Frequency and potential application of HLA antibodies from pregnant women in Mumbai

U SHANKARKUMAR*, S C GUPTE, S S GUPTE, S V PEDNEKAR, K GHOSH and D MOHANTY

HLA Department, Institute of Immunohaematology, 13th Floor, New MS Building, KEM Hospital Campus, Parel, Mumbai 400 012, India

**Corresponding author (Fax, 91-22-4138521).*

Antenatal sera from 1334 pregnant women attending the Nowrojee B J Wadia Maternity Hospital and KEM Hospital in Parel, Mumbai were collected and screened for anti HLA A and B antibodies to produce an indigenous HLA tissue typing tray. One hundred and sixty three sera (12·2%) were found positive for HLA antibodies. Nonetheless, the percentage of positive sera were almost the same in women of different parity. Moreover, the incidence of anti-HLA antibodies was correlated with the allelic frequencies in the Maharastrian population. Thus in India, collection and screening of sera from pregnant females is a simple and cost-effective method of acquiring polyclonal sera for routine use in tissue typing.

Introduction

The HLA system is generally studied from various points of view including, organ transplantation, population genetics, disputed parentage, disease association studies and to answer basic questions of immunobiology. The system consists of HLA A, B, C, DR, DQ and DP loci over a region of 3600 kb in p. 21·3 of chromosome six (ch. 6) (Campbell R D and Trowsdale J 1993 Trowsdale and Campbell 1997). Sequence studies have shown that each locus is highly polymorphic (Bodmer *et al* 1995). The products of these alleles (antigens) are expressed on various cells in the human body and are identified by specific antisera. There are many sources for obtaining these antisera namely multiparous women, transplant recipients, polytransfused patients, immunized donors and monoclonal antibodies. Antibody to HLA was first identified in the serum of a polytransfused patient (Dausset 1958); subsequently materno-foetal alloimmunization was also shown to produce anti-HLA antibodies in pregnant women (Payne and Rolfs 1958; Van Rood *et al* 1958). Pregnant women are the most common source of these antisera (though monoclonal antibodies have also been raised recently).

In contrast to the situation in India advances in the study of the HLA system have been rapid in western laboratories. The major limitation that is responsible for this situation is the non-availability of suitable antisera in the country. Consequently one has to depend on importing antisera from commercially available sources. In India, serology is the conventional technique used to define various HLA alleles. In this paper we present our results on sera from multiparous women screened for HLA A and B antibodies.

2. Materials and methods

2. *Samples*

Five to ten ml of peripheral blood were obtained from pregnant women attending various hospitals in Parel in the period 1991–1997. Their age, parity, nativity, Rh status and other relevant details were noted in questionnaires.

2.2 *Screening for antibodies*

Each "sixty well" tissue typing tray (Greiner, Germany) consisted of one positive and one negative control and 58 sera to be screened as per tray formats. A total of

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23 tray formats including all 1334 antenatal sera were prepared. Each tray was tested with lymphocytes (which were separated from each volunteer by density gradient centrifugation; Boyum 1968) with the help of the NIH standard microlymphocytotoxicity test. Thirty to fifty such reference cells were used to screen each batch of trays.

2.3 *Reference panel*

A total of 75 employees of our institute were HLA typed panel members. Their phenotypes were determined using sera from NIH, Bethesda, USA; Asia Oceania Histocompatibility Workshop 1986 sera; Kissmeiyer Neilson, Denmark; and commercial companies (Biotest Dreieich, Germany and PelFreez, Wisconsin, USA).

2.4 *Microlymphocytotoxicity test*

Peripheral blood was obtained from reference panel members by venipuncture and lymphocytes were isolated by centrifugation in a one-step density gradient (Boyum 1968). The microlymphocytotoxicity test described by Terasaki and McClelland (1964) was used for both HLA phenotyping of the reference panel member and for screening of the unknown sera. An inverted phase contrast microscope (Zeiss, Germany) was used to read the results.

2.5 *Data processing and analysis*

The reaction of each serum with each cell was correlated with the presence or absence of HLA antigen. The correlation of a given antigen (HLA antigen) with the reaction of a given serum was carried out manually using a two by two contingency table, χ^2 and correlation coefficient (r) according to standard method (Snedecor and Cochran 1968). Further analysis of serum versus serum correlations as well as serum versus antigen correlations was carried out to evaluate serum at various levels of positive sera and to obtain various parameters such as the r-value, percentage 8 positive, χ^2, Q-scores, list of cells in true positives, false positives, false negatives and true negatives.

3. Results

3. *Incidence of HLA antibodies*

Table 1 presents the total number of anti-HLA sera and their specificities identified in the present study. For comparison, frequencies of HLA alleles in the mixed population of Mumbai are also presented. The relative incidence of HLA antibodies was as expected on the basis of our knowledge of HLA antigens in general.

3.2 *Comparison of parity with incidence of HLA antibodies*

Table 2 presents the results of the correlation between the parity of the women and the incidence of the anti-HLA A and B antibodies. Irrespective of past pregnancies, the incidences of anti-HLA A and B antibodies were same. Primiparous women produced almost the same percentage (11·9%) of anti-HLA antibodies as multiparous women. These primiparous women had no past history of abortion. This incidence, however is not

Table 1. HLA specificities of HLA typing reagents detected in the present study ($n = 163$) compared to HLA antigen frequency in the population.

HLA	PF (%)	No. of sera	r value
A1	15·9	13	
A2	30·4	32	
A3	11·6	03	
A9	11·6	21	
A24	21·7	08	
A10	11·6	08	
A26	2·9	02	
A34	0·4		
A11	23·2	20	
A19	10·1	08	
A29	2·9	02	
A30	1·5	–	
A31	5·8		
A32	4·4	–	
A33	18·8	04	
A28	13·0	28	0·87
B5	7·3	03	
B51	15·9	04	
B52	5·8	02	
B7	15·9	17	
B8	2·9	09	
B12	4·4	09	
B44	14·6	04	
B13	5·8	10	
B14	4·4	02	
B15	7·3	01	
B62	10·1	04	
B17	7·3	17	
B58	7·3	–	
B18	8·7	06	
B21	1·5	01	
B49	1·5	–	
B22	1·5	01	
B55	5·8	03	
B56	2·9	–	
B27	1·5	07	
B35	24·6	09	
B37	4·4	07	
B38	4·4	–	
B40	11·6	09	
B60	4·4	–	
B61	11·6	02	
B53	2·9	02	0·89

Table 2. Parity and incidence of anti HLA A and B antibodies.

Pregnancy	Total sera	Mono *N* (%)	Duo *N* (%)	Poly *N* (%)	Total *N* (%)
First	301	15 (4·9)	12 (3·9)	9 (2·9)	36 (11·9)
Second	301	20 (6·6)	10 (3·3)	8 (2·6)	38 (12·6)
Third	521	26 (4·9)	12 (2·3)	27 (5·1)	65 (12·4)
Fourth	104	2 (1·9)	4 (3·8)	7 (6·7)	13 (12·5)
Fifth	16	2	–	–	2
Sixth	12	–	–	–	–
Seventh	63	3	4	–	7
Eighth	16	2			
	N = 1334	70	42	51	163

% $N = (N/\text{total sera}) \times 100$.

reported internationally but it has been observed at the national level.

4. Discussion

As reported by Pitchappan *et al* (1993), in India it is not feasible to collect a large quantity of blood from a pregnant woman by cubital bleeding (antenatal) especially considering her anaemic status, malnutrition and unwillingness. Further, lack of facilities for sophisticated procedures like plasmapheresis in the Primary Health Centres (PHC), government hospitals in the state and in most medical colleges suggests that post-partum collection in labour wards causes little discomfort to the women concerned and yields higher volumes of sera. Even though 5% of the sera may be wasted due to contamination, the remaining 95% will be usable. With the number of pregnant women in this country, this procedure would be best suited under the circumstances in India. Moreover this makes handling much easier for attending nurses and physicians.

In pregnant women from south India it has been reported that the incidence of antibodies positive for HLA A and B was 10% in antenatal sera and 13·4% in the post-partum sera (Pitchappan *et al* 1993). It was observed that both primiparous and multiparous women produced the same percentage of anti-HLA antibodies. Studies in sera from Venezuelan multiparous women showed that the incidence of anti-HLA antibodies in pregnant women was related to the number of pregnancies and was 4% lower in post-partum sera than antenatal sera (Simonney *et al* 1984). In another study the percentage of antibodies did not differ based on the number of past pregnancies (Rodey *et al* 1979). There is general agreement that foeto-maternal alloimmunization against HLA antigens is initiated in the first pregnancy itself (Simonney *et al* 1984). Vives *et al* (1976) observed that anti-HLA antibodies appeared by the 18th week of pregnancy in primiparous but by the 8th week in second or third pregnancies. In Mestizo women from Venezuela, the antibodies were detected only by the 34th week in primigravidas and the 17th week in a second or third pregnancy (Simonney *et al* 1984).

Wide variation in the incidence of anti-HLA antibodies in the sera of pregnant women have been reported. These are due to variations in immunization and the resultant outcomes. Values range from 7·3% to 36% (7·3%, Nymand *et al* 1971: 18·7%, Rodey *et al* 1979: 21·6%, Decary *et al* 1979: 29%, Mestizo women; and 9·6%, Warao women; Simonney *et al* 1984: 36%, Terasaki *et al* 1970 and 10·6%, Pitchappan *et al* 1993). Differences in the incidence between two different ethnic groups viz: Venezuelan of Amerindian and mixed ethnic origin have also been reported (Simonney *et al* 1984): the incidence of antibodies in post-partum sera was 2% in Warao women and 10% in women of mixed ethnic origin. In another study the incidence of antibodies in successfully completed pregnancies have been reported to be 32% compared to 10% in spontaneous abortions (Regan *et al* 1991). Various reasons can be attributed to this discrepancy in the incidence values, for example correlation coefficients (r) between the HLA antigen and antibodies, percentages of positive reactions (> 80%), the ethnic differences among the populations used with variable antigen frequencies as well as the methodology involved to detect the antibodies with appropriate HLA known panel lymphocytes.

The present study has revealed an average incidence of 12% positive sera in women at different parity which is in agreement with some of the published observations (perhaps because our sera were collected during the last trimester of pregnancy). It is possible that all the "responder" women may produce anti-HLA antibodies in the first pregnancy itself. In subsequent pregnancies the time of appearance of antibodies, titre and specificity (mono or duo) may vary. A significant observation of the present study was that the average incidence of 12% positivity was irrespective of parity.

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