# Optimization of living-related renal transplantation success through HLA genotyping, MLC stimulation cutoffs, and donor-specific blood transfusions

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A simple plan for selecting the optimal available living-related donor by means of HLA genotyping, mixed lymphocyte culture (MLC) stimulation cutoffs, and recently donor-specific blood transfusions (DST), has evolved from experience with 112 living-related donor recipient pairs. The approach was straightforward and ranked donor-recipient pairs as follows: *Group 1*: HLA-identical MLC nonstimulatory donors were preferred when available; these were almost always siblings, but HLA-identical MLC nonstimulatory parent-child combinations occurred as well.[1] *Group 2*: If no HLA-identical donor was available, then donors with low two-way MLCs (stimulation index <10) were selected.[2, 3] Finally, if only an HLA-nonidentical donor with high MLC stimulation was available, then donor-specific transfusions (DST) were used (*Group 3a*)[4] in order to improve the unsatisfactory results reported with high MLC stimulators before DSTs were introduced (*Group 3b*).[2, 3]

## Methods

Antigens of the HLA-A, B, and C loci antigens were tested by the standard microcytoxicity technique.[5] Serologic testing for DR antigens 1 through 8 was carried out with anti-human $F(ab)_2$ separation of B lymphocytes and the standard microcy-

totoxicity technique of the Eighth International HLA Workshop.[5] Platelet-absorbed recipient serum was tested at 5, 22, and 37C against separated donor T and B lymphocytes[5] before each DST, as well as before and after transplantation. Antibodies against monocytes and polymorphonuclear (PMN) leukocytes were detected with fluorochromasia.[6] Mixed lymphocyte culture (MLC) and suppressor assays were performed as previously published.[7] The suppressor assay was based on the reduction of one-way MLC stimulation by concanavalin-treated recipient lymphocytes, and the percent suppression was calculated as:

$$1 - \frac{ABm + A\ con\text{-}A}{ABm} \times 100,$$

where $A$ is recipient lymphocytes, $B$ is donor lymphocytes, $m$ represents mitomycin treatment and *con-A* is concanavalin-A treatment. If the baseline unidirectional MLC stimulation (ABm) was more than two standard deviations below the mean of all the ABm stimulations, it was found to be too low to use for testing further suppression (see Results). Survival curves were calculated by the Kaplan-Meier method.[8]

Donor-specific blood transfusions (DST) were given as follows: within 24 hours after being drawn, 200 cc of donor whole blood was transfused into the recipient at 2-week intervals 3 times prior to transplantation. Time from last transfusion to transplantation ranged from 14 to 52 days. One recipient (Case 4) with an Rh incompatibility received RhoGam without complication with each of two transfusions that had been rendered free of red cells by sedimentation with hydroxyethyl starch.[9]

## Results

In Group 1, the 50 HLA-identical MLC nonstimulatory pairs had a mean graft survival of 202.6 months (*Figure*).

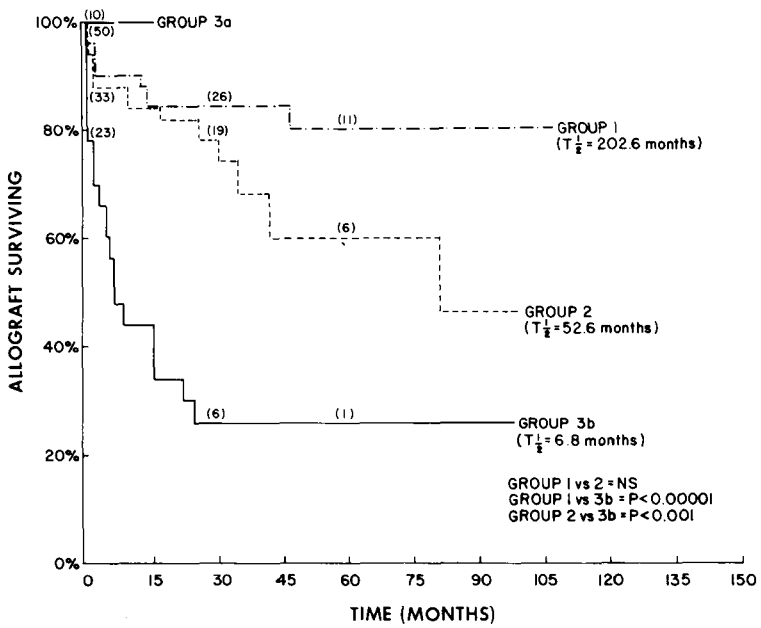


**Figure.** The numbers in parentheses ( ) represent the patients at risk at the specified time period in this Kaplan-Meier survival analysis. However, Group 3a with all allografts functioning had only 10 patients with a mean survival of just 10.9 months, and was not used for statistical comparison.

Despite this excellent graft survival there were 5 early allograft failures due to "delayed hyperacute" rejections 1–8 weeks after transplantation.[10] In these 5 failures no antibody was detected by standard or antiglobulin crossmatching against unfractionated donor lymphocytes. Similarly, no autoantibodies, donor T or B alloantibodies were detected in any of the 3 tested recipients. These 3 donor-recipient pairs were also tested for possible donor-specific antimonocyte and anti-PMN antibodies by fluorochromatic techniques.* None of the patients had pretransplantation donor-specific anti-monocyte antibody, although a weak antibody developed in one patient two weeks post-transplantation. Antidonor PMN antibody was detected before transplantation in one patient and afterwards in another. No antiendothelial antibody was detected by indirect immunofluorescence of normal kidney tissue.

In Group 2, MLC cutoffs based on the stimulation index in two-way tests were used. Twenty-eight of 33 recipients with low MLC stimulation (< 10 S.I.) have had successful allografts for at least one year, and a mean survival of 52.6 months (*Figure*).

Although only 10 patients with high MLC stimulation have had DST (*Group 3a*), all 10 allografts are functioning for a mean of 10.9 months. However, 2 of these patients have had recurrent rejections and impaired graft function (serum creatinines of 3.7 and 3.9 mg/dl, respectively). Of the 23 patients proposed for the DST protocol, 10 had already had transplantation via DST donors, 3 had been scheduled for such transplants, 5 had been previously sensitized to the intended DST donor and therefore never received any DSTs, and 5 had become sensitized after DSTs were begun and consequently were removed from the DST transplant protocol. In the latter two groups, 4 of the 10 sensitized patients have received cadaver transplants, 2 in each group.

In contrast, high MLC stimulation (> 10 SI) without DST (*Group 3b*) was associated with graft failure in less than one year in 13 of 23 cases, with a mean graft survival of just 6.8 months (*Figure*).

There was no significant difference between the HLA-identical pairs and the low MLC HLA-nonidentical pairs although their survival slopes did diverge with time. However, each of these groups had a significantly better result than the high MLC stimulatory group ($p < 0.001$) (*Figure*). The survival of all 10 allografts in DST-treated recipients, albeit short-term and small in number, is encouraging.

No significant difference was found in the frequency of transfusions or diabetes when comparing Group 1 (43/50, 4/50, respectively), to Group 2 (31/33, 2/33, respectively), Group 3a (10/10, 2/10, respectively), and Group 3b (20/23, 2/23, respectively). Also, no significant difference in recipient age was noted between any of the groups (31.0 years ± 10.3 SD, 29.1 ± 14.0, 23.3 ± 8.9, and 28.9 ± 11.5, respectively). Donor age did show a significant difference between Group 1 (30.7 years ± 9.3 SD) and Group 2 (38.9 years ± 12.4 SD), but not between any of the other groups.

Comparison of the mismatched donor-recipient DR-antigen combinations in low and high MLC groups showed that certain pairs were exclusively in one or the other category (*Table 1*). Mismatched donor-recipient DR pairs occurring more than once and exclusively in the high MLC group were DR3-DR6,

* *Performed in collaboration with Drs. John Thompson and Nancy Goeken at the University of Iowa Medical Center.*

**Table 1.** Mismatched DR antigen pairs*

| High MLC (≥10 S.I.) | Low MLC (<10 S.I.) |
|---|---|
| 1-3* | 2*-3 |
| 2*-3 | 2-5* |
| 2*-3 | 2-7* |
| 2*-B1 | 2-B1* |
| 3*-4 | 4*-7 |
| 3-5* | 4-7* |
| 3-6* | 5-7* |
| 3-6* | 5*-B1 |
| 4-6* | 6-B1* |
| 4-B1* | 6*-B1 |
| 4*-B1 | 6*-B1 |
| 4*-B1 | |
| 5-B1* | |
| 6*-7 | |
| 6-7* | |
| 7*-B1 | |

Mismatched donor antigen; the unmarked antigen is the mismatched recipient antigen.

DR4-DR Blank, and DR6-DR7, respectively. Mismatched pairs occurring more than once and only in the low MLC group were DR4-DR7 and DR6-DR Blank, respectively. No single donor or recipient DR antigen was dominant in either the low or high MLC categories.

In 10 patients with high MLCs seen within the past year, donor-specific transfusions (DST) were used. Nine of these 10 patients received 3 DSTs at approximately 2-week intervals, and one with an Rh incompatibility received two such transfusions along with RhoGam at a 2-week interval. In addition to the DSTs, 8 of 10 had received between 2 and 29 random transfusions before DST. Five of the patients received a random transfusion in the midst of the DST schedule or before transplantation while undergoing dialysis in satellite dialysis centers.

Tests for T- and B-cell antibodies, both autoreactive and alloreactive (donor-specific), were performed in the first 9 DST patients before each of the donor-specific transfusions, after donor-specific transfusions but before transplantation, and early after transplantation. In Patient 10 the T-cell crossmatches were performed at the same intervals but the B-cell antibody was tested only before DSTs were started and before transplantation. From these studies, three patterns of antibody production emerged. The first pattern was seen in 4 patients and consisted of the transient appearance of B-cell antibody. In 3 patients it appeared just before or shortly after the last DST and completely disappeared before transplantation (*Table 2*). The antibody was most reactive at 5 C but was also detectable at 22 and 37 C. It was alloreactive in all 3 and autoreactive in 2. In the fourth patient, alloreactive and autoreactive B-cell antibodies were present at the start of DST but disappeared before transplantation. Each of these patients has had excellent allograft function at 19, 17, 6, and 5 months with current serum creatinines of 1.1, 1.5, 1.1, and 1.1 mg/dl, respectively.

The second pattern, seen in 2 patients, consisted of the peritransplant appearance of allopositive B-cell antibody reactive at 5 and 22 C (*Table 3*). This antibody was detected 24 hours before to 48 hours after transplantation. Both patients have had rejections and impaired renal function with serum creatinines of 3.7 and 3.9 mg/dl, 14 and 16 months after transplantation, respectively.

The third pattern in 3 patients was one in which no B-cell antibody of any

**Table 2.** DST Antibody pattern: transient positive

| | Pre-DST | | Intra-DST | | Post-DST/Pretransplant | | | | Post-Trans-↓ plant | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Allo* | Auto | Allo* | Auto | Allo* | Auto | Allo | Auto | Allo | Auto |
| Antibody to donor B lymphocytes | | | | | | | | | | |
| 37 C | − | − | + | − | + | + | − | − | − | − |
| 22 C | + | + | + | − | + | + | − | − | − | − |
| 5 C | + | + | + | − | + | + | − | − | − | − |

Antibodies against donor T lymphocytes were absent at 5, 22, and 37 C.

* Alloreactive in all four patients, autoreactive in two.
↓ = renal transplantation.

**Table 3.** DST antibody pattern 2: peritransplant positive

| | Pre-DST | | Intra-DST | | Post-DST/Pretransplant | | | | Post-Trans-↓ plant | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Allo | Auto | Allo | Auto | Allo | Auto | Allo* | Auto | Allo | Auto |
| Antibody to donor B lymphocytes | | | | | | | | | | |
| 37 C | − | − | − | − | − | − | − | − | − | − |
| 22 C | − | − | − | − | − | − | − | − | + | − |
| 5 C | − | − | − | − | − | − | + | − | + | − |

Antibodies against donor T lymphocytes were absent at 5, 22, and 37 C.

* Alloreactive in both patients 24 hours before to 48 hours after transplantation.
↓ = renal transplantation.

**Table 4.** Suppression in DST (%): (ABm + A Con-A)

| Patient | Pre-DST | Intra-DST | Pretransplant |
|---|---|---|---|
| 1 | −3.4 | −3.6 | 74.5 |
| 2 | 25.2 | (ABm 974 cpm)* | 27.2 |
| 3 | 9.8 | 45.9 | −13.5 |
| 5 | 23.8 | (ABm 474 cpm)* | 30.3 |
| 6 | 89.4 | −16.5 | 18.0 |
| 7 | 56.6 | 37.7 | 28.0 |
| 8 | (ABm 483 cpm)* | 44.2 | 66.1 |
| 9 | 31.5 | (ABm 1376 cpm)* | (ABm 2847 cpm)* |
| 10 | 56.4 | 38.9 | 3.6 |

* These values were >2 standard deviations below the mean of stimulation in ABm combinations.

type was detectable before or after DST, or before or after transplantation. Two of these patients have had only mild reversible rejections, with current serum creatinines of 1.3 and 1.1 mg/dl, 14 and 3 months after transplantation, respectively, whereas one patient has had some deterioration of renal function (serum creatinine 2.5 mg/dl) 13 months post-transplantation.

In a suppression assay based on the reduction of one-way MLCs (ABm) by concanavalin-A treated autologous (A Con-A) lymphocytes, several features appeared in the 9 patients tested (*Table 4*). (1) In 7 of 9 recipients before DST, suppression ranged from 9.8% to 89.4%. Weak stimulation was noted in Patient 1 and such low stimulation in Patient 8 in the ABm combination (483 cpm) that autologous suppression could not be appreciated. (2) During DSTs, 2 of 9 patients (Cases 1 and 6) showed stimulation, the former unchanged and the latter as a new feature. Of the 7 patients with suppression, 3 may have expressed it as very low ABm baselines (474, 974 and 1376 cpm, respectively), and the other 4 had typical con-A suppression of high ABm responses. (3) Pretransplantation recipients who subsequently had relatively uncomplicated transplantation courses and good to excellent function showed suppression ranging from 3.6% to 74.5%. In one patient (Case 9), ABm stimulation was too low (2847 cpm) to evaluate autologous suppression. (4) The patient with recurrent rejection was the only one with stimulation just before transplantation. This is the same patient who had had biopsy-proved accelerated cellular rejection 48 hours post-transplantation when the B-cell antibody first appeared. Unfortunately, on the other recipient undergoing rejection we had insufficient data for comparison.

## Discussion

The approach outlined here has provided an effective means of improving allograft survival, with 6-month survivals of 90% or better for every type of living-related transplant, i.e., HLA-identical siblings, nonidentical recipients with low MLCs, and nonidentical recipients with high MLCs given DSTs. Our center has confirmed the basic findings originally presented by the San Francisco transplantation team.[4, 11] To those findings can now be added data to suggest that certain pairs of mismatched DR antigens are associated with either high or low MLC activity, specifically, DR3-DR6, DR4-DR Blank, and DR6-DR7 in the former group, and DR4-DR7 and DR6-DR Blank in the latter.

Additionally, certain patterns of B-cell antibody and concanavalin-A stimulated suppressor cell activity may indicate a stable or unstable condition for transplantation and predict either a smooth or a rejection-filled course. Since suppressor activity existed before as well as after DST, it is clear that DST did not basically alter pretransplantation status, with the exceptions of Patient 3 (loss of suppression) and Patients 1 and 6 (gain of suppression). One could even ask whether DST was necessary in patients with persistent suppression. Similarly, no significant alterations in MLC or CML activity have been noted after DST.[4] Consequently, the basic change induced by DST is still not understood, although patterns of persisting or altered suppressor activity correlate with the ensuing clinical course. We should emphasize, too, that the DST-treated recipients were a small group with relatively short follow-up.

The one patient suffering serious rejection who could be studied lost suppressor activity just before transplantation. This patient and another with recurrent rejections and the most severe impairment of renal function developed B-cell antibody for the first time 24 hours before to 48 hours after transplantation. The time between the last transfusion and transplantation in these two patients with serious rejection was 21

and 22 days, respectively. In contrast, the successful allograft recipients who had either no B-cell antibody or only a transient episode and some degree of suppression before transplantation had received transplants either earlier (14, 15, 16 and 19 days) or later (50 and 52 days). This period corresponds generally to the time when suppressor cells are detectable after experimental allografting, viz., up to 15 days and after 40 days.[12] The intervening time from 2 to 6 weeks after grafting is a period in which immunocompetent cells recover in the transplant and counteract the suppressor cell population.[12]

Because of possible variations in the timing of this suppressor-cytotoxic-suppressor cell sequence due to individual differences as well as to the overlapping of changing lymphocyte subsets when DSTs are given 2 weeks apart, monitoring of the patient with suppressor assays may assist in better timing of transplantation in order to avoid such early rejections. However, the duration of the suppressor assay makes pretransplantation changes still possible. Although the sudden perioperative appearance of B-cell antibody also indicates a poor prognosis, our ability to use it in a preventive fashion is limited by the fact that it may not appear until shortly after transplantation. Even if the B-cell antibody itself were not harmful, its initial appearance may coincide with a time when cytotoxic cells become predominant over suppressor cells. From a practical point of view, it may be appropriate to avoid transplantation between approximately 2½ to 6 weeks after DSTs given 2 weeks apart if no monitoring is possible. In another approach, one could consider giving the DSTs at a closer interval and transplanting within one to 2 weeks after the last transfusion in order to sustain suppressor dominance.

## Acknowledgment

We are grateful to Richard Greenstreet, Ph.D. for his assistance in performing the biostatistical analyses.

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