HUMAN LEUCOCYTE ANTIGEN (HLA) CLASS I AND II FREQUENCIES IN SELECTED GROUPS IN LEBANON

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ABSTRACT

Human Leucocyte Antigen class I (HLA-A and B) profiles were determined in 461 Lebanese subjects. These included 88 Sunnites, 149 Shiites, 50 Druze, 129 Maronites and 45 Greek Orthodox. HLA class II (HLA-DR and DQ) profiles were determined in 250 Lebanese subjects. These included 50 from each of the mentioned religious groups. The Complement Dependent Micocytotoxicity assay was used for these determinations. Frequencies for HLA epitopes in each religious sect were calculated and are presented. Such data may be important for those who are in the transplantation field and those who wish to study HLA-Disease associations. The HLA epitope frequencies in the different religious groups studied were compared. The degree of similarity in frequencies ranged from 77% to 96%.

Key words : HLA, Frequencies, Religious groups, Lebanon

INTRODUCTION

Human Leucocyte Antigens (HLA) or Major Histocompatability Complex (MHC) Antigens are glycoproteins expressed on the surface of nucleated cells. Their genetic determination, production and physico-chemical properties have been the subject of several review articles and chapters in immunology textbooks (Abdelnoor, 1997; Leffell, 1997; Salazar and Yunis, 1995). In humans, their production is determined by genes located in a region called the MHC on chromosome number 6. Within this region there are several loci (A, B, C, DR, DP, DQ and others). Collectively, these loci are called a haplotype. An individual has two haplotypes, inherited in a Mendelian fashion, one of paternal and the other of maternal origin. Each locus within a haplotype is governed by a codominant multiple allelic system. HLA encoded by A, B, and C alleles are called MHC-class I antigens and those encoded by DR, DP and DQ alleles, MHC-class II antigens.

HLA may play a major role in allograft rejection (Opelz, 1988; Cecka *et al*, 1988.), they are involved in antigen processing/presenting and the generation of an immune response (Abdelnoor, 1997), and certain HLA epitopes have been shown to be associated with susceptibility or resistance to a disease (Salazar and Yunis , 1995; Thorsby, 1995). There is a high degree of HLA profile variation within populations. With the exception of identical twins and in certain cases, siblings, it is very difficult to find two individuals that are HLA-identical. Moreover, some HLA epitope frequencies vary among different ethnic or religious groups. For these reasons, it is useful to define HLA epitope frequencies in an ethnic or religious group. In this communication, HLA epitope frequencies in Sunnites, Shiites, Druze, Maronites and Greek Orthodox in Lebanon are presented.

MATERIAL AND METHODS

Subjects:

HLA-A and –B (Class I) profiles were determined for 88 Sunnites, 149 Shiites, 50 Druze, 129 Maronites and 45 Greek Orthodox. HLA-DR and –DQ (Class II) profiles were determined for 50 individuals in each of the religious sects. They were selected at random based on their religion and sect. To the best of knowledge, off - spring of intermarriage were excluded. In the case of some groups consanguineous marriage is a religious custom. Twenty ml blood were collected into citrated tubes from each individual to be tested.

Complement dependent microcytotoxicity assay:

Thirty two Class I (A and B) and 21 class II (DR and DQ) antigens were determined.

The procedure as described by the manufacturer of the reagents (Hoecsht and One Lambda) was followed. Initially, antisera and rabbit complement used for the determination of HLA Class I profiles were obtained from Hoechst diagnostics (Germany) . Later, reagents used for the determination of both Class I and II profiles were obtained from One Lambda (California).

Mononuclear cells were separated from citrated blood by centrifugation on a ficol-hypaque gradient and used in the microcytotoxicity assay to determine HLA-A and B profiles. B-lymphocytes separated from citrated blood using Fluorobeads B (One Lambda) were used to determine HLA-DR and DQ profiles. Briefly, mononuclear cells to be tested were dispensed into wells of a microplate containing the HLA antisera (HLA-A, B antisera and HLA-DR, DQ antisera were obtained pre-dispensed in separate plates from the manufacturer). The plate was incubated at room temperature for 30 minutes. Rabbit complement was then added to each well and the microplate was again incubated at room temperature for 60 minutes. Cell viability was determined using ethidium bromide/acridine orange stain (One Lambda) and visualizing using a Lecica ultraviolet light inverted microscope. Dead cells appeared orange and viable cells green in color.

Statistical analysis:

Class I and II epitope frequencies were determined for each religious sect and compared using EpiInfo version 6. A p-value of less than 0.05 was considered as significant difference. Degree of HLA frequency similarity between groups was calculated by dividing the number of epitopes where the frequencies were similar in the two groups compared by the total number of epitopes that were tested and multiplying by 100. Degree of similarity is expressed in percent.

RESULTS

The frequencies of Major Histocompatability Complex (MHC) class I and class II antigens obtained in the five Lebanese religious sects are presented in tables 1, 2 and 3. The frequencies obtained when the Lebanese were considered 1 group are given in table 4. Those antigens that are not listed in the four tables were not tested. Moreover, some of the splits were not tested, or if tested negative results were obtained in some cases where the parent antigen was positive. Hence, it may be observed that in some cases the sum of the frequencies of the splits do not add up to the frequency of the parent antigen.

Table 1. HLA – A Frequencies and percentages in Lebanese religious sects**.**

Frequencies and percentages of epitopes in each Sect															
HLA epitope	Sunnite			**Shiite**			Druze			**Maronite**			Greek Orthodox		
	+	–	%	+	–	%	+	–	%	+	–	%	+	–	%
A1	23	65	***26***	36	113	***24***	12	38	***24***	40	89	***31***	7	38	***16***
A2	45	43	***51***	63	86	***42***	14	36	***28***	50	79	***39***	12	33	***27***
A3	20	68	***23***	28	121	***19***	5	45	***10***	22	107	***17***	6	39	***13***
A9	26	62	***29***	49	100	***33***	16	34	***32***	55	74	***43***	14	31	***31***
A23	12	76	***14***	21	128	***14***	2	48	***4***	5	124	***4***	3	42	***7***
A24	13	75	***15***	22	127	***15***	12	38	***24***	50	79	***39***	10	35	***22***
A10	11	77	***12***	15	134	***10***	8	42	***16***	17	112	***13***	7	38	***16***
A25	4	84	***4***	4	145	***3***	1	49	***2***	1	128	***1***	0	45	***0***
A26	7	81	***8***	10	139	***7***	1	49	***2***	15	114	***12***	4	41	***9***
A11	6	82	***7***	10	139	***7***	8	42	***16***	8	121	***6***	4	41	***9***
A19	18	70	***20***	37	112	***25***	13	37	***26***	36	93	***28***	12	33	***27***
A29	8	80	***9***	7	142	***5***	5	45	***10***	5	124	***4***	2	43	***4***
A30	5	83	***6***	10	139	***7***	3	47	***6***	12	117	***9***	4	41	***9***
A31	1	87	***1***	6	143	***4***	0	50	***0***	4	125	***3***	0	45	***0***
A32	4	84	***4***	13	136	***9***	3	47	***6***	15	114	***12***	3	42	***7***

***** 88 Sunnites ,149 Shiites ,50 Druze,129 Maronites ,45 Greek Orthodox were tested.

Table 2. HLA – B Frequencies and percentages in Lebanese religious sects **.**

* 88 Sunnites, 149 Shiites, 50 Druze, 129 Maronites, 45 Greek Orthodox were tested.

Table 3. HLA – DR and DQ Frequencies and percentages in Lebanese religious sects..

50 individuals from each sect were tested.

Table 4. HLA class I and II antigen Frequencies (percent) in Lebanese.

HLA class I profiles were determined in 461 Lebanese subjects.

HLA class II profiles were determined in 250 Lebanese subjects. Differences and similarities in antigen frequencies were observed among the groups. Antigens for which statistically significant differences were observed are presented in table 5. The following observations can be highlighted;

1- Comparing the Sunnites and Shiites, there were significant differences in frequencies of two class I (B40 and B22) antigens . The frequencies of class II antigens in the two groups were similar.
2- Comparing the Druze and Shiites, there were significant differences in frequencies of one class I (B35) antigen and four class II antigens (DR1, DR10, DQ1, DQ4).
3- Comparing Druze and Sunnites there were significant differences in frequencies of four class I (A2, B35, B41, B22) and four class II antigens (DR10, DQ1, DQ2, DQ4).
4- Comparing other groups with each other, the number of antigens with significant different frequencies ranged from nine to twelve.
5- The frequency of DR10 in Druze was significantly higher than that in other groups studied. Moreover, the high frequency of DQ4 in Druze can be observed. This frequency would need confirmation later, possibly by allele typing .

It was mentioned that the panel of antigens that were detected included 32 class I antigens and 21. class II antigens. Thus, the degree of similarity among the groups expressed in percent is presented in table 6.

Table 5. HLA Class I and Class II Specificities where frequencies differed among religious groups in the Lebanese population*.

Table 6. Degree of similarity in MHC antigen frequencies among the different Lebanese groups.

Groups compared	Degree of similarity expressed in percent
Shiite / Sunnite	96%
Druze / Shiite	90%
Druze / Sunnite	85%
Orthodox / Sunnite	83%
Orthodox / Shiite	81%
Orthodox / Druze	81%
Maronite / Sunnite	80%
Maronite / Shiite	79%
Maronite / Druze	77%
Maronite / Orthodox	77%

DISCUSSION

The role of ethnic group HLA epitope frequencies in transplantation may be controversial. However, the genetically close the donor and recipient are, the longer the survival of a graft. The degree of similarity in HLA frequencies between religious groups may be an indirect indication of genetic similarity. One study indicated that race or ethnic group might effect the outcome of cadaveric renal transplants (Vaidya et al,1993). The five-year kidney graft survival was compared

among 3 different ethnic groups; Whites, Blacks and Hispanics. When donors and recipients were Black, the 5-year survival was 81%, when donors were Black and recipients Hispanic it was 51% and when donors were Black and recipients White, it was 70%. Our results indicate that the degree of similarity in HLA frequencies among the different religious groups ranged from 77% to 96%, the highest being among the Sunnites, Shiites and Druze . Consanguineous marriages in certain religious groups may account for the observed differences. These results may be of value to the transplant surgeon and immunologist in selecting an appropriate donor/recipient.

In certain ethnic groups, the presence of some HLA epitopes has been associated with certain diseases (Salazar and Yunis, 1995 ;Thorsby 1995,). However, an established HLA-disease association in one ethnic group does not necessarily mean that it applies to other ethnic groups.The establishment of an HLA epitope-disease association depends among other factors, on the frequency of the epitope in the normal (non-disease) ethnic population. A high degree of variation in HLA epitope frequencies exist among the different ethnic groups because of the polymorphism that characterizes the HLA system (The Central Data Analysis Committee, 1991) . A strong association exists between the presence of HLA-B27 and susceptibility to ankylosing spondilitis in European Caucasians. Based on the frequency of HLA-B27 determined in Lebanese it was suggested and later supported by clinical observations that such a strong association does not exist in Lebanese (Abdelnoor and Heneine, 1985; Abdelnoor and Abdelnoor, ; 1993; Malak and Abdelnoor, 1997; Abdelnoor, 1997; Abdelnoor, 1998; Abdelnoor, 1998). Hence, the reported frequencies in this communication may serve those rheumatologists or other clinical specialists who wish to study HLA epitope-disease associations in Lebanon.

Other religious or ethnic groups in Lebanon were not studied due to lack of funds. Moreover, it may be argued that each township in Lebanon should be studied separately. This may be true but again financial aspects among other factors would hinder such a study. Nevertheless, we observed a certain degree of consistency within a religious sect regardless of the township.

In conclusion, most of our earlier reports dealt with class I antigen frequencies in Lebanese. This communication includes Class II antigen frequencies as well. Moreover, frequencies in the different sects are reported and compared. During the course of this investigation, allele typing (DNA typing) was gradually introduced into tissue typing laboratories either as a complementary test to, or replacement for, the Complement Dependent Microcytotoxicity test. We have

introduced allele typing in our laboratory and future communications will include the use of this test.

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