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Functional Evaluation of Isolated Islets from Baboon Pancreata

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ALLOTRANSPLANTATION of pancreatic islets has been used with variable success to treat insulin-dependent Type I diabetes mellitus (IDDM).[1] The recent reports of long-term insulin-free existence following encapsulated[2] or delayed[3] islet cell transplantation in diabetic recipients have paved the way for its successful clinical application. However, its global implementation will be impeded by the acute shortage of cadaveric donors, which has fomented our interest in the search for alternative sources of pancreata. Nonhuman primates (chimpanzees, baboons, etc.) by sharing many physiological and genetic characteristics with *Homo Sapiens*, are a reasonable alternative as donors for xenogeneic islet cell transplantation. The present study was designed to investigate the feasibility of using baboon pancreata to obtain viable and functional islets for future xenotransplantation.

MATERIALS AND METHODS

Animals

Nine healthy juvenile baboons (*Pappio annubis*) screened for all known viral and microbial infections were obtained from the Southwest Foundation for Biomedical Research, San Antonio, Texas. They were housed at the University of Pittsburgh, Montefiore Hospital Animal Facilities. Male athymic nude (nu/nu) mice were obtained from Harlan Sprague-Dawley, Indianapolis, Ind, maintained in the pathogen-free facility at the University of Pittsburgh, and used at 6 to 8 weeks of age.

Isolation of Pancreatic Islets

Islets were isolated by a modification of the automated method described elsewhere.[4] They were subsequently purified by density centrifugation. The methods of dithizone staining and trypan blue dye exclusion were used to assess islet purity, viability, and yield. Dynamic perifusion assays were used to further assess their viability and in vitro functional capacity.

Reversal of Diabetes in Nude Mice

Hyperglycemia was induced in nude mice by a single IV injection of streptozotocin (STZ; 185 mg/kg/animal). The animals were considered diabetic when their blood glucose levels were found to be >250 mg/dL. Freshly isolated baboon islets (600 IEq) were transplanted 2 to 3 days post-STZ treatment beneath the left renal capsule. A reduction in blood glucose levels to <150 mg/dL was considered as an indication of reversal of diabetes. An intraperitoneal glucose tolerance test (IPGTT) was performed approximately 5 weeks after islet cell transplantation to evaluate their function under stress. Unilateral nephrectomy of the graft-bearing kidney was eventually carried out to verify the function of the transplanted islets and the chemical destruction of the native pancreas. Insulin and glucagon content of the transplanted islets within the nephrectomized specimens were assessed by immunohistochemistry and by Northern blotting.

RESULTS AND DISCUSSION

Islets were isolated from the pancreata of nine baboons who, based on their age, have been arbitrarily divided into two groups; group I: donors <2 years of age; group II: donors >4 years of age (Table 1). Pancreatic islet cell yields from baboons in both groups were comparable to those obtained form human cadaveric donors.[3] When tested in in vitro dynamic perifusion assays, islets isolated from the pancreata of animals in group II responded efficiently to both glucose and caffeine stimulation. On the contrary, islets isolated from the pancreata of group I animals responded only to caffeine but not to glucose challenge in in vitro functional assays. This observation is similar to that made previously in the islets obtained from human pediatric donors. Implantation of baboon islets under the renal capsule of diabetic athymic nude mice led to restoration of euglycemic status in 36 of 40 (90%) of mice, with prompted reversal of hyperglycemia following intraperitoneal glucose challenge (IPGTT). The functional status of transplanted islets was further confirmed by unilateral nephrectomy of

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Table 1. Cell Yield and Purity of Islets Isolated From Baboon Pancreata

Groups	Donor age (range: yr)	n	Pancreas weight (g)	CIT* (h)	Islets (IEq)† (×10⁵)	Islets/gram (×10³)	Purity (%)
I	1–2	7	31.2 ± 16.2	4.0 ± 2.0	1.46 ± 0.5	5.3 ± 1.3	85.0 ± 10.0
II	4–18	2	11.1 ± 0.76	5.5 ± 3.4	0.80 ± 0.1	6.9 ± 0.4	86.4 ± 2.8

Results are expressed as $\bar{x}$ ± SD.
*Cold ischemia time.
†Estimated number of islets with an average diameter of 150 μ.

the graft-bearing kidney, which precipitated reappearance of diabetes. Examination of transplanted islets in the nephrectomized specimen revealed the presence of granulated β cells that stained positively for insulin and glucagon. Furthermore, RNA obtained from transplanted islets, when immobilized onto a PVDF membrane and hybridized with insulin-specific peroxidase-based chemiluminescent probe, showed positive bands suggesting the presence of insulin mRNA.

Because viable islets with acceptable purity and yield can be obtained from baboon pancreata, it is tempting to speculate that barring immunologic impedance, they may be considered for xenotransplantation to treat humans with IDDM.

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