

## HLA and renal transplantation: yet another approach\*

**SUMMARY** Renal allografts obtained from living related donors have considerably better chances of success than allografts from cadavers. The genetic factors that influence graft survival are linked to the HLA gene complex. Although results of cadaver transplants have not improved significantly when donor and recipient are matched for HLA-A and -B antigens, accumulating data suggest that matching for the more recently recognised HLA-DR antigens may produce increased graft survival.

The first successful human renal transplant was performed between identical twins in 1954.<sup>1</sup> As the transplanted kidney was an isograft, and thus not recognised as foreign tissue by the recipient, rejection did not occur. Since that time, it has become apparent that renal allografts obtained from living related donors have considerably better chances of survival than allografts from cadavers. This finding has stimulated many investigators to study the genetic factors that influence graft survival.

More than 20 years ago, Dausset in Paris,<sup>2</sup> van Rood *et al* in Leiden,<sup>3</sup> and Payne and Rolfs in San Francisco,<sup>4</sup> demonstrated that sera from multiply-transfused patients or from multiparous women were capable of agglutinating leucocytes from some, but not all, normal subjects. Thus it was appreciated that nucleated cells have an antigen system separate from the recognised ABO red cell antigens. In 1967 this new system was designated HLA (H = Human, L = Leucocyte, and A for the first system discovered). During the intervening years it has been shown that most cells of the body, including T lymphocytes and platelets, possess three series of antigens, HLA-A, -B, and -C antigens, whereas the D and the closely-linked DR antigens are expressed on relatively few cell types, principally on B lymphocytes, monocytes, and macrophages. HLA-A and -B antigens are reasonably well characterised. HLA-D and -DR antigens are, however, less well defined but

may be related to the Ia antigens of the mouse and thus are potentially of great importance in the control of the immune response.

The gene loci for all these HLA antigens are located on the short arm of the human chromosome 6. As the genes are co-dominant, each cell expresses on its surface two antigens from each series, one inherited from the father and the other from the mother. The genes for factor B and both C2 and C4 components of the complement cascade are also located on chromosome 6. The fact that so many of the important determinants of the immune response are located on a segment of one chromosome has stimulated considerable interest. Indeed, in all vertebrate species investigated to date a single chromosomal complex (known as the major histocompatibility complex or MHC) codes for the histocompatibility antigens. As the human HLA complex appears to be similar to the well-documented mouse MHC, the immune response genes, which determine the ability of an organism to mount an antibody response to a specific antigen, are also probably located within this region.

What evidence is there to indicate that HLA antigens are important in clinical renal transplantation? The principle evidence comes from the results of transplants from living related donors. The data may be considered in four parts.

- (1) Because episodes of rejection cannot occur, isografts between identical twins have excellent results with a 3-year graft survival rate of 90% or more (that is, 90% of such grafts continue to function 3 years after transplantation).
- (2) Graft survival results of transplantation between HLA identical sibs (two haplotype matches) are almost as good.<sup>5</sup>
- (3) However, results of transplantation between sibs who have no HLA antigens in common (no haplotype match) are not significantly better than the results of cadaver transplantations and produce a 3-year graft survival of approximately 60%.<sup>5</sup>
- (4) Three-year graft survival for transplantation between sibs who have one haplotype in common is around 75%<sup>6</sup> and thus lies between (2) and (3) above.

These data not only show that for living related donors graft survival is directly related to the degree of HLA matching within the family, but also serve

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to emphasise the importance of the HLA complex in renal transplantation.

Unfortunately, the results obtained from the numerically important group of cadaver transplants are more difficult to interpret. To date most studies have investigated the effect on renal allograft survival of matching donor and recipient for HLA-A and -B antigens only. The results are inconsistent. In general the large multicentre analyses have failed to demonstrate any significant benefit from matching for HLA-A and -B antigens. These data, however, have been criticised because they conceal the differences in patient selection and therapeutic policies endorsed by the individual transplant centres. Results from single centre studies have tended to show that graft survival is better when donor and recipient share all four HLA-A and -B antigens than when they have no HLA-A and -B antigens in common. However, in contrast to living related donor studies, a graded response relating allograft survival to the degree of HLA and A and B antigen matching cannot be consistently demonstrated.

In part this disappointing clinical response to the matching of cadaver donor and recipient for HLA-A and -B antigens may result from the marked polymorphism of the HLA gene complex. Already 20 alleles have been identified at the HLA-A locus, 42 at the B, eight at the C, 12 at the D, and ten at the DR.<sup>7</sup> A total of 92 identifiable specificities have thus been demonstrated. As more and more antigens are characterised, the problem of matching recipient and donor becomes more, rather than less, difficult.

For this reason, and supported by results of animal experiments, several centres began to examine the importance of matching donor and recipient for HLA-D and -DR antigens. As the HLA-D antigen can only be defined by mixed lymphocyte culture techniques, which take up to 6 days to perform, matching donor and recipient prospectively for HLA-D antigens is not generally possible in cadaver donor transplantation. HLA-DR antigens, however, are detectable by serological techniques. Although preparation of the necessary B lymphocyte suspension from the donor is technically difficult and time-consuming to perform, matching of cadaver donor and recipient for the HLA-DR antigens before transplantation is feasible.

Matching for HLA-DR antigens has another advantage over the conventional testing of T lymphocytes for HLA-A and -B antigens. Of the ten HLA-DR antigens that have been identified to date, eight are reasonably well characterised. Using suitable antisera only to these eight antigens, the gene frequencies (%) obtained from a normal European population already total 80%. In other words, it

would appear at present that the HLA-DR gene locus may prove to be less polymorphic than the HLA-A and -B loci. If it could be established that matching cadaver donors and recipients for HLA-DR antigens alone led to improvements in allograft survival rates, it would prove much easier to match for the antigens of the HLA-DR locus with ten alleles than for the 92 specificities associated with five gene loci recognised at present.

What data are currently available concerning the effect of matching donors and recipients for HLA-DR antigens? Once again the results are not conclusive but certainly provide hope that HLA-DR matching may produce improvements in allograft survival rates. Several centres have now reported that graft survival is considerably better when donors and recipients share both HLA-DR antigens than when they have no HLA-DR antigens in common.<sup>8,9</sup> This beneficial effect has been shown by one group to be independent of any influence of HLA-A and -B antigen matching.<sup>8</sup> It remains unclear whether the improvements in graft survival believed to be caused by HLA-DR matching are over and above the increases produced by pretransplant blood transfusion. As might have been anticipated in this rapidly developing area of research, the results of the first large multicentre analysis have failed to show any significant benefit from HLA-DR matching.<sup>10</sup>

Currently, one-year allograft survival rates are approximately 60% although there is a considerable variation between different transplant centres. As immunological rejection remains the single most important cause of graft failure, it is essential that the recipient's immune response be modified if graft survival rates are to improve. Pretransplant blood transfusions clearly achieve this, although their mechanism of action remains obscure. At the present time it would appear that matching recipients and donors for HLA-DR antigens may also produce significant increases in graft survival rates and moreover prove technically possible in the limited time available before cadaver renal transplantation.

ALISON M MACLEOD,  
ROBERT J MASON,  
AND GRAEME R D CATTO  
*Department of Medicine,  
University of Aberdeen,  
Foresterhill, Aberdeen AB9 2ZD*

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Requests for reprints to Dr G R D Catto, Department of Medicine, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD.

## Cherchez les femmes (or the personal touch in the laboratory)

A recent development in the prenatal diagnosis of neural tube defects has been the discovery that the qualitative assay of amniotic fluid cholinesterase and acetylcholinesterase (AChE) may be an even better diagnostic tool than  $\alpha$ -fetoprotein.<sup>1</sup>

In the course of evaluating this new development, I (M) and my collaborators (K and B) encountered a problem. The method<sup>2-4</sup> is relatively simple, consisting of protein separation by polyacrylamide gel 'disc' electrophoresis and then enzyme localisation by incubation in enzyme substrate. Initially, the method worked successfully in this laboratory without even any teething problems, and a whole study was completed by two of us (M and K)<sup>5</sup> on the AChE of a large number of normal and abnormal amniotic fluids. But when subsequently the method was introduced into our routine diagnostic programme, the third person (B) could not make it work. B is a competent and clever technician and she did not appear to be doing anything wrong. Thus it was decided that some experiments must be performed in order to determine the cause of the failure.

### EXPERIMENT 1

*Hypothesis.* That, since B works in a large open laboratory which is colder than the small rooms where M and K work, the lower ambient temperature

was inhibiting enzyme activity. B performed the enzyme assay in K's room. Result: failure.

### EXPERIMENT 2

*Hypothesis.* That the pH of one of the solutions used in the method was wrong. B checked the pH of all solutions before performing the assay. Result: failure.

### EXPERIMENT 3

*Hypothesis.* That the potency of the enzyme substrate had diminished through prolonged use of the batch. B doubled the amount of substrate used. Result: failure.

### EXPERIMENT 4

*Hypothesis.* That the batch of enzyme substrate in use had 'gone off' completely. B bought and used a new batch of substrate. Result: failure.

### EXPERIMENT 5

*Hypothesis.* That this second batch of enzyme substrate, by an unfortunate coincidence, was also faulty. B obtained from another laboratory, and used, an aliquot of enzyme substrate known to be working. Result: failure.

### EXPERIMENT 6

*Hypothesis.* That one of the other chemicals in use in the procedure was faulty. B bought and used a complete set of new chemicals. Result: failure.

### EXPERIMENT 7

*Hypothesis.* That new plastic tubes used for the incubation step were inhibiting the enzyme. B changed to glass tubes. Result: failure.

### EXPERIMENT 8

*No hypothesis*, just desperation. M performed the whole technique without trying particularly hard. Result: success.

A more detailed scientific investigation was obviously necessary.

The procedure can be broken down basically into three steps: (1) preparation of the solutions, (2) gel making and the electrophoresis, and (3) enzyme localisation.

### EXPERIMENT 9

M did steps 1 and 3, B did step 2. Result: success. Conclusion: B had no problem with gel making and electrophoresis.

### EXPERIMENT 10

B did step 1, M did steps 2 and 3. Result: success. Conclusion: B had no problem with preparation of solutions.

It appeared, therefore, that the error was occurring