1845

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

Si M. Pham, MD, Robert J. Keenan, MD, Abdul S. Rao, MD, DPhil, Paulo A. Fontes, MD, Robert L. Kormos, MD, Kareem Abu-Elmagd, MD, Adriana Zeevi, PhD, Akihiko Kawai, MD, Brack G. Hattler, MD, PhD, Robert L. Hardesty, MD, Anthony J. Demetris, MD, Massimo M. Trucco, MD, Gayle L. Rosner, PhD, John J. Fung, MD, PhD, Thomas E. Starzl, MD, PhD, and Bartley P. Griffith, MD

Pittsburgh Transplantation Institute and the Departments of Surgery and Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania

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.

(Ann Thorac Surg 1995;60:1015–20)

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

Presented at the Thirty-First Annual Meeting of The Society of Thoracic Surgeons, Palm Springs, CA, Jan 31–Feb 2, 1995.

Address reprint requests to Dr Pham, Suite C-700, PUH, 200 Lothrop St, Pittsburgh, PA 15213.

0003-4975/95/$9.50
SSDI 0003-4975(95)00579-A

Ann Thorac Surg
1995;60:1015–20

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 × 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°C for 6 days in 5% CO_2 in air. (^{3}H)-thymidine (1 μCi) 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, phytohemogglutinin-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-

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	0.70 (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	*Candida albicans* (137)	None	None
12	SL	227	Alive	...	2.59	1.85 (191)	20	10	0	*Candida albicans* (221)	AR II (8)	None
13	DL	87	Alive	...	1.95	1.65 (51)	2	5	0	*Aspergillus fumigatus* (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

[a] Numbers in parentheses are postoperative day.

Aza = azathioprine; DL = double lung; FEV_1 = 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.

corporeal membrane oxygenator because of primary graft failure.

Rejection

In the 10 heart-bone marrow recipients, the rate of rejection (grade ≥ 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 (≤ 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.

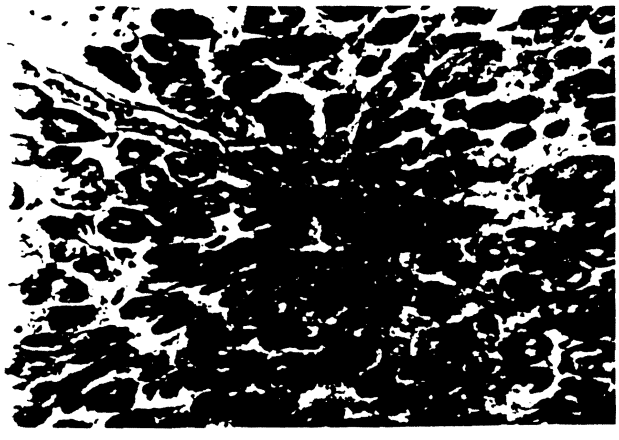

Fig 1. Histopathology of an endomyocardial biopsy specimen of a bone marrow and heart transplant recipient obtained after death at 267 days after transplantation. There was no cellular infiltration, suggesting that the death of this patient was due to nonimmunologic causes. Although the exact cause of death is still unknown, a relatively large thromboembolus in the pulmonary artery was detected at autopsy. (× 40 before 51% reduction.)

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

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] POD 7–15	POD 20–35	POD 50–70	POD 105–135	POD 150–220	PCR (POD)
1	Heart	Yes	NF	NF	NF	NF	NF	− (239)
2	Heart	Yes	NF	NF	NF	NF	NF	+ (302)
3	Heart	Yes	NF	NF	NF	NF	NF	− (225)
6	Heart	Yes	NF	NF	NF	NF	NF	− (220)
4	Heart	Yes	0.7	1.8	<0.5	<0.5	<0.5	+ (274)
5	Heart	Yes	2.7	1.1	2.1	ND	6.3	+ (246)
7	Heart	Yes	1.3	0.7	0.8	0.9	1.0	+ (199)
8	Heart	Yes	1.7	1.2	1.2	0.8	ND	+ (109)
9	Heart	Yes	1.0	0.8	ND	ND	ND	+ (11)
10	Heart	Yes	0.8	ND	ND	ND	ND	+ (35)
11	SL	Yes	1.3	2.8	1.7	2.1	1.1	+ (183)
12	SL	Yes	1.4	1.6	1.5	0.9	0.7	− (185)
13	DL	Yes	2.7	1.3	0.8	ND	ND	+ (66)
14	SL	Yes	1.1	1.5	0.9	ND	ND	+ (36)
15	SL	Yes	1.7	2.5	ND	ND	ND	+ (36)
16	Heart	No	<0.5	ND	ND	ND	ND	ND
17	Heart	No	ND	ND	<0.5	ND	ND	− (107)
18	Heart	No	ND	0.5	<0.5	ND	<0.5	− (231)
19	Heart	No	ND	ND	ND	<0.5	<0.5	− (205)
20	SL	No	2.8	<0.5	ND	ND	ND	− (36)

[a] Expressed as percent of donor cells detected in peripheral blood monocytes of the recipient.

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.

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

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

Patient No.	Graft	Bone Marrow	Before Transplantation D	TP	D/TP (%)	Postoperative Day 15–60 D	TP	D/TP (%)	70–120 D	TP	D/TP (%)	130–240 D	TP	D/TP (%)
1	Heart	Yes	86.7	137.9	63.0	ND	ND	ND	37.6	61.5	61.0	20.4	53.5	38.0
3	Heart	Yes	87.9	88.8	99.0	83.4	49.7	168.0	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	52.0	30.6	30.9	99.0	69.1	75.4	92.0
5	Heart	Yes	34.5	63.6	54.0	22.1	29.2	76.0	ND	ND	ND	1.6	4.2	37.0
6	Heart	Yes	21.8	9.3	234.0	68.7	18.0	381.0	49.2	19.7	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	49.7	91.0	25.3	37.3	68.0
8	Heart	Yes	44.8	27.7	162.0	27.4	22.7	121.0	43.7	64.5	68.0	ND	ND	ND
Mean					123.4			147.7			194.5			83.7
SD					62.8			121.2			209.5			43.7

[a] Counts per minute ($\times 10^3$).

D = donor cells; ND = not done; SD = standard deviation; TP = third-party.

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.

We thank Michael Amaya, BSN, Ann Lee, MSN, Terri L. Rapp, BSN, Jan D. Manzetti, PhD, Gerene S. Bauldoff, MSN, and Wendy Donato-Deis, BSN, for their clinical assistance; Troy S. Seskey, BS, Joshua E. Phipps, BS, and Charles M. Vasko for processing bone marrow and blood samples for chimerism study; Mary C. Pavlick, BA, and Richard A. Banas, MS, for their help in immune monitoring; Merrit L. Lutz for her help with data collection; Jo B. Harnaha, BA, for her help with preparation of the manuscript; and Rhonda J. White and Aurelia M. Tambourine for their excellent secretarial assistance.

Support in part by the American College of Surgeons Faculty Fellowship to Si M. Pham, and National Institutes of Health grant DK 29961 to Thomas E. Starzl.

References

1. Starzl TE, Demetris A, Thrice M, et al. Systemic chimerism in human female recipients of male livers. Lancet 1992;340: 876–77.
2. Starzl TE, Demetris AJ, Trucco M, et al. Chimerism and donor specific nonreactivity 27 to 29 years after kidney allotransplantation. Transplantation 1993;55:1272–7.
3. Schlitt HJ, Hundrieser J, Hisanaga M, et al. Patterns of donor-type microchimerism after heart transplantation. Lancet 1994;343:1469–71.
4. Keenan RJ, Zeevi A, Banas R, et al. Microchimerism is associated with a lower incidence of chronic rejection after lung transplantation. J Heart Lung Transplant 1994;13:532.
5. Starzl TE, Demetris AJ, Murase N, et al. Cell migration, chimerism and graft acceptance. Lancet 1992;339:1579–82.
6. Starzl TE, Demetris AJ, Trucco M, et al. Cell migration and chimerism after whole organ transplantation. Hepatology 1993;17:1127–52.
7. Fontes P, Rao AS, Demetris AJ, et al. Bone marrow augmentation of donor-cell chimerism in kidney, liver, heart, and pancreas islet transplantation. Lancet 1994;344:151–5.
8. Armitage JM, Kormos RL, Morita S, et al. Clinical trial of FK506 immunosuppression in adult cardiac transplantation. Ann Thorac Surg 1992;54:205–10.
9. Billingham ME, Cary NRB, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. J Heart Transplant 1990;9:587–92.
10. Yousem SA, Berry GJJ, Brunt EM, et al. A working formula-

tion for the standardization of nomenclature in the diagnosis of heart and lung rejection: lung rejection study group. J Heart Transplant 1990;9:593–601.
11. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature 1953;172:603–6.
12. Ildstad ST, Sachs DT. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. Nature 1984;307:168–70.
13. Slavin S, Strober S, Fukes Z, et al. Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. J Exp Med 1977;146:34–48.
14. Caridis DT, Liegeois A, Barret I, et al. Enhanced survival of canine renal allografts of ALS-treated dogs given bone marrow. Transplant Proceed 1973;5:671–4.
15. Monaco AP, Clark AW, Wood ML, et al. Possible active enhancement of a human cadaver renal allograft with antilymphocyte serum (ALS) and donor bone marrow: case report of an initial attempt. Surgery 1976;79:384–92.
16. Barber WH, Mankin JA, Laskow DA, et al. Long-term results of a controlled prospective study with transfusion of donor specific bone marrow in 57 cadaveric renal allograft recipients. Transplantation 1991;51:70–5.
17. McDaniel DO, Naftilan J, Hulvey K, et al. Peripheral blood chimerism in renal allograft-recipients with donor bone marrow. Transplantation 1994;57:852–6.
18. Rolles K, Burrough AK, Davidson BR, et al. Donor-specific bone marrow infusion after orthotopic liver transplantation. Lancet 1994;343:263–5.
19. Kahn DR, Hong R, Greenberg AJ, et al. Total lymphatic irradiation and bone marrow in human heart transplantation. Ann Thorac Surg 1984;38:169–71.

DISCUSSION

DR BRUCE A. REITZ (Stanford, CA): Doctor Pham should be congratulated for his presentation and Drs Griffith and Starzl for this very difficult clinical trial. They have demonstrated in their patients in these early results an increased incidence of microchimerism when compared with concurrent controls, and their hypothesis is that this chimerism will result in better long-term acceptance of the graft and perhaps a decrease in chronic rejection. Most previous work on bone marrow infusion at the time of transplantation has attempted to create donor-specific tolerance, perhaps an unacceptable or unobtainable clinical goal at the present time, but when bone marrow has been given it has usually been accompanied with some type of induction therapy, such as total lymphoid irradiation, whole body radiation, or antithymocyte globulin. My first question for Dr Pham is why induction therapy was not used in these patients. Even antithymocyte globulin alone would provide some induction, and antithymocyte globulin has been used as part of the lung transplant protocols at Pittsburgh in the past.

Second, Dr Pham, can you comment on how you might achieve a decrease in chronic rejection if there has been no significant decrease in the early rejection frequency or a significant increase in donor-specific hyporeactivity? I think your data show trends in this regard but the manuscript clearly shows that at this point there is no statistical difference.

Third, would you share with us some of the studies of FK 506 and donor bone marrow infusion and how this combination compares with cyclosporine in terms of chimerism induction?

Finally, the lack of graft-versus-host disease is very encouraging in this study, but I would give a word of caution in that in experiments that we have done with bone marrow infusion, the exact combination of accompanying immunosuppression is extremely important and minor changes can result in either hypersensitivity or, on the other hand, the induction of tolerance. So it is very important to have a very specific protocol and to follow it very closely. Doctor Pham, please comment on graft-versus-host disease in recipients of liver transplant and how those protocols might differ from your heart protocol.

Again, congratulations on what is a very difficult clinical study. We look forward next year or perhaps the year after to getting the long-term follow-up to see if your hypothesis has been validated.

DR JOHN R. BENFIELD (Sacramento, CA): While Dr Pham is coming to the microphone to respond to the questions, my sense of history allows me to comment that Dr David Blumenstock of Cooperstown, NY, and Dr Thomas of bone marrow transplant fame investigated the concept of chimerism as an adjunct to lung transplantation in inbred beagles almost 30 years ago.

DR PHAM: Thank you, Dr Reitz, for your comments. We did not use induction therapy such as antithymocyte globulin or radiation because we did not know what the antithymocyte globulin would do to the bone marrow stem cells. Doctor Barber and his associates from Birmingham, Alabama, had used antithymocyte globulin induction in their combined kidney-bone marrow trial and had observed an increase in the level of chimerism. However, their protocol was different from ours in that they had infused frozen bone marrow cells approximately 21 days after the patient had received a kidney transplant. We did not use preoperative radiation because of our concern for subjecting the patients to a higher risk of graft-versus-host-disease, and because of the difficult logistics due to the time constraint.

On your question about chronic rejection, the only available data on chimerism and chronic rejection are those reported by our group. We had shown that lung recipients with microchimerism had a lower risk of obliterative bronchiolitis development. These data form the basis for the current trial.

We have not used cyclosporine in recipients of combined bone marrow and heart or lung transplants. To date, we have not seen graft-versus-host disease in heart, lung, or kidney recipients who received concurrent bone marrow infusion. However, mild graft-versus-host disease developed in 2 of the 28 liver-bone marrow recipients. The graft-versus-host disease resolved spontaneously in 1, and with an increase in the prednisone dose from 7.5 mg/day to 15 mg/day in another.