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Perioperative Donor Bone Marrow Infusion Augments Chimerism in Heart and Lung Transplant Recipients

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Abstract

Background—We and others have demonstrated that a low level of donor cell chimerism was present for years after transplantation in tissues and peripheral blood of heart and lung recipients; it was associated, in the latter, with a lower incidence of chronic rejection. To augment this phenomenon, we initiated a trial combining simultaneous infusion of donor bone marrow with heart or lung allotransplantation.

Methods—Between September 1993 and January 1995, 15 nonconditioned patients received either heart (n = 10) or lung (n = 5) allografts concurrently with an infusion of unmodified donor bone marrow (3.0×10^8 cells/kg), and were maintained on an immunosuppressive regimen consisting of tacrolimus and steroids.

Results—There was no complication associated with the infusion of donor bone marrow. Chimerism was detectable in 73% of bone marrow-augmented patients up to the last sample tested. Of the 5 control recipients who did not receive bone marrow infusion, only 1 had detectable chimerism by flow on postoperative day 15, which dwindled to an undetectable level by postoperative day 36. None of the patients had evidence of donor-specific immune modulation by mixed lymphocyte reaction.

Conclusions—The combined infusion of donor bone marrow and heart or lung transplantation, without preconditioning of the recipient, is safe and is associated with an augmentation of donor cell chimerism.

We and others have recently demonstrated that a low level of donor cells was detectable in the peripheral blood and tissues of long-surviving recipients of liver [1], kidney [2], heart [3], lung, and heart-lung [4] allografts. This phenomenon of donor cell chimerism, which occurs by seeding of the host's tissues with cells from the graft [5,6], was associated with a lower incidence of chronic rejection in lung recipients [4]. To augment donor cell chimerism, we initiated a prospective trial combining the simultaneous infusion of unmodified donor bone marrow and transplantation of heart or lung allografts into nonconditioned recipients. Reported herein is the outcome of the first 15 patients in this study.

Patients and Methods

Between September 1993 and January 1995, 15 patients received combined infusion of donor bone marrow and transplantation of either heart (n = 10) or lung allografts (n = 5). The mean age of the recipients was 46.3 ± 9.1 years (range, 23 to 57 years) with a mean follow-up of 175 ± 102 days. These patients, all primary transplant recipients of cadaveric organs, were not conditioned by cytoablative or cytoreductive regimen before transplantation. Furthermore, all recipients had a panel reactive antibody titer of less than 10%, and none had a positive lymphocytotoxic crossmatch. The mean number of HLA mismatches was 4.5 ± 1.2 (range, 3 to 6), with no patient having complete HLA compatibility with the donor.

Bone Marrow Preparation and Infusion

Details of bone marrow preparation are described elsewhere [7]. Briefly, thoracolumbar vertebrae were retrieved from the donor. Marrow cells from chipped-off cancellous bone were passively released into a processing medium, filtered, washed, and resuspended in a suspension medium at a concentration of 2×10^7 cells/mL. The cell suspension was stored at 4°C until infusion. Cell viability was determined by trypan blue dye exclusion, and samples of processed bone marrow cells were retained for microbial testing and routine progenitor cell assays. When the recipient was ready to receive bone marrow, a total of 3.0×10^8 unmodified cells/kg of body weight were resuspended in 200 mL of the suspension medium and infused over a period of 15 to 20 minutes via a central venous line. The bone marrow was usually infused between 6 to 10 hours after revascularization of the transplanted organ.

Immunosuppression

Immunosuppression consisted of tacrolimus (FK506 [Prograf]; Fujisawa USA, Deerfield, IL) and steroids, as previously described [8]. During the first postoperative month, the dosage of tacrolimus was targeted to maintain whole blood trough levels of 15 to 20 ng/mL which, depending on the side effects and history of rejection, was gradually reduced to achieve levels of 5 to 15 ng/mL. Methylprednisolone (1 g) was given intraoperatively before revascularization of the organ. Except in the first heart recipient, this was followed in all other patients by a short course of steroid recycle starting on postoperative day (POD) 1 with 200 mg/day and tapering to 20 mg/day by POD 5. Further steroid reductions were individually tailored according to allograft function. Azathioprine was added if there was recurrent or recalcitrant rejection, or when renal dysfunction necessitated the administration of a lower than required dose of tacrolimus. Rejection was treated initially with steroid boluses (1 g methylprednisolone/day $\times$ 3), whereas OKT3 was reserved for steroid-resistant rejection.

In Vitro Monitoring

Mononuclear cells from recipients' peripheral blood (PBMC) were obtained preoperatively and biweekly in the first postoperative month, and bimonthly thereafter for detection of donor cells and for immunologic monitoring.

Detection of Chimerism

flow cytometry—For immunocytochemical staining, primary mouse–anti-human monoclonal antibodies directed against the polymorphic epitopes of either HLA class I or class II (to distinguish donor from recipient HLA alleles) were used. These primary monoclonal antibodies were labeled by either fluorescein isothiocyanate- or phycoerythrin-conjugated goat–anti-mouse secondary antibodies. The specificity and optimal dilution of these antibodies were determined using donor splenocytes and the recipient's pretransplantation PBMC. Single or two-color flow cytometric methods were used to identify the donor cells and their lineage,

respectively. Fifty thousand events were acquired at each determination, and the frequency of donor cells less than 0.5° was considered below the reliable detection threshold.

polymerase chain reaction—In addition to the flow cytometric analysis, polymerase chain reaction, as previously described [1,3,5], was used for detection of donor DNA in the recipient's PBMC. This method is more sensitive than the flow cytometric technique: it can reliably detect one donor cell within 10^4 to 10^5 recipient cells [7]. Oligonucleotides for either the sex determining region of the Y chromosome or the appropriate mismatched HLA alleles were used as primers.

Immune Monitoring

The in vitro immune status of the recipients, before and after transplantation, was assessed by the proliferative response of their PBMC to mitogens (concanavalin A, phytohemagglutinin), and recall antigens (tetanus toxoid) by mixed lymphocyte reactions (MLR) and by cell-mediated lymphocytotoxicity assays. The MLR cultures were carried out using γ -irradiated donor splenocytes and third-party PBMC as stimulators (5×10^4 cells), and recipient PBMC as responders (5×10^4 cells). The cells were cultured at 37° for 6 days in 5% CO_2 in air. (^3H)-thymidine ($1 \mu\text{Ci}$) was added to each well at the beginning of the final 20 hours, and its degree of incorporation was determined by liquid scintillation counting. For cell-mediated lymphocytotoxicity assays, phytohemagglutinin-activated ^{51}Cr -labeled donor splenocytes and third-party PBMC were used as targets to evaluate the effector function of 5- to 6-day MLR-cultured recipient's PBMC. Various effector:target ratios ranging from 10:1 to 40:1 were used.

Results

Clinical Course

bone marrow augmented patients—The infusion of donor bone marrow was well tolerated. None of the 15 recipients had graft-versus-host disease or complications related to the infusion of donor bone marrow. All patients, except 1, are alive with good allograft function (Table 1). The single death occurred in a heart recipient (patient 5) who died at home of a pulmonary embolus on POD 267. One week before his death, a routine right heart catheterization revealed normal cardiac function. At autopsy, there was no evidence of acute or chronic rejection in the transplanted heart (Fig 1). In 1 heart recipient (patient 10), who had been receiving aspirin preoperatively, a duodenal perforation developed on POD 3. In another heart recipient (patient 1), a benign duodenal ulcer developed on POD 237 that was successfully treated, whereas an additional heart recipient (patient 8) had *Acinetobacter* sepsis from a pneumonia on POD 92, which resolved after appropriate therapy.

Furthermore, two single-lung recipients (patients 14 and 15), who received an allograft from the same donor, suffered moderate to severe primary graft dysfunction (preservation injury), which in 1 (patient 14) necessitated support with an extracorporeal membrane oxygenator for 3 days. Two other lung recipients had *Candida albicans* in bronchoalveolar lavage on POD 137 and 221 respectively, and 1 had *Aspergillus fumigatus* (patient 13) on POD 58. All were successfully treated.

controls—Heart or lung recipients for whom bone marrow was not available, due to our inability to obtain permission to retrieve cadaveric vertebral bodies, were used as contemporaneous controls. All 4 heart recipients are alive with good graft function, whereas the single lung recipient (patient 20) died on POD 104 due to complications related to preservation injury. This lung recipient required perioperative support with an extra-corporeal membrane oxygenator because of primary graft failure.

Rejection

In the 10 heart-bone marrow recipients, the rate of rejection (grade $\geq 3A$ [9]) during the first 100 days after transplantation was 0.5 episodes, as compared with 1.0 ($p = 0.27$ by Fisher's exact test) in a historical control group of 26 heart recipients, who received an identical immunosuppressive regimen without bone marrow infusion. Only 1 heart recipient (patient 5), who did not receive steroid induction during the perioperative period (no steroid recycle during the first 5 postoperative days), had steroid-resistant rejection that required a 5-day course of OKT3 for its resolution. Furthermore, 3 additional heart recipients required azathioprine because of persistent low-grade ($\leq$ grade 2) rejection and high serum creatinine levels that precluded the use of therapeutic doses of tacrolimus. Of the 5 lung-bone marrow recipients, 3 had mild to moderate rejection (grade $> II$ [10]) on POD 11, 32, and 53 respectively, whereas 2 had a rejection-free postoperative course.

Donor Chimerism

Detection of donor cells was feasible in all bone marrow-augmented and nonaugmented patients by either flow cytometry or polymerase chain reaction. Control patients had no evidence of donor-cell chimerism in their PBMC at the most recent sample tested (Table 2). On the contrary, 11/15 study patients (73%) exhibited stable donor cell chimerism for up to 220 days after transplantation. It must be emphasized, however, that bone marrow-augmented recipients who were negative by polymerase chain reaction for donor cell chimerism in the last sample tested were positive in all previous analyses.

In Vitro Immune Testing

The unavailability of donor splenocytes precluded in vitro immune monitoring in 8/15 (53%) of bone marrow-augmented recipients. None of the bone marrow-augmented ($n = 7$) or nonaugmented ($n = 4$) patients in whom testing was feasible exhibited any evidence of donor-specific immune modulation (by MLR) for up to 210 days after transplantation (Table 3). It is nevertheless noteworthy that there was a trend in the bone marrow-augmented recipients toward a lower response to donor as compared with third party alloantigens when tested serially during the first 6 months after transplantation.

Comment

The use of bone marrow-derived cells (splenocytes) to achieve donor-specific transplantation tolerance in neonatal mice was first reported by Billingham and associates [11]. Subsequently, chimerism with donor-specific transplantation tolerance was achieved in adult animal models by preconditioning the host with different regimens, which have included, among others, total body irradiation [12], total lymphoid irradiation [13], and the use of antilymphocyte globulin [14].

The clinical use of donor bone marrow to prolong the survival of organ allografts was first attempted in kidney transplant recipients. Monaco and associates [15] first reported the use of antilymphocyte globulin and delayed (25 days after organ transplantation) donor bone marrow infusion in a kidney transplant recipient. The patient had no rejection during the postoperative follow-up until she died 8 months after transplantation of fatal peritonitis, secondary to perforated sigmoid diverticulitis. There was evidence of donor red cell chimerism; however, it did not persist after the first month, and no white cell chimerism was detected for up to 2½ months after transplantation. Barber and associates [16] used a similar regimen in recipients of cadaveric donor kidneys who were preconditioned with antilymphocyte globulin, cyclosporine, azathioprine, and prednisone before adjuvant donor bone marrow infusion. Graft survival in the bone marrow-augmented patients was significantly better than in contemporaneous controls, and other clinical evidence of benefit was also present, including

a reduced need for immunosuppression and a lower incidence of rejection in bone marrow–augmented chimeric recipients. Furthermore, donor cell chimerism was detected in 50% to 56% of patients 3 to 12 months after transplantation [17]. However, using a similar approach, Rolles and colleagues [18] were unable to show any distinct advantage that was afforded by delayed bone marrow transplantation to liver allograft recipients.

In 1984, Kahn and co-workers [19] reported the combined simultaneous infusion of donor bone marrow to 6 heart transplant recipients who were preconditioned (7 to 13 days earlier) with a total of 5.4 to 6.0 Gy of total lymphoid irradiation. Four patients died within 7 months of either primary graft failure (n = 1), chronic rejection (n = 1), or infection (n = 2). Furthermore, in the 2 patients who survived for 2 to 4 years after transplantation, donor-specific hyporeactivity (by MLR) was evidenced in 1, whereas it was not tested in the other [19]. The higher incidence of infection in patients in this study was attributed to a combination of high dose of steroids and total lymphoid irradiation.

These clinical studies were based on the premise that “space” needs to be created by preconditioning of the host with cytoreductive or cytoablative therapy, thus allowing for engraftment of donor bone marrow with subsequent establishment and perpetuation of chimerism. However, we and others have recently demonstrated that donor chimerism is a naturally occurring phenomenon after transplantation of solid organs, including the liver [1], kidney [2], heart [3], and lung [4]. This low level of donor cell chimerism was present in all long-term surviving kidney [2] and liver [1] recipients. Its beneficial effects were most noticeable in lung allograft recipients, in whom donor cell chimerism was associated with a lower incidence of bronchiolitis obliterans [4]. These observations provided the foundation for the initiation of the current study, in which unmodified MHC-mismatched donor bone marrow cells were infused into heart or lung recipients at the time of organ placement without any preconditioning of that host or deviation from the routine drugs and their therapeutic doses that were required for maintenance of adequate immunosuppression.

The preliminary data from this pilot study indicate that the infusion of unmodified donor bone marrow concurrently with heart or lung transplantation is safe, and is associated with an increased level of donor cell chimerism. Furthermore, the early immunologic events after cardiac transplantation appear to have been altered by the infusion of donor bone marrow. Although not statistically significant, there was a trend toward a lower incidence of rejection within the first 100 days after transplantation in the bone marrow–augmented heart recipients as compared with similarly treated historical controls. However, there was no in vitro evidence of donor-specific hyporeactivity in the serial MLR analysis performed during the first 6 months after transplantation.

Although the eventual effect of the augmented chimerism remains speculative, it is conceivable that its presence would enhance the acceptance and survival of the graft and reduce the incidence of chronic rejection.

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Fig 1.

Histopathology of an endomyocardial biopsy specimen of a bone marrow and heart transplant recipient obtained after death at 267 days after transplantation. There was no cellular infiltration, suggesting that the death of this patient was due to nonimmunologic causes. Although the exact cause of death is still unknown, a relatively large thromboembolus in the pulmonary artery was detected at autopsy. ($\times 40$ before 51% reductions.)

Table 1
Outcome of Heart and Lung Recipients Receiving Donor-Specific Bone Marrow Infusion

Patient No.	Graft	POD	Status	Graft Function			Immunosuppression			Complication ^a	Rejection ^a	GvHD
				LVEF ^a	FVC (L)	FEV ₁ ^a (L)	FK506 (mg/day)	Pred (mg/day)	Aza (mg/day)			
1	Heart	303	Alive	ND	...	...	8	10	0	Duodenal ulcer (237)	None	None
2	Heart	303	Alive	ND	...	...	10	5	0	None	None	None
3	Heart	274	Alive	0.88 (14)	...	...	8	10	100	None	None	None
4	Heart	274	Alive	0.07 (14)	...	...	18	15	100	None	None	None
5	Heart	267	Dead	0.66 (25)	...	...	...	...	...	Pulmonary embolus (267)	3A (13)	None
6	Heart	262	Alive	0.55 (11)	...	...	28	5	50	None	3A (15)	None
7	Heart	240	Alive	0.73 (25)	...	...	6	10	0	None	None	None
8	Heart	199	Alive	0.85 (10)	...	...	6	10	0	Gram-negative sepsis (92)	None	None
9	Heart	50	Alive	ND	...	...	36	20	0	None	3A (40)	None
10	Heart	44	Alive	ND	...	...	12	15	0	Duodenal perforation (3)	3A (24)	None
11	SL	227	Alive	...	1.76	1.29 (191)	6	5	0	<i>Candida albicans</i> (137)	None	None
12	SL	227	Alive	...	2.59	1.85 (191)	20	10	0	<i>Candida albicans</i> (221)	AR II (8)	None
13	DL	87	Alive	...	1.95	1.65 (51)	2	5	0	<i>Aspergillus fumigatus</i> (58)	None	None
14	SL	59	Alive	...	ND	ND	8	20	0	Preservation injury (1)	AR III (32)	None
15	SL	59	Alive	...	1.4	0.81 (28)	10	20	0	Preservation injury (1)	AR III (11)	None

Aza = azathioprine; DL = double lung; FEV₁ = forced expiratory volume in the first second; FVC = forced vital capacity; GvHD = graft versus host disease; LVEF = left ventricular ejection fraction; ND = not done; POD = postoperative day; Pred = prednisone; SL = single lung.

^aNumbers in parentheses are postoperative day.

Table 2

Detection of Donor Leukocytes in Recipient's Peripheral Blood Monocytes by Flow Cytometry and Polymerase Chain Reaction

Patient No.	Graft	Bone Marrow	Flow Cytometry ^a							PCR (POD)
			POD 7-15	POD 20-35	POD 50-70	POD 105-135	POD 150-220			
1	Heart	Yes	NF	NF	NF	NF	NF	NF	- (239)	
2	Heart	Yes	NF	NF	NF	NF	NF	NF	+ (302)	
3	Heart	Yes	NF	NF	NF	NF	NF	NF	- (225)	
6	Heart	Yes	NF	NF	NF	NF	NF	NF	- (220)	
4	Heart	Yes	0.7	1.8	<0.5	<0.5	<0.5	<0.5	+ (274)	
5	Heart	Yes	2.7	1.1	2.1	ND	6.3	6.3	+ (246)	
7	Heart	Yes	1.3	0.7	0.8	0.9	1.0	1.0	+ (199)	
8	Heart	Yes	1.7	1.2	1.2	0.8	ND	ND	+ (109)	
9	Heart	Yes	1.0	0.8	ND	ND	ND	ND	+ (11)	
10	Heart	Yes	0.8	ND	ND	ND	ND	ND	+ (35)	
11	SL	Yes	1.3	2.8	1.7	2.1	1.1	1.1	+ (183)	
12	SL	Yes	1.4	1.6	1.5	0.9	0.7	0.7	- (185)	
13	DL	Yes	2.7	1.3	0.8	ND	ND	ND	+ (66)	
14	SL	Yes	1.1	1.5	0.9	ND	ND	ND	+ (36)	
15	SL	Yes	1.7	1.5	ND	ND	ND	ND	+ (36)	
16	Heart	No	<0.5	ND	ND	ND	ND	ND	ND	
17	Heart	No	ND	ND	<0.5	ND	ND	ND	- (107)	
18	Heart	No	ND	0.5	<0.5	ND	<0.5	<0.5	- (231)	
19	Heart	No	ND	ND	ND	<0.5	<0.5	<0.5	- (205)	
20	SL	No	2.8	<0.5	ND	ND	ND	ND	- (36)	

DL = double lung; ND = not done either due to short follow-up or lack of adequate volume of sample; NF = not feasible; POD = postoperative day; SL = single lung.

^aExpressed as percent of donor cells detected in peripheral blood monocytes of the recipient.

Table 3
Mixed Lymphocyte Response of Recipient's Lymphocytes Against Donor and Third-Party Splenocytes^a

Patient No.	Graft	Bone Marrow	Before Transplantation			Postoperative Day							
			D	TP	D/TP (%)	15-60		70-120		130-240		D/TP (%)	D/TP (%)
						D	TP	D	TP	D	TP		
1	Heart	Yes	86.7	137.9	63.0	ND	ND	37.6	61.5	20.4	53.5	38.0	38.0
3	Heart	Yes	87.9	88.8	99.0	83.4	49.7	13.6	2.3	598.0	118.2	88.1	134.0
4	Heart	Yes	36.1	34.2	105.0	18.0	34.5	30.6	30.9	69.1	75.4	92.0	92.0
5	Heart	Yes	34.5	63.6	54.0	22.1	29.2	76.0	ND	1.6	4.2	37.0	37.0
6	Heart	Yes	21.8	9.3	234.0	68.7	18.0	381.0	49.2	250.0	24.0	18.1	133.0
7	Heart	Yes	92.0	62.6	147.0	12.4	14.2	88.0	45.5	25.3	37.3	68.0	68.0
8	Heart	Yes	44.8	27.7	162.0	27.4	22.7	121.0	64.5	ND	ND	ND	ND
Mean					123.4							194.5	83.7
SD					62.8							209.5	43.7

D = donor cells; NO = not done; SD = standard deviation; TP = third-party.

^aCounts per minute ($\times 10^3$).