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EVIDENCE THAT CANINE PANCREATIC ISLETS PROMOTE THE SURVIVAL OF HUMAN HEPATOCYTES  
IN NUDE MICE<sup>1</sup>

In the last 15 years there have been numerous reports demonstrating that transplanted hepatocytes can retain hepatocellular functions (1), correct congenital enzyme deficiencies (2-4), and improve survival rates in animal models of acute hepatic failure (5-7). Recently, Moscioni et al. (8) showed that human liver cells attached to collagen-coated microcarriers (9, 10) were able to correct metabolic defects following transplantation into mutant rat recipients that were genetically deficient in either bilirubin-uridine diphosphate glucuronosyltransferase activity or albumin synthesis. Interestingly, the intraperitoneally injected hepatocyte-microcarriers that were well vascularized were almost exclusively found on the surface of the pancreas. This apparent cellular tropism raises questions about the presence and mechanism of action of pancreas-derived hepatotropic factors and their role in the maintenance of hepatocyte integrity (11-14).

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. Human hepatocytes were obtained from segments of preserved cadaver donor livers that had been discarded during size reduction procedures before transplantation of the residual fragments to pediatric recipients. A modification of the automated procedure for isolation of human pancreatic islets (15) was used for the isolation of hepatocyte aggregates. After cannulation of its portal branch, the liver segment was perfused with 200 ml of a solution of 1 mg/ml collagenase (Boehringer-Mannheim, type P) in Hanks solution (28°C) and placed into a digestion chamber. Separation of hepatocellular clusters was accomplished by the same separation procedure used previously for isolation of human pancreatic islets (15). Between 80% and 95% of the harvested hepatocytes were viable, as assessed by trypan blue exclusion. Collagen-coated microcarriers (Cytodex 3, Pharmacia Fine Chemicals, Upsala, Sweden) were prepared as described by Demetriou et al. (9). Pancreatic islets were obtained from the

pancreata of mongrel dogs (18-25 kg) by a modification (16) of the automated method for human islet isolation (15).

Male BALB/c nude mice (20 g) were used as recipients in three groups (n=6 in each group). In group 1 (control) approximately 4000 hepatocyte clusters of an average diameter of 150  $\mu$  were placed beneath the left renal capsule as previously described (17). In group 2 (secondary control), hepatocyte clusters were transplanted in combination with 400 collagen-coated microcarriers. In group 3 the same number of hepatocyte clusters was transplanted without microcarriers but in combination with 400 pancreatic islets of 150  $\mu$ m average diameter.

Thirty days after transplantation the nude mice were sacrificed and the morphologic integrity of the renal subcapsular grafts was determined histopathologically. Hematoxylin-eosin stain was used in all sections. In addition, immunostain for insulin (immunoperoxidase) was used in sections of combined islet-hepatocyte transplants to distinguish the islets from the hepatocellular implants.

The pathologist, who was blinded to the experimental groups from which the specimens came, correctly and without exception matched the tissues to the groups as follows. No hepatocytes were detectable in the renal subcapsular implants 1 month after transplantation of hepatocyte clusters alone (group 1). In group 2, the addition of collagen-coated microcarriers to the hepatocellular aggregates resulted in the survival of a thin rim of epithelioid cells attached to the microcarriers, while the remaining hepatocytes degenerated, leaving only fibrosis and a nonspecific mononuclear cell infiltrate around the microcarriers in the transplant site (Fig. 1). This suggested a minor augmentation of hepatocellular survival by the microcarrier. In contrast, when islets were included at the transplant site (group 3) multilayers of human hepatocytes were found. The surviving hepatocytes were healthy, in large clumps, and glycogen-containing, PAS-positive, and diastase-sensitive. These results are consistent with previous demonstrations that pancreatic-derived hepatotropic factors are essential for maintenance of hepatocyte integrity (11-14). Scattered pancreatic islets were detected (Fig. 2).

In past experience, when hepatocytes were transplanted by

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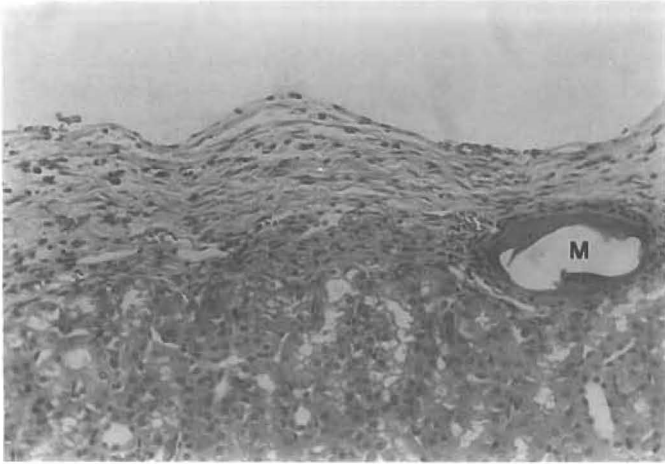


FIGURE 1. Photomicrograph of combined human hepatocytes and collagen-coated microcarriers 4 weeks after renal subcapsular transplantation. Degeneration of the hepatocytes occurred, leaving only a thin rim of epithelioid cells attached to the microcarriers (M) (H&E stain; original magnification  $\times 120$ ).

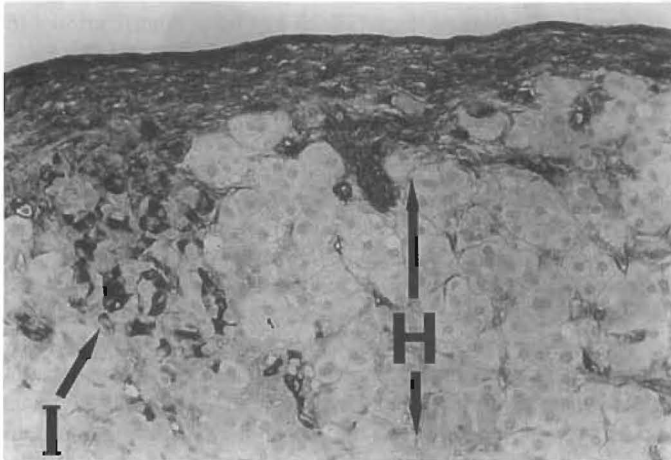


FIGURE 2. Photomicrograph of combined human hepatocytes and dog pancreatic islets 4 weeks after renal subcapsular transplantation in nude mice. Insulin immunoperoxidase (dark) stain distinguished islets (I) from the human hepatocytes (H), which appeared well preserved in the combined transplants (original magnification  $\times 180$ ).

themselves in the presence of an intact host liver, no evidence of hepatocyte proliferation could be observed at any time after transplantation (18). Whenever cells without nuclei and multinucleated cells were found at the transplant site (19), hepatic degeneration had occurred. Similarly, it was not possible to detect the hepatocytes at either 2 or 4 days after intrasplenic transplantation (20), except when there was a proliferative stimulus provided by 70% recipient hepatectomy. Using the model of Demetriou et al. (9, 10), the collagen-coated hepatocyte microcarriers, the microcarriers may have promoted the survival of a small number of hepatocytes. In the earlier experiments of Moscioni et al. (8), the selective survival of the intraperitoneally injected hepatocyte-microcarrier aggregates on the surface of the pancreas was congruent with the possible role of factors produced from the pancreas in the maintenance of normal hepatocytes. Direct evidence supporting this concept

(11-14) has come from rodent experiments in which combined hepatocyte-islet clusters were placed in ectopic sites (21, 22).

The present study has established that human hepatocytes are subject to the hepatotrophic influence of islet cell factors, and because dog islets were used with human hepatocytes in the nude mouse recipient, it could be shown that the hepatotrophic effect was not species-specific. These findings will be important in defining effective procedures for the maintenance of human hepatocytes both in vitro and in vivo, and for their use in hepatocellular transplantation, artificial liver, and gene therapy applications.

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