Major histocompatibility class I antigens in the Lebanese population

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مستضدّات التوافق النسيجي الرئيسية من الصنف الأول في المجتمع اللبناني

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خلاصة : لم تجر دراسات حول مستضدات التوافق النسيجي الرئيسية في المجتمع اللبناني باستثناء تقريرين يتعلقان بالمهاجرين اللبنانيين . وهذه المقالة تصف تواتر وتوزع مستضدات التوافق النسيجي الرئيسية من الصنف الأول الموجودة في المواضع A ، B ، C ، على أساس المعطيات التي جمعت من 200 فرد لا قرابة بينهم من مناطق مختلفة في لبنان . ووجد أن أعلى التواترات الجينيه كانت كما يلي : A2 (24.8%) ، B35 (17.9%) ، CW4 (18.6%) ، وهكذا يصبح هذا النمط الفردي أكثر الأنماط شيوعا . وأظهرت مقارنة المسافات الجينية نمطا أقرب إلى القوقازيين منه إلى المنغولين أو الشرقيين أو الأمريكيين ذوي الأصل الأفريقي .

ABSTRACT Except for two reports on Lebanese immigrants, there have been no studies on the major histocompatibility (MHC) antigens in the Lebanese population. We describe the frequency and distribution of MHC class I antigens present in the A, B and C loci based on data obtained from 200 healthy unrelated individuals from different parts of Lebanon. The highest gene frequencies were as follows: A2 (24.8%), B35 (17.9%) and Cw4 (18.6%), making this haplotype the commonest. Comparison of genetic distances revealed a pattern closer to the Caucasoid population than to the Mongoloid, Oriental or Black populations.

Les antigènes de la classe I du complexe majeur d'histocompatibilité dans la population libanaise

RESUME A l'exception de deux rapports sur des immigrants libanais, il n'y a eu aucune étude sur les antigènes du complexe majeur d'histocompatibilité dans la population libanaise. Nous décrivons la fréquence et la répartition des antigènes de la classe I du complexe majeur d'histocompatibilité présents dans les loci A, B et C en fonction des données obtenues auprès de 200 sujets sains n'ayant aucun lien de parenté et originaires de différentes parties du Liban. Les fréquences géniques les plus élevées étaient les suivantes: A2 (24,8%), B35 (17,9%) et Cw4 (18,6%), ce qui rend cet haplotype le plus courant. La comparaison des distances génétiques a mis en évidence un schéma plus proche de la population caucasienne que des populations mongoliennes, orientales ou afro-américaines.

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Introduction

Lebanon and its neighbouring Arab countries are situated at a crossroads between Europe, Africa and Asia, and historically have been invaded and populated by different peoples originating from these three continents. Although the predominant racial constitution is Caucasoid, it is still poorly defined as there have been very few anthropomorphic population studies on this region. Another feature of the population in this part of the world is the high frequency of consanguineous marriages, which continue to be common. Although polygamy occurred in the past, it has become rare in the past half century.

Data on histocompatibility antigens in patients and in population groups are becoming increasingly important, not only in the ever-expanding field of organ transplantation, but also in the area of human histocompatibility leukocyte antigen (HLA) association with a number of diseases. The latter vary in pathophysiology, from the different types of autoimmune disorders (e.g. ankylosing spondylitis, insulin-dependent diabetes mellitus, Graves disease) to some heritable disorders such as 21-hydroxylase deficiency. Information on HLA antigens can be utilized to assess genetic risk in such disorders.

Only two studies have been reported on Lebanese expatriates living in either Australia or the United States of America [*1*,*2*], and there have been a few reports about different Arab communities, mainly in Egypt [*3*–*6*] and Israel [*7*]. Beside these, there have been no published studies on the HLA profiles of the Lebanese and their immediate Arab neighbours. In this work, we describe antigen and gene frequencies of the A, B and C loci of the major histocompatibility (MHC) class I gene in a sample of 200 healthy Lebanese individuals. The results, including the haplotypes and linkage disequilibria, are compared to patterns reported for other populations in the world.

Subjects and methods

The population sample studied was chosen randomly from healthy individuals from different parts of Lebanon belonging to the two major religions. Their ages ranged from 20 years to 40 years and there were 100 males and 100 females.

Ten millilitres of venous blood were collected into tubes containing 10 000 IU of preservative-free heparin and, within 15 minutes, isolation of the lymphocytes was started using Ficoll–Hypaque density gradient as described by Boyum [*8*]. HLA class I antigens were determined immediately using commercially available trays with mono- and polyclonal antisera purchased from Hoechst Laboratories (Behring, Behringwerke AG, Marburg, Germany). The usual grading scale of 1 to 8 was used, in which 8 represented the positive control with nearly total cell kill and 1 was the negative control with the lowest number of dead cells (normally not more than 1%–2%). For the C locus, the antibodies included on the trays were restricted to the first five only; the rest were not available.

All analyses were made using the *SPSS* program with *Quatro-Pro* spreadsheet software. Phenotype frequencies were calculated by direct count. Gene frequencies were calculated using the formula

$$G_f = 1 - \sqrt{(1-A_f)}$$

where G_f is the gene frequency and A_f is the antigen frequency, based on the assumption of Hardy–Weinberg equilibrium and as described by Ishawar et al. [*9*].

The differences in phenotype frequencies were tested by contingency χ^2 and, wherever possible, by Fisher exact probability. The linkage disequilibrium parameters between the three loci were calculated using the following formula given by Piazza [*10*]:

$$\Delta(i,j,k) = p(i,j,k) - p(i)p(j)p(k) - p(i)\Delta(j,k) - p(j)\Delta(i,k) - p(k)\Delta(i,j)$$

where $p(i,j,k)$ is the haplotype frequency of alleles i, j and k.

Genetic distances between our population sample and four other populations were computed using the following formula given by Cavalli-Sforza et al. [*11*] and Bodmer et al. [*12*]:

$$d(j,k) = \sqrt{1 - \sum_i \sqrt{P(i,j)P(i,k)}}$$

where $P(i,j)$ and $P(i,k)$ are the gene frequencies in the populations of alleles j and k respectively.

Results

The phenotype and gene frequency distribution in the study sample are summarized in Table 1. In Table 2, comparison is made of the results on the Lebanese population with data published on Caucasoid, Black and Oriental populations [*13*]. Table 3 lists the genetic distances of the different antigens between the Lebanese sample and the Caucasoid, Black, Indian and Mongoloid populations.

The haplotype frequency and linkage disequilibria expressed by delta values [*10*] are shown in Table 4. A comparison of our findings with those published on Lebanese immigrants [*1,2*] and on other Arab populations [*7*] is shown in Table 5

Discussion

The results show that there was no antigen whose frequency was high enough to be considered a distinguishing feature of the Lebanese population (Table 1). Comparison with other populations revealed that, for the antigens coded by the A locus gene, there was no significant difference. As for the common antigens at the B locus, with the exception of antigens B35 and B41, which had the highest frequency in our sample, they were generally closer to the reported pattern of HLA class I markers in the Caucasoid population than to the reported frequencies in the Black and Oriental populations (Table 2). This conclusion is also supported by the data on genetic distance (Table 3).

On the other hand, a review of the antigens which were rare or of zero frequency in the Lebanese people (Table 2) revealed that while many of them are universally of low prevalence, the frequency of some seem, at face value, to be lower than that found in the rest of the groups reported. However, this apparent difference in the frequency between our population and the populations shown in Table 2 is misleading since the values reported for several loci such as A9, B5, B12, B16 and B21 for the Caucasoids, Blacks and Orientals represent the sum of the frequencies of their individual epitope components. In our study, we distinguished between phenotypes exhibiting portions of an antigen (epitope) and others expressing the entire antigen molecule. As an illustration, the frequency of A9 for the Caucasoid population is reported as (frequency of A23 + frequency of A24 = 22.4). In our study, the value considered for A9 represents situations where A9 alone was present (to the exclusion of both A23 and A24 epitopes). Whether or not this

frequency represents the third epitope for A9 (A2403) is not yet clear. Hence, if we were to follow the same procedure, then our list of rare antigens would be much shorter and would be restricted to B15 and B40 (including epitopes B62, B63 and B60, B61 respectively).

Table 1 **Distribution of HLA antigens and gene frequencies of 200 Lebanese**

Antigen positive	Number	Antigen frequency	Gene frequency
A1	46	0.230	0.123
A2	81	0.405	0.229
A3	50	0.250	0.134
A9	5	0.025	0.013
A10	2	0.010	0.005
A11	21	0.105	0.054
A23	13	0.065	0.033
A24	41	0.205	0.108
A25	0	0.000	0.000
A26	14	0.070	0.036
A28	12	0.060	0.030
A29	13	0.065	0.033
A30	16	0.080	0.041
A31	3	0.015	0.008
A32	11	0.055	0.028
A33	8	0.040	0.020
A36	8	0.040	0.020
A74	3	0.015	0.008
A2403	3	0.015	0.008
B5	1	0.005	0.003
B7	17	0.085	0.043
B8	9	0.045	0.023
B12	2	0.010	0.005
B13	13	0.065	0.033
B14	18	0.090	0.046
B15	1	0.005	0.003
B16	0	0.000	0.000
B17	5	0.025	0.013
B18	15	0.075	0.038
B21	3	0.015	0.008
B22	2	0.010	0.005
B27	6	0.030	0.015
B35	71	0.355	0.197
B36	1	0.005	0.003
B37	2	0.010	0.005
B38	14	0.070	0.036
B39	1	0.005	0.003
B40	3	0.015	0.008
B41	21	0.105	0.054
B44	27	0.135	0.070
B45	7	0.035	0.018
B47	2	0.010	0.005
B49	17	0.085	0.043
B50	8	0.040	0.020
B51	31	0.155	0.081
B52	14	0.070	0.036
B54	4	0.020	0.010
B55	9	0.045	0.023
B56	3	0.015	0.008
B57	3	0.015	0.008
B58	5	0.025	0.013
B60	2	0.010	0.005
B61	5	0.025	0.013
B62	0	0.000	0.000
B63	4	0.020	0.010
B65	1	0.005	0.003
B73	4	0.020	0.010
B4005	7	0.035	0.018
Bw4	106	0.530	0.314
Bw6	152	0.760	0.510
Cw1	10	0.050	0.025
Cw2	16	0.080	0.041
Cw3	15	0.075	0.038
Cw4	65	0.325	0.178
Cw5	6	0.030	0.015

Source: reference [13]

Table 2 **Frequency distribution of common (> 5% positive) and uncommon (< 5% positive) HLA antigens in the Lebanese population in comparison to three principal populations**

Antigen	Per cent positive			
	Lebanese	Caucasoid	Black[a]	Oriental[a]
Common antigens				
A1	23.0	26.4	15.5	2.0
A2	40.5	49.4	31.9	48.3
A3	25.0	24.7	13.0	3.0
A11	10.5	12.2	3.8	22.0
A23	6.5	2.8	15.4	0.2
A24	20.5	19.5	9.4	52.9
A26	7.0	6.3	8.8	13.9
A28	6.0	9.2	18.8	4.2
A29	6.5	5.7	9.6	0.8
A30	8.0	6.9	20.8	4.5
A32	5.5	7.6	4.5	0.8
B7	8.5	21.7	22.7	9.2
B13	6.5	5.7	3.2	7.5
B14	9.0	7.3	5.8	0.4
B18	7.5	10.7	8.2	0.6
B35	35.5	19.9	13.7	19.4
B38	7.0	4.9	3.2	1.4
B41	10.5	1.8	4.5	0.2
B44	13.5	23.1	14.6	11.6
B49	8.5	3.6	4.5	0.6
B51	15.5	12.0	3.8	15.0
B52	7.0	4.0	1.2	14.1
Cw2	8.0	8.0	22.4	2.0
Cw3	7.5	23.6	15.9	47.1
Cw4	32.5	21.7	26.0	10.3

Antigen	Per cent positive			
	Lebanese	Caucasoid	Black[a]	Oriental[a]
Uncommon antigens				
A9	2.5	22.4	24.6	53.1
A10	1.0	11.6	19.3	15.5
A25	0.0	4.7	–	0.0
A31	1.5	5.7	3.2	10.1
A33	4.0	2.8	7.6	11.6
A36	4.0	0.2	6.3	0.2
Aw66	0.0	0.4	0.6	1.0
B5	0.5	16.0	5.0	29.1
B8	4.5	18.3	10.7	0.4
B12	1.0	23.9	19.3	11.6
B15	0.5	13.2	8.9	18.3
B16	0.0	8.9	3.2	2.2
B17	2.5	5.7	5.7	3.6
B21	1.5	5.8	5.7	1.2
B22	1.0	5.6	0.6	20.2
B27	3.0	6.7	3.8	3.2
B37	1.0	3.2	2.6	1.2
B39	0.5	4.0	0.0	0.8
B40	1.5	11.7	7.5	34.6
B45	3.5	0.8	4.5	0.2
B47	1.0	0.4	0.0	0.8
B50	4.0	2.2	1.2	0.6
B54	2.0	0.2	0.0	13.0
B55	4.5	3.2	0.0	4.2
B56	1.5	2.2	0.6	3.0
B57	1.5	5.7	5.7	1.4
B58	2.5	0.0	0.0	2.4
B60	1.0	7.5	4.5	12.6
B61	2.5	4.2	3.0	22.0
B62	0.0	11.8	5.1	18.3
B63	2.0	1.4	3.8	0.0
B65	0.5	5.1	3.2	0.4
B73	2.0	0.2	0.0	0.4
Cw1	5.0	6.5	2.0	29.9
Cw5	3.0	13.3	5.9	1.2

[a]*Source: reference [13]*

These findings may prove to be of particular relevance to some HLA-associated diseases. In fact, determination of the class I antigens in patients with Graves disease did reveal a bias in favour of one of these rare antigens (B39), which in these patients exhibited an odds ratio of 9.6 [*14*].

The prevalent HLA haplotypes in the Lebanese population (Table 4) do not show any unusual combinations.

Some minor differences in the A and B loci were observed between our results and those previously reported on Lebanese immigrants [*1*,*2*] and other Arab communities [*7*] (Table 5). These could be partly due to a possibly higher rate of consanguinity in the immigrant communities, which tend to be more close-knit.

Table 3 **Genetic distances (×10 000) using gene frequencies in the A, B and C loci between Lebanese and four other populations**

Population	A locus	B locus	C locus	Average
Caucasoid	1878	2872	1654	2135
Black	2263	3375	1424	2354
Indian	3202	4022	3208	3477
Mongoloid	3413	4528	3550	3830

Table 4 **HLA haplotype frequencies in the Lebanese population**

Haplotype	Haplotype frequency (×1000)	Δ value (×1000)
A2, B35, Cw4	75	105.9
A1, B35, Cw4	60	50.3
A3, B35, Cw4	50	58.2
A24, B35, Cw4	40	58.9

Source: reference [10]

Table 5 **Comparison of the distribution of gene frequency in the sample to gene frequency in Lebanese immigrants and two other Arab groups**

Anti-gen	Present sample	Lebanese Aus.[a]	Lebanese USA[b]	Arabs Gaza[c]	Arabs Gal.[c]
A1	0.123	0.11	0.25	0.19	0.16
A2	0.229	0.18	0.08	0.27	0.20
A3	0.134	0.14	0.11	0.11	0.14
A9	0.013	0.18	0.28	0.12	0.10
A10	0.005	0.03	0.05	0.04	0.06
A11	0.054	0.08	0.05	0.04	0.07
A28	0.030	0.04	0.06	0.05	0.08
A29	0.033	(0.17)	0.14	(0.11)	(0.16)
B5	0.003	0.08	0.08	0.11	0.20
B7	0.043	0.04	0.03	0.02	0.03
B8	0.023	0.02	0.02	0.02	0.04
B12	0.005	0.05	0.05	0.11	0.05
B13	0.033	0.04	0.00	0.03	0.02
B14	0.046	0.05	0.03	0.05	0.03
B15	0.003	0.01	0.00	0.02	0.01
B16	0.000	0.07	0.06	0.06	0.05
B17	0.013	0.06	0.05	0.06	0.04
B18	0.038	(0.07)	0.05	0.04	0.05
B21	0.008	0.08	0.02	0.16	0.06
B22	0.005	0.01	0.02	0.01	0.05
B27	0.015	0.03	0.05	0.01	0.01
B35	0.197	0.24	0.31	0.16	(0.05)
B40	0.008	0.02	0.03	0.04	0.05

Aus.: Australia; Gal.: Galilee
[a]Source: reference [1]
[b]Source: reference [2]; a significance test was not conducted since the sample size was not mentioned in the reference
[c]Source: reference [7]
Numbers in brackets were found to be not significant

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