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Augmentation of Chimerism With Perioperative Donor Bone Marrow Infusion in Organ Transplant Recipients: A 44 Month Follow-Up

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WE HAVE previously documented the long-term persistence of donor cell chimerism after successful organ transplantation (Tx) and have postulated that it plays a seminal role in allograft acceptance and in the induction and perpetration of donor-specific tolerance.[1,2] To further enhance the salutary effects of chimerism we have augmented this phenomenon, since December 1992, by infusing unmodified donor bone marrow (BM) into 190 organ allograft recipients; the unavailability of consent to retrieve vertebral bodies has resulted in accrual of 110 contemporaneous controls. We present here an interim analysis of these patients at their most recent follow up.

MATERIALS AND METHODS

Patients

Since December 1992, 183 patients have simultaneously received transplants of ABO compatible donor BM and liver (n = 54), kidney (n = 35), kidney + pancreas (n = 32), kidney + islets (n = 7), heart (n = 21), lungs (n = 18), small bowel (n = 13) and multi-organ (n = 3). Additionally, since the initiation of a modified protocol in April 1996, 7 (liver n = 3, kidney n = 3, kidney + delayed islets n = 1) allograft recipients have received multiple infusions of donor BM consecutively from days 0 to 4 post Tx. The mean follow up was 498 ± 326 days and the mean recipient and donor ages were 42 ± 15 and 30 ± 15 years respectively. Furthermore, 110 (liver n = 31, kidney n = 40, heart n = 21, lungs n = 10, small bowel n = 7 and multi-organ n = 1) allograft recipients for whom donor BM was unavailable were monitored as controls. Their mean follow up was 552 ± 325 days and mean recipient and donor ages were 45 ± 15 and 33 ± 18 years respectively. The outcome in BM augmented (n = 8) and non-augmented (n = 2) kidney + islet recipients is detailed elsewhere in this issue (see Rastellini, et al).

BM Isolation

Cells from the vertebral bodies of cadaveric donors were isolated by a method described previously.[3] In patients receiving a single perioperative dose following organ revascularization, 3 to 5 × 10^8 unmodified cells/kg body weight were infused via a central intravenous line. In those who received multiple inocula of donor BM, 1 × 10^8 cells/kg body weight/d were infused consecutively from days 0 to 4 post Tx. The BM cells used in the latter protocol were maintained in culture and not cryopreserved prior to infusion.

Immunosuppression (IS)

The recipients were not subjected to any cytoablative or cytoreductive conditioning regimen prior to organ Tx and were maintained on routine IS which included tacrolimus and steroids. Minor adjustments in the doses of routine IS were made to treat all episodes of rejection. Only when steroid-resistant rejection was encountered was therapy with a short course of OKT3 or ATG deemed necessary.

In Vitro Studies

The immune status of the recipient was monitored serially by mixed leukocyte reaction, limiting dilution assay, proliferation against recall antigens, ConA and PHA; the methodology for which has been reported previously.[4] The results of these assays are reported elsewhere in this issue (see Zeevi, et al). For qualitative assessment of chimerism, recipients' PBMC were used in which the presence of donor cells was detected by cytofluorography and PCR. Serial quantitative evaluations of donor cell chimerism in a selected cohort of study and control patients was performed using a modified limiting dilution PCR assay, the details of which are reported elsewhere.[5,6] The multilineage character of chimerism was also ascertained by PCR detection of donor DNA in lineage$^+$ cells sorted from recipients' PBMC. The evidence for the presence of donor dendritic cell progenitors and therefore of engraftment was obtained by propagation of recipients' PBMC in rhGM-CSF and rhIL-4-enriched cultures using a protocol described previously.[7] Subsequent to enrichment for lineage^{null}/MHC class II$^+$ population, the presence of donor DNA within the sorted cells was confirmed by PCR analysis.

RESULTS AND DISCUSSION

The adjuvant BM infusion was safe and no complications uniquely ascribed to this procedure were witnessed in any of

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Table 1. Graft Function and Percent of BM-Augmented and Nonaugmented Organ Recipients Who Are Off Steroids and Are at Least 12 Months Posttransplantation

Organs Tx	n	Graft Function (X ± SD) TBili mg/dL	Creatinine mg/dL	Cardiac Output (L/min)	FEV_1 (L)	Off Steroids (%)
Liver						
Study	34	0.5 ± 0.3	—	—	—	23/34 (68)
Control	26	0.3 ± 0.2	—	—	—	18/26 (54)
Kidney						
Study	45	—	1.5 ± 0.5	—	—	32/45 (71)
Control	27	—	2.0 ± 0.9	—	—	13/27 (48)
Heart						
Study	10	—	—	4.9 ± 0.9	—	2/10 (20)
Control	9	—	—	5.0 ± 1.2	—	1/9 (11)
Lung						
Study	6	—	—	—	1.8 ± 0.5	1/6 (16.67)
Control	7	—	—	—	1.9 ± 0.9	0/7 (0)

the 190 organ allograft recipients. All but 17 of 190 (9%) BM augmented patients are alive as compared to 13/110 (12%) control recipients, who have died during the course of this follow up. It is noteworthy that no deaths in the augmented group were related to BM infusion. Additionally, grafts in nine (kidney n = 4, kidney + pancreas n = 1, pancreas n = 4) study patients were lost to causes unrelated to BM infusion. Similarly grafts in five (kidney n = 1, kidney + pancreas n = 1, pancreas n = 3) control patients were lost during the course of this follow up. No evidence of any complications was witnessed in any of the seven patients who had received multiple BM infusions during the course of their follow-up (19–114 d). All of the surviving patients have adequate graft function (Table 1).

The tempo, severity and cumulative incidence of rejection was comparable (~60%) in the patients in the study and control group. Graft versus host disease was only witnessed in 2 (1%) BM augmented recipients (both of liver) whose outcome has been reported previously.[3] In patients who are at least 12 months post Tx, a steroid-free existence has been achieved in 61% study and 46% control patients. Interestingly, a statistically higher percentage of kidney allograft recipients in the study group (71%) are off steroids as compared to that to controls (48%; Table 1). No patient is completely off IS.

Having previously reported that the incidence of chimerism is much higher in the study than in the control group,[3,9] we embarked on developing an assay for its serial quantitative assessment in a selected cohort of study and control patients. Using LDA-PCR assay, the levels of chimerism were found to be at least 10 to 100 fold higher in BM augmented recipients at up to 2 to 3 years post-Tx. The augmented levels of chimerism in the PBMC of study patients were indeed of multilineage character for the presence of donor DNA by PCR was confirmed in sorted T ($CD3^+$), B ($CD14^+$) and NK ($CD56^+$) cells. Unequivocal evidence for BM engraftment was obtained when the presence of donor DNA was confirmed within the $lineage^{null}$/class II^+ cells enriched from colonies harvested from cytokine-rich cultures which allows for selective propagation of dendritic cell progenitors.

Taken together these data suggest that single and multiple perioperative infusion of BM is safe and is associated with augmentation of chimerism. Although a higher incidence of steroid-free existence was witnessed in study as compared to control patients, it is too early to speculate if this observed outcome would sustain itself in a longer follow up. It is indeed interesting to note that the salutary effects of augmented chimerism on the incidence of evolvement of chronic rejection are already discernible in lung transplant recipients for 2 of 7 (29%) surviving control patients have evidence for the development of obliterative bronchiolitis, a complication yet not witnessed in the study population who have a comparable duration of follow up.

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