID 6	F	21	3.0	89	310	ab	480	6800	34	65	0.0	0.0	Pneumonia, Sinusitis, Otitis, Skin infection, Anemia
CVID 7	M	17	6.3	72	800	52	300	9200	48	38	6.0	3.0	Bronchiectasia
CVID 8	M	9	2.2	63	430	ab	42	3600	62	38	0.0	0.0	Otitis, Diarrhea, Gingivitis, FTT
CVID 9	M	12	28.7	64	90	10	17	10700	54	43	1.0	1.0	Resp. infection, Sinusitis, Otitis
CVID 10	M	10	15.5	62	380	3	25	8990	48	41	4.0	5.0	Resp. infection, Diarrhea, Sinusitis
CVID 11	M	14	NI	NI	450	24	25	NI	NI	NI	NI	NI	Resp. infection, Hepatosplenomegaly, Pyelonephritis
CVID 12	M	18	5.3	85	530	50	35	5800	40	59	1.0	0.0	Diarrhea, Sinusitis
CVID 13	M	4	21.9	67	310	18	48	6500	52	45	4.0	3.0	Pneumonia, Otitis
CVID 14	M	10	5.7	78	320	20	40	4200	51	47	1.0	1.0	TB, Chicken pox, Hepatitis, Splenomegaly, Pyelonephritis
CVID 15	F	17	6.0	58	580	20	60	9200	29	59	6.5	2.6	Resp. infection
CVID 16	F	13	20.7	68	520	23	20	NI	NI	NI	NI	NI	Otitis, Pneumonia, Resp. infection
Total *	M/F	14.6 (5.4)	10.6 (7.6)	73.2 (11.4)	408 (159.5)	32.5 (28.3)	137.3 (167.6)	7454.2 (2849.9)	40.8 (11.7)	49.1 (15.2)	3.8 (3.2)	2.1 (1.9)	

M = male; F = female; WBC = white blood cells; Lymph = lymphocytes; PMN = polymorphonuclear cells; FTT = failure to thrive; NI = not identified; Mono = monocytes; Eosin = eosinophils; Resp = respiratory; * mean (SD).

0.05) within CVID patients.

Discussion

Although the exact etiologic factor responsible for CVID remains unknown, extensive cellular and molecular investigations have revealed involvement of a variety of immunologic elements, including defects in CD27,[17] CD40 ligand,[18] and CD86 expression,[19] impaired antibody affinity maturation,[20] B-cell memory compartments,[21] enhanced T-cell apoptosis,[22] and imbalanced cytokine production.[23–25] A number of genetic abnormalities have also been frequently reported in this disease.[26–31]

CVI1D represents a defect in either B-cell development or function. Although some CVID and IgA deficiency syndrome patients have minimal numbers of circulating B-cells,[32, 33] most have normal numbers of IgG-, IgA-, and IgM-bearing B-cell precursors in their blood.[34] This is reflected in our results (Table 1).

Therefore, the defect most likely affects differentiation of mature circulating B-cells into antibody-secreting plasma cells. Help from T-cells and interaction with co-stimulatory molecules on antigen-presenting cells (APCs) are obligatory for differentiation of B-cells responding to a T-cell dependent antigen. Defective expression of the CD40 ligand has been identified in a small proportion of CVID patients.[18, 35] The CD40-CD40 ligand interaction is essential for B-cell development and immunoglobulin class-switching. However, apart from this small group of patients,

Table 2. Frequency of HLA class-I antigens in Iranian CVID patients.

HLA antigen	Controls n = 85 (%)	Patients n = 16 (%)	RR	EF	PF	Association	χ^2	p Value
A2	3 (3.5)	4 (25)	9.1	0.22	—	PA	6.6	< 0.02
A3	2 (2.3)	0 (0)	0	—	ND	NA	2.6	NS
A24	50 (58.8)	12 (75)	2.1	0.4	—	PA	0.9	NS
A25	1 (1.1)	0 (0)	0	—	ND	NA	3.3	NS
A28	27 (31.7)	3 (18)	0.5	—	0.16	NA	1.8	NS
A33	1 (1.1)	3 (18)	19.4	0.2	—	PA	6.8	< 0.001
B22	9 (10.5)	4 (25)	2.8	0.2	—	PA	1.4	NS
Bw4	58 (68.2)	8 (50)	0.5	—	0.36	NA	2.9	NS

RR = relative risk; EF = etiologic fraction; PF = preventive fraction; χ^2 = Chi-square with Yate`s correction; PA = positive association (EF > 0.15); NA = negative association (PF > 0.15); NS = non significant; ND = not determined due to insufficient number of subjects.

HLA antigens could be implicated in failure of B-cell differentiation in most patients.

HLA class-II molecules, which are expressed on the surface of APCs, present processed peptide fragments to T-lymphocyte receptors (TCR) and thereby restrict T-cell and B-cell responses to specific antigens.

A variety of HLA class-I, -II and -III alleles and haplotypes have been reported to be associated with CVID in different ethnic populations (Table 4).[8,13,30,36,45] This variation may reflect the variation observed in the prevalence of this disease among different ethnic groups.[11, 36 – 38] This indicates the importance of studies to identify the associated HLA antigens in a large number of ethnic populations.

In the present study, the association between HLA antigens and CVID was investigated in Iranian patients. Of the HLA class-I antigens, A2 and A33 were significantly increased in our patients. A lower frequency of A2 antigen expression in CVID patients was reported by other investigators,[8] but no reports have been cited in the literature on the association between A33 and CVID. The A1 and particularly B8 antigens have frequently been reported to be increased in CVID patients from other ethnic backgrounds,[8, 10, 11, 13, 30, 38, 40] but the prevalence of these antigens was not significantly different in our patients.

As for the HLA class-II antigens, similar findings to ours on the significant association with DR7 and DQ2 have also been reported.[8, 13] An association with the HLA-A1-B8-DR3 haplotype also became evident from previous studies.[7 – 12, 30, 38, 39]

Our results suggest that the identified HLA class-I antigens are positively associated with CVID, conferring higher susceptibility to the disease in Iranian patients. However, the class-II antigens were mostly negatively-associated with the disease, suggesting induction of a resistance to the disease in the normal population.

Finally the methodology employed in the present study to detect HLA class-II antigens is remarkable. EBV transformation results in overgrowth of B-lymphocytes, eliminating the tedious steps of separation for enrichment of B-lymphocytes. It also leads to significantly increased expression of both HLA class-I and particularly class-II antigens,[48] which subsequently

Table 3. Frequency of HLA class-II antigens in Iranian CVID patients.

HLA antigen	Controls n = 85 (%)	Patients n = 16 (%)	RR	EF	PF	Association	c2	p Value
DR2	22 (25.8)	1 (6.2)	0.2	—	0.2	NA	4.2	< 0.05
DR7	19 (22.3)	0	0	—	ND	NA	6.0	< 0.001
DR11	18 (21.1)	6 (37.5)	2.2	0.2	—	PA	0.6	NS
DR52	43 (50.5)	4 (25)	0.3	—	0.4	NA	4.7	< 0.05
DR53	43 (50.5)	5 (31.2)	0.4	—	0.3	NA	2.9	NS
DQ2	24 (28.2)	1 (6.2)	0.2	—	0.2	NA	4.8	< 0.05
DQ3	44 (51.7)	13 (81.2)	4.0	0.6	—	PA	3.6	NS
A9, DR11, DQ3	9 (10)	5 (31)	3.8	0.2	—	PA	3.2	NS

See footnotes to Table 2. NI = not identified.

Table 4. Statistically significant HLA antigens or alleles expressed in CVID* and IgA deficiency† patients from different countries.

Country	HLA	Patients *n*	Controls *n*	*p* Value	Ref.
UK*	A1, B7, B8, Cw7 0701, Cw7 0702 DR15, DR17, DQB1 0201, DQB1 0602	150	605	0.03, 0.0008, 0.001, 0.004, 0.0007, 0.001, 0.03, 0.01, 0.004	8
Spain*	DR4	42	334	0.05	12
Sweden*	DR1, DQw5	86	250	0.0001	37
	DR7, DQw2			0.05	
	DRw15, DQw6			0.001	
	DRw17, DQw6			0.01	
	DQw2			0.001	
Spain†	DR3, DR5, DR7, DR8 DQB1 0201, DQB1 0301	96	334	0.05	12
USA†	B8, DR3	4	30	0.04	30
Sweden†	DR1, DQw5	69	150	0.05	38
	DR7, DQw2			0.0001	
	DRw8, DQw4			0.05	
	DRw15, DQw6			0.001	
	DQw17, DQw2			0.0001	
	DQw7			0.05	
UK†	A1, B14	37	191	0.0007	40
	A28, B14			0.002	
	B14			0.0000006	
	A28			0.0007	
Canada†	A1, B8, B17	62	608	0.05, 0.0005, 0.005	41
Finland†	A1, B8, B13, Cw6	62	3445	0.0005	42
	B15			0.02	
	DR2, DR3			0.004, 0.0007	
	DR7			0.01	
Sweden†	DR1, DQw5	95	100	0.005	43
	DRw15, DQw6			0.001	
	DRw17, DQw2			0.0001	
	DR7, DQw2			0.02	
Sweden†	B8, DR3	36	272	0.001	44
	B40	35	272	0.05	
Sweden†	A1, A28, B8	19	272	0.05, 0.05, 0.01	45
	DR3	21		0.0005	
Italy†	A33, B8, B12, B14, B35 DR1, DQw1	44	NI‡	0.03,0.03,0.01, 0.0000008, 0.005, 0.00001, 0.0001	46
Hungary†	A1, B8	26	60	0.05	47
Iran	A2, A33	16	85	0.02, 0.001	Present study
	DR2, DR7, DR52, DQ2			0.05, 0.001, 0.05, 0.05	

‡ = not identified

enhances the precision and sensitivity of the classical HLA class-II detection assay.

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