INTRODUCTION

FOR the past few years the policy in most transplantation centres has been to allocate cadaver kidneys to recipients whose tissues are antigenically similar to the donor’s. Tissue similarity has been assessed by comparison of the HL-A antigens identifiable on lymphocytes (not kidney or other tissue cells) from donor and recipient. The technique of HL-A typing is not in itself difficult. Briefly, lymphocyte suspensions are prepared from freshly drawn peripheral blood by flotation on a Triosil-Ficoll solution (Harris and Ukaejiofo, 1969). After washing with buffered saline, the suspensions are added to tissue typing plates which have been primed with sera and covered with liquid paraffin to prevent evaporation. Each plate has spaces for up to 72 sera. After incubation, complement, in the form of a mixture of rabbit and human serum, is added to each test and a further incubation carried out. In those tests where lymphocyte antigens have reacted with the sera, complement activation results in cell death. Microscopic examination after the addition of trypan blue (which stains the killed cells but is excluded from living ones) enables positive tests to be identified.

In common with most of the tissue typing laboratories in Great Britain, we receive our sera from the National Tissue Typing Reference Laboratory (N.T.T.R.L.) in Bristol. This has the advantage that donors and recipients throughout Britain are, in the main, typed with the same sera. The HL-A antisera in use are mainly obtained from women during pregnancy who become immunised to HL-A antigens inherited by the foetus from the father. Antibody-containing sera are identified by the same basic method as for HL-A typing, except that cells of known antigen content from a panel of volunteers (members of staff) are tested against sera of unknown antibody content. Most tissue typing laboratories, including our own, carry out a screening programme on pregnancy sera as part of their routine work. Potentially useful sera are submitted to N.T.T.R.L. who carry out further tests, and may request further supplies of the test sera.

In parallel with the antibody screening programme, serum samples from prospective kidney recipients are also tested. This is of importance, since antibodies in a recipient’s serum may cause immediate rejection of an incompatible transplant. They are in general weak antibodies, and are difficult to characterise.
The work of the tissue typing laboratory thus comprises three parts, namely HL–A typing for transplantation, serum supply, and transplant recipient screening for HL–A antibodies. We present below some of our findings during the three year period, September 1971–August 1974.

HL–A Typing

During the three year period 64 potential kidney recipients were typed. A further 48 patients, potential kidney donors, were also typed; and in the establishment of the antibody screening programme cells from a staff panel numbering 108 were tested, most of them during late 1973 and early 1974. We were, therefore, able to type the staff panel with an improved range of sera for antigens in the W19 complex, earlier tests being relatively insensitive for these factors. Since there is at present much interest in the distribution of the HL–A antigens in various diseases and conditions of stress, we have compared the antigen frequencies in our prospective kidney recipients and potential donors, and in our staff panel with the Bristol blood donor panel reported by Nelson, Darke and Tovey (1974). Table I shows the antigen frequencies in the four groups.

### Table I

**HL–A antigen frequencies in kidney recipients, donors, and hospital staff, compared with a blood donor panel (Nelson et al, 1974). Chi squared values greater than 8.0 (with Yates' correction) are indicated**

| HL–A | Kidney Recipients (Actual + Potential) | Kidney Donors (Actual + Potential) | Staff Panel | Bristol Blood Donor Panel |
|------|----------------------------------------|-----------------------------------|-------------|---------------------------|
|      | N=64                                   | N=48                              | N=108       | N=1036                    |
|      | No. %                                  | No. %                             | No. %       | No. %                     |
| 1    | 25 39                                  | 20 42                             | 42 39       | 348 34                    |
| 2    | 38 59                                  | 24 50                             | 52 48       | 536 52                    |
| 3    | 14 22                                  | 14 29                             | 25 23       | 265 26                    |
| 9    | 11 17                                  | 13 27                             | 15 14       | 190 18                    |
| 10   | 6 9.4                                  | 4 8.3                             | 6 5.6       | 87 8.4                    |
| 11   | 6 9.4                                  | 2 4.2                             | 12 11       | 106 10                    |
| W28  | 0                                      | 2 4.2                             | 9 8.3       | 38 3.7                    |
| W19  | 10 5.6                                 | 2 4.2                             | 26 24       | 175 17                    |
| 5    | 3 4.7                                  | 3 6.3                             | 9 8.3       | 87 8.4                    |
| 7    | 19 30                                  | 19 40                             | 32 30       | 265 26                    |
| 8    | 22 34                                  | 16 33                             | 23 21       | 260 25                    |
| 12   | 16 25                                  | 13 27                             | 146 42      | 254 24                    |
| 13   | 1 1.6                                  | 1 2.1                             | 3 2.8       | 45 4.3                    |
| W5   | 8 13                                   | 6 13                              | 6 7         | 114 11                    |
| W10  | 8 13                                   | 6 13                              | 15 14       | 116 11                    |
| W14  | 5 7.8                                  | 4 8.3                             | 3 2.8       | 72 7                      |
| W15  | 9 14*                                  | 1 2.1                             | 11 10       | 50 4.8                    |
| W17  | 7 11                                   | 4 8                               | 11 10       | 79 7.6                    |
| W18  | 5 7.8                                  | 3 6.3                             | 2 1.91      | 35 3.4                    |
| W21  | 1 1.6                                  | 1 2.1                             | 1 0.9       | 18 1.7                    |
| W22  | 2 3.1                                  | 1 2.1                             | 3 2.8       | 55 5.3                    |
| W27  | 5 7.8                                  | 2 4.2                             | 10 9.3      | 77 7.4                    |

*Chi squared=8.39; 0.005>p>0.001; after correction 0.115>p>0.023  
†Chi squared=15.56; p<0.0005; after correction <0.015
When the same population samples are compared several times, the chance of finding a "statistically significant" difference increases with the number of comparisons. Accordingly, in assessing the significance of inter-sample differences, the p value obtained must be corrected by multiplying it by the number of comparisons (Grumet et al., 1971). In this instance where we have made 23 comparisons, statistical significance will only be maintained if p before correction is less than 0.002; (Chi squared greater than 9.548).

Under these criteria, the antigen frequencies in our patients and staff showed few differences from the Bristol blood donors. Surprisingly, the expected reduction in W19 antigens of the kidney recipients and donors did not achieve statistical significance; the only finding of significance was that HL–A12 occurred more frequently in our staff panel than in the Bristol blood donors (Chi squared = 15.56). The fact that our staff panel is, by definition, a selected population sample and not a random one is the most obvious explanation of the increase in HL–A12, particularly since population sampling errors tend to be more striking in relatively small samples.

**PREGNANCY SERUM TESTING FOR HL–A ANTIBODIES**

Since October 1973 we have screened samples of ante-natal sera kindly supplied by the Northern Ireland Blood Transfusion Service, and by the Laboratory, Craigavon Area Hospital. The sera are obtained from blood grouping specimens taken routinely during pregnancy, and the test for HL–A antibodies does not involve taking extra blood from the patients. The frequency with which HL–A antibodies are found in the serum increases with maternal parity, from approximately 5 per cent in primigravidae, to around 40 per cent in women who have had five or more pregnancies. Antibodies formed during a first pregnancy are usually weak, although often of narrow specificity. In subsequent pregnancies, the antibodies may be of higher titre, and are often directed against more than one antigen. Sera in which antibodies are found are re-tested in an attempt to define their specificity and titre. When the antibody is of good titre and monospecific, a further sample (30 ml) is requested through the courtesy of Col. Field (Northern Ireland Blood Transfusion Service). The sera from these larger samples are submitted to the National Tissue Typing Reference Laboratory for confirmation of our findings. If suitable, the sera are used as HL–A typing reagents. Table II presents a brief statistical summary of the ante-natal serum screening programme.

**ANTIBODY TESTING OF KIDNEY RECIPIENTS' SERUM**

A small proportion of patients awaiting a transplant already have, or may develop, HL–A antibodies following blood transfusion. Transplantation of an incompatible kidney into these patients is usually followed by hyper-acute rejection. In many cases the antibodies are weak; indeed they may become detectable only after a pyrexial episode (Nelson et al., 1971). It is therefore important to test recipients’ serum regularly and to carry out a "cross match" using patient’s serum and donor lymphocytes prior to transplantation. By this means, the possibility of a hyperacute rejection reaction can be avoided.
TABLE II  
Results of Pregnancy Serum Screening  
October 1973 – August 1974  

| Number of Pregnancy sera tested | 3,700 |
|---------------------------------|-------|
| Number with HL–A antibodies      | 460   |
| Per cent with HL–A antibodies    | 12.4  |
| Larger sample requested for      |       |
| further investigation            | 50    |
| Samples sent to N.T.T.R.L.       | 36    |
| Specificities:                   |       |
| HL–A1 (5)                        |       |
| HL–A2 (5)                        |       |
| HL–A5 (2)                        |       |
| HL–A7 (8)                        |       |
| HL–A8 (4)                        |       |
| HL–A12 (6)                       |       |
| W5 (2)                          |       |
| W10 (2)                         |       |
| W15 (1)                         |       |
| W32 (1)                         |       |

During the three year period under review, serum samples from 58 patients were tested for HL–A antibodies. Eleven (19 per cent) were found to be positive, and of these, seven were awaiting a second transplant. No positive cross matches have been found and nine of these eleven patients have now received a transplant without evidence of hyperacute rejection.

COMMENT

The place of HL–A matching for transplantation has not yet been fully assessed; and while recent reports have suggested that HL–A identity between donor and recipient confers some benefit (Oliver et al, 1972; Van Hooff et al, 1973) there is no doubt that many kidneys transplanted to recipients of widely different HL–A types will function for a long time (Belzer et al, 1974). It may be that the emphasis should be placed on ensuring that kidney recipients are immunised by their transplants only to low frequency antigens. This policy would have obvious benefits in the event of a second transplant becoming necessary, because a second donor would be unlikely to present the recipient with an antigen to which he had already been immunised.

In the pregnancy serum screening programme our experiences have been similar to those of Stastny (1972). So far we have identified sera with activity against the more common HL–A antigens, but we hope in due course to uncover also examples of the rarer antibodies. Attempts to provide useful sera for our own or for national use have become more important following the demonstration by Brewerton et al (1973) of a striking relationship between ankylosing spondylitis and the presence of the antigen HL–A27. This antigen, present in about 7.5 per cent of healthy people, is found in more than 90 per cent of sufferers from ankylosing spondylitis; its presence or absence may be a helpful diagnostic clue, and we can now provide tests for the presence of this antigen when requested. The use of HL–A typing tests in various disease states will almost certainly increase during the next few years, but we hope also for an increase in the number of potential (and actual)
kidney donors, since successful transplant operations are of benefit not only to the recipients but may also permit new patients to receive dialysis treatment more readily.

SUMMARY

The Tissue Typing Laboratory deals mainly with the immunological tests carried out in relation to renal transplantation. These include HL–A typing of prospective recipients and donors and testing of recipients' serum for the presence of antibodies which might cause hyperacute rejection of a transplant. The antisera used for HL–A typing are usually obtained from pregnant mothers, and most tissue typing laboratories also test pregnancy sera for potentially useful antibodies by observing their reactions with cells of known HL–A type.

Between September 1971 and August 1974, 64 potential kidney recipients and 48 possible donors were typed, as were 108 members of staff at the Belfast City Hospital whose cells were used in antibody detection and identification. The HL–A antigen frequencies in these three groups were compared with each other and with a large population of blood donors from the Bristol area. HL–A12 was identified more frequently in members of staff than in the other groups. This finding is attributed to the sampling error inherent in selected and relatively small population samples.

HL–A antibodies were found in 460 (12.4 per cent) of 3700 pregnancy sera; 36 of these were potentially valuable as typing reagents and aliquots were sent to the National Tissue Typing Reference Laboratory for confirmation of our findings. The urgency of the task of serum procurement is made more acute by the increase in requests for tissue typing which has followed recognition of the association between HL–A27 and ankylosing spondylitis.

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