

1682

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XENOTRANSPLANTATION, BABOON, PANCREATIC ISLETS

BABOON PANCREATIC ISLET ISOLATION AND TRANSPLANTATION

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INTRODUCTION

Given the acute shortage of human cadaveric donors, attempts are being made to search for other sources of organs and tissues for future clinical use. Xenotransplantation across concordant species (baboons --> humans) offers a reasonable alternative, since non-human primates share many physiological and genetic characteristics with man. Furthermore, non-human primates have already been used as donors to treat end-stage organ failure in humans with variable success (1-3).

Allotransplantation of pancreatic islets has been used to treat insulin-dependent type I diabetes mellitus (IDDM). We were therefore interested to explore the possibility of using baboons' pancreas to isolate functionally active islets for future xenotransplantation. We report the feasibility of isolating islets from baboon pancreas, their purity, viability, yield, *in vitro* function and finally their use to reverse experimental diabetes in athymic nude mice.

MATERIALS and METHODS

Animals: Healthy juvenile baboons (*Papio anubis*) were obtained from Southwestern Primate Reserve, San Antonio, TX. They were rigorously screened for all viral and microbial infections before and after arrival at the University of Pittsburgh, Montefiore Hospital Animal Facilities, which is approved by the American Association For Accreditation of Laboratory Animal Care (AAALAC). Inbred male athymic nude mice (nu/nu) were obtained from Harlan Sprague Dawley, Indianapolis, IN. They were maintained in the pathogen-free facilities at the University of Pittsburgh, provided with food and water *ad libitum*, and used at 6 - 8 weeks of age.

Pancreatic Islets Isolation and *In Vitro* Functional Assays: The islets were isolated by a modification of an automated method described previously (4). Their purity, viability and yield was determined by staining with dithizone (DTZ) and trypan blue. Furthermore, viability was also assessed by their capacity to secrete insulin in *in vitro* perfusion assays performed according to a method described elsewhere (5).

Islet Transplantation: Ten nude mice were made diabetic by injecting 185 mg/kg/animal of Streptozotocin intravenously. Forty-eight hours later, baboon islets (600 equivalent islet number [EIN]/animal) were transplanted beneath the left kidney capsule. Six weeks after islet placement, the graft-bearing kidney was removed, and processed for immunohistochemical staining. Blood glucose levels were routinely monitored before and after nephrectomy, and the animals were sacrificed after their return to the diabetic state.

RESULTS and DISCUSSION

A total of 5 baboon pancreatic islet isolations were performed, yielding $\sim 1.92 \times 10^5$ islets/pancreas, giving an equivalent islet number (EIN) of 8.8×10^4 for islets of $> 150 \mu$ in diameter (4×10^3 EIN/gram of pancreas). The purity was $\sim 70\%$ as determined by DTZ staining. The islets when tested for their viability and *in vitro* function, responded with > 5 fold increase in glucose-induced (27.7 mM) insulin release in a standard perfusion assay as compared to baseline control. Insulin secretion returned to basal levels at the end of this secretory challenge, and could be further stimulated by a subsequent caffeine exposure (10 mM).

To further test their viability and capacity to reverse diabetes *in vitro*, 600 EIN of pancreatic islets were transplanted beneath the kidney capsule of an immunocompromised nude

mouse, who had been rendered diabetic by previous exposure to streptozotocin. Diabetes was reversed in 8/10 mice (80%) and their blood glucose remained normal for the duration of the experiment (42 days). Six weeks after transplantation, a unilateral nephrectomy of the graft-bearing kidney was performed, resulting in reappearance of diabetes within 24 hours. This observation indicates that baboon islets were viable and functionally competent following *in vivo* transplantation. However, in the two remaining mice, islet transplantation was not able to reverse experimental diabetes, and the cause for this failure is unclear. When transplanted islets in the nephrectomized specimen were examined by immunohistochemistry, they had granulated beta-cells which stained positive for insulin and glucagon.

Taken together these observations suggest that baboon pancreas can be used as a reliable source of islets for xenotransplantation to treat humans suffering from IDDM. However, more research needs to be done to make xenotransplantation a reality.

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