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## Survival of Human Hepatocellular Aggregates in Athymic Mice

C. Ricordi, Y. Zeng, B. Carr, P. Carroll, H.L.R. Rilo, A.J. Demetris, R. Alejandro, D.R. Bereiter, J.J. Fung, A. Tzakis, and T.E. Starzl

RECENTLY, Moscioni et al<sup>1</sup> showed that human liver cells attached to collagen-coated microcarriers (CCM)<sup>2</sup> were able to correct metabolic defects following transplantation (Tx) into recipient mutant rats. Interestingly, the intraperitoneally injected hepatocyte microcarriers were found on the surface of the pancreas. This apparent cellular tropism raises questions about the presence and mechanism of action of pancreas-derived hepatotrophic factors and their role in the maintenance of hepatocyte integrity.<sup>3</sup>

The aim of this study was to determine the effect of pancreatic islets on the survival of human hepatocytes in an ectopic site in nude mice.

### MATERIALS AND METHODS

Human hepatocytes were obtained from segments of preserved cadaver donor livers that were discarded during size reduction procedures before transplantation of the residual fragment to pediatric recipients. A modification of the automated procedure for isolation of human pancreatic islets<sup>4</sup> was used for the isolation of hepatocyte aggregates (HA), the main difference being the collagenase solution (Boehringer-Manheim, type P, 1 mg/mL) that was injected into a branch of the portal vein. Between 80% and 95% of the harvested HA were viable, as assessed by trypan blue exclusion. Pancreatic islets (PI) were obtained from the pancreata of mongrel dogs.<sup>4</sup>

Male Balb/c nude mice (20 g) were used as recipients: group 1 (control, n = 6): Tx of 4000 HA the left renal subcapsular (RSC) space;<sup>5</sup> group 2 (secondary control, n = 6): Tx of HA plus 400 CCM;<sup>2</sup> group 3 (n = 6): Tx of HA without CCM but with 400 PI.

Thirty days after Tx, the mice were sacrificed and the morphologic integrity of the grafts was determined.

### RESULTS AND DISCUSSION

No HA were detectable in the RSC 1 month after transplantation of HA alone (group 1). In group 2, the addition of CCM to the HA resulted in the survival of a thin rim of epithelioid cells attached to the CCM, while the remaining HA degenerated, leaving only fibrosis and a nonspecific mononuclear cell infiltrate around the microcarriers in the transplant site. In contrast, when PI were included at the transplant site (group 3), multilayers of healthy-looking human HA were found. Scattered PI were detected (immunoperoxidase). These results are consistent with previous demonstrations that pancreas-derived hepatotrophic factors are essential for maintenance of hepatocyte integrity.<sup>3</sup>

Using the model of Demetriou et al,<sup>2</sup> CCM may have promoted the survival of a small number of hepatocytes. In the earlier experiments of Moscioni et al,<sup>1</sup> the selective survival of the intraperitoneally injected hepatocyte-CCM

**Table 1. DNA Synthesis in Monolayer Cultures of Adult Primary Rat Hepatocytes: Effect of Islet Cell Conditioned Medium**

Condition	DNA Synthesis (cpm/g protein)
0	23
EGF	67
20% islet cell CM + 0	71
20% islet cell CM + EGF	378

aggregates on the surface of the pancreas was congruent with the possible role of factors produced from the pancreas in the maintenance of normal hepatocyte. Direct evidence supporting this concept has come from rodent experiments in which combined PI-HA were placed in ectopic sites.<sup>6,7</sup>

To explain the observed in vivo phenomenon, conditioned medium from primary cultures of PI was incubated in the presence of primary monolayer cultures of adult rat hepatocytes. Results of DNA synthesis experiments (Table 1) showed that PI secrete a hepatocyte mitogen, suggesting a putative mechanism for the hepatotrophic effect.

These findings will be of assistance for human hepatocyte culture, transplantation, artificial liver, and gene therapy applications.

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From the Transplant Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, and the Diabetes Research Institute, University of Miami, Miami, Florida.

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Address reprint requests to Dr Camillo Ricordi, The Transplant Institute, University of Pittsburgh School of Medicine, 3601 Fifth Avenue, Pittsburgh, PA 15213.

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