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ARTICLE

HLA Antigens in Iranian Patients with B-cell Chronic Lymphocytic Leukemia

Seyed M MOAZZENI,[1,2] Ali A AMIRZARGAR,[3] Fazel SHOKRI[2]

[1]Department of Immunology, School of Medical Sciences, Tarbiat Modarres University, Tehran,
[2]Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran,
[3]Department of Immunology, Ahwaz University of Medical Sciences, Ahwaz, Iran

The frequency of HLA class-I and class-II antigens was investigated in 32 Iranian patients with B-cell chronic lymphocytic leukemia (B-CLL), using the microlymphocytotoxicity method. A significant increase in the HLA-B13 (P<0.01) and DR53 (P < 0.05) and a significant negative association with the A11 (P < 0.05), B35 (P < 0.05), Cw3 (P < 0.05), and DR1 (P < 0.02) antigens were observed in these patients, compared to the control normal population. These results suggest involvement of some HLA antigens in the multifactorial process of predisposition to B-CLL. (Pathology Oncology Research Vol 5, No 2, 142–145, 1999)

Keywords: HLA antigens, chronic lymphocytic leukemia

Introduction

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disease characterized by monoclonal proliferation and accumulation of relatively mature appearing lymphocytes in peripheral blood.[3,20] The leukemic cells are mostly originated from the B-lymphocyte pool (B-CLL) with T-lymphocytes being implicated in less than 5% of cases.[1,3,8] CLL is the most common type of the chronic leukemias in Western populations, but it is relatively rare in Orient and also in Iran.[9,12] Based on familial incidence and epidemiologic studies, the risk of familial leukemia is greater in CLL than any of the other leukemias, suggesting implication of some unknown genetic factors in etiology of CLL.[9,13,14] A number of genetic abnormalities have been reported in CLL including high expression of some oncogenes.[18] We have recently demonstrated a biased rearrangement of immunoglobulin variable region genes and chromosomal aberrations in CLL patients.[12,22] Different profiles of HLA antigens have also been reported in B-CLL patients from Western populations.[7,10,13] However, little is known about the pattern of expression of HLA antigens in this malignancy in the East.

In the present study, the frequency of HLA class-I and class-II antigens was investigated in 32 Iranian patients with B-CLL using the microlymphocytotoxicity assay.

Materials and Methods

Clinical samples

Heparinized peripheral blood was collected from 32 Iranian CLL patients attending the Oncology Clinic of Imam Khomaini Hospital, Tehran University of Medical Sciences. The age range at the onset of disease was 45 to 85 with a mean of 61 years. Twenty four patients were male and 8 were female, yielding a male/female ratio of 3. Diagnosis and staging of CLL were accomplished by criteria outlined by Rai et a1.[19] The major clinical, hematological and physical findings identified in our CLL patients are summarized in *Table 1*. The B-cell origin of leukemic cells was confirmed by immunophenotyping using monoclonal antibodies specific for CD19, CD20, CD5, CD3 and immunoglobulin heavy and light chain isotypes, using the methodology reported elsewhere.[22]

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Correspondence: Dr. F. SHOKRI, Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, P. O. Box : 14155-6446, LR. Iran.
Fax: +98 21 6462267; Tel: +98 21 8853021 (1)

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HLA typing

HLA typing was performed using standard microlymphocytotoxicity method.[25] Briefly, mononuclear cells (MNC) were separated from 5 ml peripheral blood by density gradient centrifugation using Ficoll-Paque (Pharmacia, Sweden) and suspended in RPMI-1640 medium (Sigma). $2.5x10^3$ of suspended MNC were added to each well of Terasaki microtiter plates (NUNC, Denmark) containing various anti-HLA class-I and class-II antisera (Biotest, Germany and Behring, Germany) under a thin layer of mineral oil. Following incubation at room temperature (RT) and addition of rabbit complement, cell viability was assessed under inverted microscope using 5% eosin dye (Merk, Germany). Normal serum from AB blood group, anti-lymphocyte globuline and anti-HLA DR (polyspecific) antibodies were used as negative and positive controls for HLA class-I and class-II microplates, respectively. The results were assessed and compared with the control group, as outlined by Sullivan and Amos.[23] The control group consisted of 100 healthy individuals who had HLA typing performed by Nikbin et al.[15]

Data analysis

Statistical significance of differences in HLA antigens expression between patients and controls was determined using Chi-square test with Yate's correction. Woolf's relative risk (RR), etiologic fraction (EF) and preventive fraction (PF) were calculated as reported elsewhere.[24]

Table 1. **Major clinical and hematological findings in Iranian B-CLL patients**

Patients	*Sex*	*Age (yr)*	*WBC count ($x10^6$/ml)*	*Lymphocyte percent*	*RBC count ($x10^9$/ml)*	*HTC*	*Hb (g/dl)*	*Plt. count ($x10^6$/ml)*	*Clinical symptoms*	*Disease stage*
CLL1	M	62	85	80	3.5	35	10.7	100	Hepat., Splen.	II
CLL2	M	52	64.3	94	4.9	46	17	163	Hepat., Splen.	II
CLL3	M	55	22.8	69	4.2	39.4	12.1	162	Lymph., Hepat., Splen.	II
CLL4	M	63	34.5	99	NI	NI	NI	89	NI	IV
CLI5	M	73	82.7	83	4.0	38	12.7	119	Lymph., Splen.	II
CLL6	M	46	9.9	77	6.2	40.7	13.6	240	Lymph.	I
CLL7	M	60	NI	66	4.4	39.8	14.8	206	Lymph.	I
CLL8	F	66	55.7	97	4.3	41.3	13.2	85	NI	IV
CLL9	M	65	87.6	95	5.3	46.2	15	131	None	0
CLL1O	F	54	17.7	91	4.9	46.2	13.8	105	Lymph., Splen.	II
CLL11	M	65	190	98	3.4	33	11	91	Lymph.	I
CLL12	M	63	24	78	5.3	48.3	15	243	None	0
CLL13	F	60	25	80	0.7	8.6	3.5	76	Lymph., Hepat.	IV
CLL14	M	63	39	94	5.7	59	16.5	143	None	0
CLL15	M	51	72	80	5.5	46	14.2	102	None	0
CLL16	M	75	23	97	6.0	47	15.6	150	Splen.	II
CLL17	F	50	27	80	4.4	35.7	12	122	Lymph., Hepat., Splen.	II
CLL18	F	63	58	98	4.8	45	14.8	109	None	0
CLL19	M	47	182	99	4.4	36	14.8	252	None	0
CLL2O	M	85	100	86	2.8	24	8.1	78	Splen.	IV
CLL21	M	66	26.5	80	3.8	37.5	12.3	174	Lymph.	I
CLL22	F	55	16.9	79	5.0	41.7	13.8	320	Lymph., Splen.	II
CLL23	M	60	8.1	78	5.4	45.3	15.3	106	None	0
CLL24	M	80	29.6	89	4.6	41	14.7	186	None	0
CLL25	F	60	11.3	80	5.5	46.7	14.7	154	Lymph., Hepat., Splen.	II
CLL26	M	45	21.9	80	4.8	44.5	14.3	169	Lymph., Hepat., Splen.	II
CLL27	M	63	27.4	81	5.3	41.7	14.3	141	Hepat., Splen.	II
CLL28	M	65	27.3	78	4.2	38.8	11.7	200	Lymph., Splen.	II
CLL29	M	57	143	98	4.5	44.2	14.6	120	Lymph.	I
CLL3O	M	65	14.1	79	5.2	48	14.7	197	Lymph., Splen.	II
CLL31	M	58	100	81	4.4	38.5	9.9	104	Lymph., Hepat., Splen.	III
CLL32	F	71	43.2	89	4.9	41.1	14	343	None	0

– M: male, F: female, WBC: white blood cells, RBC: red blood cells, HTC: hematocrite, Hb: hemoglobin, Plt: platelet, Lymph.: lymphadenopathy, Splen.: splenomegaly, Hepat.: hepatomegaly, NI: not identified.

The association was considered positive in those cases when the calculated EFs were higher than 0.15 and negative when calculated PFs were more than 0.15. [24] p values of less than 0.05 were considered significant.

Results

The results obtained for the frequency of expression of HLA class-I and class-II antigens in the CLL and control groups are shown in *Tables 2 and 3*.

Table 2. **Frequency (%) of HLA Class-I antigens associated with B-CLL in Iranian patients**

HLA Antigen	*Controls n=100*	*Patients n=32*	*RR*	*EF*	*PF*	*Xc^2*	*Pc*	*Association*
A2	35	56	2.39	0.33	–	3.71	NS	PA
A9	35	19	0.46	–	0.18	3.80	NS	NEA
A11	32	15.6	0.39	–	0.20	4.08	P < 0.05	NEA
B13	6	25	5.22	0.20	–	7.33	P<0.01	PA
B17	10	25	3	0.17	–	3.44	NS	PA
B35	35	12.5	0.26	–	0.26	7.03	P < 0.01	NEA
Cw3	38	18.7	0.12	–	0.57	4.95	P < 0.05	NEA
Cw5	4	0	NA	–	–*	3.06	NS	NEA

– RR: relative risk, EF: etiologic fraction, PF: preventive fraction, Xc^2: Chi-square with Yate's correction, Pc: p value of Chi-Square test, PA: positive association (EF>0.15), NEA: negative association (PF>0.15), NS: non-significant difference, NA not applicable, * due to inability to determine RR, the PF value can not be calculated. – Only the results showing either positive or negative association are given.

As outlined in *Table 2* an increase in the frequency of expression of HLA-B13, B17 and A2 was observed in the CLL patients, though only the former one being statistically significant.

Lower frequencies compared to the control group were found for the HLA-A9, A11, B35, Cw3 and Cw5 antigens, howewer, only those of the A11, B35 and Cw3 were statistically significant.

Among HLA class-II antigens, the frequency of HLA-DR53 was significantly higher in CLL patients than the control group.

Frequencies of HLA-DR52 and DQ1 were also higher in CLL patients but the differences were not statistically significant (*Table 3*). The frequencies of HLA-DR1 and DR2 were lower in CLL patients, but only the negative association with HLA-DRl antigen was statistically significant.

Table 3. **Frequency (%) of HLA class-II antigens associated with B-CLL in Iranian patients**

HLA Antigen	*Controls n=100*	*Patients n=32*	*RR*	*EF*	*PF*	*Xc^2*	*Pc*	*Association*
DR1	12	0	NA	–	–*	5.80	P < 0.0 2	NEA
DR2	37	25	0.57	–	0.16	2.13	NS	NEA
DR52	66	81	2.23	0.45	–	2.00	NS	PA
DR53	55	78	2.92	0.51	–	4.50	P < 0.05	PA
DQ1	65	81	2.33	0.46	–	2.28	NS	PA

– RR: relative risk, EF: etiologic fraction, PF: preventive fraction, Xc^2: Chi-square with Yate's correction, Pc: p value of Chi-Square test, PA: positive association (EF>0.15), NEA: negative association (PF>0.15), NS: non-significant difference, NA: not applicable, * due to inability to determine RR, the PF value can not be calculated. – Only the results showing either positive or negative association are given.

Discussion

The influence of MHC on development of malignancy has long been known in experimental animals.[16,17] More than one MHC genes are thought to be involved in this complex multifactorial process.[5,16] Similar studies have been carried out in human, investigating the role of HLA complex in malignancies. The haemopoietic malignancies have been the most extensively studied ones.[2,6,7,11] However, no consistent HLA associations have been established in these malignancies by serology. In B-CLL fen studies have been reported, with conflicting results.[2,6,7,10,13]

In the present study, the association between HLA antigens and B-CLL was investigated in Iranian patients. Of the HLA class-I antigens, B13 was found to be significantly increased in our patients, whereas the frequencies of A11, B35 and Cw3 were significantly lower in these patients compared to the control group, suggesting their protective roles in this leukemia. Apart from B35, association of the other three antigens has not already been reported in B-CLL. Surprisingly, contrary to our finding, B35 was reported to be significantly increased in a population of Jewish B-CLL patients of European origin.[2] A signif-

icant increased frequency of A1, A2, B8, B12 and Cw6 have also been reported in CLL patients, by some investigators,[7,11,13] though not confirmed by our results and others.[6] The frequency of A2 was also higher in our patients, but the difference was not statistically significant.

Our findings of the significant positive association with DR53 has also been reported by Dorak et al.[6] However, this association was more profoud in patients with early onset of disease. Furthermore, increased susceptibility was evident only in patients with homozygous, but not heterozygous genotypes for DR53. This latter finding which can not be clearly attested by the current serological methodology and requires molecular and genetic tools, magnifies our results. Indeed, if homozygosity is examined, the observed significant, but relatively weak DR53 association in our CLL patients, could be stonger. Interestingly, susceptibility to a number of other acute and chronic leukemias, including AML,[21] ALL[5] and CML,[4] has also been demonstrated to be linked to the DR53 antigen. Therefore, the DR53 antigen could be regarded as a common target, together with some other unidentified etiologic factors, providing susceptibility to a variety of hemopoietic malignancies. This does not rule out involvement of some environmental and health factors in the mutifactorial process of predisposition to B-CLL in our patients, which requires further epidemiological investigation.

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