Pancreatic Islet Transplantation Using the Nonhuman Primate (Rhesus) Model Predicts That the Portal Vein Is Superior to the Celiac Artery as the Islet Infusion Site

Boaz Hirschberg,1 Sean Montgomery,1 Michael G. Wysoki,2 He Xu,1 Doug Tadaki,1 Janet Lee,3 Kenneth Hines,3 Jason Gaglia,1 Noelle Patterson,1 John Leconte,4 Douglas Hale,1 Richard Chang,4 Alan D. Kirk,1 and David M. Harlan1

We’ve established a nonhuman primate islet allotransplantation model to address questions such as whether transplanting islets into the gut’s arterial system would more safely and as effectively support long-term islet allograft survival compared with the traditional portal vein approach. We reasoned that islets make up <2% of pancreatic cell mass but consume an estimated 20% of arterial blood flow, suggesting an advantage for the arterial site. Access to the arterial system is also easier and safer than the portal system. Pancreatectomized rhesus macaques were transplanted with allogeneic islets infused into either the portal vein (n = 6) or the celiac artery (n = 4). To prevent rejection, primates were given daclizumab, tacrolimus, and rapamycin. In five of six portal vein experiments, animals achieved normoglycemia without exogenous insulin. In contrast, none of the animals given intra-arterial islets showed even transient insulin independence (P = 0.048). Two of the latter animals received a second islet transplant, this time to the portal system, and both achieved insulin independence. Thus, intraportal islet transplantation under conventional immunosuppression is feasible in primates and can result in long-term insulin independence when adequate immunosuppression is maintained. Arterial islet injection, however, does not appear to be a viable islet transplantation technique. Diabetes 51:2135–2140, 2002

Type 1 diabetes results from the immune-mediated destruction of the insulin-producing pancreatic β-cells, located in cell clusters called the islets of Langerhans (1,2). The field of islet transplantation recently emerged as a promising way to restore patients with type 1 diabetes to insulin independence (3–5). Before islet transplantation can be developed as a clinically viable treatment for type 1 diabetes, however, important issues need be addressed. These include formulating guidelines to predict an appropriate islet dose, identifying reliable markers of islet quality, generating a renewable islet source, determining the best islet infusion site, promoting islet viability and growth, and improving ways to monitor and prevent rejection. We reasoned that many of these questions could best be addressed using a preclinical nonhuman primate model. Nonhuman primates are highly relevant because of their phylogenetic relationship to humans (6) and because many of the newer immunomodulatory agents (antibodies and receptor fusion proteins) are specific for human epitopes, many of which cross-react with the corresponding primate epitopes. (5,7,8).

We sought to establish a nonhuman primate model to closely mimic a recently reported steroid-sparing regimen (5,9). Currently, isolated islets are transplanted in the clinical setting via a catheter percutaneously placed into the recipient’s portal vein to the liver (5). We asked whether transplanting islets to an arterial bed (e.g., the celiac tree) versus the portal vein would more safely and as effectively support long-term islet allograft survival. We reasoned that because pancreatic islets comprise only ~2% of the pancreas cell mass but consume 20% of the arterial blood flow, the arterial site might improve islet survival and function (3,10–13). Furthermore, we hypothesized that gaining access to the celiac artery for the islet infusion would be easier and safer than portal vein cannulation because portal cannulation requires percutaneous transhepatic access, which risks intra-abdominal bleeding and portal vein thrombosis (4,5).

RESEARCH DESIGN AND METHODS

General. The procedures described in this study were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animals Resources, National Research Council, Department of Health and Human Services, pub. no. (National Institutes of Health [NIH]) 86-23 (1985) and were approved by the Animal Care and Use Committees of both the NIH and the Armed Forces Radiobiology Research Institute.

Rhesus macaques underwent total pancreatectomy to induce diabetes and were transplanted with allogeneic isolated islets. Pancreata were procured from donor primates, and islet isolation was performed using the automated and Liberase-based method for human islet isolation (14). Islets were infused into either the portal vein or the celiac artery. After transplantation, primates were given daclizumab, tacrolimus, and rapamycin to prevent islet rejection (5).
Rhesus macaques. Recipients were male and female rhesus macaques with an age range of 3–5 years and a weight range of 2–6 kg. Serological testing was performed to ensure that animals were negative for herpes B virus, simian retrovirus (SRV), simian T-cell leukemia virus (STLV), and simian immunodefi-
ciency virus (SIV). Pancreatic islet donors were 6–27 years old and weighed 4–12 kg. All animals had continuous water supply and, until the time of surgery, were fed regular primate diet supplemented with fresh fruits twice daily.

Diabetes induction. Spontaneous type 1 diabetes has not been described in nonhuman primates (15). Therefore, we performed total pancreatectomy to induce diabetes (16). Islet recipients initially underwent a spleen- and duodenum-preserving total pancreatectomy. The procedure was performed through a midline laparotomy under general anesthesia with isoflurane. The pancreas and duodenum were dissected free from the splenic vessels, pancreatic-duodenal artery, superior mesenteric artery, and portal vein with preservation of these structures. All small branching vessels leading into the pancreas from those vessels and bridging vessels from the duodenum were divided and ligated, leaving the spleen and duodenum intact. The common bile duct was preserved, whereas the main and accessory pancreatic ducts were ligated. Buprenorphine hydrochloride (0.05 mg/kg intramuscular every 12 h) (Reckit Colman Pharmaceuticals, Richmond, VA) was used for postoperative analgesia.

Islet isolation and transplantation

Pancreas procurement. Donor pancreata were procured for islet isolation as a terminal procedure. Under general anesthesia with isoflurane, a midline laparotomy was performed. The short gastric vessels were ligated, and the spleen and distal pancreas were dissected en bloc from the surrounding tissues up to the level of the portal vein with preservation of the pancreatic blood supply. Sterile ice was then packed around the pancreas. The infrarenal inferior vena cava was exposed, and 100 ml blood was drawn and used for the islet isolation procedure. The pancreas was rapidly (within 10 min) excised from the duodenum and surrounding vessels. The animal was then euthanized.

Islet isolation. Because we sought to establish a nonhuman primate model that closely mimics the recently reported human clinical studies, we elected to use the same isolation technique described by that group (5,9). Primate islet isolation was performed using a modification of the automated Liberase-based method for human islet isolation (14,16–18). Once the pancreas was chemically and mechanically disrupted into small pieces, the digest was loaded onto a continuous bioflow gradient and centrifuged in a COBE 2991 blood cell processor (COBE, Lakewood, CO). To determine islet number, volume, and purity, a 50-μl sample taken from the 50-ml islet suspension was stained with dithizone and then counted and graded for purity. These data were mathematically converted to the total number of islets, with an average diameter of 150 μm (islet equivalent [IEQ]) (19).

Islet transplantation. We transplanted pancreatic islets into either the portal vein or the celiac artery.

1) Surgical islet infusion. Islets were infused either immediately after removal of the recipient pancreas (n = 5) or during a second procedure requiring a small midline laparotomy (n = 1 as an initial transplantation, and n = 1 after a failed celiac artery transplantation; see below). In either case, a tributary of the portal vein, usually the middle colic vein, was isolated, and a 16-gauge angiocath was advanced toward the portal vein. Islets were then infused via gravity in a 20-ml volume followed by a wash with 50 ml M199 (Gibco/BRL, Life Technologies, Grand Island, NY) and 5% donor serum. When islets were infused into the celiac artery via the open surgical approach (n = 1), the aorta and celiac artery were exposed from the pancreatic bed. Occlusive clamps were placed on the aorta immediately proximal and distal to the celiac artery. A 3-inch 16-gauge angiocath was advanced through the aorta into the celiac artery. Islet and wash solutions were infused as per the portal vein infusions, except that both required gentle external pressure on the infusion syringe instead of the gravity approach, which was used for the portal vein infusions.

2) Invasive radiology (angiographic) transplantation of islets into the celiac artery (n = 3). Vascular access: the animal’s abdomen was cleaned, shaved, and prepped in a sterile manner. Surgical exposure of the common femoral artery and vein was performed. The common femoral artery was identified, and two 2/0 silk sutures were placed under the artery for hemostatic control. The femoral artery was then accessed with a 5-cm 21-gauge needle. Once arterial blood flow was identified in the needle, a 0.018-inch floppy guidewire guide (Cook, Bloomington, IN) was advanced through the needle and into the aorta. In some cases, guide wire passage into the aorta was not feasible, and manipulation with a 0.018 Glide wire (Cook) under fluoroscopic guidance was necessary. Once the wire was placed in the aorta, the needle was removed and a 4F coaxial catheter (Cook) was advanced into the aorta. The inner dilator was removed, and injection of contrast was done under fluoroscopic guidance to confirm the position of the catheter. A 0.035-inch floppy wire (Cook) was then advanced through the dilator, and the dilator was exchanged for a 4F angled glide catheter (Angiodynamics, Queensbury, NY).

Visceral angiography: the catheter was selectively placed in the celiac artery and contrast material (Omnipaque [iohexol 240 mg/ml]; Nycomed, Princeton, NJ) was injected to identify the celiac artery and its branches (splenic, hepatic, and gastric). The internal diameter of the catheter was 0.9 mm. As with islets injected using the surgical approach, islets were slowly injected via gentle pressure (over 20 min) on a 20-ml syringe into the celiac axis through the 4F glide catheter. After infusion, a small amount of contrast was injected to confirm catheter placement in the celiac axis.

3) Accessing the portal system in the monkey through minimally invasive methods (without surgery) for islet cell transplantation. Islet transplantation into the portal vein using radiologic techniques (n = 1) was performed by first gaining vascular access, performing portal venography to confirm catheter placement, and finally islet infusion.

Vascular access: the right upper quadrant was shaved, cleaned with Betadine, and then draped in a sterile manner. Under ultrasonographic guidance, a 21-gauge needle was inserted into the liver through a right subcostal or intercostal approach to the portal vein system. An 0.018 wire (Cook) was inserted into the portal system and maneuvered toward the main portal vein and into the superior mesenteric vein or the splenic vein. The needle was removed, and a 3F dilator (Cook) was inserted under fluoroscopic guidance over the wire and into the portal vein.

Portal venography: iodinated contrast material (Omnipaque) was injected to demonstrate portal anatomy.

Iset transplantation: after confirmation of catheter placement, islets were slowly injected over 20 min using a 20-cc syringe, followed by infusion of wash to clear the lines. The catheter was then removed, and hemostasis was achieved by manual compression.

Assays of islet function. Islet function was assessed by daily glucose measurements and by periodic arginine-stimulated C-peptide levels. Fasting and postprandial blood glucose levels were monitored up to three times per day (prebreakfast, prelunch, and at 6:00 P.M.) via a tail stick, followed by blood testing with a Glucometer Elite (Bayer, Elkhart, IN).

The C-peptide secretory response to intravenous arginine provides an accurate reflection of β-cell mass (20). After an overnight fast, blood samples of 1.0 ml each were collected at 5 and 0 min before the infusion of 2 g arginine (as a 10% solution) by intravenous infusion into the cephalic vein. Subsequent samples were collected from the contralateral femoral artery at 2, 3, 4, 5, 7, 9, and 10 min after the arginine infusion. Restrained bleeding and arginine stimulation procedures were achieved with an intra muscular injection of 10 mg/kg ketamine (Fort Dodge Laboratories, Fort Dodge, IA).

Immunosuppression. Dacizumab (Zenapax; Roche) was administered at 2 mg/kg i.v. on the day of transplantation and every 2 weeks for a total of five doses. Tacamycin (Rapamune and sirolimus; Wyeth-Ayerst) was given twice a day orally to target 24-h trough levels of 10–15 ng/ml. Levels were determined by Mayo Medical Laboratories (Rochester, MN) using liquid chromatography and tandem mass spectroscopy. The average daily dose required was 6 mg/kg. Tacrolimus (Prograf, Fujisawa) was given orally twice daily at 2.5–5 mg with dosage adjusted to achieve 24-h trough levels of 4–6 ng/ml. Tacrolimus levels were measured by the Clinical Chemistry Laboratory at the NIH.

Statistical analysis. The two-sided Fisher’s exact test was used to determine whether graft function and insulin independence after intraportal infusion differed from that achieved after intra-arterial islet infusion. The only animals used for this statistical analysis were those receiving their first dose of transplanted islets. That is, animals given islets via intra-arterial injection (which failed) and then given islets via injection into the portal vein were included in the intra-arterial group but not in the intraportal group.

RESULTS

Iset isolation results. The semiautomatic isolation technique with Liberase HI and continuous density separation consistently yielded pure and viable islets suitable for transplantation. The average yield was 51,304 ± 22,311 IEQ (range 17,000–93,153) (Table 1). We found no correlation between the donor age, weight, or pancreas weight and the final isolation results, although a trend for higher yield and improved viability was observed as we gained experience with the procedure. The glucose stimulation index did not correlate with the islet yield, viability, or with final in vivo function. Others have found that islet functional assays in vitro do not correlate with in vivo function (21).

Immunosuppression. We tested whether the immunosuppressive cocktail recently used in human islet trans-
plantation studies (5) could be used to answer important questions in a primate model. In the first few animals studied accordingly, we were unable to achieve immuno-suppressive agent target drug levels for several reasons. First, we found that the primates appeared to metabolize the rapamycin and tacrolimus faster than humans (data not shown). Second, and more importantly, we had great difficulty getting the primates to take the medications. Efforts to sedate the primates twice daily so that the agents could be administered by nasogastric tube were logistically difficult and were associated with animal wasting. We overcame these problems by using much higher doses of the agents (when compared with typical doses for humans, a 60-fold higher dose for rapamycin, on a per kilogram body weight basis, and an 80-fold higher dose for tacrolimus). In addition, both medications were mixed in the animal’s daily food together with Viakose (Axcan, Birmingham, AL) into Primaburger (Bio-Serv, French-town, NJ) primate chow.

Glycemic control and graft survival. Long-term insulin independence of up to 7 months was demonstrated when adequate immunosuppressive drug levels were achieved and maintained (Figs. 1 and 2). Interestingly, short periods of hyperglycemia, lasting up to 5 days, were observed in some of these primates. During instances of hyperglycemia, the animals maintained C-peptide secretion and the hyperglycemia resolved spontaneously (Fig. 2). The cause for these fluctuations is not clear, although it may have been secondary to transiently elevated tacrolimus levels.

When target immunosuppression drug levels were not attained in animal 2 (Table 2), the graft was rejected 11 days after transplantation. Another primate rejected its graft 40 days after transplantation. This primate maintained target rapamycin levels but inconsistent and, at times, zero tacrolimus levels. A third primate rejected its islets 41 days after transplant and, although therapeutic tacrolimus levels were maintained in that animal, rapamycin levels were not. These data strongly suggest the importance of maintaining appropriate immunosuppressive agent levels, although because the animals did not undergo tissue typing, we cannot rule out the possibility that allograft survival differences could be accounted for by the degree of tissue mismatching. Of note, the current approach to patients receiving clinical islet allografts is to ensure that the donor and patient have compatible blood groups, but tissue matching is not performed prospectively.

**Portal vein infusion versus celiac artery infusion.** Five primates were given six islet infusions into the portal vein (one monkey was transplanted twice), and four primates were infused with islets into the celiac artery. In five of the six portal vein infusion experiments, the animals achieved at least temporary (i.e., ≥5 days) normoglycemia without requiring exogenous insulin. Maintaining therapeutic immunosuppressive agent blood levels was very difficult, however, and was achieved in only two of the six experiments (see above). Those two animals achieved long-term insulin independence. In contrast, none of the islets transplanted into the arterial system resulted in any animal achieving even temporary insulin independence.

### Table 1

<table>
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<tr>
<th>Isolation</th>
<th>Donor weight (kg)</th>
<th>Donor age (years)</th>
<th>Pancreas weight (g)</th>
<th>IEQ</th>
<th>Viability</th>
<th>100% Viability</th>
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NA, not applicable.

**FIG. 1.** Fasting glucose levels of four representative primates. Day 1: induction of diabetes. Fasting glucose levels are shown only for the four animals achieving prolonged (>30 days) insulin independence after transplantation (T).

**FIG. 2.** Arginine-stimulated C-peptide in a representative primate. Lower limit of detection of the test: 0.5 ng/ml. At the 13-week period, the primate had several high glucose levels that spontaneously normalized.
independence. The probability that our results were due to chance was < 0.048 (two-sided Fisher’s exact test). Two of the animals that failed to achieve euglycemia after arterial islet transplantation received a second islet infusion to the portal system. Both achieved long-term insulin independence (Table 2).

**Histopathological studies.** Liver sections were available for study from five primates: one insulin-independent primate that died 5 days after transplantation due to aspiration pneumonia, one primate that was killed on day 30 due to wasting but with normoglycemia without the need for exogenous insulin, one primate that rejected 2 months after transplantation associated with low tacrolimus levels, and two primates that rejected after celiac artery transplantation. Interestingly, the intrahepatic islets observed in the animal transplanted 30 days prior already demonstrated an abundant vascular supply (Fig. 3). Islets observed in the primate transplanted 5 days before its death did not display evidence for such vascularization. We could not find any islets in the primates transplanted through the celiac artery, but each animal had lost evidence for any islet function at least 4 weeks before euthanasia.

**DISCUSSION**

We have established a clinically relevant nonhuman primate (rhesus macaque) model of islet transplantation. We transplanted pancreatic islets into nine rhesus monkeys: six infusions were given into the portal vein (one monkey was transplanted twice), and four infusions were given into the celiac artery. In five of the six portal vein infusion experiments, the animals achieved at least temporary normoglycemia without requiring exogenous insulin. Maintaining therapeutic immunosuppressive agent blood levels was logistically difficult, however, and was achieved in only two of the six animals. Those two animals achieved long-term insulin independence. In contrast, none of the islets transplanted into the arterial system resulted in an animal achieving even transient insulin independence or any evidence of graft function. Two of the latter animals were given a second islet dose, this time by infusing the cells into the portal system, and both achieved long-term insulin independence.

Although islet cell transplantation is a promising way to restore insulin independence to patients with type 1 diabetes, additional research is required to address myriad remaining questions. Thus, the first aim of this study was to establish a nonhuman primate model of islet transplantation using the islet isolation techniques and immunosuppressive cocktail recently described by the Edmonton group (5). Once we established such a model, we sought to test whether infusing the islets into an arterial bed such as that supplied by the celiac tree would be safer and still have the ability to support long-term islet allograft survival. Emphasizing the safety issue, one animal transplanted with intraportal islets via ultrasound-guided invasive radiological technique suffered a significant intra-abdominal bleed requiring a transfusion. Recalling that portal blood is desaturated relative to arterial blood (10,12) and that portal blood contains various substances absorbed from the gut (e.g., high fat concentrations) that may prove toxic to the islets, we asked whether transplanting islets into an arterial bed would better support long-term islet allograft survival. We elected the celiac tree so as to place the islets in an anatomical location such that the insulin secreted would drain via the portal veins to the liver (mimicking the normal situation). Furthermore, the pancreas originates during embryogenesis from the pancreatic bud in the second part of the duodenum, and the celiac tree perfuses that part of the intestine. Additionally, when ectopic pancreatic tissue is found, it is most typically found in the wall of the stomach or the duodenum (22). To our surprise, however, islet function after infusion into the arterial system was poor relative to infusion into the portal system.

The poor islet function observed after intra-arterial infusion might have been the result of several, non-mutually exclusive factors. 1) Arterial blood pressure is higher than portal vein pressures. Although the infusion to the portal vein is accomplished with gravity, we had to use pressure greater than arterial pressure to inject the islets into the artery. This pressure may have compromised islet integrity. 2) Islets infused into the arterial system ultimately lodge in an end arterial, possibly resulting in low flow to that bed and subsequent islet ischemia. This possibility may have been exacerbated by the surgical pancreatectomy used to induce diabetes. That is, the islet infusion was performed several weeks after a total pancreatectomy. During the pancreatectomy, efforts were made to preserve the duodenum blood supply. Nevertheless, the arterial supply to this region may have been compromised, thus further decreasing the anastomoses available to ensure an adequate local supply of arterial blood. 3) Differences in donor-recipient major histocom-
patibility complex (MHC) matching. When these studies were initiated, we did not routinely type the animals for MHC. Although it is possible that the results presented herein are due exclusively to differences between the donor and recipient tissue types, our data strongly argue against that possibility for several reasons. First, we were unable to detect islet function, even transiently, in any of the animals given islets into their arterial system. Except in cases of hyperacute rejection, usually due to antidonor preformed antibodies, allografts typically function for at least 5–7 days. Second, using two animals that had received intra-arterial islets as recipients of a subsequent portal vein islet infusion, we observed good islet function posttransplant. If those animals had rejected the islets infused intra-arterially earlier, they would likely have been sensitized to the subsequent allogeneic islets, but the animals given islets into the portal vein were rendered insulin independent. Third, donors were typically much older than recipients and were obtained from many different sources (usually animals that had to be killed for other health reasons), so the likelihood of a close match between any donor and recipient pair is quite remote.

The aim of this study was not to compare islets transplanted to the hepatic artery versus the portal vein. Even so, angiography flow data suggest that 20–50% of the islets (different in each primate) transplanted via the celiac artery should have flowed to tissue supplied by the hepatic artery. Still, no islet function was observed in any of these primates (data not shown), suggesting that hepatic artery infusion may fail as well, although specific infusion of islets into the hepatic artery should be assessed in the future.

This study also suggests that at least during the early weeks after islet transplantation, the cells are quite susceptible to rejection. It has been suggested that transplanting cells into the portal system may in itself have immunological effects so as to abrogate rejection (23). Our data support the view that aggressive immunosuppression is critical, since any reduction in either the tacrolimus- or rapamycin-circulating blood level was associated with prompt graft rejection.

In conclusion, we have established a relevant preclinical nonhuman primate islet transplantation protocol at the NIH. Diabetes was induced by total pancreatectomy. The
Edmonton approach to islet transplantation is feasible in nonhuman primates and results in long-term insulin independence when adequate levels of immunosuppression are achieved and maintained. The traditional portal vein infusion site is superior to arterial infusion.

ACKNOWLEDGMENTS
The authors are grateful to Drs. Norma Sue Kenyon and Camillo Ricordi for their teaching of primate isolation techniques and diabetic primate care and to Drs. Mark St. Clair and John Bacher for veterinary support during the angiographic procedures at the NIH. The authors gratefully acknowledge the expert veterinary care provided by Cpt. Christopher Keller, Sgt. Angela King, Sgt. Tanya Lewis, HM3 Justin Berning, SPIC Brian Kosling, and Sgt. Donald Randolph.

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