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Ibrahim I. Ghobrial
Alan G. Morris
Lionel J. Booth

Clinical significance of in vitro donor-specific hyporesponsiveness in renal allograft recipients as demonstrated by the MLR

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I.I. Ghobrial (✉)[1] · L.J. Booth
Renal Unit, Walsgrave Hospital,
Coventry, CV2 2DX, UK

A.G. Morris
Department of Biological Sciences,
University of Warwick, Coventry,
CV4 7AL, UK

Present address:
[1] Department of Medical Education,
St. Francis Medical Center, 400 45th Street,
Pittsburgh, PA 15201-1198, USA,
Fax: +1 412 622 71 38

Abstract A longitudinal study was carried out on 19 recipients of cadaveric renal allografts, monitoring their anti-donor and anti-third party responses in the mixed lymphocyte reaction (MLR) at the time of transplantation and at 3, 6, and 12 months post-transplant. Two patterns of responses were identified: in the first ($n = 11$), patients showed, or later developed, donor-specific hyporesponsiveness, and in the second ($n = 8$), patients had persistent anti-donor and anti-third party responses. After 1 year, the serum creatinine, number of episodes of acute rejection and biopsy findings were compared in both groups. In the first group, the mean serum creatinine was 136.4 mmol/l, the total number of acute rejection episodes was three and in nine of the ten available biopsies, there were minimal cellular infiltrates and normal appearance of the glomeruli, tubules and blood vessels. In the second group, the mean serum creatinine was 163 mmol/l, the total number of acute rejection episodes was 12 and in five of the seven biopsies available, evidence of ongoing rejection was obtained. The difference in mean serum creatinine was not statistically significant ($P > 0.05$), but the difference in the numbers of acute rejection episodes was ($P < 0.05$). It is concluded that in some renal allograft recipients, a state of donor-specific hyporesponsiveness develops, and this state may be associated with better graft outcome at 1 year. These data may be useful in selecting patients for reduced immunosuppressive therapy.

Key words Hyporesponsiveness, kidney recipients, MLR · Kidney recipients, hyporesponsiveness, MLR · MLR, hyporesponsiveness, kidney recipients

Introduction

Monitoring of the immune responses in allograft recipients is necessary in order to reduce patients' morbidity by identifying those in whom rejection activity has diminished or ceased. Such patients can be regarded as relatively hyporesponsive to donor tissue antigens and may therefore benefit from reduced immunosuppressive therapy. Conversely, patients who are at high risk of losing their grafts to rejection may be identified at an early stage so that more effective immunosuppression can be instituted before the development of irreversible immunological graft damage [26]. Monitoring should also improve our understanding of the rejection process, especially its chronic form.

Transplant biopsy is commonly used to assess the state of the graft, yet core biopsies can be associated with significant morbidity [42] and differentiation between the various causes of graft malfunction may be difficult [8]. The significance of various types of cellular infiltrates is still not certain [5, 15]. Analysis of cellular aspirates obtained by fine needle aspiration biopsies is restricted by the need for an experienced histopathologist and by the limited usefulness of the procedure after 1 month post-

transplant due to the development of fibrosis within the graft [23].

Because of this, efforts have been made to monitor changes in peripheral blood lymphocytes (PBL) as a window to the immune system. In view of the central role played by CD4 + cells in initiating the rejection process [14, 21], CD4 + cells appear to be a logical target for immunologic monitoring. Measurement of the CD4 +/CD8 + ratio of PBL, as initially described by Cosimi et al. [13], has not proven very helpful because of the lack of specificity of the findings [8, 10, 40, 43], nor has phenotypic analysis of lymphocytes been obtained from within the graft [17]. Studying the alloreactivity of CD4 + cells in proliferation assays would therefore appear to be necessary. Obtaining such cells from within the graft is difficult because of the large core of tissue needed to obtain sufficient number of cells and because of the possible alteration of functional reactivity after in vitro propagation [17].

Several groups have monitored their patients using the mixed lymphocyte reaction (MLR) and reported the presence of donor-specific hyporesponsiveness [4, 33, 34]. Flechner et al. [18] and Chow et al. [11] adopted a similar approach in order to select patients for reduction of immunosuppressive therapy. In the former study, patients were treated with cyclosporin and in the latter, by total lymphoid irradiation. The main problem with this approach is the lack of confirmation as to whether changes in PBL reflect changes occurring within the graft [22, 41]. We have undertaken this present study in order to confirm the reality of the phenomenon of donor-specific hyporesponsiveness and to correlate the in vitro findings with graft function as assessed by serum creatinine, the number of episodes of acute rejection and biopsy findings at the end of the study period.

We identified the presence/development of a state of donor-specific hyporesponsiveness in some renal allograft recipients, a state that correlated with good graft performance at 1 year. To understand the mechanisms that govern long-term graft acceptance, the study was extended to cover two further aspects. In patients who developed donor-specific hyporesponsiveness, the role of suppressor CD8 + cells was examined by repeating the MLR after depleting the recipients' PBL of CD8 + cells. In patients with good graft function despite high anti-donor reactivity in the MLR, the presence of blocking antibody activity was sought.

Materials and methods

Patients

Nineteen patients (13 males and 6 females) who received cadaveric renal allografts at the Walsgrave Hospital, Coventry, during the period from October 1990 to March 1992 were entered consecutively into the study. The aetiology of renal failure was: small kidney disease ($n = 6$), reflux nephropathy ($n = 4$), diabetic nephropathy ($n = 3$), polycystic kidney disease ($n = 2$), obstructive nephropathy ($n = 2$), IgA nephropathy ($n = 1$), and mesango-capillary glomerulonephritis ($n = 1$). There were six female patients, all but one of whom had had two or more pregnancies. Six patients did not receive blood transfusions prior to transplantation; the rest received two or more. For two patients this was the third transplant, for one the second, and for the rest of the patients it was the first transplant. A negative current T-cell crossmatch was a prerequisite for transplantation.

All patients received immunosuppression based on triple therapy (cyclosporin, prednisolone and azathioprine). In four patients azathioprine had to be withdrawn for clinical reasons. Diagnosis of rejection was based on rising serum creatinine after excluding other causes of deterioration of graft function. Percutaneous needle biopsy was done in doubtful cases. Rejection was treated by steroid pulses and OKT3 in resistant episodes. Biopsies were also performed at 1 year post-transplant for evaluation of the state of the graft. Histologic examination was carried out and reported by an independent histopathologist completely blinded to the in vitro results of the study.

HLA typing was carried out at the West Midlands Blood Transfusion Service Laboratory by serology and confirmed by DNA typing (restriction fragment length polymorphism; RFLP), polymerase chain reaction (PCR) and oligonucleotide typing in doubtful cases.

Cell preparation

Donor splenocytes

Spleen tissue was obtained from the donor at the time of organ harvesting. Splenocytes were disaggregated, suspended in culture medium (RPMI 1640, Gibco, UK), supplemented with 25 mM of HEPES buffer, L-glutamine, penicillin and streptomycin, 100 μg/ml each, 5×10^{-5} mM of 2-mercapto-ethanol and 10 % of human male AB serum (Sigma, UK). Viability was confirmed by staining with trypan blue and cells were purified by centrifugation over Ficoll density gradient (Lymphoprep, Nycomed, Norway), resuspended in supplemented medium to which 10 % of DMSO was added, gradually frozen to −70 °C and cryopreserved in liquid nitrogen.

Recipient peripheral blood lymphocytes (PBL)

Thirty milliliters of heparinized blood (100 units) was collected from each recipient immediately prior to transplantation and at 3, 6 and 12 months afterwards. PBL were purified by density gradient centrifugation and cryopreserved as described above.

Third party stimulator lymphocytes

Two sources were available: PBL from a healthy volunteer with a DR type different from that of our patients and a panel of splenocytes from ten different donors. Results using both sources were repeatedly shown to be comparable and work was continued using the former.

Recovery of cryopreserved cells

Recovery of cryopreserved cells was by rapid thawing and high viability was always obtained. We have repeatedly shown that the reactivity of cryopreserved cells is comparable to fresh cells.

Table 1a MLR data of patients in group 1

Patient number	Time post-transplant	Donor-specific response	Autologous control (background)	Anti-third party response	Relative response
1	T0	1668	105	3040	53.3 %
	3 months	2830	271	10154	25.2 %
	6 months	1379	273	11443	9.7 %
	12 months	2589	1795	14022	5.7 %
2	T0	5848	2693	114683	2.8 %
	3 months	10312	3977	129107	5.1 %
	6 months	3550	1078	30755	5.4 %
	12 months	4712	3320	49343	3.0 %
3	T0	[a]	[a]	[a]	[a]
	3 months	[a]	[a]	[a]	[a]
	6 months	359	104	8147	1.9 %
	12 months	860	530	17397	2.0 %
4	T0	9065	2778	16039	47.4 %
	3 months	11765	1595	61314	17.0 %
	6 months	3511	954	29642	8.9 %
	12 months	3834	1433	34482	7.3 %
5	T0	2037	1277	28638	2.8 %
	3 months	2355	2267	12406	0.87 %
	6 months	1293	1626	28993	0.0 %
	12 months	1162	1255	23992	0.0 %
6	T0	2619	630	1214	340.5 %
	3 months	918	572	4906	8.0 %
	6 months	383	303	1114	9.9 %
	12 months	3451	1281	13984	17.1 %
7	T0	[a]	[a]	[a]	[a]
	3 months	540	266	2182	33.2 %
	6 months	6087	3142	12002	10.6 %
	12 months	1497	454	10105	12.9 %
8	T0	13406	5104	[a]	[a]
	3 months	8764	746	33765	23.7 %
	6 months	1271	592	9836	6.9 %
	12 months	1698	464	13828	8.9 %
9	T0	19617	4719	48959	33.7 %
	3 months	1013	69	11321	8.4 %
	6 months	4856	948	34723	11.6 %
	12 months	2287	782	33428	4.3 %
10	T0	13720	360	14213	96.4 %
	3 months	10660	3776	23725	34.5 %
	6 months	9381	2531	15193	54.1 %
	12 months	1146	1134	13103	0.1 %
11	T0	545	54	3942	12.7 %
	3 months	6312	304	8955	69.4 %
	6 months	6408	258	9910	63.4 %
	12 months	1633	126	13699	11.1 %

[a] Experiment could not be carried out because there was an insufficient number of cells

Mixed lymphocyte reaction

The cryopreserved PBL samples obtained at different times were thawed, washed and their viability confirmed. They were used in simultaneous MLRs with donor and third party cells as stimulators. A total of 2×10^5 of recipient PBL were mixed with 2×10^5 of irradiated donor splenocytes or third party lymphocytes (28 Gy) in a 96 round-bottomed tissue culture plates in 4–8 replicates. Three sets of control cultures were included in every experiment. The first was an autologous control, the recipient's PBL with self-irradiated PBLs (28 Gy), to measure background activity. Next was a third party responder control, PBL from a healthy volunteer known to have a tissue type different from that of the donor, incubated with irradiated donor cells to confirm that the cryopreserved donor cells were capable of stimulating potentially responsive alloreactive cells. Third, there were cultures of recipient PBL stimulated with concavalin A (5 μg/ml) as a positive control. Plates were incubated at 37 °C in a humidified atmosphere of 5 % CO_2 in air. On day 5, cells

Table 1b MLR data of patients in group 2

Patient number	Time post-transplant	Donor-specific response	Autologous control (background)	Anti-third party response	Relative response
12	T0	12428	2918	21678	50.7%
	3 months	16764	3068	33662	44.5%
	6 months	23893	1859	43551	52.8%
	12 months	30451	1317	35839	84.3%
13	T0	23386	3163	22571	104.2%
	3 months	22753	1544	30820	91.8%
	6 months	24167	3114	24995	96.2%
	12 months	43590	3023	24736	186.8%
14	T0	8499	1175	10275	80.5%
	3 months	1117	696	4127	12.3%
	6 months	11770	2363	14706	76.2%
	12 months	21615	1484	19798	11.0%
15	T0	10429	1726	14469	69.0%
	3 months	11665	1294	13606	81.8%
	6 months	4088	453	2938	146%
	12 months	10035	1506	16719	57.2%
16	T0	20852	4642	27033	72.4%
	3 months	15311	1464	25525	57.5%
	6 months	16819	1841	27607	55.7%
	12 months	21492	2129	28298	74.0%
17	T0	[a]	[a]	[a]	[a]
	3 months	6597	2129	12560	42.8%
	6 months	18571	3189	39983	41.8%
	12 months	10301	2143	13261	73.4%
18	T0	25923	326	[a]	[a]
	3 months	8321	256	7679	108.7%
	6 months	5177	1188	70531	5.7%
	12 months	28195	2125	35566	78.0%
19	T0	21336	2244	25634	81.6%
	3 months	41508	1484	41914	98.9%
	6 months	16740	1569	55125	29.4%
	12 months	41813	2768	48680	85.0%

[a] Experiment could not be carried out because there was an insufficient number of cells

were pulsed with 1 μCi ^{3}H thymidine (Amersham, UK) in 50 μl of supplemented medium/well. After 18–24 h, wells were harvested (Inotech, Switzerland) and incorporation of ^{3}H thymidine was estimated using a liquid scintillation counter (Lechman, UK). Time course experiments were carried out on different samples and confirmed that peak proliferation of lymphocytes was on day 6. Results of the MLR were expressed as a mean count per minute (CPM) and as relative response (RR) according to the formula:

$$\frac{(\text{Recipient}+\text{Donor}_x)-(\text{Recipient}+\text{Recipient}_x)}{(\text{Recipient}+\text{3rd Party}_x)-(\text{Recipient}+\text{Recipient}_x)} \times 100$$

A relative response below 20% was considered indicative of hyporesponsiveness [12].

CD8 + cell depletion

Dynabeads coated with anti-CD8 antibody (Beads M450, Dynal, UK)were added to the cell suspension prepared from the recipient's PBL in a beads to cell ratio of 10:1. The mixture was kept at 4 °C for 40 min with gentle shaking and tilting. A magnet (Dynal MPC) was then applied to the mixture for 2–3 min and CD8-free cell suspension was collected by pipetting. Cells were washed and resuspended. Complete removal of CD8 + cells was confirmed by flow cytometry using an anti-CD8 antibody (Leu 8, Becton and Dickinson, UK).

Purification of immunoglobulin G

Purification of immunoglobulin G was carried out by liquid chromatography using the protein G sepharose column that binds to all subclasses of IgG (Pharmacia, USA). The plasma sample (2 ml) was diluted 1:1 in phosphate-buffered saline (PBS). The sample was loaded to the column and washed with excess PBS. IgG was eluted from the column by glycine/HCl buffer (pH 2.7). The eluate (15 ml) was received in a bottle containing TRIS buffer (pH 9) for immediate neutralisation of the pH. It was then dialysed overnight against 2 l of PBS, concentrated to 2 ml by polyethylene glycol, then re-dialysed against 500 ml of supplemented RPMI medium to be added to the cultures. The final IgG concentration in the cultures was about 10% of the starting concentration in plasma. As a control, IgG was also purified from pooled male serum type AB (Sigma, UK) and the effect of adding the recipient's and control IgG to the MLRs was studied.

Table 2a Clinical data of patients in group 1 at 1 year post-transplant

Patient number	Serum creatinine (mmol/l)	Acute rejection episodes	Biopsy findings
1	111	3 months	Minimal cellular infiltrates. Glomeruli, tubules and vessels: normal
2	110	0	Minimal cellular infiltrates. Glomeruli, tubules and vessels: normal
3	159	0	Interstitium, glomeruli, tubules and vessels: normal
4	140	0	Sparse lymphoid infiltrates. Glomeruli: normal. Tubular atrophy present. One vessel shows hyaline changes.
5	170	0	Dense periglomerular lymphoid infiltrate. Glomeruli, tubules and vessels: normal
6	112	2 weeks	Interstitium, glomeruli, tubules and vessels: normal
7	97	0	Not available
8	110	0	Sparse focal interstitial mononuclear infiltration, minor tubular atrophy, glomeruli and vessels: normal.
9	196	10 weeks	Mild cellular infiltrates. Glomeruli, tubules, and vessels: normal. Picture remained unchanged on repeat biopsy a few months later
10	197	0	Glomeruli appear normal. Focal active tubular degeneration and regeneration. Vessels: normal.
11	103	0	Interstitium, glomeruli, tubules and vessels: normal

Statistical analysis

The significance of the differences in serum creatinine and in the number of acute rejection episodes between both groups was examined using the Mann-Whitney test.

Results

MLR data

Examination of the data indicated two distinct patterns of responses. In 11 patients (numbered 1–11 and assigned to group 1), donor-specific hyporesponsiveness was present from the time of transplant or clearly developed within the 12-month period of the study. These data are given in Table 1a (mean CPM and RR). In all patients donor splenocytes were able to stimulate third party responder PBL normally.

In eight patients (numbered 12–19 and assigned to group 2), data indicated persistence of anti-donor responses throughout the 12-month period of the study. These data are given in Table 1b (mean CPM and RR).

Patient data

The mean age for patients in groups 1 and 2 was 53.1 and 36.6 years, respectively; the difference was not statistically significant. Group 1 contained six females and five males, and group 2 eight males. The mean waiting time on dialysis was 11 and 56 months, respectively. The three patients who had received previous transplants were included in group 2. The aetiology of renal failure and the history of blood transfusions were similar in both groups. With respect to HLA matching, in group 1 patients at the A locus two patients had zero mismatches, six patients had one mismatch and three patients had two mismatches; at the B locus one patient had zero mismatches, eight patients had one mismatch and one patient had two mismatches; at the DR locus nine patients had zero mismatches and two patients had one mismatch; and at the DQ locus seven patients had zero mismatches, three patients had one mismatch and one patient had two mismatches. In group 2 patients, at the A locus six patients had zero mismatches and two patients had one mismatch; at the B locus one patient had zero mismatches, six patients had one mismatch and one patient had two mismatches; at the DR locus four patients had zero mismatches and four patients had one mismatch; and at the DQ locus four patients had zero mismatches, three patients had one mismatch and one patient had two mismatches.

Clinical outcome at 12 months

Graft and patient survival at the end of the 1-year period of the study were 100%. In group 1 patients the mean serum creatinine at 1 year was 139.7 mmol/l, the total number of acute rejection episodes was four and core biopsies showed normal appearance of glomeruli, tubules and blood vessels. In nine out of the ten available biopsies no or minimal lymphoid cell infiltrates were seen in the interstitium. In group 2 patients the mean serum creatinine at 1 year was 163 mmol/l and the total number of episodes of acute rejection was 12. In five of the seven available biopsies, evidence of ongoing rejection was obtained. In two biopsies, however, there was no evidence of rejection despite high in vitro anti-donor reactivity. Tables 2a and 2b contain the clinical data at 1 year post-transplant. The difference in mean serum creatinine between the two groups was not statistically significant ($P > 0.05$), but the difference in the number of acute rejection episodes was significant ($P < 0.05$).

Table 2b Clinical data of patients in group 2 at 1 year post-transplant

Patient number	Serum creatinine (mmol/l)	Acute rejection episodes	Biopsy findings
12	201	4 weeks	Mild cellular infiltration and fibrosis in the interstitium. Focal tubular atrophy. One sclerosed glomerulus out of 18. Vessels: normal
13	193	7 weeks, 10 weeks, and 14 weeks	Mild cellular infiltrates, interstitial fibrosis and tubular atrophy. Fibrointimal hyperplasia and elastosis of an interlobular artery
14	133	1 week	Scanty focal inflammatory cell infiltrates and tubular damage. Glomeruli, tubules and vessels: normal
15	166	1 and 3 weeks	Not available
16	103	0	Diffuse lymphocytic infiltrates and tubular atrophy. Glomeruli and vessels: normal
17	245	1 week, and 5 months	Abnormal glomeruli, atrophic tubules, dense lymphocytic infiltrates. Features of acute and chronic rejection seen
18	178	11 days and 4 weeks	Mild focal chronic inflammatory cellular infiltrates and tubular atrophy. Glomeruli and vessels appear normal
19	82	2 weeks	Glomeruli, interstitium, tubules and vessels: normal

Effect of CD8 + cell depletion

CD8 + cells were depleted as described. Adequacy of depletion was confirmed by flow cytometry. No significant difference was noted on the MLRs carried out prior to and after removal of CD8 + cells.

Effect of adding recipient's IgG to the MLR

IgG was quantified in the eluate and was found to be approximately 20 % of the concentration in the starting plasma sample. When added 1:1 to the MLR the final concentration was about 10 %. No significant effect on the MLR was noted. On the one occasion on which a significant suppression was produced, a similar effect was also obtained with the control IgG purified from pooled male serum type AB, indicating non-specificity.

Discussion

Long-term monitoring of the immune responses initiated by allograft recipients towards the graft is a potentially useful clinical tool. However, such monitoring is limited by the wide diversity of mechanisms involved in the rejection process, rendering the interpretation of the results of a single test very difficult, and by the lack of a firm correlation between in vitro findings and in vivo events.

Here we discuss the results of 12 months of monitoring 19 patients using the MLR and the correlation between these findings and the intragraft events as assessed by graft function (serum creatinine), number of episodes of acute rejection and histologic findings at 1 year.

Two patterns of responses were identified. In the first group, 11 patients exhibited donor-specific hyporesponsiveness from the time of transplantation or at variable time intervals afterwards; RR remained low or decreased. An effect of immunosuppressive therapy alone would not explain this pattern in view of the normal anti-third party responsiveness [36]. Four episodes of acute rejection were recorded in patients of this group and the mean serum creatinine at 1 year was 136.4 mmol/l. The low anti-donor in vitro reactivity of PBL correlated well with the biopsy findings: in nine of the ten available biopsies there was no significant evidence of rejection.

The mechanism of developing donor-specific hyporesponsiveness is not known. This MLR pattern could not be reversed by in vitro depletion of CD8 + cells from the recipients' PBL. This finding indicates the absence of circulating CD8 + suppressor cells but does not exclude suppression as a mechanism of donor-specific hyporesponsiveness, as Gassel et al. [19] were able to isolate CD8 + suppressor cells from the spleens but not from the thoracic ducts of tolerised animals, suggesting that CD8 + suppressor cells may not be circulating. Alternatively, suppression may be achieved by a subset of CD4 + T lymphocytes [27, 31]. A consensus of opinion is now developing that suppression is not a function of a separate lineage but is shared by different types of lymphoid cells that encounter the antigen under conditions that are different from those that lead to activation [5]. Clonal inactivation (anergy) is a likely mechanism for acquired donor-specific hyporesponsiveness [1, 24, 30]. Although there is a tendency for patients with DR, and to a lesser extent DQ, compatibility to fall into group 1, the differences in tissue typing were not statistically significant and the number of patients is too small to draw this sort of conclusion.

In a study by Flechner et al. [18], patients with low anti-donor reactivity in the MLR were selected for withdrawal of steroids, yet acute rejection episodes developed in 4 out of 21 recipients of HLA identical grafts and in 2 out of 12 recipients of haplo-identical grafts. Recommendations regarding the most suitable time to re-

duce immunosuppressive therapy should await further long-term studies that would investigate the stability of hyporesponsiveness. This view is supported in the report by Arnold et al. [3], who postulated that, in adults, the state of acquired tolerance initially varies before the development of permanent tolerance. Röcken et al. [35] and Migita et al. [28], have also demonstrated that the state of anergy can be broken.

In our second group (eight patients), anti-donor responsiveness remained high or increased during the year of the study. Patients showing this pattern had a higher mean serum creatinine (163 mmol/l) at the end of 1 year and a higher number of acute rejection episodes ($n = 12$). The difference in the number of episodes of acute rejection between the two groups was statistically significant, but the difference in serum creatinine was not. Serum creatinine is a non-specific, and maybe a late, indicator of graft malfunction and can also be affected by non-immunological factors. The 1-year biopsies of these patients showed evidence of continuing rejection in four of the seven biopsies available.

There were some variations in the clinical parameters among group 2 patients. One patient (no. 16) had continuing high in vitro reactivity, yet did not have any episodes of acute rejection and serum creatinine at 1 year was 130 mmol/l. However, the routine biopsy showed mononuclear cellular infiltration and tubular atrophy, suggestive of rejection. The maintenance cyclosporin level remained within the range of 100–120 ng/l. The presence of lymphocytic infiltrates within the graft in the absence of clinical evidence of rejection has been reported by other investigators [9]. Two other patients (nos. 14 and 19) had high in vitro reactivity with normal biopsy findings. It is likely that the immune response against the graft can be inhibited by immunosuppressive agents or by suppressor regulatory mechanisms functioning beyond the stage of helper T-cell activation [25, 44]. A role for blocking antibodies (anti-idiotypic, anti-TCR antibodies) has been suggested [4, 16, 29, 38, 39]; however, we found no evidence of blocking antibody activity on repeated testing.

It remains to be seen whether patients in group 2 are more likely to develop chronic rejection than patients in group 1. Such long-term follow-up will help to answer the question as to whether chronic rejection is the end result of continuous low-grade anti-donor response similar to the process that takes a florid form during episodes of acute rejection [2, 7].

It is not possible to predict a patient's pattern of responsiveness prior to transplantation. Neither HLA matching nor the pre-transplant MLR had a constant relation to the pattern of responsiveness after transplantation, a finding also reported by Reader et al. [32]. This may be due to the effect of discrepancies at minor transplantation antigens that require pre-sensitization in order to induce proliferation of PBL in the MLR [6, 37] or to the relative immunosuppressive effect of uraemia prior to transplantation [20].

From the data obtained we conclude that some recipients of cadaveric renal allografts on cyclosporin therapy develop donor-specific hyporesponsiveness as detected by the MLR. While the presence of this state was associated with a better graft performance over 1 year, the reverse was not always true, i.e. high in vitro donor-specific reactivity of recipients' PBL was not always associated with demonstrable rejection activity within the graft. The underlying mechanism(s) of donor-specific hyporesponsiveness is not clear, and it remains to be seen whether the presence of this state indicates long-term tolerance.

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