Encapsulation of Porcine Islets Permits Extended Culture Time and Insulin Independence in Spontaneously Diabetic BB Rats

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The ability to culture porcine islets for extended times allows for both their functional assessment and the assurance of their microbiological safety prior to transplantation. We have previously shown that agarose-encapsulated porcine islets can be cultured for at least 24 weeks. In the current study, porcine islet agarose macrobeads cultured for up to 67 weeks were assessed for their ability to restore normoglycemia, respond to an intraperitoneal glucose challenge, maintain spontaneously diabetic BB rats free of insulin therapy for more than 6 months, and for their biocompatibility. Porcine islets were encapsulated in agarose macrobeads and subjected to weekly static perifusion assays for the assessment of insulin production. After in vitro culture for either 9, 40, or 67 weeks, 56–60 macrobeads were transplanted to each spontaneously diabetic BB rat. Transplanted rats were monitored daily for blood glucose levels. Glucose tolerance tests and assessments for porcine C-peptide were conducted at various intervals throughout the study. Normoglycemia (100–200 mg/dl) was initially restored in all islet transplanted rats. Moderate hyperglycemia (200–400 mg/dl) developed at around 30 days posttransplantation and continued throughout the study period of 201–202 days. Importantly, all rats that received encapsulated porcine islets continued to gain weight and were free of exogenous insulin therapy for the entire study. Porcine C-peptide (0.2–0.9 ng/ml) was detected in the serum of islet recipients throughout the study period. No differences were detected between recipient animals receiving islet macrobeads of various ages. These results demonstrate that the encapsulation of porcine islets in agarose macrobeads allows for extended culture periods and is an appropriate strategy for functional and microbiological assessment prior to clinical use.

Key words: Porcine islets; Islet culture; Xenotransplantation; Encapsulation

INTRODUCTION

Type 1 diabetes is characterized by the loss of insulin-producing β-cells within the islets of Langerhans that are scattered throughout the pancreas (19) and the complex metabolic dysregulation syndrome that results from the consequent insulin deficiency. Exogenous insulin therapy has been the standard of care to treat type 1 diabetes since the discovery of insulin by Banting and Best in 1921. Although improvements in insulin and treatment regimens continue to better mimic nondiabetic blood glucose levels and have tremendously improved the quality of life of millions of patients, this therapeutic approach is still not optimal as exemplified by the wide range of cardiovascular, renal, and neurological complications that are often the end result of this disease (62). Whole pancreas transplantation offers the chance to immediately restore normoglycemia and to reduce or even reverse complications with 1-year morbidity and mortality rates comparable to those for kidney transplantation (20). A recent report by Marchetti et al. (36) outlines improvements in cardiovascular risk factors and diabetic retinopathy with this treatment approach. Still, the long-term use of immunosuppression required to prevent rejection of the grafted pancreas is often associated with severe and even life-threatening side effects (45). Innovative work with more specific immunosuppressive agents including the selective co-stimulation blocker, belatacept (56), and the anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) (21) offer possibilities to overcome at least some immunosuppression-related side effects.
With the recent success of human islet transplantation, but the severe constraints on human islet supply, and the failure as yet to successfully clone human islets or obtain them from stem cells, it is apparent that another source of insulin-producing cells will be required if the majority of type 1 diabetic patients are to benefit from this form of cellular replacement therapy. Isolated porcine islets are widely considered the most reasonable source of nonhuman tissue for future clinical use. Of course, the immune response to the xenogeneic islets poses a tremendous challenge that must be overcome in order to take advantage of this virtually unlimited islet source. Additional concerns regarding the biosafety of this approach must also be considered to avoid the inadvertent trans-species transmission of microbiological pathogens (15).

We have recently reported the ability of agarose-encapsulated porcine islets to maintain spontaneously diabetic BB rats insulin free for more than 3 months (17). Importantly, these animals were not immunosuppressed and remained healthy throughout the study with no evidence of adventitious viral transmission, thus indicating the functional efficacy of the porcine islet macrobeads as well as their successful immunoprotection by the agarose. However, any clinical use of porcine islets will require extensive microbiological screening prior to transplantation. The capability to maintain functional porcine islet tissue during culture would allow time to screen for microbiological safety and permit the tissue to be banked for scheduled clinical use. In the current study, we report the ability of fully xenogeneic porcine islets that have been macroencapsulated in agarose to function for more than 6 months in spontaneously diabetic BB rats after in vitro culture for up to 67 weeks, in the complete absence of pharmacological immunosuppression.

**MATERIALS AND METHODS**

**Animals**

A total of 12 male, spontaneously diabetic BB rats (Biomedical Research Models, Inc., Worcester, MA) were received at 10–15 weeks of age and with evidence of clinical diabetes for 3–16 days. A total of 23 Wistar-Furth rats were received at 7 weeks of age as normal controls (Harlan Sprague Dawley, Indianapolis, IN). All rats were housed two per cage in polysulfone cages, 10.5″ wide × 19″ deep × 8″ high, with a rim wire lid and polyester filter tops (Allentown Caging Equipment Co., Inc., Allentown, NJ) within a Micro-FLO Environmental Systems High Efficiency Particulate Filter Class 100 Air (HEPA) cabinet (Allentown Caging Equipment Co., Inc.). LabDiet 5002® (PMI Nutrition International, Brentwood, MO) and clean municipal water were provided ad libitum. Diabetic rats were maintained on porcine-zinc insulin (Blue Ridge Pharmaceuticals, Inc., Greensboro, NC) prior to islet macrobead implantation. Insulin therapy was initiated with PZI insulin after two consecutive blood glucose readings >500 mg/dl.

**Isolation of Porcine Islets and Production of Porcine Islet Macrobeads**

Donor islets were prepared from Newsham sows that were over 2 years of age and had a history of multiple parities. Islet counts were expressed as equivalent islet numbers (EIN), based on a standard islet size of 150 μm, and 500 EIN were encapsulated in agarose–agarose macrobeads as previously described (17). Briefly, following manual purification, islets were resuspended in RPMI-1640 (Gibco, Invitrogen, Carlsbad, CA) containing 25 mmol/L HEPES buffer, 2 mmol/L L-glutamine and 11.1 mmol/L glucose +10% porcine serum (PS, Biologos, Montgomery, IL), and 1% antibiotic/antimycotic (A/A, Invitrogen) to a volume of 2000 EIN/ml. One half milliliter of 1.5% agarose (SeaKem Gold, BioWhittaker Molecular Applications, Rockland, ME), at 50°C, prepared in minimal essential medium (MEM, Sigma, St. Louis, MO) plus 2.5% HEPES buffer (Invitrogen) was added to 2000 EIN/ml. One evenly mixed, the islet–agarose suspension was expelled beneath the surface of sterile mineral oil (Sigma) to make four consistent beads with a smooth surface and an equal distribution of islets. The macrobeads were removed from the oil and washed two times with RPMI-1640 + 5% PS + 1% A/A. Following the first coating of agarose, macrobeads were cultured in RPMI-1640 + 5% PS + 1% A/A at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 5–7 days. After 5–7 days, macrobeads were washed three times in RPMI-1640 + 1% A/A and a second coat of agarose was applied to each macrobead. Briefly, approximately 0.5 ml of 5% agarose prepared in MEM containing HEPES buffer at 60°C was transferred by pipette to a 0.5 teaspoon sterile plastic spoon (Bel-Art Products, Pequannock, NJ). Each macrobead core was placed into the agarose using a Teflon spatula and rolled three to five times to produce a uniform second coating of agarose. The islet macrobead was then transferred to sterile mineral oil to produce an agarose–
agarose porcine islet macrobead with a smooth outer surface. Macrobeads were removed from the oil and washed two times with RPMI-1640 + 5% PS + 1% A/A and 20 macrobeads were cultured indefinitely in 25 × 100-mm polystyrene petri dishes (Nunc, Rochester, NY) with 50 ml RPMI-1640 + 2.5% PS + 1% A/A at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The final diameter of agarose–agarose macrobeads averaged 8 mm. Culture medium was changed weekly and 24-h culture media samples were taken weekly after media change to test for insulin production, for macrobeads assessed both prior to implantation and after retrieval at necropsy.

**Porcine Islet Macrobead Implantation**

Animals were anesthetized with ketamine/xylazine/butorphanol (Fort Dodge Animal Health; Ben Venue Laboratories; Fort Dodge Animal Health, respectively) at a dose of 0.1 ml/100 g (60 mg/ml ketamine, 6 mg/ml xylazine, 3 mg/ml butorphanol) given intramuscularly. At 20–21 days following arrival, all BB rats received an implant of porcine islet macrobeads that had been in culture for either 9 weeks (n = 4 rats), 40 weeks (n = 4 rats), or 67 weeks (n = 4 rats), at a dose equivalent to 1.0 times daily insulin requirements (Table 1). Control Wistar-Furth rats received porcine islet macrobeads that had been cultured for an average of 9.1 weeks at a dose equivalent to 1.0 times daily insulin requirements (n = 5 rats, 45–49 macrobeads/rat).

In an attempt to compensate for an average weight gain of approximately 75 g, a second partial implant of islet macrobeads was performed on day 97 following the first implant procedure for BB rats. An average preimplant insulin requirement of 0.0083 U of insulin/g body weight was used to determine that an additional 17 islet macrobeads producing 39.19 mU insulin/macrobead/24 h would be required. Because four macrobeads were retrieved immediately prior to the second implant, a total of 21 islet macrobeads, which had been cultured for 19 weeks, were given to each rat during the second implant procedure. Wistar-Furth rats did not receive a second partial implant.

**Clinical Observations**

Clinical observations from individual animals were recorded daily. Observations included level of alertness and responsiveness, body coat appearance, body posture, eye aspect, and the presence of any eye or nasal secretions and were subjectively scored by the animal care staff as good, fair, or poor. Body weight and blood glucose (Accu-Chek® Simplicity™ BG monitor or Accu-Chek® ComfortCurve™ BG monitor and Chemstrips, Roche) were assessed daily while urine glucose and urine ketones (Chemstrip 10 with SG, Roche Diagnostics or Keto-Diastix, Bayer) were assessed when urine was available.

**Insulin, Glucagon, and Porcine C-Peptide Assays**

Serum for the detection of insulin, glucagon, and porcine C-peptide was routinely collected throughout the study. Intrapertitoneal glucose tolerance tests (IpGTTs, 50% dextrose in sterile water, 2.0 g dextrose/kg) were also performed. Standard radioimmunoassays from Linco Research, Inc. (St. Charles, MO) were used for the detection of insulin, glucagon, and porcine C-peptide. All samples were frozen at −12 to −20°C prior to assay and were run with samples in duplicate, and with reference standards and controls in triplicate.

**Necropsy**

Complete necropsies were performed on day 201–202 following the implant of porcine islet macrobeads into BB rats. Wistar-Furth rats were sacrificed on day 334 postimplantation. Following anesthesia, exsanguination was carried out through the collection of heart blood and the peritoneal cavity was exposed.

**Histopathology**

At necropsy, the following tissues were collected and fixed in 10% neutral buffered formalin for 24 h followed by storage in 70% ETOH: heart, spleen, liver, kidneys, brain, testes, duodenum, jejunum, ileum, adrenal glands, stomach, lungs, diaphragm, pancreas, abdominal musculature, bone (sternum), spinal cord, sciatic nerve, eyes, submandibular lymph nodes, and mesenteric lymph nodes. Islet macrobeads were also collected and fixed for 18–20 h in 10% neutral buffered formalin. Histopathology was performed by Pathology Associates International (PAI, A Charles River Company, West Chester, OH). Tissues were embedded in paraffin and 5-µm sections were stained with hematoxylin and eosin (H&E). Samples were analyzed by a Diplomat of the American College of Veterinary Pathology, and the macroscopic and histopathological findings documented. Where appropriate, findings received a severity grade based upon a scale where: N = tissue within normal histological limits, 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

**Immunohistochemistry**

Slides were deparaffinized, rehydrated, and treated with 0.3% hydrogen peroxide for 5 min. Antigen retrieval was carried out by incubating in approximately 100 µl of undiluted Pronase (Biomedia, Foster, CA) for 5 min at room temperature according to the manufacturer's instructions. Sections were incubated in blocking buffer (10% normal donkey serum in PBS) for 30 min at 37°C. Guinea pig anti-swine insulin primary antibody
(1:400, DAKO, Carpinteria, CA) or rabbit anti-human glucagon (1:150, DAKO) was followed by a secondary antibody (1:500 HRP-conjugated donkey anti-guinea pig IgG, Jackson ImmunoResearch Labs, West Grove, PA) or LSAB2 (DAKO) and DAB. Slides were counterstained with hematoxylin.

Statistics

Statistical methods were performed using StatSoft Statistica (Tulsa, OK). Porcine islet macrobeads of different ages were considered independent variables for comparison of all dependent variables. Calculations resulting in probability values of \( p < 0.05 \) were considered statistically significant.

RESULTS

Insulin Production per Islet Macrobead

During culture, islet macrobeads were assessed weekly for insulin production during a 24-h static perfusion assay. Although islets were isolated from different porcine pancreas donors, there was no significant difference between islet preparations in terms of insulin production after encapsulation in agarose macrobeads (Table 1). At the time of transplantation, the average daily exogenous insulin requirement per BB rat of 2.4 U/day was met for all BB rat groups with the grafting of 56–60 macrobeads per animal.

Ability of Islet Macrobeads to Restore Normoglycemia

To evaluate the effect of culture length on the ability of porcine islet macrobeads to function in a xenogeneic and autoimmune environment, macrobeads of various ages were implanted into diabetic BB rats and followed for 201–202 days. Average daily blood glucose values for each group (\( n = 4 \) /group) are shown in Figure 1. Following islet macrobead implantation, normoglycemia (100–200 mg/dl) was restored for approximately 1 month in all study rats from the three groups. After this time, the rats developed moderate hyperglycemia (200–400 mg/dl) that persisted throughout the remainder of the study. A consistent finding was the contemporaneous development of moderate hyperglycemia and the plateau of body weight (Fig. 1). Thereafter, body weights of rats from all three groups remained consistent, while blood glucose levels fluctuated between approximately 300 and 400 mg/dl for the remainder of the study. No significant differences in final body weights were observed between implanted animals across the three groups at study termination.

Streptozotocin-induced diabetic rats, similarly transplanted with islet macrobeads, also displayed normoglycemia for approximately 1 month (Fig. 1). As observed with the BB rats, the streptozotocin-induced diabetic WF rats developed moderate hyperglycemia for the remainder of the study.

With the intention of meeting the demands of an average body weight gain of approximately 75 g following the first macrobead implant, a second partial implant of 21 islet macrobeads was performed on day 97 for BB rats as detailed above (Materials and Methods). This second partial implant did not significantly affect daily blood glucose levels. Throughout the study, no differences in the ability to regulate blood glucose were observed between the islet macrobeads of various ages.

Porcine C-Peptide Detection In Vivo

Porcine C-peptide was detected in BB rats implanted with macrobeads of all three age groups (Fig. 2). A decrease in average porcine C-peptide, from 0.6–0.9 to 0.2–0.4 ng/ml, occurred during the first 88 days for all three groups. Increased levels of C-peptide were detected on day 116, following a partial reimplant of islet macrobeads on day 97. For the remainder of the study, porcine C-peptide was detected in BB rats from all three

| Table 1. Transplant Characteristics |

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*aPartial retransplant.
EXTENDED CULTURE AND FUNCTION OF PORCINE ISLETS

Figure 1. In vivo function of long-term cultured porcine islet macrobeads. Average daily blood glucose values of spontaneously diabetic BB rats transplanted with porcine islet macrobeads (56–60 macrobeads/rat) that had been cultured for 9 weeks (heavy black line, \( n = 4 \)), 40 weeks (light black line, \( n = 4 \)), or 67 weeks (dashed line, \( n = 4 \)) and followed for 201–202 days. All BB rats received a partial retransplant (21 macrobeads/rat) of 19-week-old islet macrobeads on day 97. Streptozotocin-induced diabetic Wistar-Furth rats (\( n = 5 \)) transplanted with an equivalent insulin dose of porcine islet macrobeads (45–49 macrobeads/rat) displayed a similar pattern of daily blood glucose levels as spontaneously diabetic rats. Mean body weight of BB rats (solid gray line) paralleled blood glucose values throughout the study.

Response to Intraperitoneal Glucose Tolerance Test

Glucose challenge procedures were performed throughout the study on all rats implanted with islet macrobeads. There were no differences in the ability of macrobeads that had been cultured for different lengths of time to respond to a glucose challenge after implantation into diabetic rats. Eleven days following the initial macrobead implant (Fig. 3A–C) starting blood glucose levels of 100–200 mg/dl were approximately doubled for all three groups following the administration of glucose. A return to baseline glycemia occurred by 120 min in 10 of the 12 animals. For all three groups, this response was similar to that observed in normal Wistar-Furth rats (Fig. 3D). Although all study animals eventually became moderately hyperglycemic, a glucose challenge on day 105 posttransplantation (Fig. 3A–C) also demonstrated an initial rise in blood glucose, followed by a return to baseline glycemia. By 200 days posttransplantation, only a slight increase in baseline glycemia was observed following glucose administration, followed by a return to baseline glycemia (Fig. 3A–C).

Necropsy

Study animals were necropsied on day 201–202 following the initial islet macrobead implantation. More than 90% of the porcine islet macrobeads were free floating in the peritoneal cavity. No broken macrobeads were found. Only two macrobeads from one animal were found to have a fibrous connection to the peritoneum. An occasional macrobead was found lodged be-
Figure 3. Intraperitoneal glucose challenge results. Spontaneously diabetic BB rats were subjected to a glucose challenge on day 11 posttransplantation (filled circles), day 105 posttransplantation (open circles), and day 200 posttransplantation (filled triangles). (A) BB rat recipients of 9-week-old macrobeads. (B) BB rat recipients of 40-week-old macrobeads. (C) BB rat recipients of 67-week-old macrobeads. (D) Normal Wistar Furth rats were challenged at 8 weeks of age.

Histopathology

Individual BB and Wistar-Furth rat histopathology findings are presented in Table 2. With the exception of the expected moderate inflammation associated with the pancreatic islets from 10 of 12 spontaneously diabetic BB rats and an unexpected finding of moderate fibrosis on the diaphragm of one BB rat, only minimal or mild pathology was reported for the various tissues and macrobeads examined for all BB and Wistar-Furth tissues examined. No clear differences among the BB rats that received islet macrobeads of various ages were identified. The minimal to mild inflammation on the surface of the peritoneum was not thought to have altered the normal function of the tissue. There was no reaction on the peritoneal surfaces of the liver or kidney. A minimal interstitial inflammatory reaction was observed in the kidney of Wistar-Furth rats, as previously reported by others following the administration of streptozotocin (2,22,37).

Mild fibrosis, with or without inflammatory cells, was present on the surface of macrobeads collected both on day 97 and during necropsy of BB rats and Wistar-Furth rats. This inflammation was minimal to mild in severity and limited to a minority (≤25%) of the macrobeads. Additional findings in the day 97 macrobeads from the BB rats included multinucleated giant cells on the surface of macrobeads from the 40-week group (2 of 4 animals) and on some macrobead surfaces from the 67-week group (3 of 4 animals). Red blood cells were also noted along the surface of a few macrobeads from the 9-week group (1 of 4 animals) and the 67-week group (2 of 4 animals). Multinucleated giant cells and
were not observed on the surface of any macrobeads at necropsy. All macrobeads examined from both the BB rats and the Wistar-Furth rats, at day 97 and at necropsy, contained both viable islet tissue and cellular debris. No difference in the amount of viable tissue in the macrobeads was observed among the BB rat groups.

Immunohistochemistry of pancreas sections did not reveal any insulin-positive cells within the native islets of Langerhans from any BB rat. Recipient pancreatic islets appeared as collapsed islets with the majority of cells staining for the presence of glucagon (Fig. 4), consistent with destructive insulitis and a state of malignant autoimmunity (16). On the other hand, encapsulated islets from all groups retained insulin-positive cells as shown in Figure 4 for a 67-week-old macrobead following retrieval at necropsy.

**DISCUSSION**

The encapsulation technique reported herein allows the culture of porcine islets for more than a year. Islet macrobeads cultured for 67 weeks were able to maintain equivalent in vitro insulin secretion as well as in vivo blood glucose regulation in spontaneously diabetic BB rats when compared to macrobeads that had been cultured for either 9 or 40 weeks. All 12 spontaneously diabetic BB rats were completely insulin free for more

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Figure 4. Immunostaining of recipient pancreas and islet macrobead sections. Images were taken of tissues retrieved 201–202 days posttransplantation from a spontaneously diabetic BB rat that received 67-week-old porcine islet macrobeads, and are shown as a representation of all immunohistochemistry results. (A) An islet of Langerhans (light appearing cell group in the middle of figure) stained for insulin does not contain any insulin-positive cells. (B) The same islet as in (A), stained for glucagon. The majority of the cells are glucagon positive. (C) An encapsulated islet stained for insulin. Insulin-positive cells were found in the encapsulated islets of all retrieved porcine islet macrobeads. Original magnification: 400x.

than 200 days, regardless of in vitro culture time, and remained healthy throughout the study period in all cases without any pharmacological immunosuppression.

It has been nearly 20 years since Ricordi reported the reproducible isolation of porcine islets using a modification of the Automated Method (43). Still, the isolation of porcine islets remains tremendously challenging. In addition to this challenge, any clinical use of porcine islets must demonstrate that the islets are free of transmissible pathogens prior to transplantation. One strategy to demonstrate microbiologically safe porcine islet tissue is to raise and house donor animals in a barrier environment: given negative findings for screened pathogens prior to pancreas procurement, islets would be assumed to be suitable for use. A second approach, and the strategy we have adopted, is to screen nonbarrier but well-characterized donor animals as well as the isolated islets themselves for evidence of microbiological safety. Perhaps the greatest hurdle with this second approach is the necessity to maintain the islets during prolonged testing. However, a significant loss of viable porcine islet tissue occurs under standard culture conditions [(29,47), our unpublished results]. Our methodology reported here demonstrates the utility of encapsulation to provide for extended in vitro, as well as in vivo, islet survival.

During the process of pancreas dissociation, the extracellular matrix of the islets is disrupted. Damage to the connections between the extracellular matrix and the cytoskeleton can lead to altered cell function and viability due to changes in the structural support of individual cells (24). To provide extracellular support to purified islets, several groups have demonstrated that culturing islets with three-dimensional matrices including collagen, Matrigel, and chitosan sponge can greatly extend in vitro viability (5–7,29,34,38,41,47,51,53,57,61). In fact, Lucas-Clerc et al. were able to culture collagen-embedded human islets for 58 days (34). Furthermore, Thomas et al. previously reported the ability of incompletely isolated rhesus islets, still attached to some exocrine tissue and therefore having an intact extracellular matrix, to survive and maintain a normal perfusion response for at least 30 days (54). Yang and Zhang have also shown rat islets encapsulated in agarose to function for 40 weeks (60). We have previously demonstrated collagen–agarose- and agarose–agarose-encapsulated rat islets to maintain viability for more than 180 days (26). Although the extreme culture period presented in the current study may never be required, the results demonstrate the robustness of porcine islets encapsulated in agarose macrobeads. Furthermore, these data suggest that the necessity of replacement grafts due to a postulated normal islet turnover may not be compulsory or perhaps infrequent.

Encapsulated porcine islets have been shown to function in streptozotocin-induced models of type 1 diabetes by a number of groups (11,30,31,33,58). However, only a few examples of long-term function in nonimmunosuppressed and spontaneously diabetic animals have been reported (25,32) including the description of functional encapsulated porcine islets by Sun et al. after grafting to spontaneously diabetic cynomologous monkeys (50). Basta et al. have shown the efficacy of alginate microencapsulated porcine islets in NOD mice (1) and Rivereau et al. demonstrated the survival of hydrogel-based hollow fiber (AN69) encapsulated porcine islets in NOD mice (44). In the present article, we document the ability of agarose–agarose porcine islet macrobeads to function in a model of spontaneous type 1 dia-
Thus, an autoimmune response to the porcine islets in to be secreted by islets of Langerhans (23). We have diabetes, in which 100% of transplanted animals remained Despite the normal response to a glucose challenge may also help prolong normoglycemia. Porcine islets are a very real solution to the inade-
posttransplantation period, as reported for AN69-encap-
is also evidence that vascular complications are reduced posttransplantation. The fact that both insulin and glucagon production are depressed in retrieved islet macrobeads, as we have recently reported, suggests a nonautoimmune etiology for the loss of islet cell function (17). Weir and colleagues have described a similar reduction in insulin content of microencapsulated porcine islets (11) and insulin secretion from rat islets (52) retrieved from mice. De Vos et al. have also previously reported a reduction in the magnitude of insulin secretion from retrieved microencapsulated islets (9). Further work from this group demonstrated that an inflammatory response directed toward just 2–10% of grafted capsules could affect the islet viability of all transplanted microcapsules (8). Finally, syngeneic islets microencapsulated in agarose have been shown to be protected from disease recurrence in the spontaneously diabetic NOD mouse (28). Thus, an autoimmune response to the porcine islets in our present study appears unlikely, but other immune factors such as prostaglandins or cytokines could account for some islet loss.

Nutritional factors are also likely to play a role in the reduced islet macrobead function as observed during the course of our current study. Pancreatic islets have been shown to be highly vascularized with the insulin-secreting β-cells receiving oxygen near the arterial level of 100 mmHg (3,4). The peritoneal cavity is known to have a low partial oxygen partial pressure, which is similar to that of venous blood (49). Encapsulated islets transplanted into the peritoneal cavity are thus subject to depressed oxygen availability, which has been shown to significantly inhibit insulin secretion by a number of groups (10,39,46).

We consider it likely that both unknown immune mechanisms and nutritional deficits contribute to some islet loss following macrobead transplantation. Exogenous insulin requirements of recipient rats were only just met with the transplantation of a known amount of insulin production per macrobead. Therefore, it may be possible to avoid the development of moderate hyperglycemia, posttransplantation, with the initial grafting of additional islet macrobeads to provide a critical islet mass (59). Exogenous insulin therapy in the immediate posttransplantation period, as reported for AN69-encapsulated porcine islets implanted in diabetic mice (40), may also help prolong normoglycemia.

Despite the normal response to a glucose challenge early after macrobead transplantation, recipient animals were unable to establish normoglycemia later in the study. Still, we are encouraged that the porcine islet macrobeads alone are able to sustain recipient animals: in the absence of exogenous insulin, spontaneously diabetic BB rats normally die within a couple of days. A delay in the glucose response of macrobead transplanted rats is expected, given the need for diffusion of glucose into the macrobead and the passage of insulin from the macrobead and into the peripheral circulation. Lanza et al. reported a delay in restoring normoglycemia in BB rats transplanted with encapsulated canine islets and challenged with IV glucose (32). The results of Weir’s group, in which only a very minimal delay in restoring normoglycemia was reported with alginate macroencapsulated syngeneic rat islets during a meal challenge and during hyperglycemic clamp studies, are particularly exciting (55). It may be that normoglycemia in macrobead recipients would be easily controlled with reduced amounts of exogenous insulin, as demonstrated with human islet recipients who have resumed exogenous insulin therapy (42).

Numerous proteins in addition to insulin are known to be secreted by islets of Langerhans (23). We have recently reported the presence of glucagon and porcine C-peptide during in vitro static perfusion of porcine islet macrobeads, and a significantly reduced incidence of glycosuria and ketonuria was observed in diabetic BB rats that were implanted with the islet macrobeads compared to diabetic rats receiving exogenous insulin therapy (17). Importantly, porcine C-peptide was only found in the serum of the islet macrobead-implanted rats. In a different study with streptozotocin-induced diabetic rats, we have shown the ability of porcine islet macrobeads to prevent neuronal simplification (35). Recent studies by Sima’s group have demonstrated a beneficial role for C-peptide in the treatment of type 1 diabetes. In these studies, the continuous infusion of C-peptide inhibited neural apoptosis and cognitive dysfunction in the spontaneously diabetic BB rat (48). Further work by this same group, also in the diabetic BB rat, has demonstrated the ability of C-peptide to reverse nociceptive neuropathy (27). Taken together, the above results are consistent with the idea that additional islet-secreted proteins may play an important role in the management of type 1 diabetes. In fact, a correlation between the presence of circulating C-peptide and improvement in kidney survival in patients receiving a simultaneous islet and kidney transplant has been reported (12) and there is also evidence that vascular complications are reduced in islet transplant patients (13,14).

Porcine islets are a very real solution to the inade-
quate supply of insulin-producing tissue for the treatment of type 1 diabetes. Although still difficult to isolate, purified, highly functional porcine islets can be obtained in large numbers [18]. The data presented in this report clearly establish the ability of porcine islet macrobeads to be cultured for extended periods, thus providing a system for banking a virtually unlimited supply of porcine islets that have been fully assessed for functionality and microbiological safety prior to transplantation. The islet macrobeads function in a fully xenogeneic and autoimmune model of human type 1 diabetes, in the absence of any pharmacological immunosuppression, and should be aggressively pursued as a clinical option.

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