Association of Renal Graft Survival with Matching at the HLA-DR Locus

KARLA J. LAUENSTEIN, M.S.,
FREDERICK R. DAVEY, M.D.,
CHARLENE HUBBELL, B.S.,
ANN BURLESON, R.N.,
RICHARD P. OATES, Ph.D.,
and RICHARD L. BURLESON, M.D.

Division of Clinical Pathology,
and Departments of Surgery, Urology, and Preventive Medicine,
SUNY Upstate Medical Center,
Syracuse, NY 13210

ABSTRACT

The influence of HLA-DR antigen matching in 70 cadaver transplants was studied for up to four years. The overall cumulative renal graft survival at one year was 62 percent, and at two and four years it was 60 percent. The one, two, and four year cumulative graft survival for patients receiving one or two HLA-DR antigen matched grafts was 74 percent, 69 percent, and 69 percent, respectively. In contrast, the one, two, and four year cumulative graft survival for patients receiving zero HLA-DR antigen matched grafts was 51 percent, 47 percent, and 47 percent, respectively. The cumulative graft survival for patients receiving one or two HLA-DR antigen matched grafts was significantly different \( p < 0.05 \) than for patients receiving zero HLA-DR antigen matched grafts. No significant differences in the distribution of other prognostic factors were observed between patients receiving one or two HLA-DR antigen matched grafts and patients receiving zero HLA-DR antigen matched grafts. The present authors conclude that matching for HLA-DR antigens exerts an independent beneficial effect on renal graft survival in transplant patients, most of whom have been previously transfused.

Introduction

In humans, the human lymphocyte antigen (HLA) genes encode for the major histocompatibility antigens which are normally present on many tissues of the body.\(^{11}\) In rodents, the major histocompatibility antigens (H2) are the primary determinants which affect the length of survival of skin, renal or tumor allografts.\(^{11}\) Therefore, it would be expected that matching for HLA antigens in humans would also enhance survival of cadaveric renal grafts. Although the effect of matching for HLA-A and B or Class I antigens has been correlated to survival of cadaveric renal grafts, the association has been weak.\(^{18}\) Other fac-
tors have also been reported to affect the survival of cadaveric renal grafts and, thus, may lessen the effect of matching for HLA-A and B locus antigens. These factors include the sex\textsuperscript{19} and age of the recipient,\textsuperscript{25} the ABO types of the recipients and donors,\textsuperscript{18} the number of transfusions the recipients have received prior to transplantation,\textsuperscript{10} the presence of lymphocytotoxic antibodies in the recipients' serum,\textsuperscript{17} and the presence of warm antibodies in the recipients' serum reacting specifically with lymphocytes from the donor.\textsuperscript{21}\n
Several reports have indicated that there is a clear association between matching for HLA-DR and survival of cadaveric renal grafts.\textsuperscript{16,26} However, other studies have not confirmed these findings.\textsuperscript{9,12} In addition, the results of a large international collaborative study involving over 2000 cadaveric transplants were disappointing.\textsuperscript{20} No significant correlation between HLA-DR matching and renal graft survival was observed when the overall data from North America and Europe were analyzed. However, when the data from North American transplant centers were analyzed alone, a weak correlation between HLA-DR matching and renal graft survival was observed. Since there exists a variation in HLA-DR typing techniques and in treatment of patients among the various participating hospital centers in large collaborative studies, several investigators have now examined the effects of HLA-DR matching on cadaveric graft survival within a single transplant center.\textsuperscript{3,13}\n
The purpose of this report is to describe the usefulness of HLA-DR typing as a prognostic factor in patients transplanted with a cadaveric renal graft at the SUNY Upstate Medical Center. This report confirms our preliminary observations indicating that DR matching is associated with increased graft survival.\textsuperscript{14}\n
Methods and Materials

Patients

The study group consisted of 70 individuals, 49 males and 21 females with an age range of nine to 61 years (figure 1), treated at the SUNY Upstate Medical Center for end stage renal disease between September 1, 1978 and December 31, 1982. Sixty-four patients received a primary renal transplant, whereas six patients received a second transplant. From 1978 to 1981, cadaveric kidneys removed in Upstate New York under the direction of the transplant team were maintained using a Belzer perfusion apparatus and a modified albumin perfusate.\textsuperscript{5} Kidneys obtained from other centers, during this period, were transferred upon arrival at the Upstate Medical Center to the Belzer apparatus. After 1981, kidneys were maintained on a MOX-100 Waters Perfusion machine.

Figure 1. Age of recipients of cadaveric renal grafts.
Immunosuppression was initiated on the day of transplantation for recipients of cadaveric kidneys. Therapy usually consisted of a combination of azathio­prine and steroids given by established protocols that provide for high initial doses with gradual tapering of the dose of steroids to low maintenance levels. After seven days, the patients were switched from methylprednisolone to prednisone. In addition, episodes of rejection were treated with one gram i.v. doses of methylprednisolone and with irradiation of the graft during the first rejection episode. After 1981, patients with sustained episodes of rejection were given antithymocyte globulin.

**HLA and ABO Typing**

If accepted as a potential renal transplant recipient, the patient's ABO erythrocyte type and the HLA-A and B lymphocyte phenotype were determined using standardized methods. HLA-A and B lymphocytic antisera were obtained.* All donor-recipient pairs were serocrossmatched immediately prior to transplant and a negative result was a prerequisite for transplantation. HLA-DR typing was performed on cadaveric lymphocytes from lymph nodes, spleen, or freshly drawn heparinized blood. Mononuclear cells were isolated from other blood elements using a Hypaque-Ficoll gradient and centrifugation at 400 × g for 30 minutes. Non-T cells were separated from T cells by incubating mononuclear cells (previously harvested from the interface layer of the Hypaque-Ficoll gradient) on nylon-wool columns. T cells were non-adherent to the nylon-wool columns, whereas non-T cells were recovered by adding three to five ml of Hanks balanced salt solution with 10 percent fetal calf serum to the column and agitating the nylon wool column. The adherent non-T cells were collected and shown to have less than five percent T cells. Non-T cells were typed for HLA-DR antigens by incubating cells in micro­titer plates with HLA-DR specific antisera obtained from the UCLA Tissue Typing Laboratories. Eight different HLA-DR antigens (HLA-DR 1–8) were identified by this procedure.

In 44 of 70 (63 percent) recipients, two HLA-DR antigens were determined and in 26 of 70 (37 percent) one HLA-DR antigen was observed. In 41 of 70 (59 percent) of the donors two HLA-DR antigens were found, and in 29 of 70 (41 percent) one antigen was observed.

**Statistical Analysis**

The distribution of known prognostic factors between HLA-DR one or two antigen matched donor-recipient pairs and zero antigen matched donor-recipient pairs was compared utilizing the chi-square test and when appropriate the Fisher's exact test.

The life table method is the analytical method of choice in prospective studies where time until the occurrence of a defined event is observed. In the present study, the endpoint was renal graft rejection. Patients entered the study at the time of their graft and were followed prospectively for varying lengths of time. The life table analysis sequentially determines the proportion of patients remaining after each time ordered event occurs. For some patients, the endpoint or event did not occur during the observation period. Censored observations such as these are included in the time ordered analysis for their entire follow-up time duration. If censored observations were excluded from the analysis, a consider­able bias would result.

In the current study, four cumulative graft survival curves (figures 3 and 4)
were compared (two at a time), i.e., patients with renal grafts having (i) zero HLA-DR antigen matched donors versus one or two HLA-DR antigen matched donors and (ii) zero HLA-DR antigen incompatibility versus patients with renal grafts having one or two HLA-DR antigen incompatibilities. When two survival curves are to be tested for a statistically significant difference, the method of choice is the logrank test which is based on the chi-square distribution. The difference between the usual chi-square calculation and the logrank test rests on the definition of observed (0) and expected (E) values. In survival analysis, 0 becomes the number of renal graft rejections observed in each group compared to E the amount of exposure to the risk of renal graft rejection in each group. Prospective data is necessary in order to determine the risk of rejection so that real differences in graft rejections between groups may be determined if they exist. The chi-square calculation involves the summation of (0-E)^2/E in the two groups. One compares this value with the standard chi-square table with one degree of freedom to determine the level of statistical significance.

**Results**

The overall graft survival of patients receiving a renal graft is given in figure 2. Thirty-four of 70 patients (49 percent) were followed for one year, 19 of 70 (27 percent) for two years, 15 of 70 (21 percent) for three years and eight of 70 (11 percent) for four years. The cumulative renal graft survival at two and four years was 60 percent. Two of 70 (three percent) patients received a two HLA-DR antigen matched graft, 38 of 70 (54 percent) received a one HLA-DR matched graft, and 30 of 70 (42 percent) received a zero HLA-DR antigen matched graft. Patients receiving a renal graft with either a one or two HLA-DR antigen matched graft had a higher cumulative graft survival than those patients receiving zero HLA-DR antigen matched graft (p < 0.05; figure 3). At one year, the cumulative graft survival for patients receiving a one or two HLA-DR antigen matched graft was 74 percent, whereas the graft survival for those patients receiving a zero HLA-DR antigen matched graft was 51 percent. At two and four years, the cumulative graft survival for patients receiving one or two HLA-DR antigen matched grafts was 69 percent where the cumulative graft survival at two and four years for those with zero HLA-DR antigen matched grafts was 47 percent.

When all of the patient-donor graft combinations with unidentifiable HLA-DR antigens were excluded from the analysis, the total number of pairs under
Figure 3. Renal graft survival for patients matched at the HLA-DR locus. The cumulative renal graft survival for patients with one and two HLA-DR antigen matched grafts is significantly greater than for patients with zero HLA-DR antigen matched grafts (p < 0.05). The number at the top of the curve represents the number of patients followed for each time period (six months).

Figure 4. Renal graft survival for patients with zero or one and two HLA-DR antigen incompatibilities. The two curves are not significant at the 0.05 level. The number at the top of the curve represents the number of patients followed for each time period (six months).

consideration was reduced to 26. The subgroup (19 individuals) with one or two HLA-DR antigen matched grafts had a 12 month cumulative survival of 72 percent, whereas the subgroup (seven individuals) with zero HLA-DR antigen matched grafts had a 12 month cumulative survival of 57 percent. Because of the small size at these subgroups, a statistical analysis could not be performed.

When HLA-DR antigen incompatibilities were analyzed, 18 of 70 (26 percent) patients had two HLA-DR incompatibilities, 35 of 70 (50 percent) had one incompatibility and 17 of 70 (24 percent) had no HLA-DR incompatibility. Recipients with no HLA-DR antigen incompatibility had a higher cumulative graft survival than those individuals with one or two antigen incompatibilities, but not at a significant level (figure 4).

To determine if a maldistribution of known prognostic factors existed between patients with one or two HLA-DR matched grafts and zero HLA-DR matched grafts, both groups were compared according to sex, age, ABO type, HLA-A and B antigen matches, number of blood transfusions, and causes of graft failure (table I). No significant differences were observed in the distribution of these parameters between patients with one or two HLA-DR antigen matched grafts and zero HLA-DR antigen matched grafts. No significant differences were observed in the distribution of these parameters when zero HLA-DR antigen incompatibility was compared to patients with one or two HLA-DR antigen incompatibilities (data not shown). In addition, no significant differences were observed in graft survival when the number of matches or incompatibilities between donors and recipients at the HLA-A and B loci were examined (data not shown). There were 18 different original diseases responsible for renal failure in this group of patients. Because of the small sample size of the subgroups, a statistical analysis regarding the prognostic value of the original disease on graft survival was not performed.

Discussion

Since 1978 there have been numerous studies correlating the association of HLA-DR matching with cadaveric renal graft survival.3,9,13,30,26 Most of these stud-
ies were performed in Europe, some were retrospective and others were extensions or continuations of previous reports. Most of the studies indicating an association between HLA-DR typing and renal graft survival were performed in single centers. In contrast, a large multicenter international study suggested that HLA-DR typing is not associated with subsequent graft survival. However, when renal transplants were performed in multiple centers and HLA-DR typings were performed by a single laboratory, a correlation between HLA-DR matching and cadaveric renal graft survival was observed. These and the current data suggest that when the variables of multicenter typing for HLA-DR matching are reduced to one center, an association between HLA-DR matching and renal graft survival is realized.

The current study confirms our former data and others indicating that patients with grafts matched for one or two HLA-DR antigens have a superior graft survival. This difference cannot be attributed to other prognostic factors such as sex, age, ABO typing, HLA-A and B antigen matching, number of transfusions, or causes of graft failure because these characteristics were found in similar proportion in both matched and non-matched groups.

Since not all of the HLA-DR determinants are known, the presence of "blank" antigens complicates the analysis of the influence of HLA-DR matching on renal graft survival. In the current study, two HLA-DR antigens were identified in 61 percent of the donors and recipients, and one HLA-DR antigen was identified in 39 percent of the donors and recipients. In this study, the percentage of individuals with one identifiable HLA-DR antigen is similar to the percentage observed in normal control individuals studied in our institution. Although HLA-DR antigens are more difficult to detect on lymphocytes from patients with end-stage renal disease or from blood of cadaveric donors than from lymphocytes of normal healthy individuals, accurate HLA-DR typing can be performed on lymphocytes from these patients.

To examine the relationship of HLA-DR matching and graft survival free of the problem of blanks, the survival data in donor-recipient pairs with two identifiable HLA-DR antigens was examined by the present authors. Although the numbers in each subgroup were small, individuals with a one or two HLA-DR antigen matched graft still had a superior graft survival compared to those with zero HLA-DR antigen matched grafts.

Some studies have indicated that transfusions improve graft survival predominantly in patients receiving DR-mismatched transplants. These data sug-

| TABLE I Correlation of HLA-DR Antigen Matched Grafts With Other Prognostic Factors |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Match No. %     | Match No. %     | p Value         |
| Sex                             |                 |                 |                 |
| Male                            | 30 75           | 19 63           | NS              |
| Female                          | 10 25           | 11 37           |                 |
| Age                             |                 |                 |                 |
| < 30                            | 15 38           | 5 17            | NS              |
| > 30                            | 25 62           | 25 83           |                 |
| ABO Type                        |                 |                 |                 |
| O                               | 16 40           | 11 37           | NS              |
| Non-O                           | 24 60           | 19 63           |                 |
| HLA-A and B Match*              |                 |                 |                 |
| 0                               | 4 10            | 1 3             |                 |
| 1                               | 7 17            | 6 20            |                 |
| 2                               | 21 52           | 15 50           |                 |
| 3                               | 5 13            | 7 23            |                 |
| 4                               | 3 8             | 1 3             |                 |
| No. Transfusions                |                 |                 |                 |
| 0                               | 1 3             | 3 10            |                 |
| 1-5                             | 14 35           | 7 23            | NS              |
| 5-10                            | 11 27           | 4 13            |                 |
| >10                             | 10 25           | 8 27            |                 |
| No. Transfusions                |                 |                 |                 |
| Not Known                       | 4 10            | 8 27            |                 |
| Causes of Failure of Grafts     |                 |                 |                 |
| Acute & chronic rejection       | 9 92            | 14 88           | NS              |
| Non-immunologic mechanisms      | 2 18            | 2 12            |                 |

*Represents the number of HLA-A and B antigens shared by the donor and the recipient.
gest that recipients, who have not been transfused, would benefit greatly from receiving HLA-DR matched grafts. In the current study, only four patients did not receive any transfusions. Thus, the detrimental effect of no transfusions on patients receiving renal grafts could not be determined. Nevertheless, a similar distribution in the number of transfusions was observed in recipients receiving one or two antigen matched grafts and in those receiving zero antigen matched grafts. Thus, the beneficial effect of HLA-DR matching in the current study appears to be independent of the number of transfusions received by the recipients.

In summary, our data strongly suggest that matching for HLA-DR antigens correlate with a beneficial renal graft survival. In addition, HLA-DR matching appears to be an independent prognostic factor.

References