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REVIEW ARTICLE

HLA AND DISEASE ASSOCIATIONS IN IRAQ

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SUMMARY

The HLA system is deeply involved in susceptibility to a variety of diseases. Relationships between HLA and diseases are of considerable interest and importance, as they provide new tools for studying the inheritance, classification, and pathogenesis of these diseases. Studies on the distribution of HLA antigens in different populations have revealed the existence of racial variation and are therefore a prerequisite for studying HLA and disease associations in different racial groups.

This study reviews six articles concerning HLA and disease in the Iraqi population.

A comparison of these associations and an analysis of overall antigen frequencies among other Arab population and different ethnic groups are included. Some of our HLA-disease associations confirm other studies reported in these racial groups, while other diseases showed different HLA antigen associations from those recorded in other racial groups.

KEY WORDS HLA Iraq Ankylosing Spondylitis Graves Disease Lymphoma Coeliac Disease Herpes Simplex Diabetes

INTRODUCTION

The first indication of MHC-disease association came with the discovery that susceptibility to virus-induced Leukaemia in mice was genetically determined, and that alleles of one of the loci responsible segregated with those of H-2 system (Lilly *et al*, 1964). In man, the first suggestive evidence of such an association was found by Amiel, (1967) who studied Hodgkin's disease which is analogous to the mouse Leukaemia, and found an association between this disease and a group of HLA-B Lous antigens (4C) which were later found to be the B5, B35, B15, and B18 cluster. During the next 25 years, a progressively larger number of diseases has been reported to have susceptibility associated with or linked to the HLA system (for review see Dausset and Svejgaad, 1977; Tiwari and Terasaki, 1985).

Studies on the distribution of HLA antigens in different populations have revealed the existence of racial variations which have been attributed to gene drift, gene flow, or possibly to linkage of certain immune response genes in such a way that individuals with certain phenotypes have better survival, i.e. selective advantage (Forbes *et al*, 1973, Hill

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et al., 1992). Therefore this racial variation in the frequency of HLA antigens may be of importance for studying HLA and disease in different racial groups. Iraq is an Arab country with a population of 16 million. Up to the present, six studies concerning disease association with HLA antigens have been accumulated.

MATERIALS AND METHODS

HLA typing was performed on lymphocytes isolated from peripheral blood by a gradient centrifugation technique (Boyum, 1968) using the two-stage lymphocytotoxicity method (Terasaki and Maclelland, 1964) or one-stage microcytotoxicity (Kissmeyer-Neilsen and Kjerbye, 1968). HLA antisera used in these studies are from the issue Typing and Clinical Immunology Laboratory of the Royal Infirmary, Glasgow. Additional sera were provided from Hoechst (W. Germany) and from local sources. Patients and controls from the same population were typed for HLA-A, -B, C. DR antigens were examined in only two studies. HLA phenotypic and genotypic frequencies in patients as compared to the controls were determined for each study. Gene frequencies (g) were estimated using the formula, $g=1-\sqrt{1-f}$, where f in the antigen frequency. Relative risk (RR) was calculated using the formula:

$$RP=\frac{\text{No. of patients positive for the antigen} \times \text{No. of controls negative for the antigen}}{\text{No. of patients negative for the antigen} \times \text{No. of controls positive for the antigen}}$$

RESULTS AND DISCUSSION

Diseases for which HLA association have been tested in Iraq are ankylosing spondylitis, Graves' disease, diabetes mellitus, coeliac disease, Lymphomas, and recurrent herpes simplex virus infection (Table 1). HLA antigens and gene frequency in the Iraqi population are shown in Table 2. When we compare antigen frequencies of the Iraqi population with other ethnic groups (Terasaki, 1980) (Table 3) it appears that HLA-A9, HLA-B5, HLA-B35 show considerably elevated frequencies within class 1 HLA antigens. Table 4 shows similar comparisons for HLA-DR antigens. DR2 shows the highest frequency (56%), DR5 and DR8 the lowest (0.04% for DR5 and 0.02% for DR8). Comparing these findings with the figures for HLA-DR antigens from other Arab populations, it appears that HLA-DR7 has a 46% frequency in Saudi Arabs (Ollier *et al.*, 1985), while it shows only 27% in this study and 25.7% in Tunisians (Ayad *et al.*, 1987). HLA-DR2 occurs in 19% of Saudi Arabs and 19.3% of Tunisians. There are no significant differences in phenotype frequencies for the other HLA-DR antigens as shown in Table 5. The frequencies of DR antigens for the three Arab populations studied are broadly similar to those reported for several other racial groups (Tables 5 and 6).

Table 1. HLA antigens and diseases studied in Iraq.

DISEASE	HLA ANTIGENS ASSOCIATED	FREQUENCY IN PATIENTS	FREQUENCY IN CONTROLS	RELATIVE RISK R.R.
1 Ankylosing spondylitis	B27	48%	2.1%	244.2
2 Graves' disease	BW40	45%	15%	4.64
3 Coeliac disease	B8	40%	5%	12.6
4 Non-Hodgkins Lymphoma	A29	26%	7.3%	4.52
	B15	21.4%	2.7%	9.93
5 Insulin dependent diabetes mellitus	A1	60%	28%	3.95
	B8	46%	11%	6.88
	DR3	58%	25%	4.14
	DR4	52%	15%	6.14
	B5	30%	57%	0.32
	DR2	4%	56%	0.03
6 Recurrent herpes simplex viral infection	A1	51%	25%	3.16
	B5	65%	45%	2.19
	DR1	39%	21%	2.29
	A3	24%	13%	2.00
	A23	25%	11%	2.37

DISEASE ASSOCIATIONS

As shown in Table 1, some of the diseases studied showed different HLA-antigens association in Iraqi Arabs when compared with other ethnic groups or, when association was with the same antigen, different relative risk values were found.

In the Iraqi population ankylosing spondylitis was observed in 0.07% (Al-Rawi *et al.*, 1978). HLA-B27 was found in 21 out of 25 patients included in this study (84%) but in only two out of 95 normal controls (2.1%) with a relative risk of 244. In Britain Brewerton and colleagues (1973) found an incidence of 96% in patients and 4% in controls. Schlosstein *et al.* (1973) found the incidence of HLA-B27 to be 88% in patients and 8% in controls in the U.S.A.

Among the auto immune diseases studied, Graves disease has a positive association with HLA-B8 in Caucasians (Grumet *et al.*, 1974, Whittingham *et al.*, 1975) while it is associated with HLA-B55 in the Japanese (Grumet *et al.*, 1976). In the Iraqi population

Table 2. Absolute number, phenotype and gene frequency of Iraqi population n=579.

HLA-Antigen	Absolute No.	Phenotype Frequency	Gene Frequency
A1	159	0.27	0.150
A2	221	0.38	0.213
A3	102	0.18	0.095
A9	143	0.25	0.134
A10	81	0.14	0.073
A11	88	0.15	0.078
A28	66	0.11	0.057
A29	84	0.15	0.078
A30	28	0.05	0.025
A31	19	0.03	0.015
A32	14	0.02	0.001
A33	28	0.05	0.025
BLANK	125	0.22	
B5	262	0.45	0.258
B7	75	0.13	0.067
B8	82	0.14	0.73
B12	63	0.11	0.057
B13	48	0.08	0.041
B14	41	0.07	0.036
B15	31	0.05	0.025
B16	8	0.01	0.005
B17	58	0.10	0.052
B18	38	0.06	0.031
B21	88	0.15	0.078
B22	23	0.04	0.022
B27	6	0.01	0.005
B35	153	0.26	0.140
B37	11	0.02	0.001
B38	2	0.003	0.015
B40	55	0.09	0.046
B41	27	0.05	0.025
BLANK	87	0.15	

Graves disease patients have a significantly increased frequency of HLA-B40 (Al-Zubaidi, 1978). In this study the frequency of B40 in patients was 45% with relative risk of 4.46. HLA-W19 and HLA-B35 antigens show significantly decreased frequencies. In Graves disease, there has been no previous report of negative associations in Caucasians (Grumet *et al.*, 1974; Whittingham *et al.*, 1975) nor in Japanese (Grumet *et al.*, 1976).

Analysis of HLA antigens among Iraqi children with coeliac disease showed a significant increase in frequencies of B8 and B12, with a relative risk of 14.05 for B8 and 4.58 for B12 (Dawood *et al.*, 1981). In this study four families were included in which one child has the disease. Four of five siblings who inherited the HLA-B8 antigen have developed coeliac disease. In one of the families both siblings have HLA-B8 but only one of them contracted the disease. Robinson *et al.* (1971) and Mylotte *et al.* (1972) have suggested that the inheritance of coeliac disease is polygenic. This was later supported by Demarchi *et al.* (1979) who found strong association with both DW3 and DW7.

A study by Ven den tweel *et al.* (1982) showed a significant association between HLA-w33 (a split of HLA-W19) and B-cell lymphoma in Caucasoids, and between HLA-AW24 and B40 and B-cell non-Hodgkins lymphoma in Negroid patients. In the Iraqi population one study on lymphomas (Jabbar and Yassin, 1984) examined the association of lymphomas with HLA-A and B locus antigens in fifty patients from the southern region. Forty-two of these lymphomas were of non-Hodgkins type and eight patients had Hodgkins disease. In our study Hodgkins disease was found to be associated with HLA-A1, B5 and B15. None of the patients carried HLA-B8. However the number of patients was very small. Increases of HLA-A29 were found in patients with non-Hodgkins lymphoma as compared with the control group. The relative risk was 4.52 for HLA-A29 and 9.93 for B15. The increased frequency of HLA-W33 reported by Ven den tweel *et al.* (1982) might be comparable to our finding of an increase in the frequency of HLA-A29 because both are regarded as splits of the compound antigen, AW19. Apart from B15, no significant association with other antigens of the 4C group were detected.

Behbehain *et al* (1987) have studied HLA-A, B, C, DR and DQ antigens frequencies in Arab patients with IDDM, and found a significant positive association with HLA-DR3 and DR4 and a negative association with DR2 and DR5. HLA-B8 and B18 also showed increased frequencies in those patients, but with a variation between the two groups studied (Gulf and non Gulf residents in Kuwait). In the Iraqi population we found highly significant associations of HLA-A1, B8, DR3 and DR4 with IDDM.

These results are in agreement with those found in Caucasoids except for the association with B15 which we could not detect. The association found for HLA-A1 may be due to linkage disequilibrium with B8. B7 and DR2 have been called protective genes since they are found with much lower frequency in diabetics than in the general population (Bhatia, 1985). In the present study we have found a significant negative association between HLA-B5 (not B7) and HLA DR2 and IDDM. As in all reported studies, there was no HLA relationship with non insulin dependent diabetes mellitus (NIDDM).

Few studies of the association between HLA antigens and recurrent herpes simplex viral infections have been undertaken. Russel and Schlant (1975) reported a significant association between HLA and recurrent herpes labialis. Volker-Dieben (1984) found that HLA-A3 was significantly more frequent in patients with recurrent herpes keratitis. Zimmerman *et al.* (1977) reported in increased frequency of HLA-B5 in that condition.

Table 3. HLA gene frequency (%) in Iraqi population compared with other population.

HLA-antigen	Iraqi Population No. 579	European Caucasoid No. 228	African Blacks No. 128	Japanese No. 195
HLA - A1	15.0	15.8	3.9	1.2
A2	21.3	27.0	9.4	25.3
A3	9.5	12.6	6.4	0.7
A9	13.4	5.6	6.5	37.2
A10	7.3	4.9	4	12.7
A11	7.8	5.1	0	6.7
A28	5.7	4.4	8.9	0
A29	7.8	5.8	6.4	0.2
A30	2.5	3.9	22.1	0.5
A31	1.5	2.3	4.2	8.7
A32	0.1	2.9	1.5	0.5
A33	2.5	0.7	1.0	2.0
BLANK	16.7	2.2	11.0	4.2
B5	25.8	5.9	3.0	20.9
B7	6.7	10.4	7.3	7.1
B8	7.3	9.2	7.1	0.2
B12	5.7	16.6	12.7	6.5
B13	4.1	3.2	1.5	0.8
B14	3.6	2.4	3.6	0.5
B15	2.5	4.8	3.0	9.3
B16	0.5	2.7	1.5	3.7
B17	5.2	6.7	16.1	0.6
B18	3.1	6.2	2.0	0
B21	7.8	2.2	1.5	1.5
B22	2.2	3.6	0	6.5
B27	0.5	1.6	0	0.3
B35	14.0	9.9	7.2	9.4
B37	0.1	1.1	0	0.8
B38	1.5	0	0	0
B40	4.6	8.1	2.0	21.8
B41	2.5	0	1.5	0
BLANK	7.8	3.6	17.9	7.6

Table 4. The absolute number, phenotype and gene frequency of HLA-Class II antigens (DR) among Iraqi individuals.

HLA Antigen	Absolute Number	Phenotype Frequency	Gene Frequency
DR1	39	0.18	0.095
DR2	119	0.56	0.330
DR3	54	0.25	0.134
DR4	32	0.15	0.078
DR5	8	0.04	0.020
DR6	23	0.11	0.057
DR7	60	0.28	0.140
DRW8	4	0.02	0.010
BLANK	93	0.43	0.245

Table 5. HLA-DR Phenotype frequencies in Iraqis, Tunisians and Saudi Arabs.

DR Antigens	Iraqis N=216	Tunisians N=109	Saudi Arabs N=100
DR1	0.18	0.183	0.160
DR2	0.56	0.193	0.190
DR3	0.25	0.284	0.270
DR4	0.15	0.202	0.300
DRW 11		0.294	
DR5	0.04		0.150
DRW 12		0.237	
DRW 13		0.156	
DR6	0.11		0.272
DRW 14		0.046	
DR7	0.26	0.257	0.460
DR8	0.02	0.027	0.020
DR9	NT	0.000	NT
DR 10	NT	0.000	0.010
BLANK	0.43	0.321	--

Table 6. HLA-DRW Locus frequencies in Iraqi population in comparison to other population.

Allele	European Caucasoids	North American Caucasoids	American Blacks	African Blacks	Japanese	American Indians	Iraqi Population
DRW1	6.2	5.2	7.3	-	4.5	-	9.5
DRW2	11.2	13.8	13.8	8.7	16.5	8.4	33.0
DRW3	8.9	12.4	12.4	11.7	-	9.1	13.4
DRW4	7.8	7.2	7.2	3.5	14.4	21.5	7.8
DRW5	15.1	15.4	15.4	7.4	5.4	6.0	2.0
DRW6	8.6	19.1	19.1	9.9	6.7	5.9	5.7
DRW7	15.6	12.0	12.0	6.6	-	3.7	14.0
DRW8	5.6	7.5	7.5	7.2	7.2	12.9	1.0
BLANK	21.1	5.3	5.3	45.0	45.3	32.5	24.5

In the Iraqi population analysis of HLA antigens in 150 patients with recurrent HSV labialis compared to 176 normal Iraqi controls showed a highly significant association with HLA-A1, B5 and DR1 (Jabbar *et al.* 1990). HLA-A23 was also significantly associated with disease. No differences in association with these HLA antigens were found when the patients were subdivided into groups according to rate of recurrence or ELISA reading of antibody titre. Several observations suggest that antibody is not primarily important in preventing recurrence of herpes labialis (Overall, 1984). However, there is some evidence for defects in cell mediated immunity, in otherwise normal patients with recurrent herpes labialis (Kirchner, 1982).

Differences between populations in the pattern of HLA associations with the same diseases support the view that the findings are really due to association with alleles of immune response genes in the HLA region controlling susceptibility through differences in the ability to respond to various immune stimuli (Benaceraff and McDevitt, 1972).

Such comparisons between genetically disparate groups can therefore have considerable impact on our understanding of disease heterogeneity and the inheritance of disease susceptibility, increasing our knowledge of the biological function of the HLA system.

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