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D. Talbot
M. White
B. K. Shenton
A. Bell
D. Manas
G. Proud
R. M. R. Taylor

Flow cytometric crossmatching in renal transplantation – outcome after five years

D. Talbot (✉) · M. White · B. K. Shenton · A. Bell · D. Manas · G. Proud · R. M. R. Taylor
Renal Transplant Unit, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP, UK

Abstract The association of a positive flow cytometric crossmatch between recipient IgG directed against donor T lymphocytes and poor outcome is well described in renal transplantation. Until now, no long-term follow-up on such patients has been available. A total of 117 renal transplant patients were followed up for a period of 5 years. Of these, 21 were known to have donor T cell-directed IgG and 5 had B lymphocyte-directed IgG. Both groups of patients with these antibodies had a significantly poorer outcome at 5 years than did the group of patients without IgG ($P < 0.0001$ Handel Maenzel test). Patients with antibody detected preoperatively were tested again, either at the time of graft failure or at 5 years posttransplantation. The sera were tested against stored donor cells and the intensity of surface IgG compared with the preoperative levels. In those recipients who lost their grafts, the levels increased in 60 % of cases but those that retained their grafts also had an increase in levels of donor-directed antibody in 50 % of cases. The changing levels of antibody therefore appeared to have little relevance to outcome. However, when IgG isotypes were considered, for those who experienced graft failure and also had a γ_3 isotype, a rise in IgG was demonstrated in all cases. Conversely, successful grafts with γ_3 had a decline in levels between preoperative and 5-year samples in three of the four cases (p not significant).

Introduction

The association of a positive flow cytometric crossmatch between recipient IgG directed against donor T lymphocytes and poor outcome is well described in renal transplantation. Most series to date demonstrate the outcome up to 1 year [1, 5, 6, 7, 8] after transplantation but no long-term data exist regarding graft survival after 5 years from transplantation.

Materials and methods

Patient group

A total of 117 renal transplants was studied retrospectively. The transplants were performed between August 1985 and April 1988 during the time when the flow cytometric crossmatch was under investigation. At this time although the results of the flow cytometric crossmatch were known by the research team, this knowledge was not shared with the clinicians involved.

The transplants were from cadaveric donors and the recipients were receiving their first graft in 81.4 % of cases, the remainder being regrafts. There was a mean of 1.53 A/B locus match and 0.83 DR match between donor and recipient human lymphocyte antigen (HLA). At that time there was a policy of not transplanting across a warm or cold, historic or current cytotoxic T cell crossmatch, immaterial of B crossmatch or panel reactivity. The mean current panel reactivity was 7.1 % for the whole group with a

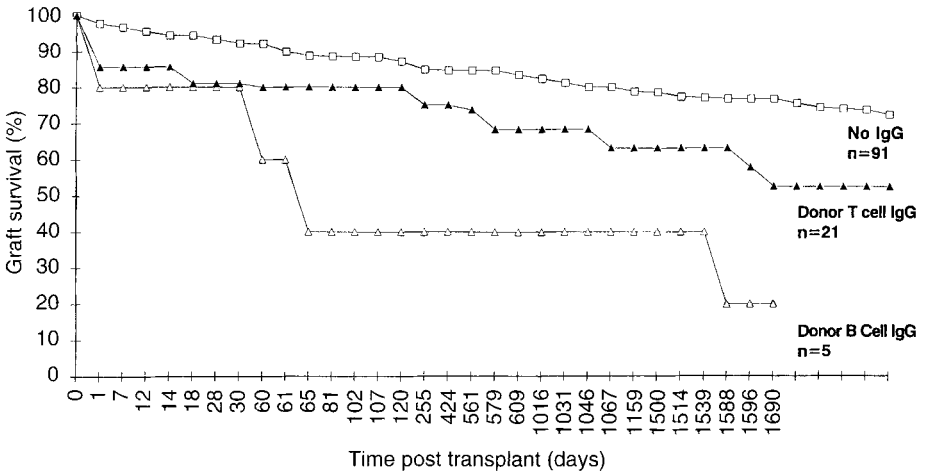

Fig. 1 Renal graft survival with and without preoperative donor-directed IgG as detected by flow cytometry

mean peak of 21.6 %. Cyclosporin was used as the base-line immunosuppression in 94 % of cases.

The flow cytometric crossmatch

This was performed as described previously [9] using donor splenocytes and current recipient sera. Six sera from healthy volunteers were used as controls. Anti-CD3 and anti-CD20 (Becton Dickinson) were used to label T and B cells, respectively, and anti-human IgG Fc (Sigma) was used to label the test or control sera. The cell/sera combinations after incubation and washings were exposed to the labelling antibodies. After further incubation and washing, the cells were analysed in a FACS 420 using Consort 30 software (Becton Dickinson).

Second serum analysis

Serum samples were obtained, when available, from the Tissue Typing laboratories where they were stored after routine periodic panel reactivity testing. When a graft failed, the nearest serum sample was obtained prior to the failure. Otherwise, the second sample of serum was obtained approximately 5 years after transplantation. The second sample was tested against the appropriate donor cells which had been stored in liquid nitrogen since the time of transplantation. In this way, 18 combinations were retested for the second sera out of the original 26 transplants known to have lymphocyte-directed IgG, as detected by flow cytometry.

With small numbers of cells viable after thawing the samples 5 or more years after transplantation, it was not possible to discriminate between B or T lymphocytes and therefore data analysis was performed on ungated samples. The 530-nm fluorescence (proportional to surface IgG) was determined for test and control sera and expressed as a ratio. The ratios determined for preoperative and 5-year samples were obtained with the flow cytometer aligned in the same way using fluorescent marker beads (Becton Dickinson).

IgG isotype detection

This has been previously described [10]. Briefly, donor splenocytes were incubated with the control or test sera. After incubation and washing, the cells were incubated with mouse anti-human γ_1, γ_2, γ_3 or γ_4 monoclonal antibodies (Serotec). The cells after washing were incubated with goat anti-mouse antibody conjugated with fluorescein isothiocyanate (Becton Dickinson). After further washing, the cells were incubated with a non-specific mouse serum to saturate spare binding sites. Finally, after more washing, the cells were incubated with anti-CD3 or anti-CD20 conjugated with phycoerythrin depending on the known affinity of the recipient sera. The cells were then analysed in the flow cytometer. The 530-nm fluorescence (fluorescein) of the 575-nm positively fluorescent cells (phycoerythrin) was compared between the recipient/donor combinations and the control/donor combinations.

Results

The outcome of the total group of 117 recipients is illustrated in Fig. 1. The total graft survival at 5 years was 59 %. There were 13 deaths with functioning grafts; if these are considered lost to follow-up, the proportion of patients with functioning grafts rises to 66.3 %. When the patients are subdivided into those without antibodies detectable preoperatively, the 5-year graft survival is 72.5 % (58/80 with 11 deaths). Those recipients with T-directed IgG as detected by flow cytometry preoperatively ($n = 21$), had a 5-year graft survival of 52.6 % (with 2 deaths). All these patients had no cytotoxic antibodies detectable by standard means and the grafts lost were for biopsy-proven rejection in 90 % of cases. When recipients with B-directed IgG are considered ($n = 5$), the 5-year graft survival is considerably less at 20 %. Both these survival curves are significantly worse than the zero antibody group, as assessed by the Handel Maenzel test ($P < 0.0001$).

Obviously, there were still some recipients with donor-directed IgG as detected by flow cytometry preoperatively who had no problem with rejection and did quite well (11 surviving at 5 years). These patients were no different regarding risk factors of tissue match (1.6 versus 1.4 match for A/B loci, 0.6 versus 0.6 match for DR, fail versus success, respectively), ischaemic times

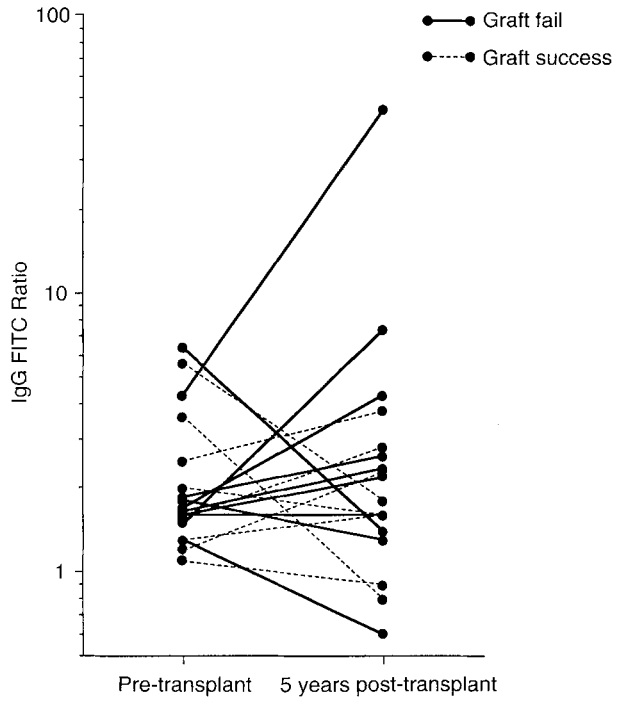

Fig. 2 Recipient/control sera ratio of IgG fluorescein isothiocyanate *(FITC)* before and after renal transplantation

(cold: 1174 versus 1156 min, warm: 34 versus 31 min, fail versus success, respectively) and regraft (42 % versus 36 %, fail versus success, respectively). This led us to retest the recipients at 5 years for the presence of donor-directed IgG. Figure 2 illustrates the changing antibody ratios with time (test/control). On first inspection of the figure it appears that the group that failed generally has an increasing level of antibody, as would be expected. However, this did not occur in all cases (60 %). Considering the converse, where grafts were retained, one would expect a decline in levels of antibody but the findings actually showed a rise in 50 % of cases. There were insufficient samples to retest for IgG isotypes to clarify the situation in the second sample but isotype testing had been performed in a number (16) of cases preoperatively. The results are summarised in Table 1. In eight cases, γ_3 only was identified and in these cases a subsequent rise always indicated failure (100 %) whereas a decline indicated that the graft would be retained in most cases (75 %). Statistical significance was not achieved but this was probably due to insufficient numbers ($P = 0.07$, Fishers exact test).

Discussion

The survival curves illustrated in Fig. 1, whilst showing a worse outcome with T- or B-directed IgG, suggest that the biggest impact for T-directed IgG occurs early. The curves then are largely parallel, suggesting that control has been achieved at a cost. This is obviously not the situation for the B-positive group where the lines diverge. Admittedly, the numbers of B-positive combinations were small ($n = 5$) but they confirm the finding of others where outcome was noted to be worse [2–4].

Why outcome should vary in the flow cytometric crossmatch combinations led us to to test other factors such as regraft, mismatch and ischaemic times, but no differences could be established between those that functioned and those that failed. Retesting for donor-directed antibody at 5 years was interesting in so far as this also did not reflect outcome. This would suggest that late rejection bears no relationship to antibody changes and that cellular events are the important factors.

However, IgG isotypes were apparently of some help, with rising IgG and γ_3 in preoperative samples indicating failure and falling levels indicating salvage. However, graft failure could still be seen in recipients with $\gamma_{1,2}$ and γ_4 isotypes, again suggesting that cellular events were of more importance.

Further conclusions could possibly have been drawn if the target of the antibodies was established. Unfortunately, there was insufficient donor material to establish anti-class I or II activity.

Table 1 Outcome after renal transplantation in flow cytometric positive combinations with known donor lymphocyte-directed IgG. The ratio of fluorescence to control is shown for preoperative samples and for sera collected prior to graft loss or at 5 years posttransplantation

Recipient	IgG target	γ Isotype	Test/control fluorescence (pretransplant sera)	Test/control fluorescence (5 years post-transplant sera)	Outcome
Jo	T	γ_3	4.3	45.8	Fail
Si	T	γ_3	1.5	7.4	Fail
Go	T	γ_3	5.6	1.8	OK
Ho	T	γ_3	1.1	0.9	OK
Sn	T	γ_3	1.6	2.2	Fail
Cr	T	γ_3	1.3	1.6	OK
Wh	T	γ_{2+3}	1.2	2.3	OK
Mc	B	γ_3	1.6	1.6	Fail
Sw	T	γ_2	2.5	3.8	OK
Da	B	γ_2	1.7	4.4	Fail
Ri	T	γ_3	3.6	0.9	OK

In summary, this study serves to confirm the original concept that a positive preoperative flow cytometric crossmatch for IgG to T lymphocytes carries a poorer prognosis for outcome. The principal effect is seen early, with the survival curves subsequently paralleling those of the zero antibody group. For B-directed IgG, the results appear to be even worse and this is now currently under evaluation in our centre.

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