IMPROVED KIDNEY GRAFT SURVIVAL IN EUROTRANSPLANT BY HLA-DR MATCHING AND PROSPECTIVELY GIVEN BLOOD TRANSFUSIONS

G. G. PERSIJN,* A. VAN LEEUWEN,** J. PARLEVLIEET,** B. COHEN,* Q. LANDBERGEN,** J. D'AMARO** and J. J. VAN ROOD**

*Eurotransplant Foundation, University Hospital, 2333 AA Leiden, The Netherlands
**Department of Immunohaematology, University Hospital, 233 AA Leiden, The Netherlands

This work was in part supported by the Dutch Organisation for Health Research (TNO), the Dutch Foundation for Medical Research (FUNGO), which is subsidized by the Dutch Foundation for the Advancement of Pure Research (ZWO), the J. A. Cohen Institute for Radiopathology and Radiation Protection (IRS) and the Dutch Kidney Foundation.

CADAVERIC kidney graft survival is influenced by many different factors. Two such factors are matching for the HLA-DR determinants between donor and recipient and blood transfusion(s) given to the recipient before transplantation. Indeed, recent reports have shown that matching for the HLA-DR determinants and the effect of pre-transplant blood transfusions play an important role in predicting kidney graft outcome in human cadaveric renal transplantation. Most of these retrospective studies were criticized because of selection criteria, insufficient accuracy, heterogeneity, patient management etc. To tackle the problem of retrospective studies as many patients as possible on the Eurotransplant waiting list were typed prospectively for the HLA-DR determinants. Kidney donors were typed before or at time of transplantation and are therefore considered as prospective.

Concerning the blood transfusion effect on kidney graft survival a prospective blood transfusion protocol was started in 1977 in the Netherlands. Non-transfused kidney patients waiting for a cadaveric kidney transplant received either one unit of washed ABO identical blood (i.e. leucocyte poor blood) or one or three units of cotton wool filtered blood (i.e. leucocyte free blood). Due to the bad kidney graft survival obtained in non-transfused kidney patients a prospective non-transfused control group is lacking.

We present here the results of the effect of matching for the HLA-DR determinants in 599 kidney transplants and the effect of prospectively given blood transfusions in 52 patients.

PATIENTS, MATERIALS AND METHODS

HLA-DR analysis

A total of 599 cadaveric kidney transplants done under the auspices of Eurotransplant were studied. Of them 72 were second transplants and six were
third transplants. All but 25 patients had had blood transfusions before transplantation. Immunosuppressive therapy mostly consisted of azathioprine and prednisone. In some transplantation centres, anti-thymocyte globulin was used as part of pilot studies. Graft survival was considered successful if the recipient could live without haemodialysis. Non-immunological and technical failures were not excluded from the analysis.

All HLA-A and -B typings of the kidney patients were performed twice, once in the regional typing center and once in the Eurotransplant Reference Center in Leiden, both with the Eurotransplant serum set which recognized all official HLA and Workshop -A and -B specificities. All donors and recipients were typed with anti-sera recognizing the HLA-DR 1-8 specificities, according to the definitions used during the Seventh International Histocompatibility Workshop in Oxford. Typing was performed before or at time of transplantation in 488 cases and is therefore considered as prospective. No specific B-cell cross-matching was done. The two-colour fluorescence serological method was used for HLA-DR typing and about half of all typings were performed twice \(^7\). All patients’ sera were screened at least once every two months for the presence of lymphocytotoxic antibodies against a panel of 50 selected HLA-typed donors. In this panel, all known HLA-A and -B antigens are represented. Cross-matching by the standard microlymphocytotoxic test was performed with the most recent serum sample of the recipient available in the donor tissue typing laboratories. Eventually, the cross-match was repeated in the transplantation center with the serum samples containing leucocyte antibody activity. A negative result was mandatory for transplantation of the recipient.

Graft survival times were estimated using the actuarial life table method \(^8\). The chi square test was used to determine the significance of the differences in the observed numbers of successes and failures in each class.

**Blood transfusion study**

In March 1977, the decision was taken in the Netherlands to stop transplanting non-transfused kidney patients waiting for a cadaveric kidney transplant. A blood transfusion protocol was introduced with two arms. One arm consisted of a group of never-transfused and/or nulli-parous patients who received 1 unit of twice-washed ABO identical blood (i.e. leucocyte poor blood). The amount of leucocytes in the blood was decreased by washing to 40-60 per cent. The choice of washed erythrocytes was based on the fact that this blood product had usually been given in our retrospective study and the risk of immunization against HLA antigens was low, especially when only one transfusion was given \(^6\).

The other arm of the protocol included patients that were given 1 or 3 units of cotton-wool filtered blood. This is a technique originally described by Diepenhorst et al. which makes the blood almost completely leucocyte free \(^9\). Blood for the transfusions was obtained from normal healthy Dutch Red Cross volunteers, who are checked at least once annually and screened by the RIA method for the presence of Hbs Ag — antigens to avoid the transmission of hepatitis B. As a rule, the blood was less than 3 days old.
Washed erythrocytes (leucocyte poor blood)

Thirty-one male and nine female patients were transfused with 1 unit of washed (i.e. leucocyte poor) erythrocytes. Their age ranged from 16 to 56 years (mean: 36 years). The haemodialysis period varied from 3 to 68 months with a mean of 19 months. All patients who were transfused according to this protocol but who later required additional transfusions prior to transplantation for medical reasons were excluded from this study. The interval between blood transfusion and transplantation varied from 21 days to 1108 days (mean: 251 days). Most of the serum samples tested after the transfusion(s) of these patients showed no detectable lymphocytotoxic antibody activity. In two cases, very weak activity amounting to approximately 5 per cent kill above background developed against part of the panel but this activity disappeared when subsequent serum samples were screened. The follow-up time in this study was at least one month. All patients received one to six transfusions, mostly of leucocyte free blood, during transplantation.

egin{figure}
\centering
\includegraphics[width=\textwidth]{graft_survival_hla-dr_matching}

\caption{Kidney graft survival and HLA-DR matching. Note that 13 patients received an HLA A, B and DR identical kidney. Kidney graft survival in his group is 92 per cent after 2 years, while the group with no mismatches for HLA-DR has 80 per cent after 2 years. The numbers between brackets are the total number of patients each group (p = 0.003).}
\end{figure}
Filtered blood (leucocyte free blood)

Six male patients received 1 unit of cotton-wool filtered, i.e. leucocyte free, blood. Their mean age was 36.5 years (range: 31-56 years). Three male and three female patients received 3 units of cotton-wool filtered blood. In this group, the mean age was 37.5 years (range: 16-50). The 1 and 3 unit group of patients are combined for analysis because of the small number of patients. The hemodialysis period varied from 3-21 months with a mean of 10 months. In the one unit group, the interval between transfusion and transplantation varied from 35 to 179 days (mean: 120 days). In the group given 3 units, this interval varied from 17 to 371 days (mean: 127 days) after the last transfusion. Here too, all patients received transfusions of leucocyte free blood, varying from 1 to 4 units, during the operation.

RESULTS

HLA-DR analysis

Figure 1 shows the results in 599, mostly prospectively HLA-DR typed donor/recipient combinations. Kidney graft survival was 80 per cent after 2 years in the group with no HLA-DR mismatches. This is significantly different from

EFFECT OF HLA-DR MATCHING
IN NON-TRANSFUSED PATIENTS

\[ (N=25) \]

\[ \begin{align*}
\text{NR} & \quad \text{OF MISM} \\
0 & \quad (N=5) \\
1 & \quad (N=10) \\
2 & \quad (N=10)
\end{align*} \]

Figure 2

The effect of HLA-DR matching in 25 non-transfused patients. Five patients who received an HLA-DR identical kidney have 80 per cent graft survival after 6 months.
graft survival in the groups with one or two HLA-DR mismatches \((p = 0.003)\). Thirteen donor/recipient pairs which had no mismatches for the HLA-DR antigens did not have mismatches for the HLA-A and -B antigens as well. Graft survival in this particular group was 92 per cent after two years. The group of recipients which had one HLA-DR mismatch with their donor had a graft survival of 60 per cent after two years. This value is the same as the results in the group with two HLA-DR mismatches.

The influence of HLA-DR matching in 25 patients who never had been transfused or who had received only leucocyte free blood is shown in figure 2. Graft survival in five patients who had no HLA-DR mismatches with their donor was 80 per cent after six months. In the non-transfused patients who had two HLA-DR mismatches with their donor, 30 per cent graft survival was obtained after six months. The group with one HLA-DR mismatch showed intermediate values namely 60 per cent after six months. These small numbers of patients do not permit us to draw any statistical conclusions from these results.

**Blood transfusion analysis**

**PROSPECTIVE ANALYSIS**

\[\text{(NO EXCLUSIONS)}\]

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{The effect of prospective blood transfusions on kidney graft survival. The numbers indicate the patients at risk. Graft survival is 80 per cent after 200 days in the group who received one leucocyte poor blood transfusion. Patients who received 1 or 3 units of leucocyte free have 33 per cent graft survival after 200 days.}
\end{figure}
Figure 3 shows that kidney graft survival in patients prospectively transfused with one unit of washed erythrocytes is 80 per cent after 200 days. Eight recipients had a graft failure. In four cases, this was due to non-immunological causes such as myocardial infarction and pneumonia. Four patients lost their kidney due to rejection.

Figure 3 also shows the surprising finding that kidney graft survival was very poor in patients given 1 or 3 unit(s) of leucocyte free blood i.e. 33 per cent after 200 days. Eight patients lost their graft due to irreversible rejection. This is significantly different from the survival in the group prospectively given one transfusion of leucocyte poor blood ( $\chi^2 = 6.41$, $p = 0.01$).

DISCUSSION

Our data show that good graft survival is obtained in unrelated donor/recipient combinations with no HLA-DR mismatches. Kidney graft survival was worse in the group who had one or two HLA-DR mismatches, but surprisingly the latter did much better than in the retrospective study.

The difference in kidney graft survival between the group with one HLA-DR and the group with two HLA-DR mismatches in that study was remarkable $^1$. In the data presented here, this difference is not observed. We have no explanation for those different findings. Perhaps we are seeing the results of improved patient management and monitoring regarding immunosuppressive therapy $^10$.

The excellent graft survival in patients who receive an HLA-A, -B and -DR identical kidney is remarkable. The role of the interaction between HLA-A and -B and HLA-DR on graft survival is not yet fully understood and remains a subject for further study. We think that, especially in patients who have developed leucocyte antibodies, HLA-A and -B matching is of overriding importance and has the first priority.

The results of this study demonstrate that HLA-DR typing and matching in the cadaveric donor selection is very feasible. More than 20 per cent of the recipients received an organ with no HLA-DR mismatches. That proportion is much higher than ever obtained in matching for HLA-A and -B antigens alone. However one has to be careful, since typing for the HLA-DR determinants is often a very difficult procedure especially with potential kidney donors. Therefore, regular quality controls and workshops regarding HLA-DR typing should be held. Special attention should be given to the different techniques and variety of donor material like spleen, lymphnode etc.

Concerning pretransplant blood transfusions, this prospective study clearly shows that a single unit of leucocyte poor blood given prior to transplantation can lead to prolonged cadaveric kidney graft survival. Data obtained in this study are reliable because they have been obtained prospectively and, without exception, they originate from dialysis centers in the Netherlands which can be considered as a homogenous group. Opelz et al. did not find a similar beneficial effect of one transfusion in corresponding groups of recipients but his study was retrospective and encompasses a heterogenous patient population from many
different centers \(^{11}\). Other authors have shown an improvement in kidney graft survival in patients who had received very few and even one blood transfusion prior to transplantation \(^{12} \,^{13} \,^{14} \,^{15}\). Since most of these reports refer to retrospective analysis it is obvious that these patients received blood transfusions due to a variety of indications and the possibility of a potentially important variable has not entirely been excluded. They received at least one transfusion prior to transplantation.

A new and unexpected finding in our study was that 8 out of 12 patients who received 1 or 3 unit(s) of leucocyte free blood rejected their transplanted kidney within 120 days. This extreme divergence from the excellent survival in the group given one transfusion of leucocyte poor blood cannot be explained by the quality of the HLA matching, because the average HLA mismatch between donor and recipient was the same for all groups, namely 1.5. The poor results obtained in the leucocyte free group are comparable to the group of patients which received no transfusions at all.

The favourable kidney outcome in patients “pretreated” with only one unit of washed erythrocytes (leucocyte poor) has many important implications for non-transfused hemodialysis patients awaiting a cadaveric kidney graft. The risk of immunization against HLA antigens of the kidney donor is minimized by the use of leucocyte poor blood. Another advantage of our policy of transfusing only one unit of leucocyte poor blood prior to transplantation is that the risk of transmitting infectious diseases such as hepatitis B and cytomegaly is reduced. So far, we have not had a single case of such a disease after transfusion.

The mechanism underlying the beneficial effect of blood transfusion in kidney allograft survival is unclear. It seems unlikely that kidney graft survival is increased by specific enhancing antibodies such as anti-HLA-DR antibodies in patients who have received only one transfusion. We do not exclude the possibility that the improved graft survival of pretransfused patients is due to the triggering of a non-specific suppressor mechanism by leucocyte poor blood transfusions. That hypothesis is already under investigation with special attention to these prospectively transfused patients \(^{16}\).

Finally, the interaction between blood transfusions and HLA-DR matching is an interesting phenomenon. The effect of pretransplant blood transfusions might be more outspoken in the group of patients who receive a cadaveric graft with two HLA-DR mismatches. Good graft survival was obtained in non-transfused patients or in patients who had received only leucocyte free blood only when they received a kidney with no HLA-DR mismatches. On the contrary, graft survival was far worse in the group with two HLA-DR mismatches. This observation confirms the finding of the Oxford group \(^{17}\). Furthermore, our group noticed, as did others, that graft survival improvement due to matching for one HLA-DR determinant alone appears also to depend on previous blood transfusions. This phenomenon is well known as kidney graft survival in parent-child combinations is much better when the recipient had received blood transfusions prior to transplantation. These combinations share, as a rule, only one HLA-DR determinant \(^{18}\).
Concluding we suggest that blood transfusions probably have an additive or synergistic effect on the influence of HLA-DR matching in renal transplantation.

This study could not have been performed without the generous support and cooperation of the physicians collaborating in Eurotransplant. We therefore wish to express our deep gratitude to them and their nursing and administrative staff. We also wish to thank the staff of the Euroransplant Foundation and the department of Immunohaematology of the Leiden University Hospital, and especially the tissue typing, screening and celluology laboratories for excellent technical help. We thank Ms. M. Groenewegen for preparing the manuscript.

REFERENCES

1. PERSIJN G G, GABB B W, VAN LEEUWEN A et al. Matching for HLA antigens of A, B and DR loci in renal transplantation by Eurotransplant. Lancet 1978; 1: 1278.
2. TING A, MORRIS P J. Matching for B-cell antigens of the HLA-DR series in cadaver renal transplantation. Lancet 1978; 1: 575.
3. MARTINS DA SILVA B, VASSALI P, JEANNET M. Matching renal grafts (letter to the Editor). Lancet 1978; 1: 1047.
4. ALBRECHTSEN D, FLATMARK A, JERVELL J et al. HLA-DR matching in cadaver renal transplantation. Lancet 1978; 1: 825.
5. VAN ES A A, BALNER H. Effect of pretransplant transfusions on kidney allograft survival. Transplant. Proc. 1979; 9: 27.
6. PERSIJN G G, VAN HOOFF J P, KALFF M W et al. Effect of blood transfusions and matching on renal transplantation in the Netherlands. Transplant. Proc. 1977; 9: 503.
7. VAN ROOD J J, VAN LEEUWEN A, PLOEM J S. A method to detect simultaneously two cell populations by two colour fluorescence. Its application for the recognition of B cell (la like) determinants. Nature 1976; 262: 795.
8. PETO R, PIKE M C, ARMITAGE P et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 1977; 35: 1.
9. DIEPENHORST P, SPROKHOLT R, PRINS H K. Removal of leukocytes from whole blood and erythrocyte suspensions by filtration through cotton wool. Vox Sang 1972; 23: 308.
10. VAN HOOFF J P, VAN ES A, PERSIJN G et al. Cadaveric graft survival, clinical course, blood transfusions, HLA (A and B) match, and DR match in adult patients transplanted in one centre. Proc EDTA Amsterdam 1979; 16: 359.
11. OPELZ G, TERASAKI P I. Poor kidney-transplant survival in recipients with frozen-blood transfusions or no transfusions. N Engl J Med 1978; 299: 799.
12. HUSBERG B, LINDGERD B, LINDHOLM T et al. Blood transfusion and kidney transplantation. Scand J Urol Nephrol 1977; 42 (suppl.): 73.
13. BRIGGS J D, CANAVAN J S, DICK H M et al. Influence of HLA-matching and blood transfusion on renal allograft survival. Transplantation 1978; 25: 80.
14. BUY-QUANG D, SOULILLOU J P, FONTENAILLE CH et al. Rôle bénéfique des transfusions sanguines et des grossesses dans la survie des allogreffes rénales. La nouv Presse med 1977; 6: 3503.
15. MORRIS P J, OLIVER D, BISSHOP, M et al. Results from a new renal transplantation unit. Lancet 1978; 2: 1353.
16. GOULMY E, PERSIJN G G, BLOKLAND E C et al. CML-studies in renal allograft recipients. Transplantation 1981 in press.
17. WILLIAMS K A, TING A, FRENCH M E et al. Peroperative blood transfusions improve cadaveric renal allograft survival in non-transfused recipients. Lancet 1980; 1: 1104.
18. BRYNGER H, FRYSK B, AHLMEN J et al. Blood transfusion and primary graft survival in male recipients. Scand J Urol Nephrol 1977; 42 (suppl.): 76.