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Combined Bone Marrow and Whole Organ Transplantation From the Same Donor

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The recognition that after transplantation resident bone marrow-derived cells migrate out of the graft into the recipient has led to an increasing acceptance that the consequent establishment of chimerism is a seminal event in whole organ allograft acceptance, and the first step towards subsequent induction of donor-specific nonreactivity.[1–6] Because the migratory interstitial cells are of hematolymphoid origin, the next logical attempt was to augment this natural phenomenon by infusing donor bone marrow cells at the time of whole organ transplantation.

The goal of this study was to induce a higher level of chimerism in unmodified recipients of liver, kidneys, heart, and pancreatic islets by perioperative infusion of unaltered bone marrow from the same donor, in an attempt to either enhance graft survival and/or decrease or ultimately eliminate the requirement for chronic nonspecific immunosuppression.

MATERIALS AND METHODS

Patients

Sixteen patients were simultaneously transplanted with donor bone marrow and various other organs (Table 1). Immediately following whole organ transplantation, and Without cytoreduction or total lymphoid irradiation (TLI) of the recipient, unmodified donor bone marrow cells (3×10^8/kg body weight) were infused through a central IV line. Additionally, in three type I diabetic patients, donor pancreatic islets ($5.7 \times 10^5 – 1.5 \times 10^6$ islets/recipient) isolated by a modification of the automated method[7] were infused intraportally after graft placement and prior to bone marrow infusion.

Immunosuppression

All patients were maintained on routine immunosuppression with FK 506 and prednisone. The FK 506 dosage was targeted to plasma trough levels of 1.5 to 2.0 ng/mL in the first postoperative month, and 0.6 to 1.2 ng/mL thereafter and adjustments made as indicated by side effects and/or rejection. Episodes of acute rejections were treated with an in increase in FK 506 or a steroid cycle of solumedrol (1 g tapered to 20 mg), whereas, azathioprine, and OKT3 were added to treat recalcitrant rejection episodes.

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Determination of Chimerism

The presence of donor cells in the recipients' peripheral blood mononuclear cells (PBMC) was evaluated weekly in the first month after transplantation and monthly thereafter by the following techniques.

Flow Cytometry—Using donor or recipient-specific fluorescein isothiocyanate (FITC)-conjugated anti-HLA class I or class II monoclonal antibodies (Mab), single color FACS® analysis was performed. Frequency of acquired cells <0.5% was considered below the detection threshold or unquantifiable.

Fluorescent In Situ Hybridization (FISH)—Cytocentrifuge preparation of PBMC obtained from four female recipients of male organs were hybridized with biotinylated Y-chromosome specific probe, which was visualized by FITC-conjugated avidin.

Polymerase Chain Reaction (PCR)—Donor DNA was detected in the recipient's PBMC by PCR, a procedure described previously.[4] Additionally, donor DNA was also quantitated by competitive PCR (cPCR) in four female recipients of male organs by a previously described technique.[4]

Immune Monitoring In Vitro—Recipients' PBMCs were used to monitor their in vitro immune reactivity prior to transplantation and monthly thereafter to mitogens (ConA & PHA), recall antigens (tetanus toxoid), mixed leukocyte reactions (MLR), and cell-mediated lymphocytotoxicity (CML) assays.

RESULTS AND DISCUSSIONS

The infusion of donor bone marrow cells was uneventful in all patients and all allografts are functioning well. Steroids have been discontinued in five of six liver and three of seven kidney recipients, whereas the remainder of the patients are on a weaning protocol. All patients are currently being maintained on FK 506 (ranging from 2 to 23 mg/d) and the two kidney and pancreatic islet recipients are also receiving additional immunosuppression with azathioprine (75 mg/d).

All patients have evidence of circulating donor cells for up to 16 months after transplantation by either flow cytometry, PCR, or FISH. Only two liver recipients exhibited asymptomatic graft vs. host involvement of the skin, which in one regressed spontaneously, whereas in the other, it required a slight increase in her routine immunosuppression (Table 2). Allografts of 10 of 16 recipients also underwent mild to moderate rejection episodes, which in each case was completely resolved by treating with a gram of steroids followed by a recycle or in one case with an additional course of OKT3.

Nine of the 16 recipients, in whom interpretation of in vitro immune testing was possible, exhibited evolving donor-specific hyporeactivity as early as 50 days after transplantation, which in most cases was sustained for up to the last sample tested (POD 225).

It is too early to predict the impact of donor bone marrow cell augmentation on either allograft survival or on our capacity to wean and/or eventually withdraw immunosuppression. Nevertheless, perioperative infusion of bone marrow was safe, which in each recipient resulted in the establishment of "macrochimerism." Furthermore, serial in vitro monitoring data suggest that simultaneous infusion of bone marrow along with the whole organ confers an advantage to these patients, for the majority of them exhibit evolving donor-specific hyporeactivity.

Acknowledgments

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Table 1

Demographic Profile of Bone Marrow and Whole Organ Transplant Recipients

	Recipients (n = 16)
Male	8
Female	8
Male — Female Transplants	4
Mean Age [y]	46
Follow-up [range]	148–471
Organs Transplanted	
Liver	5
Liver + Pancreatic Islets	1
Kidney	7
Kidney + Pancreatic Islets	2
Heart	1
HLA Matches (X ± SD)*	
Liver	0.37 ± 0.41
Kidney	1.72 ± 1.81
Crossmatch Positive	1/16

* Of a possible of six.

Table 2

GVH Reactions in Two Patients Receiving Simultaneous Liver and Bone Marrow Transplantation

	Graft vs Host Reaction*			
Case No.	**POD**	**Grade**	**Treatment (mg d^{-1})**	**Outcome**
1†	54	||‡	Prednisone (7.5 ↑ 15)	All rash was gone within 3 weeks
2§	21	<I	No treatment	All rash was gone within 1–2 weeks
	74	<I	No treatment	All rash was gone within 1–2 weeks

*Asymptomatic skin rashes without any other organ involvement.

†She is currently on 6mg/day of FK506 and no Prednisone.

‡Maculopapular skin rash involving 25–50% of body.

§She is currently on 8 mg/day of FK506 and no Prednisone.

||Mild rash involving the skin of one or the other lower leg.