INTRODUCTION

Antibodies to antigens of the HLA system are formed by a proportion of women during a pregnancy and sometimes after blood transfusion. The gestational antibodies are of no significance to the pregnancy and in most cases they are thought to disappear from the serum a few weeks after termination of the pregnancy. They are, however, of great value as HLA typing reagents and most tissue typing laboratories (including our own) rely heavily on pregnancy sera as their source of HLA antisera. Blood samples from many mothers during their pregnancies are sent to the Northern Ireland Blood Transfusion Service for red cell antibody screening and aliquots of these sera are also examined for HLA antibodies. When a serum is found to contain a monospecific antibody of potential value for tissue typing, the patient and/or her family doctor are contacted and arrangements are made to take a further 50 ml sample of blood. This serum is sufficient for our own needs but in order to facilitate the interchange of sera with other laboratories both in the United Kingdom and abroad larger volumes of sera are necessary. It is inappropriate to ask for a full standard donation of blood from a mother a short time before or after the end of a pregnancy. Large volumes of serum can be obtained by plasmapheresis, a procedure which entails no loss of red cells by the patient, but because of the time involved it is often inconvenient for the mother of a new baby to attend hospital (or the transfusion service building) to give plasma by this means.

We have therefore considered a different approach for obtaining large volume supplies of HLA typing sera. In some women, HLA antibodies may persist for years after pregnancy (Tongio, Berrebi and Mayer, 1973) and therefore a proportion of female blood donors might be expected to have these persistent and stable antibodies. It would not be difficult to obtain 'dry bottle' donations from selected regular blood donors at their next visit to the transfusion centre.

With a view to obtaining HLA typing sera in this way we screened blood samples from a group of female blood donors. This paper reports our findings.

MATERIALS AND METHODS

Blood samples (2 ml) were obtained from 7,230 female blood donors for HLA antibody screening. To simplify administrative procedures no attempt was made
to select married woman donors who had been pregnant. The sera were separated and stored at \(-20^\circ\text{C}\) for up to three weeks before screening against panels of lymphocytes selected to include all the known HLA-A and -B locus antigens. A standard two-stage microcytotoxicity test at 22°C was used throughout.

Sera showing HLA activity were, if necessary, further tested to identify monospecific antibodies. A simple questionnaire was sent to the blood donors found to have antibodies. In it we asked whether or not they had ever had a blood transfusion and/or pregnancy and if so, the dates thereof.

RESULTS

Table 1 summarises our findings in the blood donors and compares them with the results of routine screening of ante-natal sera. Of 220 pregnancy/transfusion questionnaires issued 172 (78 per cent) were returned. The answers are summarised in Tables 2 and 3.

TABLE 1: HLA ANTIBODIES PRESENT IN SERA FROM BLOOD DONORS AND ANTE-NATAL SAMPLES

|                     | Blood donors | Ante-natal |
|---------------------|--------------|------------|
| No. of samples tested | 7230         | 4756       |
| No. with HLA antibody | 220 (3.04%)  | 956 (20.1%)|
| No. with monospecific antibody | 25 (0.35%)  | 44 (0.93%) |

TABLE 2: SUMMARY OF REPLIES TO PREGNANCY/TRANSFUSION QUESTIONNAIRE

| History of Blood Transfusion | History of Pregnancy |
|------------------------------|----------------------|
| Yes                          | No                   |
| YES                          | 34                   | 0          |
| NO                           | 120                  | 18         |
|                              | 154                  | 18         |
|                              |                      | 172        |

TABLE 3: INTERVAL SINCE LAST IMMUNISING STIMULUS

| Years | Pregnancy Alone | Pregnancy and Transfusion |
|-------|-----------------|---------------------------|
| 10+   | 31              | 8                         |
| 5-10  | 25              | 10                        |
| 2-5   | 41              | 8                         |
| 0-2   | 13              | 7                         |
| Pregnant at time of sample   | 3                   | 0                         |
| Reply unclear                 | 7                   | 1                         |

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DISCUSSION

The proportion of blood donors with HLA antibodies was, as expected, much smaller than the proportion of ante-natal sera with anti HLA activity (3.04 per cent as compared with 20.1 per cent). The yield might have been improved if we had selected only blood donors who had been pregnant; our figures might then have approached those of Rodey, Anderson and Aster, (1976) who found HLA antibodies in 18.7 per cent of non-pregnant multiparae with a history of four or more pregnancies.

The replies to our questionnaire indicated clearly that transfusion alone is not a common stimulus to the production of persistent antibodies; and almost 90 per cent of the blood donors with antibodies had been pregnant at some time. Their antibodies are remarkably long lived: 74 of 154 women with antibodies (48.1 per cent) had not been pregnant nor had transfusion for 5 or more years. In Rodey's (1976) series the corresponding figure was 64 per cent, presumably due to the greater (4 or more) parity of his donors.

We define an antibody to be potentially valuable for tissue typing if it is adequately avid and if it appears to be monospecific. Ante-natal sera often contain multispecific antibodies and only a small proportion (44 of 956 = 4.6 per cent) are potential tissue typing sera. On the other hand, antibody containing sera from blood donors yield a much higher proportion of “clean” HLA antibodies (25 of 220 = 11.36 per cent) probably because the weaker and cross reacting antibodies have declined in activity to undetectable levels.

Our finding of HLA antibodies in a significant number of female blood donors confirms the possibility of obtaining HLA typing sera from this source. The vast majority of donors give blood regularly and it is a simple administrative matter to arrange for the taking of “dry bottle” donations from specially identified donors. As an alternative plasmapheresis could be considered although this would often entail a special visit to the transfusion centre and we feel it important to avoid, as far as possible, interference with the routines of blood donation and collection. In order to reduce unproductive screening of negative sera, multiparous donors could be chosen for testing and we feel that this particular group represents a viable alternative to pregnant mothers as a source of typing sera, especially for HLA laboratories located within transfusion centres.

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REFERENCES

RODEY, G. E., ANDERSON, J. andASTER, R. H. (1976). Acquisition of HL-A lymphocytotoxic antibodies. In N.I.A.I.D. Manual of Tissue Typing Techniques, ed. Ray, J. G., Hare, D. B., Pedersen, P. D. and Mullally, D. I., p. 151-160. Bethesda, Maryland: D.H.E.W. Publication number (N.I.H.) 77-545.

TONGIO, M. M., BERREBI, A. and MAYER, S. (1973). Anti-HL-A foeto-maternal immunization. Persistence of Antibodies. Tissue Antigens 3, 115-122.