1680

TRANSPLANTATION PROCEEDINGS APRIL 29, 1994

CHIMERISM, TYPE I DIABETES MELLITUS, ISLET ALLOTRANSPLANTATION;Bone Marrow

SIMULTANEOUS SOLID ORGAN, BONE MARROW AND ISLET ALLOTRANSPLANTATION IN TYPE I DIABETIC PATIENTS

Patricia B. Carroll.,[1], Paulo Fontes[2]., Abdul S. Rao[2,3]., Camillo Ricordi[4].,
Horacio L.R. Rilo[2]., Adriana Zeevi[3]., Massimo Trucco[3,5]., Ron Shapiro[2].,
Witold B. Rybka[1]., Velma Scantlebury[2]., Forrest S. Dodson[2]., Andreas G. Tzakis[2].,
William A. Rudert[5]., Roubik Behboo[2]., Theodore Karatzas[2]., Rana Khan[2].,
Anthony J. Demetris[3]., Jareen Flohr[2]., John J. Fung[2], Thomas E. Starzl[2]

Pittsburgh Transplantation Institute
and the Department(s) of Medicine[1], Surgery[2], Pathology[3], and Pediatrics[5]
University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, and
The Diabetes Research Institute[4], Miami, Florida, USA

This work was supported by NIH grant DK29961.

Address reprint requests to: Abdul S. Rao, M.D., D.Phil., University of Pittsburgh, Pittsburgh Transplantation Institute, E1551 Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15213, USA

INTRODUCTION

The unusual tolerogenicity ascribed to liver has been invariably attributed to its greater load of bone marrow-derived cells (1), which after transplantation migrate into the recipient, and play an important role in the induction and subsequent maintenance of donor-specific tolerance (1, 2). Since persistence of donor cell chimerism might confer an advantage to the recipient, a natural extension would be to augment this by infusing donor bone marrow at the time of organ transplantation.

In line with this argument, we infused donor bone marrow cells into three type I diabetics, who received pancreatic islets along with kidney ($n=2$) and liver ($n=1$) allografts. The long-term goal was to enhance allograft survival and achieve an insulin-free and possibly a drug-free state.

MATERIALS & METHODS

Patients: Three type I diabetic patients having a mean age of 34 $\pm$4.7 years were transplanted with donor bone marrow, pancreatic islets and kidney ($n=2$) and liver ($n=1$) allografts. Each patient received 3 x 10^8 bone marrow cells/kg body weight, which were infused through a central intravenous line, after whole organ transplantation but prior to islet infusion. The ABO compatible donors had HLA antigen mismatches with the recipients of 6 and 5 for the kidney and 3 for the liver. All patients received routine immunosuppression with FK506 and prednisone, whereas kidney-islet recipients additionally received Imuran. All episodes of acute rejection were treated with a transient increase in their routine immunosuppression.

Islet Isolation & Transplantation: Human pancreatic islets were obtained by a modification of an automated method described previously (3). Shortly after the allografts were

revascularized, a dose of 5.7 x 10^5 - 1.5 x 10^6 fresh islets (3.2 x 10^5 - 8.6 x 10^5 islet equivalents) from the organ donor were infused into the portal vein.

Post-Transplant Monitoring of Islet Function: Islet function was monitored by routine plasma glucose and C-peptide levels. A Sustacal challenge test was done at four weeks after transplantation, and every three months thereafter, with glycosylated hemaglobin (HbAlc) levels being measured every six weeks after transplantation.

Detection of Chimerism & *In Vitro* Immune Monitoring: Peripheral blood mononuclear cells (PBMC) were used to detect the presence of donor cells and to monitor the *in vitro* immune status of the recipients by techniques listed in Table 1, and described in detail elsewhere (4).

RESULTS AND DISCUSSION

The infusion of bone marrow and pancreatic islets was uneventful and all whole organ allografts are functioning well. The plasma C-peptide activity in a liver-islet-bone marrow recipient increased from 0.05 pmoles/ml before transplant to 0.83 pmoles/ml at POD 54, falling eventually to 0.02 pmoles/ml at POD 131. This precipitous fall was triggered by the involvement of the colon with post-transplant lymphoproliferative disorder, which required a complete withdraw of immunosuppression resulting in an episode of acute cellular rejection (mild to moderate) on POD 86. To achieve normal blood glucose levels, this patient is currently being maintained on 29 U/day of insulin, down from 68 U/day, which was required prior to transplantation. He is currently receiving 10 mg/day of FK506 and 5 mg/day of steroids, and has normal liver function. He is chimeric and remains reactive against the donor in *in vitro* MLR assays for up to the last sample tested (POD 131).

One kidney-islet-bone marrow recipient who started with no detectable pre-transplant plasma C-peptide activity, exhibited a level of 0.11 pmoles/ml on POD 276 after reaching a level of 0.35 pmoles/ml on POD 45. He is 307 days post-transplant and is receiving 30 U/day of insulin as compared to 56 units/day prior to islet transplantation. He had one mild acute cellular rejection episode on POD 16, and is currently maintained on 26 mg/day of FK506, 2.5 mg/day of steroids and 75 mg/day of Imuran. The level of chimerism in peripheral blood is relatively high (3%) and he exhibits 42% reactivity against the donor as compared to that to third party on the last date tested (POD 275).

The other kidney-islet-bone marrow recipient had a circulatory C-peptide activity of 0.56 pmoles/ml on POD 42, which was elevated from 0.04 pmoles/ml baseline level prior to transplantation. Her last C-peptide activity on POD 266 was 0.44 pmoles/ml. She is currently being maintained on 36 U/day of insulin while she received ~32 U/day prior to transplantation. She is receiving 9 mg/day of FK506, 15 mg/day of steroids and 75 mg/day of Imuran. Despite two rejection episodes, she is still chimeric. Furthermore, the donor-specific response in *in vitro* MLR assay on POD 257 was significantly higher as compared to that to third party.

Combined bone marrow and pancreatic islet-allotransplantation was unable to reverse diabetes in all 3 patients. All patients are chimeric and two-thirds (66%) exhibit evolving donor-specific hyporeactivity. Current C-peptide activity is not high enough to sustain an insulin-free existence in two, whereas in the third, a kidney-islet-bone marrow recipient, it may be high enough to result in eventual insulin-withdrawal. The causes of poor islet function are unclear

and may be attributed to transplantation of a low critical islet cell mass, to rejection or to an inappropriate site of islet transplantation.

REFERENCES

1. Qian S, Demetris AJ, Murase N, et al., *Hepatology,* **19:**916, (1994).

2. Starzl TE, Demetris AJ, Murase N, et al., *Lancet,* **339:**1579, (1992).

3. Ricordi C, Lacy PE, Fink EH, et al., *Diabetes,* **37:**413, (1988).

4. Fontes P, Rao AS, Demetris AJ, et al., *Lancet,* (1994 (in press)).