MIXED LYMPHOCYTE CULTURE (MLC) TEST AND LIVING RELATED DONOR KIDNEY TRANSPLANTATION

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SUMMARY

The MLC tests performed prospectively was correlated with graft survival in 73 living, related grafts. The MLC was expressed as the relative response (RR), and graft survival assessed at six months, one year and two years. The 1-haplotype matched grafts with high RR had poorer graft survival at one and two years than those with low RR, but this difference was not statistically significant.

INTRODUCTION

Previous reports have indicated that HLA-D disparity as reflected by the mixed lymphocyte culture (MLC) test is important in living related renal transplantation, and this test could predict the outcome of 1-haplotype matched renal transplants. The clinical outcome of 73 patients who received living related grafts between 1978 and 1981 was reviewed, and graft survival correlated with the MLC tests performed prospectively.

METHODS AND MATERIAL

73 renal transplants performed between December 1978 and March 1982 were included in this review. They were all first transplants. There were 32 sibling donors and 41 parent or offspring donors. All had unidirectional MLC testing performed prior to transplantation by an established technique with unrelated controls. No transplant with MLC prospectively tested for was excluded. The degree of stimulation of the MLC was expressed as the relative response (RR).

Mixed lymphocyte culture

Medium RDMI - 1640 (Flow Labs, Rockville, USA) is used. The powdered medium is dissolved in one litre of glass-distilled water, incorporating 0.85 g/l of NaHCO₃, penicillin 100 i.u./ml and streptomycin 100 µg/ml. The medium is sterilised by millipore filtration and stored in aliquots of 100 ml at 40 °C. Serum is added aseptically to the medium before use. The serum is obtained from a pool of ten healthy nontransfused male donors and tested to exclude lymphocytotoxin antibodies and stored frozen at 20 °C until use.

Peripheral blood was obtained from the donor, recipient and related controls into vacutainers containing preservative free heparin. The blood is mixed with an equal volume of medium containing heparin and this mixture is layered over 3 ml of Ficoll-Isopaque in a tube. The tubes are centrifuged at 400g for 20 minutes. The lymphocyte-rich layer is harvested and the cells washed three times with
medium containing 5% serum and finally resuspended in medium containing 20% serum.

The stimulating cells are treated with 25 μg/ml of mitomycin C (Kyowa, Japan) for 30 minutes at 37°C and then washed three times in medium containing 5% serum and finally resuspended in medium containing 20% serum.

The concentration of both stimulating and responding cells are adjusted to give 10⁶/0.075 ml of medium. The cells are delivered into round-bottom plastic microculture trays with 96 wells from a 2.5 ml plastic disposable syringe mounted to a Hamilton repeating dispenser.

All combinations are put up in triplicate, and appropriate controls of both responder and stimulator cells are included in each culture. The microculture tray is placed in a humidified water jacketed incubator with 5% CO₂ and 95% air at 37°C and incubated over six days. Eighteen hours before termination of the culture, 1 μc of ³H thymidine is added to each well and the tray is reincubated. The cells are harvested by a semi-automated cell harvester and deposited on to glass fibre filter mats. Filter discs containing the cells are punched out and transferred to 2 ml of scintillation fluid, and are counted for 10 minutes in a Packard scintillation spectrometer.

The MLC response is expressed as the relative response (RR). All patients received immunosuppressive treatment with azathioprine and steroids. Graft and patient survival were computed by actuarial methods, and statistical significance calculated by the chi square test. All deaths were included as graft loss. Graft loss was taken from the period the patient had to return to recurrent dialysis.

RESULTS

An arbitrary dividing line was taken at RR of 20% with results above this value taken as HLA-D incompatible. Among five 2-haplotype matched sibling transplants, two had RR greater than 20% and three less than 20%. From the rest of the 68 1-haplotype matched transplants, 40 had RR greater than 20% and 28 less than 20%. Different degrees of stimulation in the MLC test appeared to be associated with differences in graft survival, however there was no difference in patient survival.

Of the 40 patients with the higher RR, 16 (40%) rejected their kidneys, whereas seven (25%) out of 28 with the lower RR rejected their grafts. Graft survival for those with low RR was 79% at six months, 73% at one year and 62% at two years compared with 80%, 60% and 37% at corresponding periods for those with higher RR. Graft survival for the 2-haplotype matched transplants was 80% as a results of sudden death in one patient. Renal function was normal at time of death. The differences in patient and graft survival at six months, one year and two years between the groups with high and low RR was not statistically significant.

DISCUSSION

There are several methods of expressing MLC reactions. In this study it was expressed as the relative response, which when greater than 20% was arbitrarily taken as indicating HLA-D incompatibility. Our results showed that the higher RR on the MLC test was associated with poorer graft survival, with only 37% of grafts functioning at two years. This observation is consistent with other reports where a higher RR was associated with poorer graft survival, although the MLC stimulation were expressed in different ways, and the cut-off point for the high MLC response differed in the various published reports. In our experience the main difference in graft survival between high and low responders appeared at two years, but this was not statistically significant.

From this experience 1-haplotype grafts with high RR did no better than cadaver type, and it would be preferable to use cadaver grafts for transplantation. Unfortunately cadaver grafts are not obtainable in Malaysia, and living related donor grafts are the only ones that can be used for transplantation. The MLC is useful to help identify the best suitable living related kidney donor from among several 1-haplotype matched potential donors. It would be ideal for a complete family study to be done with HLA-A, B serotyping and MLC testing, however in practice, usually only the potential donor is prepared to undergo these tests.
In our circumstances where cadaver kidneys are not available, a 1-haplotype matched donor with high RR on MLC will often be the only option for treatment for many patients. We feel that a high RR does not disqualify a family member as a donor if there is no other possible donor in the family. Although HLA serotyping and MLC testing are valuable in helping to select the best donor recipient match, some recipients with HLA-D incompatible grafts do very well, and it is important to identify additional loci and other factors such as donor specific blood transfusions enhancing antibodies, etc., which determine graft survival, to justify transplanting 1-haplotype matched donor kidneys with HLA-D incompatibility.

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REFERENCES


