# Bone Marrow Augmentation for Heart, Liver, and Small Bowel Transplantation: Prolongation of Graft Survival and Incidence of Graft-Versus-Host Disease

1740

N. Murase, A.J. Demetris, S. Fujisaki, M. Tanabe, Y. Qing, S. Todo, and T.E. Starzl

THE persistence of donor leukocytes in recipients after transplantation (chimerism) has been postulated as the basis for graft acceptance.[1,2] There have been direct efforts to augment chimerism in order to increase the chance of graft acceptance, even though the mechanism of the bidirectional modulation between host and chimeric cells to achieve donor-specific hyporeactivity is not clear. In this study we examined the influence of donor bone marrow infusion at the time of solid organ transplantation on the levels of chimerism and the outcome of transplants.

## MATERIALS AND METHODS

ACI ($RT1^a$) and Lewis (LEW, $RT1^l$) rats (Harlan Sprague-Dawley, Indianapolis, Ind.) were used as donors and recipients, respectively. Orthotopic small bowel, liver, and heterotopic abdominal heart transplants were performed using the methods previously described.[3,4] Intramuscular FK 506 (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) was used at a daily dose of 1 mg/kg on days 0, 1, and 2. Bone marrow cells were obtained from tibias and femurs of naive ACI rats and $250 \times 10^6$ cells were infused to each recipient on the day of solid organ grafting. Whole organ graft survival and the incidence of graft-versus-host disease (GVHD) were examined with or without simultaneous bone marrow infusion. The percentages of donor phenotype lymphocytes in the recipient blood were analyzed by flow cytometry using MAb specific for donor (ACI) major histocompatibility complex (MHC) class I (MN4-91-6, Serotec, UK).

## RESULTS

Median heart graft survival of animals treated with a 3-day course of FK 506 alone was 48 days (n = 6). The addition of donor bone marrow infusion on the day of heart grafting significantly ($P < .01$) improved graft survival, and all animals survived more than 100 days (n = 6). FK 506 treatment alone prolonged small bowel graft survival to a median of 57.5 days (n = 6). These animals died exclusively of rejection without signs of GVHD. Interestingly, 5 of 8 animals developed GVHD (skin rash) between 20 and 35 days after the simultaneous small bowel and bone marrow transplantation. GVHD was fatal in 4 of 5 animals and death occurred between 25 and 43 days. One animal recovered from GVHD and survived for 200 days, at which time histopathological evidence of chronic rejection was noticed. The remaining 3 animals in this group were free from GVHD; however, rejection was the primary cause of animal death between 41 and 51 days after grafting. Finally, a median small bowel graft survival of 42 days with bone marrow infusion was slightly shorter than that without bone marrow infusion. All FK 506 treated recipients of liver grafts lived more than 100 days regardless of bone marrow infusion. When the liver and bone marrow were transplanted together, 2 of 5 recipients showed temporary GVHD, which spontaneously resolved.

Flow cytometric analysis revealed that bone marrow infusion increased the numbers of donor cells in the recipient circulation, when compared with those after solid organ transplantation alone. Detection of donor cells by flow cytometry was difficult in recipients of heart grafts but relatively easy after liver transplantation. After small bowel grafting, especially with bone marrow infusion, donor cells remained in the recipients for a long time. They rapidly increased to 20% to 60% in GVHD animals, but were not detected after recovery from GVHD.

## DISCUSSION

The engraftment of allogeneic cells with a state of donor-specific tolerance has been achieved in cytoablated recipients of bone marrow transplantation.[5] Consequently, the strategic use of donor bone marrow to establish acquired transplantation tolerance was commonly applied in combination with ALG.[6] As seen in this study and in the results of a clinical trial,[7] bone marrow cell infusion along with solid organ transplantation affected the graft outcome through the engraftment of bone marrow cells under conventional immunosuppression. The graft that profits most from this procedure may be the organ, which has relatively poorly distributed passenger lymphocytes, since the best result was obtained in the heart graft in this study.

Development of GVHD in small bowel plus bone marrow recipients suggests that the quality, as well as the quantity, of whole donor inoculum affects the subsequent host-graft relationship. Further strategies to improve graft survival may be necessary.

## REFERENCES

1. Starzl TE, Demetris AJ, Murase N, et al: Lancet 339:1579, 1992

2. Starzl TE, Demetris AJ, Murase N, et al: Immunol Today 14:326, 1993

From Pittsburgh Transplant Institute, Departments of Surgery and Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania.

Address reprint requests to Noriko Murase, Pittsburgh Transplantation Institute, 4C Falk Clinic, 3601 Fifth Ave, Pittsburgh, PA 15213.



0041-1345/95/$3.00/+0

3. Ono K, Lindsey ES: J Thorac Cardiovasc Surg 7:225, 1969
4. Murase N, Demetris AJ, Kim DG, et al: Surgery 108:880, 1990
5. Main JM, Prehn RT: J Natl Cancer Inst 15:1023, 1955
6. Monaco AP, Wood ML, Russell PS: Ann NY Acad Sci 129:190, 1966
7. Fontes P, Rao AS, Demetris AJ, et al: Lancet 344:151, 1994