Correlation Between MLC Stimulation and Graft Survival in Living Related and Cadaver Transplants

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"Multiple MLC's" (parallel tests in recipient, donor and globulin-poor plasma) were performed in 211 consecutive transplant donor-recipient pairs. The two-way MLC's were performed on patients' lymphocytes before immunosuppression. All grafts regarded as "successful" were at risk for at least six months. Patients with a low MLC (Stimulation Index less than 8 times controls) usually had successful grafts (graft survival was 83% in related transplants and 76% in cadaver transplants). Patients with high MLC's had poor graft survival (0% graft survival in related transplants and 32% in cadaver transplants). An adjusted graft survival was calculated to exclude patients who died with normal renal function (serum creatinine less than 2 mg%). The adjusted graft survival was 91% for living related transplants and 88% for cadaver transplants. Falsely low MLC's occurred when the recipient's plasma contained low-titer cytotoxic antibodies. In 15 recipients of cadaver kidneys, the MLC in recipient plasma was significantly lower than MLC's in donor or globulin-poor plasma. Since the MLC when using cadaver donors was necessarily retrospective, the results were not known pre-transplant and all 15 grafts were rejected. In living related pairs, however, we were able to screen for such antibody activity and could avoid humoral presensitization and cellular compatibility.

THE MOST RECENT REPORT of the Kidney Transplant Registry shows graft survival at one year to be 74% when sibling donors are used, and 76% when parental donors are used. Although this appears to be a significant improvement over the 50% survival obtained with cadaver organs, it still means that one of four living related grafts fails within the first year. Obviously, there is a great need for improved, pre-transplant matching of donor-recipient pairs. At present, there are two techniques used for such matching: HLA tissue typing and the mixed lymphocyte culture test, or MLC. Although HLA typing has proved of great value in identifying HLA identical siblings, it has not been useful in selecting between less well-matched related donors such as parents and siblings who share one haplotype or less with the recipient. Because the Mendelian odds for HLA identity are not very high and because non-HLA factors are strongly indicated by most graft survival data, interest in mixed lymphocyte culture testing is increasing.

The data presented here will summarize the results of preoperative MLC/s performed on 212 consecutive transplants at the University of California School of Medicine, San Francisco (i.e. 59 living related pairs and 153 unrelated pairs). This group includes patients who could be considered "poor risks," i.e. patients receiving secondary grafts; small children; diabetics; patients with lupus erythematosis; etc. A graft must have been at risk for at least six months to be represented as "successful" in our data. Graft loss on the other hand, includes not only graft rejection but death of the patient from any cause. All MLC tests were performed pre-transplant; however, when cadaver organs were used, the results were available only after transplantation because of the five-day incubation period needed to perform the MLC and the present limitations of organ preservation.

Methods

Sixty milliliters of heparinized blood were obtained from the donor and the recipient before transplantation and before any immunosuppressive drugs were administered to the recipient. The blood was then separated

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on a Ficoll/Hypaque density gradient to obtain a purified lymphocyte population (90–95% mononuclear cells contaminated by less then 5% erythrocytes and granulocytes). Platelets were removed during the subsequent rinsing of the lymphocytes (three times in balanced salts). The purified and washed lymphocytes were cultured in two-way MLC's which consist of 5-replicate mixtures and triplicates of each of the appropriate controls. Mixtures contained 1×10^6 donor lymphocytes plus 1×10^6 recipient lymphocytes in 4 ml of medium. Control cultures contained 2×10^6 donor or recipient lymphocytes, again in 4 ml of medium. The medium used was tris-buffered Eagles' MEM-S with antibiotics and L-glutamine.[8] Parallel MLC tests were cultured in 10% recipient plasma, 10% donor or AB plasma and 10% plasmanate. This battery of tests is called the "multiple MLC." After 5 days in culture the cells were labeled with tritiated thymidine (5 microcuries per 4 ml. of culture; 6.7 curies per m mole) for 3 hours. The acid insoluble DNA fraction was precipitated and collected on filters which were then burned on a Packard tricarb oxidizer for liquid scintillation counting. The counts of replicate cultures were averaged and expressed as counts per minute per culture. The degree of stimulation in each test is expressed as a ratio between the counts of the mixture and those of the appropriate controls; this is the Stimulation Index or SI.

$$SI = \frac{\text{Counts Per Minute Mixture (A + B)}}{\text{CPM of A control + CPM of B control} \div 2}$$

The counts per minute of the replicate cultures seldom varied more than 10% from one another.

Results

The results of the MLC in 59 related transplants are given in Table 1. We have divided the patients into two groups: Those with a SI lower than 8 and those with a SI of 8 or above. The MLC data in this and other tables refer to MLCs done in recipient plasma. Fifty-six recipients of living related grafts had low MLCs with their donor (i.e., SI of less than 8). Of these grafts, 47 (83%) are functioning normally at present (from 6 months to 3 years post-transplant). Five patients died with functioning kidneys. Only one of these patients had an elevated serum creatinine (2.4 mg/100 ml) at the time of death. If the 4 patients who died with "normal" serum creatinines (i.e., less than 2 mg/100 ml) are not considered graft losses, graft survival would be 91% or the adjusted graft survival.

Only three patients who had high MLCs with their related donors received transplants. All of these grafts were rejected.

The two-year graft survival for patients with low MLCs is 80% (i.e., 24 out of 30 patients have normally functioning grafts). The adjusted graft survival for this group is 92%.

The correlation between our MLC results and graft survival in 15 patients who received cadaver organs is shown in Table 2. Eighty-five patients had SIs of less than 8. Sixty-five of these patients (76%) still have functioning kidneys and seven have rejected their kidneys. Thirteen patients died, but only 4 of the 13 had elevated serum creatinines, or had been treated for rejection within 6 months prior to death. If the 9 patients who died with normal kidney function are not considered graft losses, the adjusted graft survival is 87% for cadaver recipients with low MLCs. Fifteen recipients of cadaver grafts had falsely low MLCs in their own plasma. This was detectable only by an analysis of the multiple MLC. These falsely low MLCs occurred when the recipient's plasma contained humoral cytotoxic antibodies against the donor. All 15 patients had very low MLCs (SI less than 1.8) in their own plasmas. However, the parallel MLCs in 10% donor plasma and 10% plasmanate were significantly higher. Since MLC tests are retrospective when cadaver organs are used due to the 5-day incubation period, these results were known only post-transplant and all 15 grafts were rejected. All 15 recipients had had negative pre-transplant cytotoxic cross-matches with their respective donors' cells. How-

TABLE 1. *Correlation of MLC Results and Graft Survival in Related Transplants*

MLC SI*	Number of Patients	Graft Survival	Adjusted Graft Survival
<8	56	83%	91%
>8	3	0%	0%
	Two-Year Graft Survival		
<8	31	80%	92%

* Stimulation Index in 10% recipeint plasma.

TABLE 2. *Correlation of MLC Results and Graft Survival in Cadaver Transplants*

MLC SI*	Number of Patients	Graft Survival	Adjusted Graft Survival
<8	85	76%	88%
(False Neg)	15	0%	0%
>8	53	32%	33%
	Two Year Graft Survival		
<8	28	71%	87%
>8	21	23%	23%

* Stimulation Index in 10% recipient plasma.
(False Neg) = False negative MLC

ever, the recipients did develop cytotoxic antibodies after their rejected kidneys were removed.

Fifty-three recipients of cadaver kidneys had high MLCs and only 17, or 32%, of these patients now have functioning grafts. One patient died from myocardial infarction one month post-transplant. This patient had a serum creatiine of 1.3 at the time of death. If this patient is not considered a graft loss, the adjusted graft survival in patients who had high MLCs with their cadaver donors is 33%.

The two-year graft survival for the cadaver recipients with low MLCs was 71%. Twenty of the 28 patients in this category have serum creatinines of less than 2 mg/100 ml. The adjusted graft survival for this group was 87%. Only 5 of the 21 patients with high MLCs have functioning grafts at two years post-transplant.

Discussion

The survival of allogeneic grafts depends upon the degree of genetic disparity between donor and recipient. At present, there are two methods of selecting human donor-recipient pairs: 1) serological HLA phenotyping; and 2) matching by mixed lymphocyte cultures tests (MLC). Many studies have been published on the correlation between matching for HLA antigens and renal allograft survival. There is general agreement that a graft from a HLA identical sibling has a 90% chance or better for long-term survival, in contrast to the significantly lower survival rate of mismatched sibling grafts. However, when unrelated donors are used, a good correlation between HLA matching and graft survival has not been demonstrated.[6,11] This would indicate a major participation by non-HLA factors in graft rejection. Since the description of the mixed lymphocyte culture reaction (MLC) by Bain,[5] several investigators have tried to demonstrate a relationship between this phenomenon and histocompatibility.[1,3] Studies by Bach and Amos[2] initially showed that MLC reaction between siblings corresponded to the HLA match between them. However, the same investigators later found an HLA identical sibling pair that stimulated in MLC.[1] Studies by Yunis and Amos,[16] and by Eijsvoogel *et al.*,[9] have shown that the MLC reaction is an expression of disparity at a genetic locus which is distinct from, but closely linked to, the serologically defined HLA locus.

The MLC test was first suggested as a method for the pre-transplant selection of donor-recipient pairs by Bain in 1965[5] and by Bach and Amos[2] in 1967. Early reports on the correlation between the degree of stimulation in MLC and the length of skin graft survival were made by Ceppellini *et al.*,[7] and Russell *et al.*[14] Bach[4] and Hamburger[12] later demonstrated a correlation between a positive or negative reaction in the two-way MLC and the clinical results of their living related renal transplants.

We have previously reported a correlation between the results of our two-way MLCs and graft survival in our living related transplants.[8] In contrast to most investigators, we have continued to use the two-way test in which both donor and recipient cells are potentially able to participate. This is because we and others[4,12] have found that tests rendered one-way by treatment of the donor cells with either Mitomycin C or X-irradiation have not correlated as well with actual graft prognosis. Although it could be argued that a two-way MLC could just as well reflect the perhaps irrelevant response of donor to recipient, this would not interfere with the identification of low reactors.[10] In fact, we have demonstrated that the MLC can show some response and still be predictive of good graft survival. However, if the MLC reacted over a certain degree (i.e., SI over 8), graft survival was poor.

For the first time we were able to demonstrate a positive correlation between MLC results and graft survival in cadaver transplants. This was later confirmed by Kashiwagi *et al.*[13] In addition, Van Rood[15] has also demonstrated a significant correlation between MLC results and skin graft survival in unrelated donor-recipient pairs.

The data presented here give further support to the important role of MLC matching in renal transplantation. The 56 related donor-recipient pairs with low MLCs had an overall 83% graft survival and a two-year graft survival of 80%. This is to be compared with the 67% to 69% survival for grafts between living related pairs reported by the Transplant Registry. The three living related pairs with high MLCs who were transplanted had poor graft survival.

The MLC results were also predictive of unrelated graft survival. The cadaver recipients with low MLCs had an overall graft survival of 76% and a two-year graft survival of 71%. These results are a considerable improvement over the 46% two-year graft survival reported by the Registry. Cadaver recipients who had high MLCs with their donors had a graft survival rate of only 32%, which decreased to 23% at two years.

We have previously reported on the use of the multiple MLC (i.e., parallel MLCs in recipient, donor, and globulin-poor plasma) to detect presensitized patients.[8] We have now identified 15 cadaver recipients who were presensitized and also had false-negative MLCs. These patients all had secondary grafts and had had very low values when the MLC was done in their own plasma, but had higher values when the same MLC was done in donor plasma or a non-specific protein source. This rise in MLC value was particularly impressive when

TABLE 3. *Cause of Death After Transplantation in Patients With a Low MLC*

	Cause of Death	No. of Patients
1. Patients dying with normal renal function. (Serum Creatinine < 2; mean value 1.4)	Myocardial Infarction	2
	Suicide	1
	Pulmonary Emboli	2
	Non Bacterial Infection	
	Viral Encephalitis	1
	Cytomegalovirus	1
	Pneumocystis	1
	Toxoplasmosis	1
	Bacterial Infection	1
	Hepatitis	1
	G. I. Bleeding	1
	Hyperparathyroid Crisis	1
		13
2. Patients dying with Serum Creatinine > 2 or recently treated for rejection.	Non Bacterial Infection	
	Cytomegalovirus	1
	Pneumocystis	2
	Bacterial	1
	Fungal	
	Nocardia	1
		5

it occurred in 10% plasmanate. Since plasmanate is virtually free of gamma globulin, it loses a good deal of its supportive value for the metabolism of the MLC, and tests on it are usually quite low. However, if the patient has alloantibodies, and therefore a falsely low MLC in his own plasma, the concomitant test done in plasmanate gives a higher value. Thus, the inhibitory effect of recipient plasma with antibodies against the donor can be so great that even a medium which gives less than optimum support can yield higher stimulation. The importance of detecting circulating antibodies by this method can not be over emphasized since all of these patients rejected their kidneys.

Since prolonged immunosuppression can have profound psychological and/or physiological effects, we feel that the death of any transplant recipient could be transplant-related. However, when a patient dies with a perfectly functioning kidney, it is difficult to regard it as an immunological graft loss. We therefore have calculated an "adjusted graft survival" to separate graft loss due to immunological causes from graft loss due to the patient's death. The adjusted graft survival data have been calculated for both living related and cadaver recipients. We have divided the patient deaths into two groups (Table 3). Group 1 includes those patients who had a stable serum creatinine value of less than 2 mg/100 ml for at least 6 months prior to death. These patients were on maintenance immunosuppressive therapy and had not been treated for rejection within the 6 months prior to death. Group 2 included patients with elevated serum creatinine values, and patients who died with a normal creatinine but who had been treated for rejection within 6 months preceding death. If the patients dying with normal renal function (Group 1) are not considered graft losses, we obtained an "adjusted graft survival" value for living related recipients who had low MLCs with their donors. This was more than 90% even at two years. Similarly adjusted data give a two year graft survival of 87% for unrelated transplants with low MLCs.

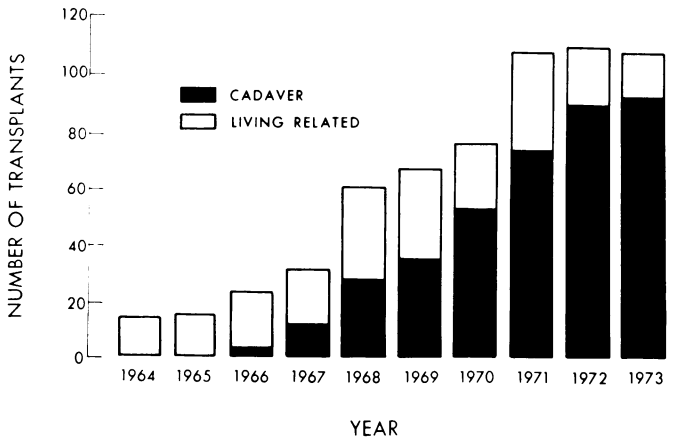

FIG. 1. Renal transplants by year and donor source.

In September of 1972 we made it our policy to transplant only those living related donor-recipient pairs who had consistently low MLCs. Recipients having demonstrably false negative MLCs, or consistently high MLCs were placed in the computerized recipient pool to await a cadaver organ. Patients receiving related transplants under this policy have had a graft survival rate of 88% for up to 1½ years post-transplantation. In an attempt to provide kidneys for those recipients denied related transplants by this policy, we have had to rely on our already active organ procurement program.

Figure 1 illustrates the total number of renal transplants performed at the University of California School of Medicine at San Francisco over the past 10 years. Because large numbers of cadaver organs are available we have been able to give kidneys to 8 of the 9 patients referred to the cadaver program because of their poor MLC results.

In an effort to make cadaver transplantation as safe as possible we have adopted a more conservative immunosuppressive program for our transplant recipients. Using this new regimen, we have been able to increase patient survival from 85% to 93% for the recipients of cadaver organs and from 91% to 100% for the recipients of related kidneys.

Since HLA identity in a random population is so unlikely, HLA matching for the use of cadaver organs

is not only inadequate but also impractical. The first because it has not been shown to correlate well with clinical graft survival; the second because it necessitates such a large recipient pool to achieve good antigenic matches. A reliable pre-operative MLC for the recipients of cadaver kidneys would enable the use of a significantly smaller recipient pool. The test could be used as a sort of "cellular cross-match" in which a pool recipient could be tested against every available donor with an appropriate blood type and a negative cytotoxic cross-match. Our data already indicate that almost 50% of our recipients have acceptable MLC reactions with their donors.

We are very encouraged by our success in using the MLC as a screening device for related transplants. It now remains to adapt the test so that it can be used pre-operatively for the recipients of cadaver organs, or to improve organ preservation so that enough time is available to perform the present MLC.

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Discussion

Dr. John Sarkis Najarian (Minneapolis): Unfortunately, as Dr. Cochrum has pointed out, the studies with MLC in the cadaver donors were done retrospectively, since the tests takes five to seven days to perform; it showed significance. Therefore, there is an urgent need for a MLC test that can be performed in 24 to 48 hours. The aspect I'm interested in, however, is the related donor side of transplantation. Cochrum and Belzer report three patients with related donors, who had high MLCs before transplansplant. All three transplants failed. Apparently, since that time, or since 1972, they have not done related donor transplants with a high MLC. That's three out of some 57, so it's less than 6%.

Dr. Cochrum, how often have you seen a high MLC in a related donor since 1972? Is it still running about 5 or 6%, or is the figure higher?

In addition, you didn't mention HL-A, and I am curious to know what the HL-A typing of the three failures were. Were they really bad matches by HL-A, or were they reasonably good matches?

The overall result in the cadaveric group, as I read it, was around 54 to 58%, 1- and 2-year survival, which is quite consistent with the national survival. So if you could get rid of that 33% survival group, you would really do very well. Maybe Dr. Cochrum can tell us if you have been able to find a rapid MLC that most of us can use to help us select these patients.

Dr. G. Melville Williams (Baltimore): I'd like to ask two questions. The first question has to do with the bad results obtained by the current investigators when preoperative serum was found to block the mixed lymphocyte reaction between donor and recipient. It had been my impression from previous reports by these and other investigators that the MLC blocking in the absence of cytotoxic antibody to the donor frequently led to good results rather than disasterous results. I wonder if the authors could clarify the discrepancies between this report and previous ones.

The second question has to do with the whole concept of what the MLC reaction measures. I have been struck in our small experience with delayed hypersensitivity testing of patients waiting