The application of DNA technology to tissue typing

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SUMMARY
A new method of determining class II HLA antigens by genotyping with HLA DNA probes is described. This method compares favourably to the traditional serological methods.

INTRODUCTION
HLA cell-surface antigens are involved in various functions of the immune system, such as cooperation between lymphocyte subsets, antigen presentation and allograft rejection.1 Some HLA antigens have been shown to be associated with susceptibility to certain diseases.2 Bone marrow transplantation usually requires an identical HLA match between donor and patient for class I (HLA- A, -B, -C) and class II (HLA-DR, -DQ, -DP) antigens.3 Although a complete match is not an absolute requirement in kidney transplantation, there is a significant improvement of graft survival between the best and worst matches for HLA -A, -B and -DR antigens.4,5,6

HLA typing is traditionally performed according to immunological methods using antibodies reacting with HLA gene products on the cell surface. These methods rely on obtaining monospecific alloantisera from multiparous females.7 In addition for class II typing suitable B lymphocyte preparations are required.

It is now possible to apply techniques of molecular biology to the problems of HLA typing. Restriction endonucleases that recognise specific nucleotide sequences are used to digest DNA. The resulting DNA fragments are then separated according to size by electrophoresis in agarose gels and transferred to nylon membranes. Radiolabelled complementary DNA probes are used to identify genes for class I and class II regions. Each probe hybridises with several fragments of DNA, some of which are constant in the general population whereas others called allogenotypes are present in some persons but absent in others. We describe the use of a single enzyme/single blot/multiple reprobe system of HLA-DR and -DQ (developed in this laboratory in conjunction with Dr J Bidwell, United Kingdom Transplant Service, Bristol) to assign by analysis of allogenotypes the HLA-DR and DQ specificity at the DNA level. We have applied this technique to typing renal patients in order to assess any differences obtained by this technique when compared to conventional serological methods.

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MATERIALS AND METHODS
The renal population consisted of 160 patients either transplanted consecutively since May 1986 or awaiting a transplant. DNA was prepared from peripheral blood leucocytes which were isolated from EDTA blood by two erythrocyte lysis steps using 2–3 volumes of red cell lysis buffer RCLB (10 mM Tris-HCl pH 7.6/5 mM MgCl2/10 mM NaCl). The leucocytes were resuspended in 2 ml RCLB followed by the addition of 13 ml white cell lysis buffer (10 mM Tris-HCl pH 7.6, 10 mM EDTA pH 8.0, 50 mM NaCl, 0.2% sodium dodecyl sulphate, 200 μg/ml proteinase K), mixed gently and incubated overnight in a shaking water bath at 42°C. Proteins were removed by two extractions with 15 ml phenol/chloroform/isoamylalcohol (25:24:1) followed by two 15 ml extractions with chloroform/isoamylalcohol (24:1). DNA was precipitated from the aqueous layer by the addition of 300 μl 3 M NaCl and 15 ml isopropanol. The DNA was harvested using a sealed glass Pasteur pipette, washed three times in 4 ml 70% ethanol, dried and resuspended in TE (10 mM Tris HCl pH 7.6, 1 mM EDTA pH 8.0). The DNA was assayed spectrophotometrically and stored at a final concentration of 1–2 μg/μl at 4°C.

Samples of genomic DNA (8 μg) were digested to completion with the restriction endonuclease Taq 1 (Bethesda Research Laboratories, 5 units/μg DNA) and resolved using agarose gel electrophoresis for 18 hr at 40 V in an agarose gel (25 x 20 x 0.6 cm, 0.7% w/v agarose (Bethesda Research Laboratories) in TAE electrophoresis buffer (40 mM Tris-acetate, 1 mM EDTA containing 0.5 μg/ml ethidium bromide)). The DNA was denatured, transferred to a nylon membrane (Amersham Hybond·N) and hybridised. Membranes were hybridised sequentially with the following radiolabelled exon-specific cDNA probes; HLA–DRβ pRTV1, HLA–DQB pII–β–19 and HLA–DQα pDCH1. After autoradiography the membranes were dehybridised by washing, with gentle agitation, at 42°C in 500 ml 0.4 M NaOH for 30 min followed by 500 ml 0.2 M Tris·HCl pH 7.6 0.5% SDS for 30 min prior to rehybridisation.

RESULTS
The figure shows the allogenotypes revealed using the DRβ cDNA probe pRTV1. The allogenotypes DRβ1, DRβ2, DRβ3, DRβ4, DRβ7, DRβ8, DRβ9, DRβ11, DRβ12, DRβ13, DRβ14 are associated with the corresponding HLA–DR serological specificities. Allogenotypes are also observed which are subtypes of individual DR specificities not previously differentiated by serological methods i.e. DRβ31, DRβ32 and DRβ13a1, 13a2, 13a3, 13a4. The figure also shows the results of rehybridising the membrane with the DQB and DQα cDNA probes respectively. Using the DQB probe, six DQB allogenotypes are identified (β1a, β1b, β2a, β2b, β3a, β3b). These allogenotypes correlate with known serological types of DQ i.e. β1a and β1b with DQw1 serotypes; β2a and β2b with DQw2 serotypes; β3a and β3b with DQw3 serotypes.

Using the DQα probe, five DQα allogenotypes are identified (α1a, α1b, α1c, α2 and α3). These describe another allelic series which also correlates with serological types of DQ, though to a lesser degree than the absolute correlation seen with DQB allogenotypes. Thus DQα1a, α1b and α1c correlate with DQw1 serotypes though α1b is also associated with DQw3, and α2 and α3 allogenotypes are both associated with DQw2 and DQw3 serotypes.
When the results obtained by allogenotyping were compared to those obtained by serology, there was a difference in 25 (16%) of the patients. In 5 patients an incorrect antigen had been assigned by serological typing. On two occasions DRw13 had been assigned instead of DR3 and on a further two occasions instead of DRw12. On one occasion DRw12 was assigned instead of DRw13. In 20 patients an antigen detected by allogenotyping was not detected by serology. The antigen HLA–DRBr was not detected in 11 patients, HLA–DRwB was not detected in 2 patients, HLA–DRw14 was not detected in 2 patients and the following antigens were not detected on one occasion in separate patients; HLA–DR1, –DR3, –DR4, –DR9, –DRw13.

**DISCUSSION**

The methods described in this report permit the assignment of HLA–DR and –DQ specificities by the sequential use of short DRβ, DQβ and DQα DNA probes. The DRβ, DQβ and DQα allogenotypes in heterozygotes are interpreted by the summation of patterns demonstrated by homozygous control cells.

A major application of DNA typing is in the interpretation of HLA–DR and DQ specificities where serological assignment is not possible or where the results are equivocal. Serological assignment is influenced by poor quality or low numbers of circulating B cells and by the lack of reliable monospecific alloantisera for certain specificities.

Our results have shown that the majority (20/25) of differences between the serological and allogenotyping method are due to the detection by allogenotyping.
of antigens which are not detected by the serological method. The remaining five discrepancies arose due to the difficulties involved in the serological typing of DRw13, an antigen for which it is difficult to obtain monospecific sera. Thus all the differences that have occurred are due to the unavailability of suitable sera. On all occasions when differences between the allogenotype and the serological type are found, it is possible, using the allogenotype result, to assess the quality of the HLA sera.

We can now assign DQ specificities using allogenotyping, which we could not do by serological means because all the DQ sera available to this laboratory are contaminated with DR antibodies. For example, all available DQw1 sera always contain antibodies against one or more of the DR antigens associated with DQw1 (DR1, DR2 and DRw6).

There are two main disadvantages at present to the allogenotyping: cost and length of time to perform the technique. The difficulties in cost are due to the initial setting-up of the technique. Once the techniques are established then the cost of DNA typing is very similar to the cost of serological typing. The technique is not readily applicable to cadaver donor typing since it is incompatible with the clinical urgency set by organ ischaemia time. In the future it should be possible to shorten the time taken by using allele specific probes.

The DNA methods can also be applied to disease studies. At present we are investigating multiple sclerosis patients with a variety of endonucleases to determine any differences in the size of the resulting DNA fragments between patients and controls. Previous disease studies, using Taq 1 restriction enzyme and DRβ and DQβ probes, have identified a restriction fragment pattern observed in 11 of 12 DR3/4 patients with insulin dependent diabetes mellitus but absent in all 12 DR-matched controls.¹¹

A further possible application of DNA typing is the replacement of the mixed lymphocyte culture for measuring differences between donor and recipient in bone marrow transplantation. Using a variety of endonucleases and probes, it should be possible to determine if there are any differences between recipient and donor. This would be extremely useful in those instances where donor and recipient sibling are separated by geography or where the use of a non-related HLA identical donor is contemplated.

Already DNA typing has proved most useful in this laboratory. As the technique is developed, it should be possible to identify splits of DR antigens and more generally to provide further insight into the genetic complexity of the human major histocompatibility complex.

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