HLA-DR AND -DQ ANTIBODIES IN THE SERA OF SOUTH INDIAN PAROUS WOMEN

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HLA-A, -B positive sera of south Indian parous women were screened for the presence of HLA-DR and -DQ antibodies. The sera were absorbed with pooled platelets in a micro-method and screened against HLA-DR and -DQ typed B-lymphocytes panel. Out of fifty two sera, twenty were found to contain HLA-DR and -DQ antibodies. Seven sera were demonstrated to be specific for well defined DR and DQ antigens.

Introduction

The HLA (Human Leucocyte Antigen) complex has been localized to the short arm of the chromosome number 6 in the distal portion of 6p 21.3 band. This complex consists of at least seven well estimated loci which are designated as HLA-A, -B, -C, -D, -DR, -DQ and -DP. HLA-A, -B and -C antigens are known as class I antigens and HLA-D, -DR, -DQ and -DP antigens are known as class II antigens (1).

HLA-phenotyping of class I and some class II antigens is being carried out by serological typing method using allospecific human antisera and HLA-genotyping by restriction fragment length polymorphism (RFLP) using DNA probes for the HLA region of the human genome (2) and/or polymerase chain reaction (PCR) amplified human DNA (HLA genome) with sequence specific oligonucleotide (SSO) probes (3).

It has been well established that the sera of pregnant women are the most suitable and easily obtainable source for HLA antibodies. Further, it is evident that HLA-A, -B, -C antisera also frequently contain anti-HLA -DR (and -DQ) antibodies; specific -DR (and -DQ) antisera without MA-ABC antibodies have also been observed though rarely (4 - 6). In our earlier reports (7, 8), data on the screening for HLA-A, -B (class I) antibodies in the sera of South Indian parous women were presented. In the present study HLA-A, -B (class I) positive sera of the previous studies were screened for the presence of HLA-DR and -DQ (class- II) antibodies and the results are discussed.

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Materials and Methods

(i) Collection of parous women sera

Postpartum Mood dots were collected immediately after delivery (primiparous and multiparous) and the sera were separated and stored at -90ºC as described elsewhere (7,9).

(ii) Screening of sera for HLA-A. -B antibodies

A total number of 1161 sera were screened against HLA-A, -B typed cell panel members and the results have been published in our earlier reports (7,8).

(iii) Screening of sera for HLA-DR and -DQ antibodies

The HLA-A, -B positive sera were screened for the presence of HLA-DR, and -DQ antibodies against 32 normal individuals.

(iii)a. Elimination of HLA-A, -B, -C antibodies

It is well established that platelets contain HLA-A, -B and -C antigens on their surface (adsorbed antigens). Platelets from 20 different individuals were separated and pooled. The pooled platelets were used to absorb the anti-HLA antibodies from the HLA-A, -B positive sera. The method described by Cambon- Thomsen et al (9) was followed. In brief, all the HLA-A, -B positive sera (1µl) were plated in 60 well Terasaki plates. The pre-dotted walls were added with 1.5 x 10^6 pooled platelets (10.5 x 10^9/ml) in 1 µl saline and stored at -20°C until use.

(iii)b. T and B cell separation

T and B cells from peripheral blood mononuclear cells were separated by nylon wool column adherence method (10). In brief, plastic drinking straws approximately 8cm height were packed with 75 to 100 mg of nylon wool for 4 to 5 cm height. The columns were sterilized (10 psi) and used for T and B-cell separation. The nylon wool columns were rinsed with TC 199 medium containing 10% foetial calf serum (FCS) and antibiotics. Approximately 10 - 12 x 10^6 peripheral Mood mononulear cells (Ficoll-Hypaque separated) in 1 ml medium containing 10% FCS was loaded on the top of the column and incubated for 45 minutes. Afterwards the nylon wool nonadherent cells (enriched T-cell population) were eluted using 7ml of warm medium. Nylon wool adherent cells (enriched B-cell population) were eluted with 5 ml medium by pressing and squeezing the nylon wool column and used for HLA-DR and -DQ serological typing.

(iii)c. HLA-DR and -DQ typing in normal volunteers (for cell panel)

HLA-DR and -DQ typing of normal volunteers (32 individuals) was performed by two stage NIH standard microlymphocytotoxicity (antibody dependent complement mediated cytotoxicity) assay (11). A total number of 38 well defined HLA-DR and -DQ sera (Biotest, West Germany) covering DR 1 to DR 9 and DQ 1 to DQ 3 antigen specificities were used (Table 1).

(iii)d. Screening of sera for HLA-DR and -DQ antibodies

The plates containing the HLA-A, -B positive sera (pre- dotted) and pooled platelets were incubated at room temperature for 1 h with two or 3 times vortexing; and then the plates were used for -DR and -DQ antibody screening. For screening procedure the two stage NIH standard microlymphocytotoxicity (antibody dependent complement mediated microlymphocytotoxicity) assay was followed (11). In brief, one µl aliquot containing 2000-3000 (nylon wool column separated enriched) B-lymphocytes was added to each well. The plates were incubated for 1 h at 25°C; 5 µl of rabbit complement (absorbed with white cells) was added and incubated further for another 2 h. Then the reaction was arrested by adding 5 µl of 3% eosin and neutralized formaldehyde. The plates were read under phase contrast microscope.

Results

Our earlier reports on the screening of parous women sera for HLA-A, -B antibodies revealed 59 sera out of the total 1161 to be positive (Table 2) (7,8).

Out of these 59 HLA-A, -B positive sera, 52 were screened presently for HLA-DR and -DQ antibodies. Out of the 52, only 20 exhibited positive reaction on B lymphocytes when tested on the HLA-DR and -DQ typed B cdl panel (32 individuals) (Table 1). Eighteen sera were found negative and 14 sera still elude exact assignment warranting further analysis (Table 3).

Out of the 20 HLA-DR and -DQ positive sera, 7 were found to be either monospecific or duospecific to well defined DR and DQ antigens; the rest of the sera were multispecific (Table 4).

Discussion

The present study reveals that the frequency of HLA-DR and -DQ antibodies is less (20 + 14 =34, out of 1161 sera) (2.9%) (Table 3) when compared to the frequency of HLA-ABC antibodies (5.08%) (7,8) (Table 2).

Further, it also indicates that anti kHIA-DR and -DC typing reagents can be expected from multiparous sera (from post- partum blood clots) containing anti HLA-A or -B antibodies (38.5%; Table 3). Sera without ABC antibodies have also been reported to contain HLA-DR and -DR antibodies; however, the frequency of class II antibodies is less in these sera when compared to the sera that are positive for HLA-ABC antigens (12).

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Table I

Screening of cells (thirty two) for HLA-DR and -DQ antigens (cell panel)

	Specificity (number of cells positive)
HLA-DR	DR 1 (1); DR 2 (17); DR 3 (4); DR 4 (8); DR 5 (5); DR 6 (2); DR 7 (12); DR 8 (4); DR blank (11)
HLA-DQ	DQ 1 (25); DQ 2 (12); DQ 3 (14); DQ blank (12)

Table 2

Frequency of HLA - A, - B antibodies in parous women sera*

	Total sera	Percentage
Sera screened for HLA-A, -B, -C antibodies	1161	–
Positive sera	59	5.08%
a) Monospecific sera	16	1.38%
b) Multispecific sera	43	3.7%
Negative sera	1102	94.9%

*Based on our earlier reports (7,8)

Table 3

Frequency of HLA-DR and -DQ antibodies in HLA-A, -B positive sera of parous women

	Total sera	Percentage
Sera screened for HLA-DR and -DQ antibodies	52*	–
Positive sera	20	38.5%
Negative sera	18	34.6%
Still under analysis for assignment	14	26.9%

* HLA-A, -B positive sera of parous women

Table 4

Analysis on the behaviour of HLA-DR and -DQ positive sera of parous women

Serum number	Specificity	Positive	False positive	False negative	Negative	r-value
CDM 741	DR 2	15	2	2	11	0.73
CDM 1111	DR3	3	0	1	26	0.84
CDM 1217	DR3	3	0	1	26	0.84
CDM 1501	DR2	13	7	4	6	0.24
CDM 1521	DR 8	2	0	2	26	0.68
CDM 1593	DR 7, DQ 2	8	7	4	11	0.27
CDM 1622	DQ2	6	2	6	16	0.43