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Induction of donor-specific tolerance or sensitization as measured by sequential MLC reactivity up to 24 months after renal transplantation

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Introduction

The majority of recipients of a cadaver renal allograft retain good graft function following a reduction in the maintenance immunosuppressive therapy. In some patients it is theoretically possible to withdraw immunosuppressive therapy completely. This suggests that immunological adaptation or donor-specific hyporesponsiveness occurs following transplantation [1–4]. In contrast a small number of patients reject their grafts within 6 to 12 months following renal transplantation, despite maintenance immunosuppression. These patients appear to become sensitized to the donor antigens.

In this study we investigated the induction of donor-specific hyporesponsiveness or sensitization using sequential MLC reactivity up to 24 months following renal transplantation.

Patients and methods

Patients

Sequential MLC studies were performed on 29 renal transplant recipients. All patients were recipients of first cadaver transplants and were studied up to two years postoperatively.

Immunosuppression

The immunosuppression protocol consisted of cyclosporine (10 mg/kg/day in 2 divided doses), methylprednisolone (24 mg/day) and azathioprine (1–2 mg/kg/day). The cyclosporine dose was adjusted to maintain a whole blood trough level of 300–500 ng/ml. Rejection episodes were diagnosed clinically and treated with intravenous methylprednisolone 500 mg on four consecutive days. Steroid resistant rejection was treated with the monoclonal antibody OKT3.

Mixed lymphocyte cultures

Donor lymphocytes were obtained from the donor spleen. Blood samples were collected from the recipients preoperatively and at 3, 6, 12, 18 and 24 months postoperatively. The donor and recipient lymphocytes were frozen in 10% dimethylsulphoxide and stored in liquid nitrogen until the time of assay.

The one-way MLC test was performed as described previously [5]. Donor spleen cells were used as stimulators against recipient lymphocytes. Cells from three healthy unrelated volunteers were used as third party controls. Stimulator cells from the donor (D), recipient (A) or third party controls (C) were irradiated and mixed with recipient responder cells (R). The culture medium consisted of RPMI 1640, 10% fetal calf serum, 2 mmol/l glutamine and antibiotics. Cell proliferation was assessed after 5 days by (^{3}H)-thymidine incorporation during an 18-h period.

The relative response was calculated after subtraction of the autologous response. The relative donor-specific response (DSR (r)) was calculated by expressing the recipient response to donor lymphocytes as a percentage of the recipient response to third party controls $((R*D)-(R*A)\times 100/(R*C)-(R*A))$.

Results

Two groups of patients were identified. Group 1 consisted of 17 patients who either experienced no rejection episodes (RE) ($n=8$) or who experienced 1–2 RE during the first 3 months ($n=9$) following transplantation. Twelve patients in Group 2 were either treated for RE in both the early and late (>12 months) postoperative period ($n=5$) or lost their grafts between 6 and 24 months postoperatively ($n=7$). The changes in the individual DSR's are shown in Table 1.

In 6 of the 8 patients who never had any RE, the DSR decreased gradually over the 2 year period; in the remaining two patients the DSR remained unchanged. Of the 9 patients who only experienced early rejection, the DSR decreased in six and remained unchanged in three.

Of the five patients who had both early and late RE, two had an increasing DSR. In the remaining three patients the DSR was high pre-transplant and remained unchanged in one and decreased slightly in two.

Seven patients lost their grafts between 6 and 24 months postoperatively. The DSR increased gradually in four patients, remained unchanged in one and decreased in two patients.

Table 1 The trends in the individual donor-specific responsiveness as measured by the sequential MLC in patients who had no rejection early rejection, early and late rejection, and graft loss following rena transplantation

	Donor specific response (DSR)		
	Increased	Unchanged	Decreased
No rejection ($n=8$)		++	++++++
Early rejection ($n=9$)		+++	++++++
Early and late rejection ($n=5$)	++	+	++
Graft loss ($n=7$)	++++	+	++

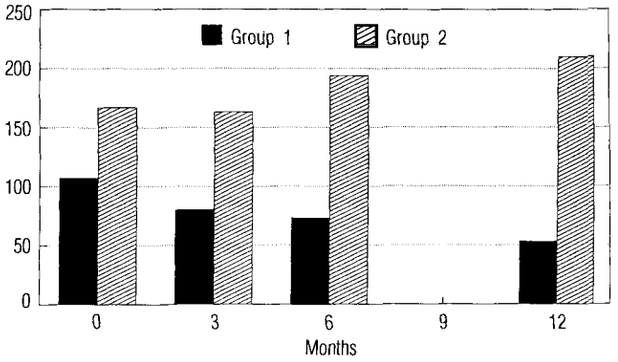

Fig. 1 The mean donor-specific response in the patients with no or early rejection (Group 1) and the patients with early and late rejection or graft loss (Group 2) at 0, 3, 6, 12, 18 and 24 months following renal transplantation

The mean DSR's for the patients are shown in Fig. 1. The mean pre-transplant DSR of the patients in Group 1 was lower than in Group 2. The mean DSR decreased over the 2 year period in Group 1 and increased in Group 2. The mean DSR of the patients in Group 1 was significantly lower at each time point than the DSR of the patients in Group 2.

Discussion

In this study patients who had significant problems with rejection following renal transplantation (Group 2), were hyperactive towards the donor antigens preoperatively and they tended to become more responsive with time following transplantation. In contrast, in the patients who experienced few problems related to rejection, "in vitro" donor-specific hyperresponsiveness was noted pre-

operatively, and the reactivity towards donor antigens decreased even further with time. These results confirm that immunologic adaptation or proliferative hyporesponsiveness to donor antigens does occur in over 50% of renal transplant recipients [3, 4]. These patients may be candidates for reducing immunosuppression.

References

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