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Successful transplantation of kidneys bearing previously mismatched HLA A and B locus antigens

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Abstract. Transplantation of kidneys bearing HLA antigens to which recipients have previously been exposed is generally avoided, and such prudence is a well-documented means of preventing early graft loss. Prior exposure and subsequent reactions can, however, take a wide variety of forms, and blanket avoidance may prevent many deserving patients from being transplanted. In our region, operating through a single tissue-typing laboratory, we follow a consistent policy of allowing retransplantation with kidneys bearing previous mismatches, provided no relevant antibody response has been detected. Twenty-one of 34 such transplants remain functioning at time periods ranging from 7 months to 7 years. Four were lost due to rejection within the 1st month, and the remaining 9 functioned for periods ranging from 2 months to 8 years. Three were lost for reasons other than rejection. Our antibody screening policy and our criteria for a negative crossmatch results in the exclusion of two-thirds of all repeat mismatch transplantations. The results indicate that in the remaining third, transplantation can be performed across a repeat mismatch with excellent long-term results, provided our defined crossmatch policy is adhered to strictly.

Key words: HLA - Kidney - Transplantation.

In renal allografting it has been traditional practice to avoid previously mismatched HLA antigens when performing second or subsequent renal transplants. This practice has evolved in the expectation that anamnestic responses to the previously mismatched antigens would result in rapid antibody-mediated destruction of the current graft. There is good evidence that previous allografting, regardless of the presence of anti-HLA antibodies, has an independent, deleterious effect on graft survival [4]. However, it has not been demonstrated that reduced survival is due to the presence of mismatched antigens. A recent study has documented the early loss of 9 out of 14 grafts performed across previous HLA mismatches, and it goes on to suggest that transplantation across previous mismatches should be avoided [2]. A further study, involving 11 patients, reported the loss of four grafts within 2 weeks [6].

In 1983, Cardella et al. [3] demonstrated that it was feasible to perform crossmatches with current sera while ignoring positive findings with historical sera. Since that time, this practice has increasingly been followed in many transplant centres. Despite this, most units are faced with an increasing proportion of sensitised patients, many of whom have had previous transplants [5]. A strict policy of excluding previously mismatched antigens when considering retransplantation in this cohort of patients would inevitably lead to the extension of an already long wait for a crossmatch-negative kidney. The increased use of whole or segmental pancreatic allografts in diabetic recipients who have a currently functioning or previously failed renal allograft also raises important questions as to the importance of previously mismatched antigens present on the donor pancreas. We therefore felt it appropriate at this time to reexamine the problem of previous HLA mismatches and their role in the cyclosporin-A era.

Despite changes in clinical and laboratory procedures over the past 12 years, we have maintained

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a policy of allowing retransplantation across previous mismatches, provided two criteria are met:

1. The recipient has at no time produced a specific cytotoxic antibody to the mismatched antigen.

2. A prophylactic course of antilymphocyte globulin (ALG)/antithymocyte globulin (ATG) is administered for 10 days post-transplantation.

The specificities of cytotoxic antibody responses are allocated on the basis of a sophisticated screening procedure which is constantly updated. Prior to 1981, all relevant sera were used in the crossmatch, but subsequent to that time, only sera taken in the 6 months prior to transplantation were tested. This change in policy and the development of procedures to identify IgM non-HLA lymphocytotoxic (MALT) antibodies, which are often autoreactive [1], has resulted in an increased ability to perform transplants in patients who had previously lost their grafts. We are therefore in a position to address the problem of retransplantation across previous HLA mismatches in a relatively large cohort of patients. We are also able to discuss its implications for high-quality screening procedures and the availability of staff of sufficient calibre to enable error-free implementation of such policies.

Materials and methods

Patients

Data sets on all health service patients receiving second and subsequent grafts in our three centres over the past 12 years were examined. Those grafts that included HLA A or B antigens identical to or strongly crossreactive with previous mismatches were selected to be the subject grafts of this study. No distinction was made between cadaver grafts and those from living related persons.

Antibody screening policy

Panel reactivities of serum samples were determined by screening against 66 randomly selected HLA A, B, and Cw-typed normal cells. Any kill was regarded as significant, and the cell panel was changed every month to allow specificity patterns to emerge. A MALT antibody which reacted with about 60% of the panel was used on each screening plate as an aid in defining those sera which contained such antibodies. Samples from patients on dialysis were screened at regular intervals (usually every 3 months) and additionally at 10 days after a blood transfusion or documented viral infection. Additional samples were screened at the time of transplantation or subsequent graft failure. In cases where serum samples displayed consistent, broad reactivity, selected samples were rescreened at dilution(s). Titering of samples against three or more random cells was used as an aid in determining the screening dilution(s).

Crossmatch policy

MALT antibodies that were detected by screening, autologous cell killing, or treatment of the crossmatch serum with dithiothreitol (DTT) at the time of transplant were ignored. It is important to stress that all MALT antibodies were defined by prior screening and that since 1983, DTT is only used at the time of transplant when the presence of such an antibody has already been proven. Restricted use of DTT allows newly formed IgM anti-HLA antibodies, which may prove to be damaging to the graft, to be detected by a positive crossmatch test. Since February 1981, only serum samples taken in the previous 6 months have been used for crossmatching in fully screened patients.

Two microlitres of serum per well was used for crossmatches in a two-stage dye exclusion test (1 h at each stage). Spleen cells were used for cadaver donors and peripheral T and B lymphocytes for living related donors. Any positivity was normally considered a bar to transplantation unless it was caused exclusively by a MALT antibody. Fluorescent activated cell sorter backup for the above crossmatch tests is now routine for highly (>50% peak panel reactivity) sensitised recipients of cadaver grafts and for all sensitised recipients of transplants from living relatives.

A previous mismatch was avoided if the patient had produced a response to it or may have produced a response to it (e.g. if transfer had been made from another centre and adequate screening data were not available). A previous mismatch was allowed if no response to it had been detected but additional immunosuppression was given. An antibody response which could have arisen from pregnancy was treated as a response to a previous graft mismatch. If the patient had formed an antibody to a donor antigen caused exclusively by transfusion, then the transplant was allowed to proceed, but with prophylactic ATG/ALG.

Prior to February 1981, screening was carried out on 66 HLA A and B locus-typed cells. Dilution and rescreening were not used for additional determination of specificity, and all non-current sera were used in crossmatch tests. However, due to the time taken to build up accurate screening data, some overlap of both procedures occurred for 2 years.

Results

All three tables have the same format. Listed are the subject grafts, HLA mismatches, and survival times for all additional grafts. Those individuals who had two repeat mismatch grafts are entered twice where appropriate. In the repeat mismatch column, certain antigens are enclosed in parentheses. These indicate crossreactions or shared epitopes. Thus, we consider an A2 graft into a patient who has previously lost an A28 mismatched graft as a repeat mismatch. Similarly, we surmised that the third member of the HLA 1, 3, 11 crossreactive group would be important if the patient had been previously mismatched for the other two. The antibody column is necessarily simplified since some patients had over 100 serum samples screened during the time period covered here. In the first transplant row, the figure relates to the peak panel reactivity observed pretransplant. The remainder define the peak panel re-

Table 1. Grafts bearing repeat mismatches that have survived 3 or more years. Tx, Transplant number; CyA, cyclosporin A; Ab%, panel reactivity; N, no; Y, yes

Subject graft	Tx	CyA	Mismatch	Survival time (months)	Repeat mismatch	Ab%	Specificity
	1	N	2, 12, 9	3	-	0	
1	2	N	28, 12	94	(28), 12	0	
(19)	3	Y	2, 28, 62	>21	2, 28	0	
	1	N	2, 19, 5, 12	13	-	3	
2	2	N	2, 32, 44, 15	>95	2, 44	16	
	1	N	3, 29, 27	2		0	
3	2	N	29, 44	>86	29	33	3, 27
	1	N	1, 3, 8, 62	2	-	4	
4	2	N	3, 18	58	3	4	
	3	Y	31, 60	>30	-	51	1, 8+
	1	N	1, 5, 17	16	-	0	
5	2	N	1, 3, 14, 57	>48	1, 57	6	
	1	N	3, 28, 7	14		4	
6	2	Y	2, 60, 62	>47	(2)	75	7, 27+
	1	Y	28, 23, 55, 27	18		0	
7	2	Y	2, 31, 51, 7	>47	(2)	0	
	1	N	2, 3, 44, 35	48	-	11	27
8	2	Y	2, 29, 44, 55	>45	(2), 44	21	Cw4
	1	Y	2, 28, 62	1	-	0	
9	2	Y	2, 31, 7	>43	2	0	
	1	N	2, 28, 12	16	-	0	
	2	N	26, 16, 37	10	-	45	1+
10	3	Y	2, 62	>41	2	62	1+
	1	N	1	15	-	0	
	2	N	3	58	-	0	
	3	N	0	74	-	14	
	4	N	35	23	-	5	
11	5	Y	3, 7, 35	>41	3, 35	0	
	1	N	2, 23, 7, 22	23	-	0	
12	2	Y	2, 23, 27, 61	>39	2, 23	0	
	1	N	28, 29, 17	1	-	1	
13	2	Y	2, 23, 62, 50	>39	(2)	46	29

activity between transplants. In the specificity column, the figures indicate those HLA antigens that the patient reacted against in the time period covered by the antibody column. Rescreening of old samples has allowed all the results quoted to arise from the newer and more rigorous screening policy.

Table 1 details the grafts that have survived over a long term or that show every indication of doing so. Of the 13 grafts in this category, 11 are still functioning at periods ranging from 3 to 8 years; 1 failed at 94 months.

Table 2 shows the grafts that have survived from 1 to 3 years. Of the 10 grafts in this group, 7 are still functioning; 1 was lost at 28 months through noncompliance. Graft 21 was removed at 13 months after showing declining function for 4 months. Although biopsy specimens indicated an element of vascular rejection, no antibody to the mismatched antigen could be detected. It is now 3 months after graft failure and recent screening still fails to detect any antibody to the HLA B7 mismatch.

Table 3 shows the grafts that have survived for up to 1 year. Two of them (nos. 32 and 33) should not have been transplanted according to our policy because both had previously produced antibodies (1 and 3 years earlier, respectively) to graft antigens. The severe vascular rejection (termed "delayed hyperacute") observed in both patients was accompanied by high-titre antibodies to B8 and A9, respectively. Repeat crossmatches (on stored donor spleen cells) were negative, as was a retrospective

Table 2. Grafts bearing repeat mismatches that have survived 1-3 years. Tx, Transplant number; CyA, cyclosporin A; Ab%, panel reactivity; N, no; Y, yes

Subject graft	Tx	CyA	Mismatch	Survival time (months)	Repeat mismatch	Ab at Tx	Specificity
	1	N	51, 50	20	-	0	
	2	N	32, 44, 27	3	-	0	
(31)	3	N	26, 7, 44	1	44	37	
14	4	Y	11, 7, 51	>35	7, 51	14	
	1	N	3, 7	12	-	0	
	2	N	2, 35, 57	1	-	8	
15	3	Y	2, 25, 27	>34	2	0	
	1	Y	1, 11, 22, 35	6	-	0	
16	2	Y	3, 30, 35, 13	28	35	78	1+
	1	N	2, 3, 5, 27	22	-	0	
17	2	N	3, 49, 27	22	3, 27	0	
18	3	Y	11, 25, 18, 62	>23	(11)	83	2,3
	1	N	2, 12, 9	3	-	0	
(1)	2	N	28, 12	94	(28), 12	0	
19	3	Y	2, 28, 62	>21	2, 28	0	
	1	N	2, 26, 7, 44	16	-	2	
20	2	Y	26, 35	>18	26	88	2, Cw5, wk (24, 27)
	1	N	2, 11, 39	2	-	0	
	2	N	3, 7, 62	56	-	74	2++
21	3	Y	25, 7	13	7	76	2++
	1	N	11, 44	24	-	0	
	2	N	1, 18, 55	2	-	0	
22	3	Y	7, 44	>12	44	0	
	1	N	3, 11, 35	25	-	0	
23	2	Y	3, 31, 8, 60	>12	3	16	Cw4, wk (51, 44)

cell-sorter crossmatch for graft 32. A third graft (no. 34) was also lost due to a proven anti-donor secondary antibody response. Here the patient had previously received an HLA B5 mismatched graft and produced an antibody to both HLA B5 and B53 prior to his third graft, which was mismatched for HLA B35. Delayed hyperacute rejection occurred, and antibody screening revealed a high-titre anti-B53, which crossreacted strongly with HLA B35. This observation has prompted us to modify our policy to take more account of crossreactions.

In a comparable period, 10 repeat mismatch grafts and 92 other second or subsequent grafts were carried out before the introduction of cyclosporin A (CyA); 24 repeat mismatch grafts and 84 additional retransplants have been carried out since then. It is apparent that there has been a significant increase in the relative numbers of repeat mismatch grafts in recent years. Although 18/24 repeat mismatch and 60/84 additional second or subsequent grafts remain functioning with CyA as the primary immunosuppressive agent and the survival figures appear to be similar, accurate comparisons cannot be made. This is because we use selected immunosuppression regimes for different categories of non-mismatch retransplants.

Discussion

There has been a traditional reluctance to transplant kidneys bearing previously mismatched HLA antigens because of the expected accelerated rejection of the new graft. We have demonstrated that when certain conditions are met, accelerated rejection is uncommon: of 34 mismatched grafts, only 4 were lost in the first month post-transplantation. In 3 of these cases, there was serological evidence of anti-HLA class I antibody-mediated graft destruction. One produced an antibody to a crossreactive group, which included an antigen present on the new graft. The other two patients were mistakenly transplanted with grafts carrying antigens to which they had previously mounted an antibody response. Thirty grafts functioned for 2 months or more, and 21 continue to function at periods ranging from 6 months to 8 years. Actual graft survival at 1 year

Table 3. Grafts bearing repeat mismatches that have survived less than 1 year. Tx, Transplant number; CyA, cyclosporin A; Ab%, panel reactivity; N, no; Y, yes

Subject graft	Tx	CyA	Mismatch	Survival time (months)	Repeat mismatch	Ab at Tx	Specificity
	1	N	1	3	-	0	
	2	N	3, 19, 35	3	-	0	
24	3	N	1, 30, 13	11	1	0	
25	4	Y	11, 8	2	(11)	0	
	5	Y	26	3	-	88	1, 3, 11, 8, 35, 37, 19
	1	N	3, 7, 35	2	-	0	
	2	N	2, 40	47	-	15	
26	3	Y	2, 62	>8	2	15	
	1	N	1, 8, 40	83	-	7	
27	2	Y	29, 8	>8	8	7	
	1	N	1, 11, 22, 35	20	-	92	?
28	2	N	1, 7, 13	7	1	51	51++
	1	N	51	8	-	0	
	2	N	11, 27	0	-	11	51
	3	Y	29, 17	1	-	14	51
	4	Y	3, 23, 7	1	-	14	51+wk 11
29	5	Y	3, 7, 60	4	3, 7	0	
	1	N	3, 24, 38, 44	2	-	0	
30	2	N	3, 30, 49	2	3	13	
	1	N	51, 50	20	-	0	
	2	N	32, 44, 27	3	-	0	
31	3	N	26, 7, 44	1	44	0	
(14)	4	Y	11, 7, 51	>36	7, 51	0	
	1	N	3, 30, 18, 35	3	-	0	
	2	Y	8	3		0	
32	3	Y	8	1 wk	8	23	3, wk 8
	1	N	3	14		0	
	2	N	24, 5, 7	2		0	
33	3	Y	23, 8, 56	2 wk	9	25	9, wk 51
	1	N	24, 5, 19	3	-	8	wk 2
	2	N	44	3	-	29	57, wk (2, 25)
34	3	Y	35, 40	0	(53)	76	44, 57, 53, 49, wk (5)

for the group as a whole was 73%, which is a creditable result in a group of high-risk patients such as this, some of whom had their transplants in the pre-cyclosporin era.

A recent report by Barger et al. documented the early loss of 9 out of 14 grafts performed across previous mismatches and, on the basis of these results, advised against this practice [2]. There are three possible reasons for the discrepancy between our results and theirs. Application of our screening and crossmatch policies to the data of Barger et al. [2] would have excluded at least 3 of the 9 failed grafts. Our policy is, therefore, more selective. All of our patients received prophylactic ALG/ATG for 10 days post-transplantation whereas none of their patients did. Finally, whenever possible, we avoided transplantation for 6 months subsequent to the loss of the previous graft to enable adequate screening for anti-HLA antibodies and to define their specificity. This avoids the possibility of performing a transplant while antibody responses to the previous graft are still rising.

We have not included data on DR typing or anti-class II antibodies for several reasons. During the 12-year period which this study embraced, there were many changes in DR typing technology and, hence, in the ability to distinguish class II antibody specificities. Spleen cell suspensions are used as crossmatch material, however, and the presence of current anti-class II antibodies therefore results in a positive crossmatch and precludes transplantation. Our current policy is to carefully define DR and Cw specificities but to ignore repeat mismatches at these loci for the purposes of transplantation.

There has been an increasing use of grafts bearing previously mismatched antigens at our centres

in recent years, and we attribute this to changes in our crossmatch policies and in our immunosuppressive protocols. Since 1981, only sera taken in the 6 months prior to transplantation have been used for the crossmatch. This has allowed transplantation of a greater proportion of our sensitised patients. However, its full impact was not felt until adequate screening data became available for all waiting list patients, and this took 2 years to acquire. As a result, the availability of cyclosporin A, the ability to identify and ignore MALT antibodies, and full implementation of our new crossmatch policy all took place in 1983. Twenty-four of the 35 grafts included in the above study were done between 1983 and 1987. We are of the opinion that the increase is due to a growing confidence in the screening results and in the recognition of the superior immunosuppressive effects of cyclosporin A.

Reliance on screening data is less foolproof than the past practice of using all previous sera in the crossmatch test. It is, however, at least as sensitive as the increasingly common practice of using current serum and peak-positive sera in crossmatches. One concern about our approach is the complexity of the decision-making process at the time of crossmatching involving, as it does, the scanning of up to 100 sets of complex screening results in addition to the performance and interpretation of the actual crossmatch. It is therefore perhaps not surprising that the study uncovered two transplants that contravened the guidelines in operation at the time. This exemplifies how the interpretation of large numbers of data sets in a laboratory (we handle 300 kidneys per annum) can result in crucial errors.

We conclude that transplantation of kidneys bearing antigens present on grafts that have failed previously is attended by satisfactory graft survival rates provided certain criteria are met, namely that the patient has not produced an antibody to the mismatched antigen or to a crossreacting group, and that prophylactic ALG/ATG is administered for 10 days post-transplantation. The policy is crucially dependent on accurate and high-quality screening and, while it defines a set of conditions in which transplantation across repeat mismatches is feasible, it may exclude some patients in whom transplantation is also possible. For example, we and other groups perform transplants in recipients who have developed anti-HLA antibodies due to blood transfusion but which are directed at antigens present on the donor kidney. It may be that a subset of patients who have developed antibodies through parity or prior transplantation may also be transplantable. Efforts are currently being directed at the identification of this subset.

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