Serial Evaluation of Immune Profiles of Simultaneous Bone Marrow and Whole Organ Transplant Recipients

A. Zeevi, M. Pavlick, S. Lombardozzi, R. Banas, A.S. Rao, P. Fontes, A.J. Demetris, R. Shapiro, M. Jordan, F. Dodson, S. Pham, M. Trucco, J. Fung, and T.E. Starzl

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BONE MARROW (BM) augmentation of donor-derived chimerism was achieved in recipients of kidney, liver, heart, and pancreas islet transplantation.[1] We have proposed that these donor-derived cells are essential for graft acceptance and the induction of donor-specific nonreactivity.[2,3] The first 15 BM-augmented transplant recipients and 16 nonmarrow controls who were more than 120 days posttransplant underwent sequential in vitro immunological evaluations to determine the development of donor-specific hyporeactivity.[4] Based on proliferative responses (pre- and posttransplant) of peripheral blood mononuclear cells (PBMCs) to mitogens and alloantigens, the recipients were classified into four categories: donor-specific hyporeactive, intermediate, responsive, and suppressed.[4] Fifty-three percent of BM treated recipients (eight of 15) exhibited progressive modulation of antidonor responses, whereas only 12% of nonmarrow controls showed donor-specific hyporeactivity. However, the follow-up time in the nonmarrow control group was shorter (112 ± 10 days) as compared with that of patients in the study group (282 ± 113 days).[4] In this report, we reanalyzed the immune profile of BM-treated and nonmarrow controls 3 months later, and the results were compared with the earlier report.

METHODS

Case Material

Since December 1992, 64 patients have received simultaneous BM-positive whole-organ transplants, whereas 53 recipients of whole organs alone have been monitored as contemporaneous controls. However, the present study included 17 of 19 BM-augmented recipients (kidneys, n = 6; kidney plus islets, n = 2; livers, n = 7; liver plus islets, n = 1; and heart, n = 1) and 22 of 25 nonmarrow controls (kidneys, n = 6; livers, n = 14; and hearts, n = 2) who were more than 120 days posttransplant and in whom in vitro immune monitoring was possible. Immunosuppression was similar in both groups and consisted of FK 506 and prednisone routinely used at our center.[1] Using HLA-specific markers and probes for Y chromosome (in male to female transplant recipients), all patients were examined for donor chimerism by fluorescence-activated cell sorting (FACS), fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR).[1]

In Vitro Immune Monitoring

Recipients' PBMCs were prepared via a method described previously, were tested for proliferative responses induced by mitogens (phytohemagglutinin [PHA] and concanavalin A [Con A]) and by alloantigens (donor and third-party panel cells) in mixed lymphocyte reaction (MLR) assays, the details of which were summarized elsewhere.[5,6]

Based on the proliferative responses of recipients' PBMCs pre- and posttransplantation, we have classified them into four categories.[4]

Category I. Donor-specific hyporeactive: significant decrease (over 70%) in posttransplant versus pretransplant donor-specific MLR responses while maintaining adequate reactivity to control third party stimulator cells and mitogens.

Category II. Donor-specific intermediate: reduction (40% to 70%) in posttransplant donor-specific alloreactivity as compared with pretransplant baseline control, with no attenuation of responsiveness to third-party stimulators or mitogens.

Category III. Reactive: minimal change in pretransplant versus posttransplant donor-specific reactivity.

Category IV. Suppressed: global nonreactivity to all in vitro stimuli.

RESULTS

Distribution of Donor-Specific MLR Patterns in BM-Augmented and Control Transplant Recipients

The overall percentage of donor-specific hyporeactive/intermediate responders in the BM-augmented and control groups remained similar to that reported 3 months earlier (53% and 12% versus 41% and 18%, respectively) (Table 1). In the current analysis, five of eight BM/liver recipients (62%) exhibited modulation of donor-specific reactivity (four hyporeactive and one intermediate), whereas in the control group only three of 14 patients (21%) showed a reduction in antidonor reactivity (three intermediate). BM/kidney and kidney control recipients had a similar distribution of donor-specific nonreactivity (25% and 16%, respectively). Furthermore, all control recipients who previously exhibited global nonreactivity (kidneys, n = 2; livers, n = 2) regained their in vitro immune responsiveness and became reactive to donor cells (Table 1).

From the Pittsburgh Transplantation Institute and the Departments of Pathology (A.Z., M.P., R.B., A.S.R., A.J.D.), Pediatrics (S.L., M.T.), and Surgery (A.S.R., P.F., R.S., M.J., F.D., S.P., J.F., T.E.S.), University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Adriana Zeevi, Ph.D., Department of Pathology, Biomedical Science Tower, Room W1551, University of Pittsburgh, Pittsburgh, PA 15261.

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Table 1. Donor-Specific MLR Patterns in BM-Augmented and Control Transplant Recipients

Report Period	Groups (POD ± SD)*	n†	Distribution of MLR Profiles (%)			
			Hyporeactive	Intermediate	Reactive	Suppressed
April 1994	With BM	15	20	33	47	0
	(150 ± 60)		(1K, 2L)‡	(2K 3L)‡	(4K, 2L, 1H)‡	
	Without BM	16	12	0	63	25
	(112 ± 10)		(1K, 1L)‡		(2K, 8L)‡	(2K, 2L)‡
July 1994	With BM	17	29	12	59	0
	(285 ± 119)		(1K, 4L)‡	(1K, 1L)‡	(6K, 3L, 1H)‡	
	Without BM	22	0	18	82	0
	(153 ± 74)			(1K, 3L)‡	(5K, 11L, 2H)‡	

*Postoperative day when last sample was tested.
†Number of recipients per group.
‡Number of transplant recipients for each organ: K, kidney; L, liver; H, heart.

Immune Reactivity, Immunosuppression, and Chimerism

As shown in Tables 2 and 3, the number of patients off steroids was higher in the kidney and liver (66% and 75%, respectively) transplant recipients who also exhibited diminished donor-specific MLR responses than in the donor-reactive patients (0% and 43% respectively) in both BM-augmented and nonaugmented groups.

Chimerism in the BM-augmented group was detectable by flow cytometry for up to 18 months after transplantation and ranged between 0.5% and 2.6% in both the hyporeactive and donor-reactive recipients, whereas in the majority of the control recipients detection of donor cells by FACS analysis yielded negative results 30 to 60 days posttransplant (donor-derived cells accounted for less than 0.5%) (Tables 2 and 3).

Shift in Donor-Specific Reactivity

Four BM-treated recipients (two livers, one with pancreatic islets; and two kidneys, one with islets) who exhibited early evidence of donor-specific hyporeactive or intermediate reactivity reverted into reactive against the donor when last tested. In three of four patients, we also have evidence of an increase (three- to 10-fold) in circulating donor-specific helper T cells (data not shown). All four recipients experienced late rejection episodes. Heightened donor-specific reactivity was maintained in three of four (liver plus islet, n = 1; kidney, n = 1; and kidney plus islet, n = 1) recipients after resolution of their ongoing rejection, whereas one liver patient reverted to being hyporeactive to the donor.

The kidney recipient who was treated for recurrent late rejection episode exhibited antidonor alloreactivity before initiation of antirejection and after steroid recycle. The specificity of this response was confirmed when lymphocyte cultures propagated from pre- and posttherapy samples in bulk MLRs also exhibited vigorous proliferation to the donor-mismatched HLA class II DR1 antigen (data not shown). Furthermore, the prerejection lymphocyte culture exhibited cell-mediated lymphocytotoxicity against donor lymphoblastoid B cells (data not shown).

DISCUSSION

The recognition that after whole-organ transplantation resident bone-marrow derived cells migrate out of the graft into the recipient and persist has led to the concept that establishment of chimerism is a seminal event in allograft acceptance and subsequent induction of donor-specific hyporeactivity.[2,3] This spontaneous chimerism was safely augmented by simultaneous infusion of bone-marrow along with whole-organ transplants.[1] The in vitro immune reactivity profile of patients in the BM-augmented group was reminiscent of earlier observations in recipients of long-

Table 2. Correlation Between Immune Reactivity, Immunosuppression, and Chimerism: Kidney Transplant Recipients

MLR Reactivity Profile	Immunosuppression		Chimerism (%)	
	FK Level (ng/mL)	Off Prednisone	FACS*	PCR†
Hyporesponsive/intermediate				
Study (n = 2)	0.1, 0.8	1/2	1.9, 0.5	100
Control (n = 1)	0.9	1/1	0.83	NF
Responsive				
Study (n = 6)	1.0 ± 0.5	0/6	0.5–2.6	100
Control (n = 5)	0.9 ± 0.4	0/5	<0.5	60

*Level of circulating donor cells.
†Percentage of patients positive.
NF, not feasible.

Table 3. Correlation Between Immune Reactivity, Immunosuppression, and Chimerism: Liver Transplant Recipients

MLR Reactivity Profile	Immunosuppression		Chimerism %	
	FK Level (ng/mL)	Off Prednisone	FACS*	PCR†
Hyporesponsive/intermediate				
Study (n = 5)	0.4 ± 0.3	3/5	0.5–2.5	100
Control (n = 3)	0.4 ± 0.1	3/3	NF	66
Responsive				
Study (n = 3)	0.3 ± 0.1	1/3	0.5–2.3	100
Control (n = 11)	0.7 ± 0.2	5/11	1.3, <0.5	56

*Level of circulating donor cells.
†Percentage of patients positive.
NF, not feasible.

term functioning allografts who exhibited donor-specific hyporeactivity.[7–9] However, in the BM-treated recipients these changes occurred more frequently and earlier than in the contemporaneous controls.[4]

Longer follow-up of both BM- and non–marrow-treated liver recipients demonstrated that the BM augmentation has confirmed an advantage because five of eight (62%) progressed to stable donor-specific hyporeactive status, whereas only 21% of recipients of liver allograft alone exhibited donor-specific intermediate responses. These results suggest that the inherent tolerogenecity of liver allografts can be further enhanced by simultaneous BM infusion.

In contrast to liver recipients, BM-enriched and control kidney recipients had a similar pattern of donor-specific hyporeactivity that was maintained over the course of our follow-up. Nevertheless, the graft function of marrow-augmented kidney recipients was significantly better as compared with that of the controls.[10]

Of note is that none of the BM-treated or control recipients when last tested exhibited universal nonreactivity and all of the previous recipients in the control group who were classified as suppressed reverted to become donor reactive.[4]

In summary, BM infusion with whole-organ transplantation proved to be a safe procedure to augment donor chimerism in transplant recipients.[1] Serial immunologic examinations of these recipients may help us to better understand the role of both immune systems (donor and recipient) in establishing allograft acceptance and tolerance.

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