Transpl Int (1994) 7: 182–189

ORIGINAL ARTICLE

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Characterization of donor-directed antibody class in the post-transplant period using flow cytometry in renal transplantation

Received: 19 February 1993
Received after revision: 31 August 1993
Accepted: 20 September 1993

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Abstract Over the past few years there has been increasing awareness of the importance of humoral mechanisms in the rejection of renal transplants. In this study we have monitored the development of antibodies directed against donor T and B lymphocytes using the sensitive flow cytometric technique. Forty-two cadaveric renal transplants were studied both before and for a maximum of 14 days after transplantation. Donor cells were separated from spleen on the day of transplantation and stored in liquid nitrogen until required. The dual colour flow cytometric assay was used to detect IgG or IgM directed againts donor T or B lymphocytes. Using AB sera as controls, results were expressed as relative median fluorescence (RMF) and then correlated with the clinical performance of the grafts. Significant associations were found between the incidence of donor-directed antibodies and the development of clinical rejection. The magnitude of the rise in antibody levels was also related to graft performance. In patients showing severe graft rejection, high levels of antibodies of the IgG class developed before the clinical diagnosis of rejection was made. The routine use of this test allows the prediction of impending severe rejection to be made and may have important implications for immunosuppressive therapy.

Key words Renal transplantation, post-transplant antibodies
Antibodies, after renal transplantation · Donor-direct antibodies · Flow cytometry, renal transplantation, antibodies

Introduction

Whilst it is well accepted that the presence of humoral antibodies directed against donor lymphocytes prior to renal transplantation are detrimental to graft performance [10, 15, 18, 22], the significance of these antibodies in the immediate post-transplant period remains unclear. Using the micro-lymphocytotoxic crossmatch (CDC) test system, antibodies have been detected in the post-transplant period against both donor-specific and third party lymphocytes [11, 12, 17] or lyophilized renal cells [3]. An association has also been reported between the development of these antibodies and graft rejection. Using different methods, the presence of antibodies eluted from human allografts has been demonstrated by indirect immunofluorescence [13], and also a donor-specific T-cell line has been used in a binding assay to detect IgG antibodies in both the pre- and post-transplant periods from sera of renal allograft recipients [6].

However, since early graft losses have been reported in CDC-negative pre-transplantation crossmatches in renal patients [1, 4, 9, 14, 20], the sensitivity of tests for detecting humoral antibodies has been questioned. The introduction of the flow cytometric crossmatch (FCXM) technique has provided a more sensitive and precise method for detecting the presence of antidonor antibodies in renal patients. Several reports have confirmed that positive pre-transplant FCXM results are associated with both in-

Table 1 Summary of transfusion history, panel-reactive antibodies, tissue matching and ATG administration in the patient groups

Group	Transfusion history[a]	Panel-reactive antibodies		ATG	HLA mismatches		
		T	B		A	B	DR
1- No rejection	7	0/6	1/6	0/7	0.86	1.14	0.71
2- Mild rejection	14	1/14	1/14	0/14	1.1	1	0.55
3- Severe rejection	10	1/8	2/8	9/10	1.3	1.14	0.6
4- Delayed graft function	11	2/11	1/11	1/11	0.73	1	0.5

[a] All patients were transfused

creased frequency of rejection episodes and graft failure [2, 5, 7, 8, 19]. Furthermore, a report has indicated that the FCXM can detect low levels of antibodies in the post-transplant period, and a significant association between detection of IgG by flow cytometry and the number of rejection episodes in the post-transplant period has been shown [16, 21].

In the present study, we report the value of flow cytometry in monitoring antidonor-directed antibodies (T or B cells). Identification of these antibodies in the post-transplant period has been possible and we have correlated the presence of such antibodies with the diagnosis of clinical rejection. Our results indicate that a close association exists between the development of antidonor IgG antibodies in the post-transplant period and clinical rejection.

Materials and methods

Patients

Forty-two consecutive renal transplants (all receiving first grafts from cadaveric donors) with negative (on current serum samples) pre-transplant flow cytometric and conventional cytotoxic crossmatches for T cells were included in this study. All patients were transfused with a minimum of three units of blood and none had previous pregnancies. All patients were commenced on cyclosporin A monotherapy post-transplantation. Sera were collected both before transplantation and daily over the period of their stay in hospital (range 10–14 days) and were stored at −20°C until tested. Donor mononuclear cells were separated at the time of transplant and stored in liquid nitrogen.

Rejection episodes were diagnosed on well established clinical criteria (graft tenderness, influenza-like symptoms decreased urine volume) and biochemical criteria (rising serum creatinine). On the basis of clinical diagnosis, function of the renal grafts in the post-operative period and renal biopsy results, patients were divided into four different clinical groups.

Groups

Group 1 (no rejection) included patients whose kidneys showed excellent function with no signs of clinical rejection and who were not treated for rejection episodes ($n = 7$).
Group 2 (mild rejection) included patients with one rejection episode that responded to a single course of methylprednisolone steroid therapy (250 mg on 3 consecutive days; $n = 14$).
Group 3 (severe rejection) included patients with more than one rejection episode (two to four episodes), requiring both methylprednisolone steroid therapy and anti-thymocyte globulin (ATG, Merieux, Lyon France). Evidence of rejection was always confirmed by renal biopsy ($n = 10$).
Group 4 [no rejection; delayed graft function (DGF)] included patients who showed delayed graft function (requiring dialysis) and no evidence of rejection on renal biopsy ($n = 11$). No patients in this group showed any symptoms of rejection and ATG was only given to one patient in this group.

With regard to panel reactivity on immediate pre-transplant sera, number of pre-transplant transfusions, or tissue matching there were no significant differences between the clinical groups. No ATG was given to patients in the no rejection or mild rejection groups, but 9 out of 10 of the severe rejection patients and 1 out of 11 of the patients showing delayed graft function received a 10-day course of ATG at a dosage regimen of 3 mg/kg per day (Table 1).

Monitoring of IgG and IgM with the FACS

The dual-colour flow cytometric assay [19] was used for the detection of antidonor antibodies. Briefly, 50 µl (1×10^5) of donor mononuclear cells were mixed with 50 µl of recipient serum for 20 min at 37°C. Pooled sera from five different normal human sera were used as controls. After the first incubation the cells were washed using a cell washer (Ross Lab, UK). Then, 48 µl of 1:40 goat F(ab')2 anti-human IgG FITC (Sigma, UK) or goat F(ab')2 anti-human IgM FITC (Caltag Laboratories, Calif., USA) were added to detect the class of antidonor antibodies. To identify cell phenotype, 2 µl of anti-CD3-PE (anti-Leu 4 PE to detect T cells) or 2µl of anti-CD20 PE (anti-Leu 16 PE to detect B cells; Becton Dickinson, Calif., USA) were added. The mixture was then incubated for 20 min at 4°C. The test lymphocytes were then washed, resuspended in 200 µl of Isoton II (Coulter Euro Diagnostics, UK) and analyzed using the FACScan (Becton Dickinson) flow cytometer. Fluorescent excitation was produced by a 15 mW argon laser. Viable lymphocytes were gated by using the forward scatter and 90° side scatter parameters. A total of 10,000 cells were counted and only those positively stained for PE (FL2) were analyzed for their green fluorescence intensity (FL1). Using Lysys II software (Becton Dickinson) the median fluorescence channel of the gated PE-positive T or B lymphocytes for the test sera was compared with that produced by the normal AB control sera. To determine the degree of binding, results were expressed as a ratio, termed relative median fluorescence (RMF), calculated as follows

$$\frac{\text{Medican channel of gated fluorescence (FL 1) of test serum}}{\text{Medican channel of gated fluorescence (FL 1) ofcontrol AB sera.}}$$

In the study groups, the number of tests performed depended on the availability of both donor cells and recipient serum. When possible, both IgG and IgM tests were performed on all samples.

Statistical analysis

Differences between the groups were calculated using the Mann-Whitney test, Wilcoxon rank test and chi-square test.

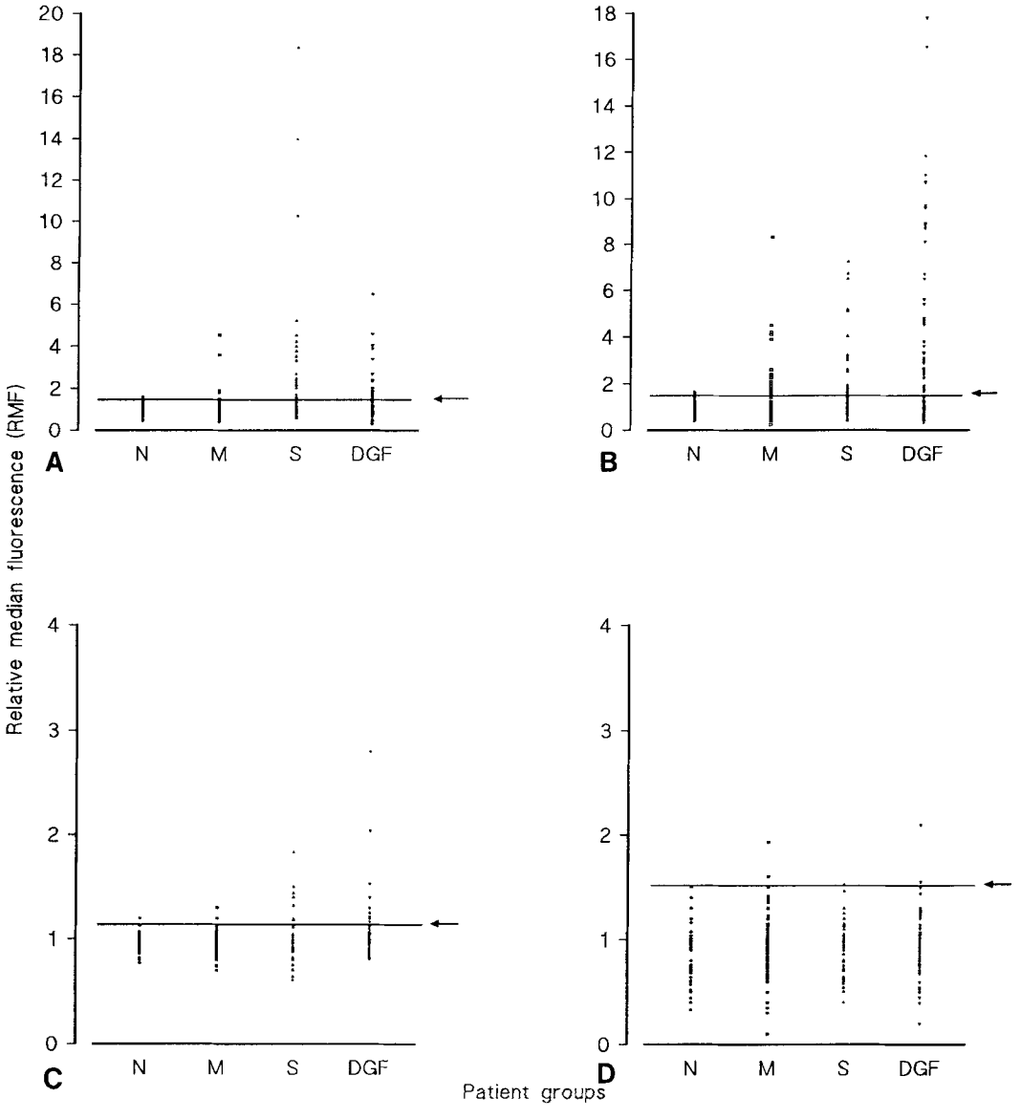

Fig. 1 A–D Distribution for all patients of relative mean fluorescence *(RMF)* for anti-donordirected antibodies in the post-transplant period:
A RMF values of IgG directed to T cells;
B RMF values of IgG directed to B cells;
C RMF values of IgM directed to T cells;
D RMF values of IgM directed to B cells.
The *arrow* indicates the mean value + 2 SD of RMF in the no rejection group. (*N* No rejection, *M* mild rejection, *S* severe rejection, *DGF* no rejection; delayed graft function)

Results

To illustrate the total distribution of RMF results within the groups of patients studied, all RMF values for donor-directed IgG and IgM are presented in Fig. 1. The groups studied comprised 7 patients with no evidence of rejection (group 1) in the immediate post-transplant period, 14 patients with mild rejection (group 2), 10 patients with biopsy-confirmed severe rejection (group 3) and 11 patients with DGF and no evidence of clinical rejection as seen on biopsy (group 4). As in the studies of pre-transplant cross-match monitoring [19], test serum results were compared with those of a panel of AB sera taken from normal healthy adult volunteers. As a discrimination index for definition of positivity, any daily values of RMF above 1.56 for T cells or 1.54 for B cells (the figures being derived from the mean RMF plus two standard deviations for the normally distributed group of patients showing no rejection) were considered to be positive and are shown plotted above the horizontal line on the Y axis in Fig. 1.

Table 2 summarises the incidence of positive sera tested in the patient groups examined. With respect to the occurrence of IgG antibodies in the post-transplant period in patients with no rejection (group 1), only 1.14% (1/88) of the samples tested showed values above control AB sera. Compared with the no rejection group (group 1), the occurrence of IgG antibodies directed against T cells was not significantly greater in those patients with mild rejection (group 2; 2.91%, $P = 0.87$) but was elevated both in patients with severe rejection (group 3; 24.04%, $P = 0.0001$) and also in the presence of DGF (group 4; 15.75, $P = 0.0002$). Whilst patients in

Table 2 Incidence of positive RMF values within each group of patients studied for IgG- and IgM-directed antibodies to donor cells. *P* value using chi-square test

Group	IgG		IgM	
	T cells (*n*) %	B cells (*n*) %	T cells (*n*) %	B cells (*n*) %
1- No rejection	1.14 (1/88)	1.14 (1/88)	2 (1/59	0 (0/50)
2- Mild rejection	2.91 (5/172)	14.4 (23/160)	4.5 (4/89)	12.4 (11/89)
3- Severe rejection	24.04 (25/104)	31.8 (27/85)	23.7 (9/38)	8.6 (3/35)
4- Delayed graft function	15.75 (23/146)	27.2 (40/147)	20.3 (15/74)	6.9 (5/73)
	T cells			B cells
1 vs 2	NS	0.0005	NS	0.0076
1 vs 3	0.0001	0.01	0.0019	NS
1 vs 4	0.0002	0.023	0.0023	NS
2 vs 3	0.001	0.01	0.002	NS
2 vs 4	0.001	0.01	0.002	NS
3 vs 4	NS	NS	NS	NS

Table 3 Comparison between all the RMF values for IgG antibodies directed to T or B cells. *P* value using Wilcoxon rank test

Group	T cells			B cells		
	(*n*)	Median	Range	(*n*)	Median	Range
1- No rejection	(88)	0.91	0.46–1.6	(88)	0.9	0.4–1.65
2- Mild rejection	(172)	0.83	0.4–4.53	(160)	0.95	0.24–8.3
3- Severe rejection	(104)	1.1	0.55–18.32	(85)	1.21	0.4–7.24
4- Delayed graft function	(146)	1.02	0.3–6.5	(147)	0.96	0.31–20.95
	T cells			B cells		
1 vs 2	0.033			0.189		
1 vs 3	< 0.001			< 0.001		
1 vs 4	< 0.001			0.015		
2 vs 3	< 0.001			< 0.001		
2 vs 4	< 0.001			0.15		
3 vs 4	0.1			< 0.001		

Table 4 Comparison between all the RMF values for IgM antibodies directed to T or B cells. *P* value using Wilcoxon rank test

Group	T cells			B cells		
	(*n*)	Median	Range	(*n*)	Median	Range
1- No rejection	(50)	0.97	0.77–1.2	(50)	0.73	0.33–1.5
2- Mild rejection	(89)	0.94	0.7–1.3	(89)	1.001	1.3–1.93
3- Severe rejection	(38)	0.92	0.61–1.83	(35)	0.9	0.4–1.52
4- Delayed graft function	(74)	0.995	0.81–2.8	(73)	0.94	0.2–2.1
	T cells			B cells		
1 vs 2	0.05			< 0.001		
1 vs 3	NS			NS		
1 vs 4	NS			0.011		
2 vs 3	NS			0.06		
2 vs 4	< 0.001			0.07		
3 vs 4	0.073			0.5		

group 2 showed a significantly lower incidence of positive sera (2.91 %; 5/172) than those in either group 3 (24.04 %; 25/104) or group 4 (15.75 %; 23/146), there was no significant difference between the incidence of positivity in groups 3 and 4 ($P = 0.988$). With respect to IgG antibodies directed against B cells in the post-transplant period, highly significant differences were found between almost all of the groups compared. The incidence of B-cell-directed IgG was always higher than that found in the same group for T-cell antibodies. As with T-cell IgG, there was no difference in the incidence of positive results between groups 3 and 4 ($P = 0.88$). The pattern of T-cell-directed

Table 5 Results of positive FACS crossmatch in the post-transplant period of IgG and IgM directed to donor cells in each group

Group	Ab	*n*	T	B	T&B
1- No rejection	IgG	7	1	1	0
	IgM	7	1	0	0
2- Mild rejection	IgG	14	0	4	3
	IgM	10	1	0	0
3- Severe rejection	IgG	10	2	3	5
	IgM	4	2	0	0
4- Delayed graft function	IgG	11	2	0	4
	IgM	8	3	0	1

IgM antibodies for the groups studied was identical to that found for the IgG T-cell antibodies with a significantly elevated incidence in groups 3 and 4 compared to group 1. As with IgG antibodies, there was no difference between groups 1 and 2 and groups 3 and 4. The percentage incidence of IgM antibodies to T cells was similar to that found in the T cell IgG groups. In contrast to the IgG pattern for the groups, the incidence of IgM to B cells was low, only the comparison between groups 1 and 2 achieving significance ($P = 0.0076$).

The results of the comparison between the calculated median for all patients in the study groups are shown in Table 3. With respect to IgG antibodies directed to donor T cells, significant differences were found between groups tested except in the comparison of groups 3 and 4 (Table 3). For B cell antibodies, the group of patients

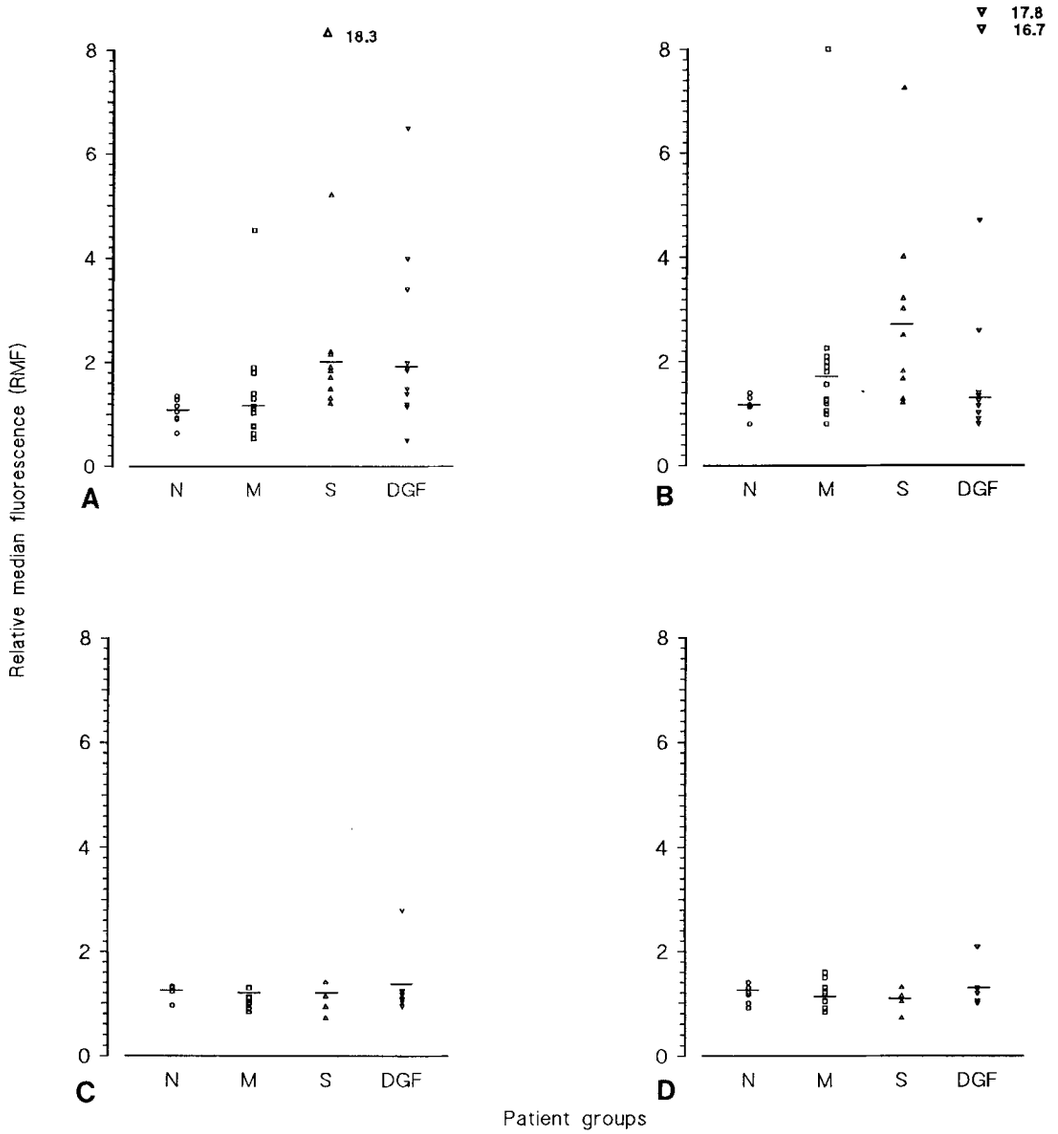

Fig. 2 A–D Distribution of maximum value of relative median fluorescence *(RMF)* for antidonor-directed antibodies in the post-transplant period: **A** maximum RMF values of IgG directed to T cells; **B** maximum RMF values of IgG directed to B cells; **C** maximum RMF values of IgM directed to T cells; **D** maximum RMF values of IgM directed to B cells. The *line* indicates the median value of RMF in each group. (*N* No rejection, *M* mild rejection, *S* severe rejection, *DGF* no rejection; delayed graft function)

Table 6 Comparison between the maximum RMF values for IgG antibodies directed to T or B cells. *P* value using Wilcoxon rank test

Group	(*n*)	T cells		B cells	
		Median	Range	Median	Range
1- No rejection	(7)	1.05	0.64–1.35	1.13	0.8–1.4
2- Mild rejection	(14)	1.11	0.52–4.53	1.68	0.8–8.3
3- Severe rejection	(10)	1.9	1.2–18.32	2.75	1.2–7.24
4- Delayed graft function	(11)	1.85	0.51–6.5	1.134	0.8–17.8
		T cells		B cells	
1 vs 2		NS		0.047	
1 vs 3		0.002		0.0028	
1 vs 4		0.018		NS	
2 vs 3		0.004		NS	
2 vs 4		0.039		NS	
3 vs 4		NS		NS	

Table 7 Comparison between the maximum RMF values for IgM antibodies directed to T or B cells. *P* value using Wilcoxon rank test

Group	(*n*)	T cells		B cells	
		Median	Range	Median	Range
1- No rejection	(6)	1.22	0.96–1.33	1.2	0.91–1.4
2- Mild rejection	(13)	1.02	0.85–1.3	1.15	0.84–1.6
3- Severe rejection	(4)	1.05	0.71–1.4	1.1	0.71–1.3
4- Delayed graft function	(18)	1.33	0.97–2.8	1.3	1.01–2.1
		T cells		B cells	
1 vs 2		0.03		NS	
1 vs 3		NS		NS	
1 vs 4		NS		NS	
2 vs 3		NS		NS	
2 vs 4		NS		NS	
3 vs 4		NS		NS	

Table 8 Correlation between the appearance of IgG antibody class and the onset of rejection episodes in patients with rejection episodes. + Rise of antibody after clinical diagnosis, – rise of antibody before clinical diagnosis

	Mild rejection		Severe rejection	
T	–	–	–1,–2	(–1.5)
B	–2, +5, +8, +10	(+5.25)	–2,–3,–1	(–2)
T&B	+5, +9, +11	(+8.3)	–2,–2,–1,–1,–1	(–1.4)
	+9, +9, +13	(+10.3)	–3,–2,–2,–1,–1	(–1.8)

showing severe rejection differed from all other groups. For IgM T cell antibodies (Table 4), only DGF patients showed an elevation in antibodies when compared to the mild group (Table 4). For IgM B cell antibodies, mild rejection patients showed a slightly higher RMF value than that found in the no rejection group.

Having shown the significant association between the presence of antibodies and renal graft malfunction, Table 5 summarises the discriminatory value of such assessment in individual patients. The overall results showed that 59.5% of patients had IgG antibodies and 27.6% had IgM antibodies; 40% of the former had an increased number of rejection episodes. Positive results are shown when the RMF values reached a maximum for each patient in the post-transplant study period. As can be seen (Table 5) two out of seven patients with no rejection had IgG antibodies and only one out of seven had IgM. Half of the patients with mild rejection showed IgG antibodies and only one out of ten of the patients showed IgM. An increased incidence of IgG was found in patients with severe rejection, all patients showing IgG ($P = 0.009$ when compared with no rejection) and two out of four IgM. Interestingly, 6/11 patients with DGF showed IgG and 4/8 had antibodies of the IgM class.

In order to study the magnitude of any rises in the levels of IgG antibodies, the RMF values within each group of patients were compared. Figure 2 summarises the maximum RMF values found for each patient within the

various clinical groups. In Table 6 the median RMF values and ranges are shown for IgG. With respect to T-cell antibodies, patients showing severe rejection (median 1.9) and DGF (median 1.85) had greater RMF values than patients with mild rejection (median 1.11; $P = 0.004$ and 0.039, respectively). No significant differences were seen between either the no rejection and mild rejection, or severe rejection and DGF groups. With regard to B-cell antibodies, the only significant differences found were between the no rejection group (median 1.13) and the mild (median 1.68, $P = 0.047$) and severe (median 2.75, $P = 0.0028$) rejection groups. With regard to donor-directed antibodies of the IgM class, no significantly elevated values were found (Table 7).

Table 8 shows the correlation between the incidence of IgG antibodies and the onset of rejection episodes. In patients showing mild rejection the rises in T- and B-cell-directed IgG always occurred after the diagnosis of clinical rejection. In contrast to the mild rejection group, patients with severe rejection always showed rises in IgG antibodies before the clinical diagnosis of rejection.

Discussion

Several studies have shown that the FACS crossmatch is a more sensitive method for detecting low levels of donor-directed antibodies in pre-transplantation sera than the conventional cytotoxic technique. The use of this method is associated with better graft prognosis, thus indicating that a positive flow cytometric crossmatch detects antibodies that appear to be deleterious to the graft if present in the sera of potential recipients [2, 5, 7, 8, 19]. Unfortunately, the significance of the antibodies post-transplantation is still unclear. Results in the present study have shown that donor-directed antibodies in the sera of transplant recipients can be monitored daily using the dual colour flow cytometric crossmatch in the post-transplant period. Serial monitoring of donor-directed antibodies in the post-transplantation period showed that 59.5% of the patients had IgG antibodies and 27.6% had IgM antibodies, 40% of the former having an associated higher number of rejection episodes.

Whilst previous reports have shown that such antibodies are detectable after transplantation, they have failed to identify the class and the target antigen for such antibodies using the standard CDC or other methods [3, 6, 11–13]. Recent reports have shown that the FACS is a useful tool for the detection of antibodies to T cells in the post-transplantation period. However, these studies have only screened for the presence of donor-directed antibodies [16, 21]. In the group of patients showing either more severe rejection episodes or DGF, an increase in the RMF value was observed, indicating that a humoral response in those patients may have an important role. A recent study [2] has shown that there is a close relationship between a positive T-cell FACS crossmatch before transplantation and immediate non-function of the renal graft. Interestingly, when antibodies were observed against both T and B cells in rejecting patients, the RMF values were higher than if antibodies were only detected against T or B cells. In the present study, the antibodies detected in all patients with severe rejection were observed before the diagnosis of severe rejection. This was in direct contrast to patients with mild rejection, who showed slightly higher levels of antibody only after the diagnosis of clinical rejection. Clearly, the specificity of the antibodies in the post-transplant situation is difficult to define. It would seem from this study that antibodies to both T and B cells, probably against MHC class I and class II antigens, show the clearest relationship with rejection, with no overlap between the elevated RMF and clinical diagnosis. Class II antibodies, against donor B cells, were found in some patients but their presence was not as clearly associated with rejection as those directed to both class I and class II antigens. T-cell antibodies alone were rarely found and their specificity may have been either to class I antigens, which may have been less exposed by the B cells of the spleen, or to another undefined antigen. Any iatrogenic effect of ATG given to DGF patients could be seen since only one of the grafts showing DGF was given ATG.

We conclude from this study that antidonor-directed antibodies play an important role in graft rejection, and by monitoring these antibodies with flow cytometric techniques, modification in immunosuppression therapy may be suggested.

Acknowledgement The author would like to thank Dr. Peter Kelly of the Department of Medical Statistics for helping in the statistical analysis of this study. This work was supported by a grant from the King Faisal Specialist Hospital and Research Centre and The Ministry of Higher Education in Saudi Arabia.

References

1. Amos D, Bashir H, Boyle W, MacQueen M, Tiilikainen A (1969) A simple microcytotoxicity test. Transplantation 7: 220–223
2. Cook D, Terasaki P, Iwaki Y, Terashita G, Lau M (1987) An approach to reducing early kidney transplant failure by flow cytometry crossmatching. Clin Transplant 1: 253–256
3. Ende N, Williamson E (1968) Humoral antibodies and human renal homograft rejection. Am J Clin Pathol 49: 155–160

4. Fuller TC, Phelan D, Gebel H, Rodey GE (1982) Antigenic specificity of antibody reactive in the antiglobulin-augmented lymphocytotoxicity test. Transplantation 34: 24–29
5. Garovoy M, Rheinschmidt M, Bigos M, Perkins H, Colombe B, Feduska N, Salvatierra O (1983) Flow cytometry analysis: a high technology crossmatch technique facilitating transplantation. Transplant Proc 15: 1939–1944
6. Huber C, Irschik E, Leiter E, Binz H, Niederwieser D, Spielberger M, Kathrein H, Schönitzer D, Margreiter R (1986) Use of donor-specific T-cell lines for monitoring of human allograft recipients. I. Demonstration of IgG binding to autologous TCL. Exp Cell Biol 54: 16–24
7. Iwaki Y, Terasaki PI (1987) Primary nonfunction in human cadaver kidney transplantation: evidence for hidden hyperacture rejection. Clin Transplant 1: 125
8. Iwaki Y, Cook D, Terasaki P, Lau M, Terashita G, Danovitch G, Fine R, Ettenger R, Mendez R, Kavalich A, Martin D, Soderblom R, Ward H, Berne T, Lieberman E, Strauss F (1987) Flow cytometry crossmatching in human cadaver kidney transplantation. Transplant Proc 19: 764–766
9. Johnson AH, Rossen RD, Butler WT (1992) Detection of alloantibodies using a sensitive antiglobulin microtoxicity test: identification of low levels of preformed antibodies in accelerated allograft rejection. Tissue Antigens 2: 215–216
10. Kissmeyer-Nielsen F, Olsen S, Petersen V, Fjeldborg O (1966) Hyperacute rejection of kidney allografts associated with pre-existing humoral antibodies against donor cells. Lancet II: 662–665
11. Manzler A (1968) Serum cytotoxin in human kidney transplant recipients. Transplantation 6: 787–792
12. Martin S, Dyer P, Mallick N, Gokal R, Harris R, Johnson R (1987) Posttransplant antidonor lymphocytotoxic antibody production in relation to graft outcome. Transplantation 44: 50–53
13. McPhaul J, Stastny P, Freeman R (1981) Specificities of antibodies eluted from human cadaveric renal allografts: multiple mechanisms of renal allograft injury. J Clin Invest 67: 1405–1414
14. Mittal K, Mickey M, Singal D, Terasaki P (1968) Serotyping for homotransplantation. XVIII: Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation 6: 913–927
15. Patel R, Terasaki P (1969) Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 280: 735–739
16. Scornik J, Salomon D, Lim P, Howard R, Pfaff W (1989) Posttransplant antidonor antibodies and graft rejection. Evaluation by two-color flow cytometry. Transplantation 47: 287–290
17. Shorter R, O'Kane H, Nava C, Hallenbeck G (1969) Lymphocytotoxins in sera from patients receiving renal allografts. Surgery 65: 793–796
18. Starzl T, Lerner R, Dixon F, Groth C, Brettschneider L, Terasaki P (1968) Schwartzman reaction after human renal homotransplantation. N Engl J Med 278: 642–648
19. Talbot D, Givan A, Shenton BK, Stratton A, Proud G, Taylor RMR (1988) The relevance of a more sensitive crossmatch assay to renal transplantation. Transplantation 47: 552–555
20. Terasaki PI, Bernoco P, Park MS, Ozturk G, Iwaki Y (1978) Microdroplet testing for HLA-A, -B, -C and -D antigens. Am J Clin Pathol 69: 103–120
21. Torlone N, Piazza A, Valeri M, Monaco P, Provenzani L, Poggi E, Adorno D, Casciani C (1992) Kidney transplant monitoring by anti donor specific antibodies. Transpl Int 5 [Suppl 1]: 676–678
22. Williams G, Hume D, Hudson R, Morris P, Kano K, Milgrom F (1968) "Hyperacute" renal-homograft rejection in man. N Engl J Med 279: 611–618