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Three Years of Follow-Up of Bone Marrow-Augmented Organ Transplant Recipients: The Impact on Donor-Specific Immune Modulation

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THE DISCOVERY of the presence of previously unsuspected microchimerism in successful long-term liver and kidney transplant recipients prompted us to postulate that these cells are essential for graft acceptance and the induction of donor-specific hyporeactivity.¹⁻³ This observation provided the basis for the initiation of a new therapeutic strategy which involved infusion of donor bone marrow (BM) cells under conventional immunosuppressive treatment with tacrolimus and prednisolone.⁴ The initial outcome of sequential in vitro immunologic evaluations performed to determine the development of donor-specific hyporeactivity in the first 15 BM-augmented and 16 contemporaneous controls has been described previously.^{5,6} We report here the immune profile of 102 BM-augmented and 57 control patients who were at least 6 months posttransplantation.

All patients were analyzed for donor chimerism by FACS and/or polymerase chain reaction (PCR) using HLA-specific markers.⁴

In Vitro Immune Responsiveness

Pre- and serially posttransplant (5, 4, and 3 times in 1st, 2nd and 3rd year respectively) monitoring of recipient's immune status was carried out by evaluating the proliferative responses of the peripheral blood mononuclear cells (PBMC) to mitogens (concanavalin A [ConA], phytohemagglutinin [PHA]), to recall antigens (tetanus toxoid), and to mixed lymphocyte reaction (MLR) with donor and third-party cells. The details of the assays were summarized elsewhere.^{6,7} The criteria developed to serially evaluate in vitro immune status were also published previously.^{6,7} Briefly, we have classified the transplant recipients based on their in vitro responsiveness as either donor-specific hyporeactive, donor-specific intermediate, reactive, or suppressed.

MATERIALS AND METHODS

Case Material

Since December 1992, 138 study patients have received simultaneous BM + whole organ transplants. Ninety-two recipients of whole organs for whom permission could not be obtained for BM procurement from cadaveric donors were monitored as controls. Sequential immune monitoring was possible in 102 study (liver n = 31, kidneys n = 27, kidney + pancreas n = 19, kidney + islets n = 6, heart n = 10, lung n = 8) and 57 control (liver n = 23, kidney n = 10, kidney + pancreas n = 7, kidney + islets n = 2, heart n = 9, and lung n = 6) patients who were at least 180 days posttransplantation and for whom donor cells were available. Immunosuppression was similar in both groups and consisted of tacrolimus and prednisolone.⁴

RESULTS AND DISCUSSION

In transplant recipients who were augmented with BM, with the exception of those receiving hearts, progressive donor-

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Table 1. Results of MLR in BM-Augmented Organ Transplant Recipients and Its Correlation With Incidence of Chimerism

Type of Transplant	No. Transplants	DSHI				Reactive			
		No. (%)	POD ± SD	% Off Steroids	Chimerism (%)	No. (%)	POD ± SD	% Off Steroids	Chimerism (%)
Liver*	29	15 (48)	872 ± 199	87	86	14 (45)	750 ± 157	50	92
Kidney†	23	10 (43)	605 ± 214	50	78	13 (56)	716 ± 207	77	75
Kidney + pancreas	19	8 (47)	482 ± 151	50	86	11 (64)	536 ± 179	44	70
Kidney + islet	6	4 (67)	891 ± 148	75	75	2 (38)	942 ± 236	50	50
Heart	10	2 (20)	824 ± 20	NA	50	8 (80)	605 ± 2703	NA	88
Lung	8	5 (62)	587 ± 190	NA	80	3 (38)	467 ± 164	NA	100

*Two recipients were suppressed (nonresponsive to both donor and third-party stimulators).

†Four organs were lost in patients who were reactive.

Table 2. Results of MLR in Non BM-Augmented Organ Transplant Recipients and Its Correlation With Incidence of Chimerism

Type of Transplant	No. Transplant	DSHI				Reactive			
		No. (%)	POD \pm SD	% Off Steroids	Chimerism (%)	No. (%)	POD \pm SD	% Off Steroids	Chimerism (%)
Liver	23	7 (30)	886 \pm 109	100	67	16 (70)	871 \pm 87	56	50
Kidney	10	5 (50)	846 \pm 75	20	60	5 (50)	674 \pm 346	60	75
Kidney + pancreas	7	2 (28)	653 \pm 103	50	50	5 (72)	453 \pm 128	20	40
Kidney + islet	2	0	0	NA	NA	2 (100)	334 \pm 68	50	100
Heart	9	2 (22)	752 \pm 190	NA	100	7 (78)	536 \pm 283	NA	43
Lung	6	2 (33)	515 \pm 23	NA	100	4 (67)	495 \pm 16	NA	50

specific immunomodulation was achieved in 50%. On the contrary, only 18 of 57 (32%) of the control patients exhibited hyporeactivity or intermediate responses to the donor (DSHI). It is noteworthy that the temporal relationship of monitoring was comparable in both study and control recipients (Tables 1 and 2).

Liver Transplant Recipients

BM-augmented liver transplant recipients developed stable DSHI within the first year posttransplantation and 13 of 15 patients retested remained DSHI at the 2-year follow-up (range 200 to 600 days). In contrast, in those who received only a liver, only three of seven recipients who exhibited DSHI previously maintained persistent immunomodulation (data not shown). Two of the BM-augmented liver recipients exhibited low proliferative responses to mitogens, donors, and third-party alloantigens and were considered suppressed. The majority of study (87%) and control (100%) recipients who displayed DSHI were off prednisolone, while weaning from steroids was achieved in only 50% of those who were reactive in these groups (Tables 1 and 2). Furthermore, a significant lower incidence of late acute cellular rejection was witnessed in BM-augmented liver recipients who had previously exhibited DSHI as compared to those who were MLR reactive. The frequency of donor cell chimerism in study recipients displaying DSHI was similar to those who were reactive (87% and 92%, respectively), whereas the frequency of donor cell chimerism detected in the control group was approximately 60%.

Kidney Transplant Recipients

The outcome in the BM-augmented and control kidney transplant recipients was comparable for all the parameters tested including immune status, percent off steroids, and incidence of donor-specific chimerism (Tables 1 and 2).⁷ Four BM-augmented kidney transplant recipients who experienced late rejection episodes lost their grafts. Two of these recipients developed DSHI during the first year post-transplant but reverted to MLR reactive status prior to evolution of the late rejection episode, ultimately leading to graft loss. It is noteworthy that a higher percent of BM-augmented kidney + pancreas and kidney + islet recipients exhibited DSHI in comparison to that of control patients.

Thoracic Organs

Most of the heart transplant recipients in the study and control group retained donor-specific responsiveness (Tables 1 and 2). On the contrary, in lung allograft recipients a twofold higher incidence of induction of DSHI (62%) was observed in BM-augmented patients as compared to that of controls (33%). The frequency of donor cell chimerism was 80% in BM-augmented and nonaugmented heart and lung transplant recipients who exhibited DSHI, whereas it was only 50% in controls who were MLR reactive. Although not statistically significant, there was a trend toward a lower incidence of rejection within the first 100 days after transplantation in the study heart recipients as compared to similarly treated historical controls.⁸ Bronchiolitis obliterans (OB), a form of chronic rejection in lung recipients, was witnessed in two of six controls and in none of the eight study patients with a follow-up of 18 months posttransplantation.

In summary, the simultaneous infusion of donor BM cells in allograft recipients was associated with an increase in the incidence of donor cell chimerism in all organ allografts, and with a twofold increase of DSHI in liver and lung recipients. Furthermore, BM-augmented transplant recipients who developed stable donor-specific hyporeactivity experienced less late allograft rejection and improved long-term outcome.

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