

HISTOCOMPATIBILITY ANTIGENS IN OMANIS: COMPARISON WITH OTHER GULF POPULATIONS AND IMPLICATIONS FOR DISEASE ASSOCIATION

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Background: This is the first comprehensive report of HLA antigens in Omanis, and the first application of HLA sequence-specific primer (SSP) DNA typing in a Gulf population. The objective was to compare the findings with other Gulf populations and assess their implications for disease association.

Patients and Methods: HLA typing was carried out on 321 healthy Omanis. One hundred and twenty-six of these were typed for Class II antigens by low-resolution SSP DNA typing. The results were compared with other HLA antigen frequencies recorded from Kuwait and Saudi Arabia.

Results: The Omani population was characterized by a very high incidence of HLA-DR2 (66%), with associated HLA-DQ1 (76%) and a reduced incidence of DR4, DR7 and DR53. The incidence of DR2 is the highest recorded worldwide. HLA-A11, A32, B17, B35 and B40 were significantly higher than in Kuwait and Saudi Arabia, and A9, B21(B50) significantly lower ($P < 0.05$). HLA-B27 is very low in the Omani population (0.3%). The high incidence of HLA-DR2 in Oman and disparities in the frequency of other antigens would indicate that there has not been any significant migration from northern Arabia. Class II DNA typing revealed that DR16 was the predominant split of DR2 (63%), with DR15 being 18% and both DR15 and 16 being found in 6%, giving a total of 87% for "DR2"-associated antigens (serology of the same individuals gave a DR2 incidence of 74%). The major disparity between serology and DNA typing was in the definition of DR4 (serology 8%, DNA 14%) and DR51 (53% vs. 70%).

Conclusion: The frequency of many HLA antigens in Omanis differs significantly from frequencies found in the populations of Kuwait and Saudi Arabia, possibly reflecting different migration patterns. The high incidence of HLA-DR2 in Oman may have important implications for disease association.

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Oman is situated on the southeast coast of the Arabian peninsula, sharing borders with the United Arab Emirates, Saudi Arabia and Yemen. It covers an area of 305,000 square kilometres and has a population of approximately two million, of which more than half are under 15 years of age. The Omani population is predominantly Arab (74%), while a proportion of the rest have connections with the Indian subcontinent and Africa.

To date, no detailed information has been published on histocompatibility antigens in Oman. Three recent studies from this department have given limited information in relation to disease association.¹⁻³

We describe here the results of a study on 321 Omanis studied by HLA serology, 126 of which were also

TABLE 1. Comparison of HLA-A antigen frequencies in Omanis with other Arabian Gulf populations.

HLA-A antigen	Percentage frequency				
	Omanis n=321	Saudis ⁶ n=407	Saudis ⁷ n=1145	Saudis ⁸ n=109	Kuwaitis [*] n=100
A1	15	14	20	13	26
A2	40	46	25	42	36
A3	10	10	17	14	14
A9	14**	24	33	29	23
A10	18	17	12	8	10
A11	20**	5	7	8	11
A19	36	26	40	43	28
A23	4	NT	NT	10	NT
A24	10	NT	NT	20	0
A25	0	NT	NT	NT	0
A26	12	NT	NT	8	NT
A28	10	22	15	21	18
A29	2	5	NT	28	8
A30	18	7	NT	13	10
A31	3	3	NT	12	1
A32	21**	2	NT	4	1
A33	8	NT	NT	14	NT
A34	0	NT	NT	NT	NT
A36	0	NT	0	1	NT

*Kuwait data (A.G. White, unpublished); ** $P < 0.02$; NT=not tested.

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TABLE 2. Comparison of HLA-B antigen frequencies in Omanis with other Arabian Gulf populations.

HLA-B antigen	Percentage frequency				
	Omanis n=321	Saudis ⁶ n=407	Saudis ⁷ n=1145	Saudis ⁸ n=109	Kuwaitis* n=100
B5	39	37	36	37	27
B7	4	14	11	15	8
B8	18	16	15	11	6
B12	5	5	7	5	12
B13	3	2	5	3	3
B14	4	6	4	6	6
B15	6	8	8	8	8
B16	4	5	5	9	NT
B17	21**	12	11	11	11
B18	6	2	4	3	1
B21	8**	32	28	39	12 [†]
B22	5	3	5	NT	4
B27	0.3	2	3	1	4
B35	30**	19	19	15	19
B37	2	2	2	2	NT
B38	1	NT	NT	5	NT
B40	15**	3	4	4	8
B41	1	NT	4	9	NT
B42	1	NT	1	3	NT
B44	3	NT	NT	5	NT
B45	2	NT	NT	NT	NT
B48	0.3	NT	0	NT	NT
B49	3	NT	NT	3	5
B50	2*	NT	NT	38	NT
B51	21	NT	NT	26	NT
B52	2	NT	NT	3	NT
B53	3	NT	0	10	NT
B55	3	NT	NT	NT	NT
B57	1	NT	NT	NT	NT
B60	0	NT	NT	2	0
B61	0	NT	NT	2	NT
B62	1	NT	NT	NT	NT
Bw4	69	NT	NT	62	NT
Bw6	79	NT	NT	83	NT

*Kuwait (A.G. White, unpublished); ** $P_c > 0.05$; [†]data not included in significance calculation; NT=not tested.

investigated for Class II DNA polymorphisms. The results are compared with HLA antigen frequencies recorded from Kuwait and Saudi Arabia.

Patients and Methods

The Omanis in this study were healthy and comprised blood donors, potential kidney and bone marrow donors from all parts of the country, and were considered representative of the normal population. None of the Omanis was related to each other.

For detection of HLA antigens by serology, HLA-A and B antigens were investigated in all 321 donors, HLA-DR and DQ antigens in 283 donors, and Class II DNA in 126 donors. For serological HLA-AB and DR typing, 15-20 mL of blood taken into EDTA was used. Lymphocytes were separated by density gradient centrifugation and then the

Class II positive cells (mainly B lymphocytes) were separated, using magnetic beads (Dynabeads, Dynal, Skoyen, Norway). The supernatant containing T cells was used for A and B typing and the purified B cells for HLA-DR (Class II) typing. HLA typing was performed using a modified two-stage cytotoxicity technique with ethidium bromide/acridine orange staining and observation with a semi-automated fluorescent microscope.¹

The following serological specificities could be determined (76 in total):

A locus: 1,2,3,9,10,11,19,23,24,25,26,28,29,30,31,32,33,34,36.

B locus: 5,7,8,12,13,14,15,16,17,18,21,22,27,35,37,38,40,41,42,44,45,48,49,50,51,52,53,55,57,60,61,62, Bw4, Bw6.

DR locus: 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,

DRw52, DRw53.

DQ locus: 1,2,3,7.

For Class II DNA typing, the DNA was extracted from 2 mL of EDTA blood using either a commercial kit (Nucleon, Scotlab, UK) or a local method using a rapid mini salting out technique described by Miller et al.⁴ The separated DNA was incubated in a thermal cycler with Class II primers defining DR β and DQ β alleles (Dynal low-resolution sequence-specific primers, Dynal, Skoyen, Norway), together with polymerase chain reaction (PCR) buffer, taq polymerase and DNA nucleotides (Gibco BRL, Paisley, UK). The products were run on 1.2% agarose gels containing ethidium bromide, examined under UV illumination and the Class II specificities were identified. The following DR and DQ specificities could be determined (35 in total):

DNA DR:

1,2(15,16),3(17,18),4,5(11,12),6(13,14),7,8,9,10.

DRw: 51,52 & 53.

DNA DQ:

1 (5.1,5.2,5.3,6.1,6.2,6.3,6.4,6.7,6.9).

2 (2.1,2.2).

3 (3.1,3.2,3.3,3.4,3.5).

4 (4.1,4.2).

The results are expressed in terms of percentage antigen (allele) frequencies. Statistical differences between the individual Arabian Gulf populations and the Omanis were obtained by determination of probabilities (P), which were obtained by calculation of the standard error of the percentages, with references to multiples of standard errors for a normal distribution.⁵ The probabilities obtained were corrected by multiplying by the numbers of antigens tested (P_c).

Results

The results of serological HLA-A, B, DR and DQ typing are shown in Tables 1 to 3. The results are compared with data published for Saudi Arabia⁶⁻⁸ and

Kuwait (AG White, unpublished data). Percentages are given to the nearest whole number.

HLA-A11, A32, B17, B35, B40, DR2 and DQ1 are all significantly more frequent in Oman than in Saudi Arabia or Kuwait ($P_c < 0.05$). HLA-A9, B21, B50, DR4, DR7 and DR53 are significantly reduced ($P_c < 0.05$). HLA-B27 is lower in Omanis (0.3%) than in Saudia Arabia or Kuwait.

Comparison of HLA Class II typing by serology with typing by SSP/DNA in 126 individuals is shown in Tables 4 and 5. There was a disparity in the definition of DR4 and DR51 between serology and SSP/DNA. In the case of DR4, this is probably due to the different subtypes of DR4 defined by serology and SSP/DNA.

There were also some inconsistencies in the SSP/DNA data. For example, those individuals who were DR15 and DR16 positive (109), based on data in other populations, would usually be DR51 positive. In fact only 88 of the 109 individuals were DR51 positive. This may be due to technical shortcomings, the quality of the typing reagents or the use of primers developed mainly for use in Caucasian populations on a new, isolated population with a high degree of consanguinity (although none of the individuals in this study were known to be related to each other).

Discussion

The major finding in this study was the very high incidence of HLA-DR2 in the Omani population. HLA SSP DNA typing on 126 Omanis revealed an incidence of DR2-associated antigens of 87%, of which 63% were DR16, 18% DR15, with 6% having both antigens. Serology on the same individuals (n=126) gave a figure of 74% DR2 positivity.

In the overall study of 283 individuals who were serologically HLA-DR typed, 66% were DR2 positive. In the literature, the highest reported incidence for DR16 (16%) was in Sardinia,⁹ with the DR15 frequency being 8%.

The common Caucasian haplotypes of HLA A1, B8, DR3 and A3, B7, DR2 occurred rarely in Oman. As might be expected as a result of the very high incidence of DR2 in Oman, the haplotypes associated with DR2 predominated. HLA-A2, B5(51), DR2(DR16) was the most common haplotype based on population and family studies.

The very high frequency of DR2 in Omanis may be a consequence of inbreeding in isolated communities which were formed by a common ancestor. The incidence of parental consanguinity in the population studied here was 40%, 25% being first cousins, 11% second cousins and 4% distantly related. It is likely that the incidence of consanguinity in earlier generations would be much higher.

TABLE 3. Comparison of HLA-DR and DQ antigen frequencies in Omanis with other Arabian Gulf populations.

HLA DR and DQ antigen	Percentage frequency			
	Omanis n=283	Saudis ⁷ n=325	Saudis ⁸ n=100	Kuwaitis* n=100
DR1	7	11	16	13
DR2	66**	18	19	26
DR3	29	25	27	28
DR4	13**	31	30	13 [†]
DR5	18	12	15	29
DR6	8	8	27	3
DR7	11	35	46	28
DR8	1	3	2	3
DR9	0	3	NT	2
DR10	4	NT	1	6
DR11	13	NT	NT	NT
DR12	1	NT	NT	NT
DR13	1	NT	NT	NT
DR14	1	NT	NT	NT
DR52	50	NT	60	74
DR53	19**	NT	64	27
DQ1	76**	NT	51	57
DQ2	36	NT	NT	12
DQ3	27	NT	46	27

*Kuwait (A.G. White, unpublished); ** $P_c > 0.02$; [†]data not included in significance calculation; NT=not tested.

TABLE 4. Class II HLA-DR antigens determined by serology and DNA typing in Omanis.

Class II DR specificity	Serology n=126		DNA n=126	
	# Positive	Antigen frequency (%)	# Positive	Antigen frequency (%)
DR1	4	3	3	2
DR2	93	74	-	-
15	-	-	22	17
16	-	-	79	63
15+16	-	-	8	6
DR3	36	29	-	-
17	-	-	34	27
18	-	-	3	2
17+18	-	-	1	2
DR4	10	8	17	14
DR5	-	-	-	-
11	14	11	17	14
12	1	1	2	2
DR6	7	6	-	-
13	2	2	8	6
14	2	2	5	4
13+14	-	-	1	1
DR7	11	9	10	8
DR8	1	1	1	1
DR9	0	0	0	0
DR10	4	3	4	3
DR51	67	53	88	70
DR52	62	49	65	52
DR53	21	17	25	20

It is of interest to note that the incidence of DR2 is much higher than the 18%-19% reported for Saudi Arabia,^{7,8} or the 26% in Kuwait (A.G. White, unpublished), possibly suggesting a different pattern of

TABLE 5. Class II HLA-DQ specificities determined by serology and DNA typing in Omanis.

Class II DQ specificity	Serology n=126		DNA n=126	
	# Positive	Antigen frequency (%)	# Positive	Antigen frequency (%)
DQ1	98	78	104	82
5.1	—	—	14	11
5.2	—	—	82	65
5.3	—	—	3	2
6.1	—	—	7	6
6.2	—	—	10	8
6.3	—	—	5	4
6.4	—	—	2	2
6.3/6.7	—	—	1	1
6.4/6.9	—	—	2	2
DQ2	39	31	43	34
DQ3	29	23	32	25
3.1	—	—	15	12
3.2	—	—	14	11
3.3	—	—	1	1
3.4	—	—	3	25
3.2/3.5	—	—	0	0
DQ4	3	2	4	3
4.2	—	—	4	3

migration of the population of Oman. It is believed that the main migrations of Arabs to Oman were in the 1st and 2nd centuries AD, and were of the Qahtani tribe of Azd from Yemen.¹⁰ Unfortunately, there is no published information on the frequency of HLA-DR antigens in the Yemeni.

HLA-DR2 has been associated with a wide spectrum of autoimmune and other diseases.¹¹ We have recently reported an increased risk of blinding trachoma in Omanis with HLA-DR2/DQ1.³ More significantly, DR2 and associated DQ1 are involved in protection against insulin-dependent diabetes mellitus (IDDM).¹² IDDM is less common in Oman, the incidence in children under 15 being about 2.5/100,000 for 1993-94.¹³ This is lower than in Europe and Scandinavia, where there is an incidence of 3.7-28.6/100,000 for 1990-91.¹⁴ More important is that the incidence in Oman is lower than that in Kuwait (3.96/100,000 for 1980/81),¹⁵ where the frequency of HLA-DR2 is 26%. The incidence of IDDM has reportedly been inversely associated with temperature, but this factor cannot explain all the reported differences in prevalence rates.¹⁴

Genetic factors are known to be of importance in IDDM and the high frequency of HLA-DR2/DQ1 in Oman and its apparently universal association with protection against IDDM may be an important factor in the low incidence of IDDM in Oman.

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