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HLA-A, -B and -DR Allele and Haplotype Frequencies in Malays

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SUMMARY One thousand four hundreds and forty-five Malays registered with the Malaysian Marrow Donor Registry were typed for HLA-A, HLA-B and HLA-DR. Fifteen HLA-A, twenty nine HLA-B and fourteen HLA-DR alleles were detected. The most common HLA-A alleles and their frequencies were HLA-A24 (0.35), HLA-A11 (0.21) and HLA-A2 (0.15). The most common HLA-B alleles were HLA-B15 (0.26), HLA-B35 (0.11) and HLA-B18 (0.10) while the most common HLA-DR alleles were HLA-DR15 (0.28), HLA-DR12 (0.27) and HLA-DR7 (0.10). A24-B15-DR12 (0.047), A24-B15-DR15 (0.03) and the A24-B35-DR12 (0.03) were the most frequent haplotypes. This data may be useful in determining the probability of finding a matched donor and for estimating the incidence of HLA associated diseases.

The major histocompatibility complex (MHC) in man comprises a highly polymorphic set of genes found on the short arm of chromosome 6. The human leukocyte antigens (HLA) which are the products of the MHC play a key role in the immune system. HLA allele frequency in the population may be used to study disease associations, carry out anthropological studies and identify useful epitopes for molecular vaccines. Clinically, HLA typing is used to identify compatible donors for solid organ and bone marrow transplants.

The association of disease with HLA alleles has been well documented.[1] The varying frequencies of the alleles in different populations worldwide may account, to some extent, for increased resistance or susceptibility to diseases in different populations. This information may be helpful in pinpointing differences that may lead to a better understanding of disease mechanisms.[2,3]

It has been established that the interaction between antigenic epitopes and the immune system of the host varies with the HLA allele involved. This information may be of use in both identifying appropriate epitopes for inclusion in molecular vaccines and determining the probable efficacy of these vaccines in a particular population.[4]

The HLA locus has been useful in determining the origin, migration and relationships between populations. Cavalli-Sforza *et al*.[5] studied 25 populations in South-East Asia and grouped them according to three main clusters. The Malaysian Malay population was placed in the same cluster with those of Indonesia and Borneo but apart from the Chinese which form a significant proportion of the popula-

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tion. Malaysia has experienced both Portuguese settlement and colonization by the British. Furthermore the Straits of Malacca was and still is a major shipping route between China and Europe. These factors may have resulted in some admixture in the local Malay population.

We report here the HLA-A, -B and -DR allele and haplotype frequencies from 1,445 Malay donors registered over a period of 1 year with the Malaysian Marrow Donor registry. There were reports[6-8] in the seventies on this population based on serology and reports for some alleles using molecular techniques for the HLA-A[9] and HLA-DR loci.[10] This is the first report of the frequency of HLA-B alleles in the Malay population using molecular techniques.

MATERIALS AND METHODS

A total of 1,445 Malays who were registered with the Malaysian Marrow Donor Registry Program were tested. Only donors whose parents were both Malay were included in the analysis. The donors were mainly students, recruits and trainees drawn from universities, the uniformed services and allied health care workers, respectively, and came from all around the country.

HLA typing was carried out using Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) (MicroSSP Generic HLA Class I and Class II ABDR DNA Typing Tray 384-SSPABDRX, One Lambda Inc., Canoga Park, CA, USA or Biotest ABDR SSP tray Cat No. 826230, Biotest AG, Germany). Interpretation of the results was carried out using the worksheets provided by the manufacturer. Briefly, 5 ml of peripheral blood was collected in EDTA. White cells were separated using Ficoll Hypaque, and the DNA was extracted using the QIAamp DNA Blood Mini Kit from Qiagen (cat. no. 51106). The purity and concentration of the DNA was estimated using an Eppendorf Bio Photometer (Hamburg, Germany). All DNA was stored at -20°C until tested.

Allele frequencies were calculated directly because all donors were tested using low to medium resolution SSP-PCR kits and all alleles could be identified. Allele frequencies were calculated by dividing the total number of occurrences of that allele by the total number of alleles at that locus in the population. Ambiguities occurred when the interpretation of the data indicated more than two possible alleles. About 3-5% of the samples were tested using another kit to clarify this. It was not found necessary to resolve ambiguities using single allele trays. Allele frequency was defined as the fraction or percentage of loci that the allele occupies within the population.[11] Where only one allele was detected we considered it as homozygous and the allele was counted twice.

Statistical analysis was carried out using Arlequinn 3.0.[12] Gene frequency results were checked using Microsoft Excel. The Hardy Weinberg equilibrium and haplotype frequencies were also calculated. All loci were tested individually for Hardy-Weinberg equilibrium and the significance was determined using the chi-squared test.[13] Maximum likelihood estimation tests were used to obtain the haplotype frequencies. Haplotype frequencies were estimated using Expectation Maximization (EM) algorithm included in the ARLEQUIN software. Standard deviations of haplotype frequencies were determined by performing 2,500 bootstrap replicates.

RESULTS

The frequencies for HLA-A, -B and –DR are shown in Fig. 1. Fifteen HLA-A, twenty nine HLA-B and fourteen HLA-DR alleles were detected. The most frequent HLA locus was HLA-A*24 (0.35) followed by HLA-A*ll (0.21) and HLA-A*02 (0.15). The highest allele frequencies for the HLA-B locus were HLA-B*15 (0.26), HLA-B*35 (0.11) and HLA-B*18 (0.10). The most common HLA-DRB1 frequencies were HLA-DR*15 (0.28), HLA-DR*12 (0.27) and HLA DR*07 (0.09).

Table 2 lists the most common haplotype frequencies in Malays: HLA-A*24-B*15-DR*12 (0.047), HLA-A*24-B*15-DR*15 (0.042) and the HLA-A*24-B*35-DR*12 (0.39).

DISCUSSION

The HLA-A allele frequencies that we found in our study are consistent with earlier serological results[6-8] as well as later reports using molecular

Table 1 Frequency of HLA-A, -B and -DR alleles in the Malay population (n = 1,445)

HLA-A*	Number	Frequency	HLA – B*	Number	Frequency	HLA-DR*	Number	Frequency
01	60	0.021	07	103	0.036	01	19	0.007
02	437	0.151	08	13	0.004	04	185	0.064
03	54	0.019	13	115	0.040	07	280	0.097
11	604	0.209	14	4	0.001	08	45	0.016
23	9	0.003	15	742	0.257	09	103	0.036
24	1015	0.351	18	295	0.102	10	68	0.024
26	27	0.009	27	81	0.028	11	81	0.028
29	23	0.008	35	326	0.113	12	780	0.270
30	28	0.010	37	20	0.007	13	124	0.043
31	28	0.010	38	139	0.048	14	157	0.054
32	13	0.005	39	19	0.007	15	810	0.280
33	373	0.129	40	205	0.071	16	106	0.037
34	171	0.059	41	2	0.001	17	128	0.044
68	21	0.007	42	3	0.001	18	4	0.001
74	27	0.009	44	180	0.062			
			45	6	0.002			
			46	55	0.019			
			48	16	0.006			
			49	4	0.001			
			50	4	0.001			
			51	192	0.066			
			52	102	0.035			
			53	4	0.001			
			54	6	0.002			
			55	21	0.007			
			56	25	0.009			
			57	38	0.013			
			58	168	0.058			
			73	2	0.001			

techniques.[9,10] Bungawan *et al.*[9] also showed that the most frequent subtypes for each of these alleles were: HLA-A*2402, A*1101 and A*0203. The frequencies of the HLA-A alleles were similar to those found in the Singapore Chinese[14] where the most common HLA-A alleles (and their frequencies) are HLA-A*02 (0.34), HLA-A*11 (0.29) and HLA A*24 (0.17). However, in the present study it was found that there was a statistically significant deviation from Hardy-Weinberg proportions for the HLA-A locus ($p < 0.001$) although both HLA-B and HLA-DR alleles were in Hardy-Weinberg equilibrium. Bungawan *et al.*[9] reported the frequency of the HLA-A alleles in the Malays (n = 148) as follows: HLA-A*24 (0.37), HLA-A11 (0.26) and HLA-A*02 (0.196), stating that there were no significant deviations from Hardy-Weinberg proportions. Reasons for deviations from Hardy Weinberg equilibrium include selection, recurrent mutation, migration, non-random mating and genetic drift. It is possible that the A lo-

Table 2 Haplotype frequencies in the Malay population

Haplotype	Frequency
HLA-A*24 B*15 DR*12	0.0047
HLA-A*24 B*15 DR*15	0.0042
HLA-A*24 B*35 DR*12	0.0039
HLA-A*33 B*44 DR*07	0.0038
HLA-A*11 B*15 DR*12	0.0038
HLA-A*24 B*18 DR*12	0.0037
HLA-A*02 B*15 DR*12	0.0036
HLA-A*34 B*15 DR*15	0.0034
HLA-A*24 B*35 DR*15	0.0034
HLA-A*11 B*15 DR*15	0.0034
HLA-A*33 B*58 DR*17	0.0030
HLA-A*24 B*18 DR*15	0.0029
HLA-A*24 B*38 DR*15	0.0029
HLA-A*11 B*18 DR*15	0.0027
HLA-A*24 B*51 DR*12	0.0025
HLA-A*02 B*18 DR*15	0.0025
HLA-A*33 B*58 DR*13	0.0021

cus is not in equilibrium because the Malays may be a non-randomly mating population.

Allele frequencies for HLA-B observed in Malays are quite different from the pattern seen in the Singapore Chinese where the most common alleles and their frequencies respectively are HLA-B*4001 (0.172), HLA-B*4601 (0.132) and HLA-B*5801 (0.104).[14] An earlier study on the aboriginal population of peninsular Malaysia reported a population frequency of 41.9% for HLA-B*1513, an allele which is purported to give protection against falciparum malaria. This pattern is not seen in the Malay population.

Our results were slightly different but largely consistent with the findings of Mack *et al.*[10] which showed that the most frequent HLA-DRB1 alleles in the Malay population were HLA-DR*12 (0.36), HLA-DR*15 (0.23) and HLA-DR*07 (0.07). The subtypes with the highest frequency for each of these groups were HLA-DRB*1202, DR*1502 and DR*0701, respectively.

The majority of bone marrow transplants carried out in Malaysia are between siblings where medium to low resolution typing is sufficient. However, the number of transplants from unrelated donors is slowly increasing. In such cases it is important to carry out high resolution typing in order to confirm compatibility. Additionally, frequencies of HLA alleles are important to determine the probability of finding a match in the registry and the size of the registry that would be necessary to be useful. Furthermore this data can be used to estimate the incidence of an HLA associated disease in the population.

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REFERENCES

1. Ryder LP, Svejgaard A, Dausset J. Genetics of HLA disease association. Annu Rev Genet 1981; 15: 169-87.

2. Hirayama K, Zaidi AS, Lokman Hakim S, *et al*. Molecular analysis of HLA-B in the Malaysian aborigines Tissue Antigens 1996; 48: 692-7.
3. Dhaliwal JS, Too CL, Lisut M, Lee YY, Murad S. HLA-B27 polymorphism in the Malays 2003. Tissue Antigens 62; 330-2.
4. Dawson DV, Ozgur M, Sari K, Ghanayem M and Kostyu DD. Ramifications of HLA Class I polymorphism and population genetics for vaccine development. Genet Epidemiol 2000; 20: 87-106.
5. Cavalli-Sfroza, Menozzi P, Piazza A. The History and Geography of Human Genes. New Jersey: Princeton University Press. 1994.
6. Ting A, Wee GB, Simons MJ, Morris PJ. The distribution of HLA-A leukocyte antigens in Singapore Chinese, Malays and Indians. Tissue Antigens 1971; 31: 258-64.
7. Chan SH, Wee GB, Srinivsaan N, *et al.* HLA antigens in three common populations in South East Asia- Chinese Malay and Filipino. Tissue Antigens 1979; 13: 361-8.
8. Sukumaran KD, Ong KJ, Ding SH, Thavamalar B, Lopez CG. HLA antigen, gene and haplotype frequencies of Malays. HLA in Asia-Oceania 1986. Proceedings of the Third Asia-Oceania Histocompatability Workshop and Conference, Sapporo, Japan. June 27-July 1, 1986. Aizawa M, Natori T, Wakisaka A, Konoeda Y, Eds. 1986; pp. 266-70.
9. Bugawan TL, Mack SJ, Stoneking M, Saha M, Beck HP, Erlich HA. HLA class I allele distributions in six Pacific/Asian populations: evidence of selection at the HLA-A locus. Tissue Antigens 1999; 53: 311-9.
10. Mack SJ, Bugawan TL, Moonsamy PV, *et al*. Evolution of Pacific/Asian populations inferred from HLA class II allele frequency distributions. Tissue Antigens 2000; 55: 383-400.
11. Leffell MS, Steinberg AG, Bias WB, Machan CH, Zachary AA. The distribution of HLA antigens and phenotypes among donors and patients in the UNOS registry. Transplantation 1994; 58: 1119-30.
12. Excoffier LG, Laval G, Schneider S. Arlequin 3.0. An integrated software packaged for genetics data analysis. Evolutionary Bioinformatics Online 2005; 1: 47-50 (accessed in October 2006).
13. Guo SW, Thompson EA. Performing the exact test off Hardy-Weinberg proportion for multiple alleles. Biometrics 1992; 48: 361-72
14. Middleton, D, Hawkins, BR, Williams F. HLA class I allele distribution of Hong Kong Chinese population based on high resolution PCR-SSOP typing. Tissue Antigens 2004; 63: 555-61.