HLA-A and HLA-B antibodies of pregnant mothers in Malaysia

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Summary

The aim of this study was to determine the frequency[1,2] and specificity of HLA-A and B antibodies in multiparous mothers in the Malaysian population. 1,100 maternal serum samples obtained during normal childbirth were screened against a panel of 100 lymphocytes with known HLA antigen types for HLA antibodies by the complement dependent lymphocyte microcytotoxicity dye exclusion test. From the total number of 1,100 samples of maternal serum that were screened for HLA antibodies only 205 specimens (18.6%) tested positive for antibodies. The percentage of maternal sera which contained HLA-B specificities (10.6%) were significantly higher than those which contained HLA-A specificities (3.0%). Sixty maternal serum samples (5.5%) had high enough titres to be utilised as tissue typing reagents. Thirty nine maternal serum samples (3.5%) contained monospecific HLA antibodies. In this study the most common monospecific HLA antibodies characterised included the following specificities: A2, B5, B17 and B40. Malaysian multiparous mothers of gravida 3, 4 and 5 had a higher frequency for producing HLA-antibodies.

Key words: HLA-A antibodies, HLA-B antibodies, multiparous mothers, tissue typing, screening programme.

Introduction

Antibodies against human[4] leucocyte antigen HLA-A, B, C, DR and DQ specificities were commonly found in the sera of women who had multiple pregnancies. In the pregnant mother, antibodies are formed as a result of natural immunisation occurring during pregnancy when foetal HLA antigens of paternal origin enter into the maternal circulation and immunise the mother after placental detachment. The aim of this study was to determine the [5,6] frequency of multigravida-multiparous mother who produce HLA antibodies which is still the main source of HLA tissue typing reagents in wide usage today. Such information will enable a HLA tissue laboratory in the planning and execution of an efficient serum screening programme to produce an excellent panel of HLA typing reagents.

Materials and methods

Collection of serum: One thousand one hundred maternal blood samples were collected in clean bottles from unrelated mothers during deliveries from the maternity wards of the hospitals. The maternal blood was centrifuged at 3,000 rpm for ten minutes at 4°C. The maternal serum

was separated, documented and preserved in 0.1% sodium azide. It was then aliquoted in 0.4ml microfuge tube and test tube (15 mm x 16 mm) and stored at –20°C.

Reference cell panel (n = 100): 15 mls of blood was collected from 100 healthy donors and volunteers with known HLA phenotypes. The lymphocytes were separated by the density gradient centrifugation method. The cells were then washed in phosphate buffered saline and standardised to[7] 1 x 10^6 cells/ml in RPMI 1640 (Rosewell Park Memorial Institute 1640) Medium for screening of HLA antibodies.

Screening of maternal sera for HLA antibodies: 0.001 ml of each maternal serum was dispensed into each well of Terasaki tissue typing tray, covered with liquid paraffin and stored at –70°C. The sera were screened against 100 reference cells by the standard lymphocyte microcytotoxicity dye exclusion test of the National Institute of Health (NIH). Rabbit complement used was obtained from Pel-Freez Biological, U.S.A. To ensure accuracy and reproducibility, each sample of serum were tested at minimum of seven times against a single HLA antigen. (e.g. while screening for A1 antibody one must have seven reference cells with A1 antigens).

Reading of results: The test was read using a phase contrast inverted microscope. The percentage of dead cells was assessed. The criteria for positive and negative reactions are based on the percentage of dead cells as assessed under a phase contrast inverted microscope.

Dead Cells		Results
0% – 40%	=	negative reaction
41% – 80%	=	positive reaction
81% – 100%	=	strong positive reaction

The strength index (S.I.) of an antibody is calculated as follows:

$$\text{S.I.} = \frac{\text{No. of strong positive reactions}}{\text{No. of positive reactions}}$$

Results and Discussion

Out of a total of 1,100 maternal sera that were screened for HLA antibodies, only 205 specimens (18.6%) were tested positive for antibodies (Table 1). This percentage compares well with results documented in screening programme of other countries (10.26%). The number of sera which contained HLA-B specificities (10.6%) were significantly higher than those which contained A specificities (3.0%). This data correlates with the findings reported by Minev[8] 1975 who suggested that antigens of HLA-B series are more immunogenic than those of the HLA-A series. Antibodies that possessed few weak reactions and had no correlation with any of HLA-A or B antigens were considered as unidentified specificities.

Sera that gave a 'r' value of 0.85 and strength index (S.I.) of 80% were considered good. Sixty (5.5%) of the maternal sera had good HLA antibodies which can be utilised as HLA typing reagents. Thirty nine (3.5%) of the HLA antibodies were monospecific (Table 2). The most common monospecific HLA antibodies characterised are shown in Table 3. This study indicates that to obtain a high yield of good HLA anti-sera for use as a typing reagent, maternal screening programmes in Malaysian multiparous mothers should be confined to women in the 3, 4 and 5 gravida (Table 3).

Table 1
HLA Antibodies Detected from the Sera of Multiparous Mother

HLA Antibodies	n	%
Total sera screened	1100	100
Positive for antibodies	205	18.6
HLA-A specificities	333	3.0
HLA-B specificities	117	10.6
Multispecificities	30	2.7
Unidentified specificities	25	2.3

Table 2
Good and weak HLA antibodies

HLA Antibodies	Number detected	Good Antibodies				Weak Antibodies
		Mono specific	Di specific	Tri specific	Total	
HLA-A Antibodies	33	16	5	3	24	9
HLA-B Antibodies	117	23	7	7	37	80

Table 3
Frequency of HLA monospecific antibodies

Specificity	A2	A3	A9	A11	B5	B7	B13	B16	B17	BW22	B27	B40
n	7	1	2	1	4	1	2	2	5	2	2	4

Table 4
Gravida with frequency of antibodies

Gravida	1	2	3	4	5	6	7	8	9	10
% of mothers with HLA antibodies.	3.2	6.6	24.7	21.2	17.2	7.1	5.8	0.5	10.0	0.5

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