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BRIEF COMMUNICATIONS

COMBINED KIDNEY/BONE MARROW TRANSPLANTATION—EVIDENCE OF AUGMENTATION OF CHIMERISM[1,2]

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Recent studies on patients who have maintained stable hepatic and renal allograft function for 10–28 years have revealed systemic microchimerism in all (*1*, *2*). With the belief that this is the essential mechanism of allograft acceptance, we sought to augment the spontaneous chimerism in newly transplanted recipients, and began a program of combined simultaneous kidney/bone marrow transplantation without pretransplant radiation or other cytoreduction therapy (*3*). Postoperative immunosuppression was with FK506 and prednisone, using these drugs in the same way as in standard practice (*4*).

Seventeen cadaver kidney recipients were entered into the bone marrow augmentation series up to June 1994. All are well. We report here the first 10, whose simultaneous kidney-bone marrow transplantations were between December 14, 1992 and December 14, 1993. Two of them also received pancreatic islets. The mean recipient age was 41.3±13.7 years (range 24.6–63.4). All patients were undergoing their first transplantation and had a panel-reactive antibody level of less than 15%. The mean number of HLA matches and mismatches was 2.4±1.3 (range 1–5) and 3.2±1.8 (range 1–6), respectively. The mean donor age was 25.0±7.5 years, and the mean cold ischemia time was 26.5±9.4 hr.

Eight control patients undergoing their first cadaveric kidney transplantation but not receiving bone marrow were also studied. The most common reason for not performing the combined procedure in the control cases was refusal of the donor family to consent to vertebral body recovery. Their mean age was 44.0±11.0 years (range 29.7–59.8). The mean number of HLA matches and mismatches was 3.5±1.9 (range 1–6) and 2.4±2.0 (range 0–5). The mean donor age was 50.3±12.4 years, and the mean cold ischemia time was 31.3±3.6 hr.

Neither irradiation nor any other kind of recipient preconditioning was used in the bone marrow recipients. All blood transfusions, if needed, were with irradiated packed red blood cells. After completion of the renal transplant procedure, 3×10^8 unmodified bone marrow cells/kg, isolated from the donor vertebral bodies, were infused via central line (*3*). Bone marrow infusion was performed at the time of closure of the transplant incision, or shortly thereafter in the recovery room. In the 2 patients who received islets, isolation was according to previously described techniques (*5*). The islets were infused into the portal vein, after completion of the kidney transplant. All of the protocols were approved by the Institutional Review Board of the University of Pittsburgh.

Just prior to transplantation, 40 ml of blood was drawn and placed in heparinized tubes. Postoperatively, blood was drawn weekly for the first month, and monthly thereafter, for chimerism studies, which included fluorescent activated cell sorter analysis (FACS), polymerase chain reaction (PCR), and Y-chromosome analysis by both competitive PCR (cPCR) and fluorescent in-situ hybridization (FISH) of cytospin samples (in the case of female recipients of kidneys from the male donors).

The mean follow-up for the 10 kidney/bone marrow recipients is 9.9±4.7 months; all are alive and well, and all have functioning allografts. The mean serum creatinine and BUN are 1.6±0.4 mg/dl, and 23±8 mg/dl. Five (50%) patients have been weaned off steroids. The two islet recipients remain on insulin; they both have evidence of C-peptide production (0.44 and 0.11 pmol/ml), but at levels insufficient to allow for insulin independence. Two (20%) patients experienced early kidney nonfunction and required dialysis during the first postoperative week. The incidence of acute rejection was 50% (5 patients). All rejection episodes were responsive to steroids and/or an increase in the FK 506 dosage. No patient required antilymphocyte therapy. Cytomegalovirus (CMV) was seen in 2 (20%) patients and was treated successfully with intravenous gancyclovir. Graft vs. host disease (GVHD) was not seen any patient.

The control recipients of a kidney only have been followed for 4.9±0.5 months; all are alive and have functioning allografts (Table 1). The mean serum creatinine and BUN are 2.4±1.0 mg/dl and 50±32 mg/dl. None of these patients has been weaned off prednisone as of yet—however, this is most likely related to the relatively short follow-up. Two (25%) patients had acute tubular necrosis (ATN), although only 1

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TABLE 1. Summary of clinical course

	Initial function	Rejection	CMV	Serum creatinine (mg/dl)	BUN (mg/dl)	FK506 (mg/day)	Prednisone (mg/day)
Kidney/bone marrow							
1	No	No	No	1.1	19	36	7.5
2	Yes	Yes	No	1.3	15	20	12.5
3	Yes	No	No	1.1	22	9	0
4	Yes	No	No	1.5	28	18	2.5
5	Yes	Yes	Yes	2.3	37	8	12.5
6	Yes	Yes	No	1.7	22	24	0
7	Yes	No	Yes	2.0	11	4	0
8	No	Yes	No	1.9	19	18	10
9	Yes	Yes	No	1.2	32	4	0
10	Yes	No	No	1.7	28	9	0
	8 (80%)	5 (50%)	2 (20%)	1.6±0.4	23±8	15±10	4.5±5.5
Kidney alone							
1	Yes	No	No	1.3	21	12	10
2	Yes	Yes	No	1.9	39	20	10
3	No	Yes	Yes	3.5	53	30	7.5
4	Yes	No	No	1.4	22	21	5
5	Yes	Yes	Yes	4.2	96	6	25
6	Yes	No	No	2.2	103	4	12.5
7	Yes	Yes	No	2.4	33	8	5
8	No	Yes	No	2.4	32	14	7.5
	6 (75%)	5 (63%)	2 (25%)	2.4±2.0	50±32	14.4±8.8	10.3±6.8

(13%) required dialysis during the first week after transplantation. The incidence of acute rejection was 63% (5 patients). All rejections were responsive to steroids and an increase in FK506. CMV was seen in 2 (25%) patients; both patients were successfully treated with gancyclovir. GVHD was not seen.

In the kidney/bone marrow patients, evidence of chimerism was present by at least one of the three modalities in 9 of 9 (100%) evaluable patients (Table 2). The tenth patient, who received a 1-B, 2-DR matched same-sex allograft, was not evaluable for microchimerism by any of these technologies. FACS was positive in 5 of 7 patients in whom evaluation was feasible, with a range of 0.9–3.0%. PCR for HLA disparities was positive in 8 of 8 patients in whom the study could be performed (Fig. 1). In the three female recipients of kidneys from male donors, Y-chromosome detection by PCR was positive in all cases and in one, competitive PCR (6) demonstrated a concentration of 0.5% donor DNA in PBMCs at 1 year. Using FISH for Y-chromosome detection, a level of 0.2–1.4% was seen (Fig. 2).

TABLE 2. Testing for microchimerism

	FACS[a]	PCR	FISH[b]
Kidney/bone marrow			
1	0.9%	+	
2	2.3%	+	
3	NA[d]	NA	
4	<0.5%	+	
5[c]	1.3%	+	
6[c]	3.0%	+	1.0%
7	2.7%	NA	
8	NA	+	1.4%
9	<0.5%	+	
10	NA	+	0.2%[e]
Kidney alone			
1	NA	NA	
2	NA	–	0%
3	<.5%	+	
4	NA	NA	
5	NA	NA	
6	NA	+	0.5%
7	0%	–	
8	<.5%	+	0.5%

[a] All determinations performed >90 days after transplantation.

[b] Fluorescent in-situ hybridization for Y-chromosome was performed in female recipients of male organs.

[c] Also received islets.

[d] NA: test not performed because of lack of feasibility (perfect DR match and lack of sex disparity or availability of antibody to mismatched HLA A, B antigens).

[e] Chimerism was also detected by competitive PCR and was found to be 0.5%.

In the kidney alone patients, 3 of the 8 patients could not be evaluated for microchimerism by any modality, because of complete DR matching and lack of donor sex disparity. In the 5 evaluable patients, 3 (60%) were positive for microchimerism by PCR, 1 by HLA disparity, and 2 by Y-chromosome analysis. However, FACS failed to detect microchimerism in any of 3 evaluable patients by 3 months after transplantation.

Donor-specific reactivity could not be accurately assessed in 3 of the ten kidney/bone marrow recipients because there

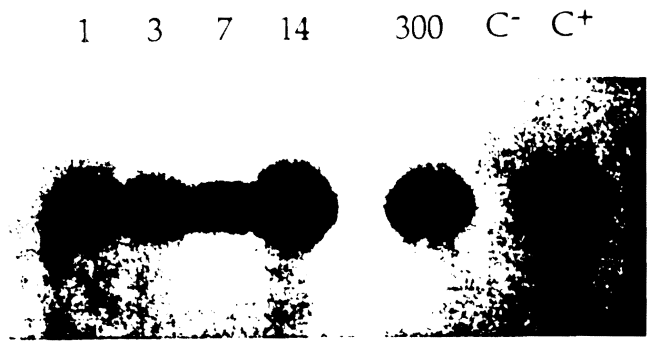

FIGURE 1. Polymerase chain reaction (PCR) in a kidney/bone marrow recipient with a disparity for HLA-A29, demonstrating persistent chimerism.

FIGURE 2. Fluorescent in-situ hybridization (FISH) of a cytospin preparation of PBMCs of a female recipient of a kidney/bone marrow from a male donor: orange represents X-chromosome; green represents Y-chromosome.

was complete DR-matching and a decreased in vitro immunologic response both pre- and post-transplantation. In the other 7 patients studied over time, there was one example of donor-specific nonresponsiveness and 2 of decreased donor-specific responsiveness that waxed and waned (Fig. 3). The other 4 patients retained donor-specific responsiveness. Only 5 of the 8 kidney-alone recipients were evaluable. One of the 5 has some evidence of decreasing responsiveness.

These results and those with 7 subsequently treated kidney-bone marrow recipients have shown that kidney/bone marrow transplantation is straightforward to perform and is safe. There were no examples of GVHD. Stable chimerism estimated to be 1000-fold more dense than that occurring spontaneously (*3*) was regularly achieved. It should be emphasized that (as was expected) bone marrow infusion did not influence the early events after transplantation. The incidence of acute rejection, the rates of early nonfunction, and the number cytomegalovirus infections were similar to those seen in patients receiving kidneys alone. However, treatment of these early problems was straightforward. After passing through this phase of convalescence, which was equally volatile with or without bone marrow augmentation, the more densely chimeric cohort of kidney-marrow recipients appeared to be an advantaged group. There was 100% patient and graft survival, and early completion of steroid weaning in half the cases. The full benefit of the chimeric state is expected to take one or 2 years, or longer. This trial was begun with patients undergoing primary transplantation who had low PRAs. We are now broadening our indications for kidney/bone marrow transplantation to include candidates undergoing retransplantation and/or those with high PRAs in order to assess the utility of adjuvant bone marrow in a more complex transplant setting.

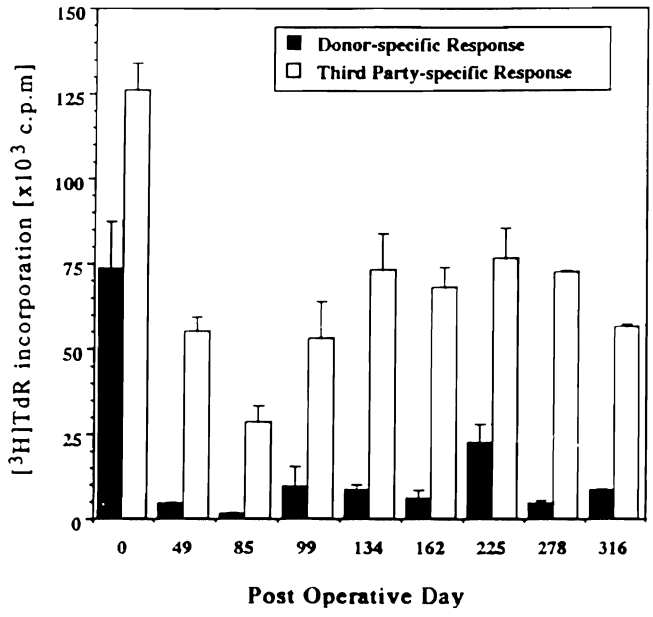

FIGURE 3. Serial MLR responses of a kidney/bone marrow recipient.

Historically important clinical attempts to facilitate kidney graft acceptance have been reported using adjunctive preoperative donor blood transfusions (*7*) or delayed (by 3 weeks) cryopreserved cadaveric donor bone marrow (*8*, *9*). Unlike the premises of these earlier trials, our hypothesis was that long-term engraftment of the infused donor cells could occur without "making space" by host preconditioning, without an undue risk of GVHD, and using the same immunosuppressive strategy that fostered the previously unrecognized spontaneous development of microchimerism (*1–3*). The observations in the kidney recipients herein reported, as well as in recipients of livers and hearts (*3*) are confirmatory of these predictions.

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INDEFINITE SURVIVAL FOLLOWING SMALL INTESTINAL TRANSPLANTATION AFTER INTRATHYMIC INJECTION OF THE DONOR WITH RECIPIENT-TYPE SPLENOCYTES IN A RAT MODEL[1]

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Due to multiple toxicities associated with chronic nonspecific immunosuppressive therapy, induction of donor-specific unresponsiveness or immune tolerance has long been a goal of the transplant community (*1*). Various tolerance induction regimens have been devised that administer donor-type antigen to the recipient following brief conditioning in which peripheral, immunocompetent lymphoid cells are depleted or rendered nonfunctional. Immunologic tolerance induction following the intrathymic (i.t.)* injection of a specific antigen has been studied extensively recently after it was demonstrated that islet allografts survive indefinitely in rodents treated with brief immunosuppression following the i.t. injection of islets from the same strain (*2*). The phenomenon of immune tolerance following i.t. injection of an antigen was actually reported much earlier in several different models (*3, 4*). The immune unresponsiveness induced by i.t. injection of an antigen has been shown to be donor-specific but not tissue-specific—i.e., i.t. injection of various donor-type cells can induce unresponsiveness to varying tissue or organ allografts (*5, 6*).

Despite intense investigation into the mechanisms involved in tolerance induction following i.t. injection of an antigen, several important questions remain to be answered. The optimal source of antigen, the optimal timing of administration and the optimal immunosuppressive regimen, if any, are all currently unknown and probably vary depending on the model studied. The need for the continued presence and influence of the thymus with injected antigen is also unknown, and with currently available transplantation models it is difficult to assess.

This report describes a new model in which we examine tolerance induction following i.t. injection of an antigen with the goal of providing new insights into the basic mechanisms involved in this type tolerance. We viewed the phenomenon of i.t. tolerance induction in a reciprocal fashion with respect to the current rejection models following transplantation. Our studies show that i.t. injection of recipient-type antigen into the donor following a single dose of antilymphocyte serum (ALS), in a unidirectional graft-versus-host disease (GVHD) model of small bowel transplantation (SBT) in the rat, results in specific unresponsiveness of donor-type lymphocytes against recipient-type antigen. This unresponsiveness is demonstrated by complete elimination of GVHD, which allows indefinite survival of untreated recipients of small bowel allografts in this model.

Lewis rats, either pretreated or naive, served as small bowel donors and (Lewis × Brown Norway) F_1 hybrid ($LBNF_1$) rats were used as recipients. This model of SBT is a unidirectional model in which only GVHD occurs (*7*). In an attempt to "tolerize" the donor lymphocytes against recipient-type antigen the donors were treated prior to graft harvest and transplantation. The six treatment groups are summarized in Table 1. Rabbit antirat ALS was given at a dose of 1 ml by intraperitoneal injection on day −14 relative to graft harvest and SBT on day 0. On day −9, Lewis donors were given splenocytes (SC) from different rat strains (depending on the experimental group) by direct i.t. injection of 50×10^6 cells in 0.1 ml RPMI medium; each thymic lobe received half the total dose. Following this donor pretreatment regimen, Lewis small bowel grafts were transplanted heterotopically into untreated $LBNF_1$ recipients as previously described (*8*). Recipients were weighed daily and assessed for clinical signs of GVHD. At the time of death, transplanted intestine, native small intestine, spleen, and skin biopsies were examined by routine histology to confirm the diagnosis of GVHD. Statistical analysis of survival data was performed by the Kaplan-

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* Abbreviations: ALS, antilymphocyte serum; GVHD, graft-versus-host disease; i.p. intraperitoneal; i.t. intrathymic; i.v. intravenous; $LBNF_1$ (Lewis × Brown Norway) F_1 hybrid; MST, median survival time; SBT, small bowel transplantation.