Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154


ABSTRACT Reported effects of anti-CD154 treatment on autoimmunity, alloreactivity, and inflammatory events mediated by macrophages and endothelial cells indicated that it might be an ideal agent for the prevention of intrahepatic islet allograft failure. This hypothesis was tested in MHC-mismatched rhesus monkeys. Transplantation of an adequate number of viable islets resulted in engraftment and insulin independence in six of six recipients treated with anti-CD154 (hu5c8) induction plus monthly maintenance therapy (post-operative day ≥125, ≥246, ≥266, ≥405, ≥419, ≥476). Anti-CD154 (hu5c8) displayed no inhibitory effect on islet cell function. For monkeys followed for >100 days, continued improvement in graft function, as determined by first phase insulin release in response to intravenous glucose, was observed after the first 100 days post-transplant. No evidence of toxicity or infectious complications has been observed. All recipients treated with anti-CD154 became specifically non-responsive to donor cells in mixed lymphocyte reactions. Furthermore, three monkeys are now off therapy (>113, >67, and >54 days off anti-CD154), with continued insulin independence and donor-specific mixed lymphocyte reaction hyporesponsiveness. In striking contrast to all previously tested strategies, transplantation of an adequate number of functional islets under the cover of anti-CD154 (hu5c8) monotherapy consistently allows for allogeneic islet engraftment and long-term insulin independence in this highly relevant preclinical model.

Islet cell transplantation for patients with type 1 diabetes can result in the reversal of hyperglycemia and normalization of metabolic control (1–7). Broad-based application of curative islet cell transplantation has been limited, however, by the inability of current, generalized immunosuppressive reagents to reliably support long-term islet graft survival and function. The CD40-CD154 costimulation pathway has proven to be a critical interaction in the generation of a T-dependent immune response (8–10), and blockade of this pathway has prevented allograft rejection (11–16), graft versus host disease (17–19), renal allograft rejection (20–29), and autoimmunity (30–36). Blockade of the CD40-CD154 co-stimulation pathway, therefore, has the potential to prevent allograft rejection, recurrent autoimmunity, and the nonspecific inflammatory events that occur on transplantation of islets into the liver, without the adverse effects of conventional, generalized immunosuppressive drugs on islet function (37). This study was undertaken to determine whether anti-CD154 (hu5c8) monotherapy would prevent the rejection of allogeneic islets in a preclinical, non-human primate model of pancreatectomy-induced diabetes.

MATERIALS AND METHODS

Rhesus Monkeys. SPF monkeys were purchased from Covance (Alice, TX) or through local sources (Charles River Breeding Laboratories). Recipients were 2–4 years old and weighed 3–4 kg at the time of transplant. Recipients either maintained weight or lost up to 0.4 kg during the initial 3–6 months post-transplant, followed by continued weight gain. Beginning and current weights are listed in Table 1. Donors were 2–4 years of age and weighed ≥5 kg. Recipients were pair housed and were fed twice daily. The experiments described in this study were conducted according to the principles set forth in the Guide for Care and Use of Laboratory Animals (38).

Restraint of Animals. Monkeys were sedated with ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) injected into the gluteus muscle at a dose of ~5–10 mg/kg. Additional ketamine was administered as needed. Because it has been demonstrated that ketamine reduces first-phase insulin release, we kept the ketamine dose as low as possible for the purposes of metabolic testing (39). Animals also were physically restrained with a restraining board during performance of intravenous glucose tolerance tests (IVGTTs).

Administration of anti-CD154 (hu5c8). Monkeys received induction therapy consisting of i.v. hu5c8 (Biogen) on post-operative days (PODs) −1, 0, 3, and 10, or on PODs −1, 0, 3, 10, and 18, at a dose of 20 mg/kg. Maintenance therapy at a dose of 10 or 20 mg/kg was initiated on POD 28 and was given every 28 days thereafter (Table 1). No adverse reactions were observed during antibody administration. Serum samples were obtained before each antibody dose, as well as biweekly, to determine hu5c8 and anti-hu5c8 levels [determined by ELISA (Biogen)]. The pharmacokinetics of the antibody in cynomolgus monkeys has been previously described by Gobburu et al. (40).

Donor Pancreatectomy and Islet Isolation. The donor pancreas was harvested on POD −1. In all cases, the procedure was performed under general anesthesia through a long vertical midline incision. The splenicocolic and splenoportal ligaments were divided so that the spleen, together with the tail of the pancreas, was mobilized. The common bile duct, the main,
and (occasionally) the accessory pancreatic ducts were identified and ligated. A 14-gauge catheter was placed in the infrarenal aorta, and the animal was exsanguinated. The head of the pancreas was excised from the second portion of the duodenum by sharp dissection. Similarly, the rest of the pancreas was excised from the surrounding tissues, leaving the division of the pancreaticoduodenal and splenic vessels at the end to minimize any ischemia to the pancreas. The pancreas was taken out, en block, with the spleen.

Rhesus monkey islet isolation was undertaken via minor modifications of the automated method for human islet isolation (41, 42) by using Liberase (Boehringer Mannheim) at a concentration of 0.47 mg/ml. A three-layer, discontinuous Euroficol gradient (densities 1.108, 1.097, 1.037) and a Cobe blood cell processor (Cobe Laboratories, Lakewood, CO) were used for purification of islets from the pancreatic digest (43). Samples of the final islet preparation were stained with dithizone (44), and the preparation was assessed by counting the number of islets with an average diameter of 150 μm and were expressed as islet equivalents (IEQ) (45).

Recipient Pancreatectomy and Intrahepatic Islet Cell Transplantation. Total pancreatectomy, without duodenectomy or splenectomy, was performed. The tail and the body of the pancreas were dissected along the splenic artery and vein, which were carefully preserved by ligating and dividing only the branches to the pancreas. The inferior mesenteric and middle colic veins were recognized and preserved during the dissection of the body of the pancreas. The portal and superior mesenteric vein were recognized and the pancreatic veins were ligated and divided.

By using a Kocher maneuver, the duodenum was mobilized. The branches of the pancreaticoduodenal vessels that enter the pancreas were ligated and divided, leaving the branches for the duodenum intact. The common bile duct was identified and preserved during the sharp dissection between the head of the pancreas and the second portion of the duodenum. The main and the accessory pancreatic ducts were ligated with nonabsorbable sutures and were divided, and the pancreas was taken out of the peritoneal cavity.

The efficacy of the pancreatectomy procedure has been verified in rhesus and cynomolgus monkeys and baboons. Control recipients (no hu58) that fully rejected their grafts, FK506 treated animals, and recipients with partial function were carefully explored at necropsy. No evidence (gross observation or histological analysis) of residual pancreas has been observed. In addition, recipients of rejected islet allografts were C-peptide-negative.

Overnight cultured islets were washed in transplant media, consisting of RPMI medium 1640 (GIBCO/BRL) supplemented with 2.5% normal donor serum, and were counted to determine the number of IEQ. The islets were pelleted and resuspended in 20 ml of transplant media supplemented with 200 units of heparin, and, for recipients treated with anti-CD154 (hu58), 20 mg of 5e8 was added. Intrahepatic islet transplantation was accomplished via gravity drainage of islets into a sigmoid or a branch of the left colic vein (draining into the portal vein), through a 24-gauge intravenous catheter.

The blood glucose level was checked with a glucometer every 15 minutes during the pancreatectomy, and every 15 minutes after the infusion of islets, to check for hypoglycemia. Hypoglycemia was treated with i.v. infusion of 5–7.5% dextrose as needed. Blood glucose was maintained between 60–120 mg/dl during the surgical procedure. Blood glucose values were routinely stabilized within 4 hours post-operation.

Post-Operative Care and Diet. On the day of islet transplantation, monkeys were given i.v. fluids. Buprenorphine hydrochloride (0.05 mg/kg, s.c., Reckitt & Colman Pharmaceuticals, Richmond, VA) was administered for pain on the day of surgery and on POD 1. Baytril antibiotic (enrofloxacin, Bayer, Shawnee Mission, KS) was given at a dose of 5 mg/kg, i.m., from POD 0–4. On the first post-operative day, the animals were given Gatorade. On POD 2, the monkeys were fed banana and grapes in the morning and afternoon. Water was administered ad libitum. The animals were subsequently fed a diet of 160 g of carbohydrate per day, consisting of a morning and an afternoon meal of High Protein Monkey Chow (product code 5045, Purina Mills, Richmond, IN) and fruit. Pancreatic exocrine insufficiency was compensated via addition of Viokase-V (Fort Dodge Animal Health, Fort Dodge, IA) to the diet.

Blood Glucose Monitoring, Insulin Administration, and Definition of Rejection. Fasting and post-prandial blood glucose levels (FBG and PPG) were monitored three times per day (pre-breakfast, pre-lunch, and at 8 PM) via heel stick, followed by blood testing with a Glucometer Elite (Bayer, Elkhart, IN). We have observed that post-prandial hyperglycemia precedes elevations in fasting blood glucose, and, based on our experience in both rhesus monkeys and baboons, we consider two consecutive FBGs >100 and/or two consecutive PPGs >150 mg/dl to be evidence of rejection in recipients with previously stable glucose values. When needed, monkeys were treated with subcutaneous insulin if FBG exceeded 100 mg/dl and/or PPG exceeded 150 mg/dl. For animals with no graft function, three insulin injections were required per day to maintain FBG in the 100- to 200-mg/dl range.

Metabolic Testing. It has previously been demonstrated that first-phase insulin release (FPIR) in response to intravenous glucose tolerance testing provides an accurate reflection of β cell mass (46). After an overnight fast, blood samples of 1.5 ml each were collected at 10, 5, and 0 minutes before the infusion of glucose, followed by intravenous administration (cephalic
nuclear cells (PBMCs) were used as responders against culture reactivity (Table 1). Recipient peripheral blood mono-recipient pairs were chosen based on positive mixed leukocyte alloreactive donor- and Anti-Third-Party Immunoreactivity. C-peptide was 0.20 ng insulin and C-peptide levels. The lower limit of detection for C-Peptide. Plumae (Roche Diagnostic) was used to assess plasma analyzer. A double antibody method (Diagnostic Products, Los Angeles, CA) was used to assess plasma glucose was measured by using a Cobas Mira glucose analyzer. Normal monkey serum was used as a negative control.

Leukocyte Subsets, Fasting Plasma Glucose, Insulin, and C-Peptide. Leukocyte subsets were obtained from the recipient before, and 1, 0, 3, and 10 or on PODs −1, 0, 3, 10, and 18 (Table 1). Maintenance doses were given every 28 days, beginning on POD 28. Subsequent to the induction of diabetes via total pancreatectomy, allogeneic islets were transplanted via the portal vein into the liver of recipient monkeys. To maximize the potential benefit of anti-CD154 (hu5c8) on the nonspecific inflammatory events that occur as a consequence of intrahepatic transplantation, 20 mg/kg hu5c8 was included in the islet preparation. The number of islets transplanted per kg and recipient anti-donor MLC reactivity (pre-transplant), as well as recipient age and weight, are given in Table 1. The monkeys were transplanted with a range of ≈10,000 to 40,000 IEQs/kg of recipient body weight (Table 1).

Transplantation of allogeneic islets into MLC mismatched monkeys treated with humanized anti-CD154 (hu5c8) resulted in islet engraftment, long-term function, and insulin independence in six of six animals (PODs >125, >246, >266, >405, >419, >476), with no evidence of rejection in five monkeys throughout the follow up period. One animal that was under 10 mg/kg maintenance therapy experienced an elevation in evening blood glucose levels, followed by a rise in fasting and post-prandial values on POD 64 (RH-05). This monkey was given antirejection therapy consisting of 20 mg/kg anti-CD154 (hu5c8) on PODs 64, 68, and 75. The animal returned to normoglycemia and has remained insulin-independent (POD >170) on 20 mg/kg maintenance therapy. Representative fasting and PPG levels for the two longest surviving monkeys are depicted in Fig. 1, and the duration of graft survival (insulin independence) for all of the monkeys is given in Table 2.

RESULTS

Anti-CD154 (hu5c8) Monotherapy Prevents Rejection of Primary Islet Allografts in Rhesus Monkeys with Pancreatectomy-Induced Diabetes. Donor pancreatectomy and islet isolation were performed on POD −1. Induction therapy, consisting of 20 mg/kg anti-CD154 (hu5c8), was administered to recipient monkeys on PODs −1, 0, 3, and 10 or on PODs −1, 0, 3, 10, and 18 (Table 1). Maintenance doses were given every 28 days, beginning on POD 28. Subsequent to the induction of diabetes via total pancreatectomy, allogeneic islets were transplanted via the portal vein into the liver of recipient monkeys. To maximize the potential benefit of anti-CD154 (hu5c8) on the nonspecific inflammatory events that occur as a consequence of intrahepatic transplantation, 20 mg/kg hu5c8 was included in the islet preparation. The number of islets transplanted per kg and recipient anti-donor MLC reactivity (pre-transplant), as well as recipient age and weight, are given in Table 1. The monkeys were transplanted with a range of ≈10,000 to 40,000 IEQs/kg of recipient body weight (Table 1).

Transplantation of allogeneic islets into MLC mismatched monkeys treated with humanized anti-CD154 (hu5c8) resulted in islet engraftment, long-term function, and insulin independence in six of six animals (PODs >125, >246, >266, >405, >419, >476), with no evidence of rejection in five monkeys throughout the follow up period. One animal that was under 10 mg/kg maintenance therapy experienced an elevation in evening blood glucose levels, followed by a rise in fasting and post-prandial values on POD 64 (RH-05). This monkey was given antirejection therapy consisting of 20 mg/kg anti-CD154 (hu5c8) on PODs 64, 68, and 75. The animal returned to normoglycemia and has remained insulin-independent (POD >170) on 20 mg/kg maintenance therapy. Representative fasting and PPG levels for the two longest surviving monkeys are depicted in Fig. 1, and the duration of graft survival (insulin independence) for all of the monkeys is given in Table 2.

![Graph](Image)

**Fig. 1.** Fasting (Left) and post-prandial (Right) blood glucose values for the two longest surviving recipients of allogeneic islets. RH-01 occasionally received a dose of 0.5 units of insulin during the first 2 weeks post-transplant to maintain PPG < 200 mg/dl but was subsequently insulin independent.
In the first 2–4 weeks post-transplant, four of the six monkeys required small doses of insulin (<1.0 units/kg/day) to maintain normoglycemia. In contrast, a pancreatectomized or streptozotocin-treated rhesus monkey requires 3–4 units of insulin per kg/day. After the first month post-transplant, the FG and PPG levels were extremely stable in all six recipients, without any need for administration of exogenous insulin.

Allogeneic Islets Are Rapidly Rejected in a Control, Non-treated Rhesus Monkey. In striking contrast to the anti-CD154 (hu5c8) treated recipients, rejection was first detected in a control, nontreated monkey as an elevated PPG on POD 6 (Fig. 2). Insulin treatment, consisting of three injections per day, was begun immediately at a dose of 2.4–2.7 units/kg/day and was rapidly increased to a dose of 4.2–4.8 units/kg/day. Despite aggressive insulin therapy, it was difficult to achieve fasting blood glucose values <120 mg/dl (Fig. 2), and the monkey suffered from occasional hypoglycemia because of intensive insulin therapy. Fasting C-peptide was normal on POD 3 and was negative from occasional hypoglycemia because of intensive insulin therapy. Fasting C-peptide was normal on POD 3 and was negative from occasional hypoglycemia because of intensive insulin therapy.

Islet Allograft Function in Anti-CD154 (hu5c8)-Treated Rhesus Monkeys. Fasting C-peptide levels, determined every other week, varied from 0.6 to 6 ng/ml range. These technical issues compared with PODs 42 and 99 (Fig. 3). Long-term maintenance of islet mass is demonstrated by the overlapping insulin response curves obtained on PODs 155, 227, and 296 (Fig. 3). The IVGTT results obtained on POD 367 are similar to those obtained before pancreatectomy and islet transplant (Fig. 3).

Retransplantation in a Monkey Treated with Anti-CD154. Preliminary results, generated in the course of establishing the macaque models (rhesus, cynomolgus), resulted in a series of recipients (n = 5) with partial islet allograft function whereas insulin independence was consistently achieved in baboons (Papio hamadryas). It became evident that the smaller donor size and age (3–4 kg, 1.5–2 years of age) of the macaques, as compared with the baboons (>20 kg and >4 years old) contributed to technical difficulties in islet isolation and fragility and subsequent transplantation of an inadequate number of functional islets. Similar to what has been observed clinically, these animals had varying degrees of partial graft function, ranging from excellent (required <1.5 units of insulin/kg/day) to good (required 1.5–2.5 units/kg/day). Monkeys with no islet allograft function are C-peptide negative and require up to 4 units/kg/day insulin to maintain blood glucose levels in the 100- to 200-mg/dl range. These technical issues were resolved by identifying older (>4 years of age) and larger (>5 kg) donors.

One anti-CD154 (hu5c8)-treated rhesus monkey (RH-07) that was transplanted with a marginal islet mass (as determined by IEQ count plus morphological assessment) was treated with 3–4 units/kg/day insulin for 169 days post-transplant. Partial graft function, as detected via IVGTT (Fig. 4), was clearly present on POD 160. Subsequent to a second islet allograft on POD 170, RH-07 required small doses of insulin for the first month post-transplant to maintain fasting blood glucose <100 mg/dl and PPG <150 mg/dl. On POD 27, the animal was taken off insulin and remained insulin-independent (Table 2). An IVGTT on POD 99 revealed a dramatic increase in FPIR subsequent to the second transplant (Fig. 4), and additional tests performed on PODs 173 and 235 demonstrated preservation of islet mass over the course of this study.

Lack of Side Effects in Anti-CD154 (hu5c8)-Treated Monkeys: Monitoring of CBC, Weight, Leukocyte Subsets, and Serum Chemistries. All recipients were monitored weekly in the first month post-transplant, and biweekly thereafter, for CBC, weight, and chemistry. Immunophenotypic analysis of peripheral blood leukocyte subsets was undertaken three times pre-transplant to obtain a baseline and monthly thereafter. No alterations in CBC or leukocyte subsets were detected. In some
respectively, with continued insulin independence and maintenance does of hu5c8 at 1 year post-transplant (Table 2). These three monkeys had been off therapy for 113, 67, and 54 days, respectively, with continued insulin independence and maintenance of the islet graft. Continued insulin independence and maintenance of anti-donor mixed lymphocyte reaction hyporesponsiveness has been observed in three recipients that have been taken off anti-CD154 therapy. Only one recipient, of four tested, has developed anti-donor antibody.

Islets must be revascularized and integrated into the hepatic architecture to become fully functional. Because anti-CD154 (hu5c8) does not adversely effect islet cell function (50) we have been able to detect subtle changes in islet cell function over time. Engraftment appears to take ~100 days in our model, as reflected by the observation of continued improvement in FPIR over the first 3-4 months post-transplant (Fig. 3). Islet mass is maintained over the long-term, and this is clearly represented by the data obtained from the longest surviving monkey (Fig. 3).

Humans with type 1 diabetes require up to 1 unit/kg/day insulin; rhesus monkeys without islet function (e.g., after islet allograft rejection or after treatment with streptozotocin) require up to 4 units of insulin per kg/day. Rhesus and human insulin are identical (51), thus suggesting that rhesus monkeys require more insulin and, by extrapolation, more islets than humans to maintain normoglycemia. In humans, the minimal number of IEQ/kg required to achieve insulin independence (obtained only in a small number of patients) has been reported to be ~6,000 (52). This number has been determined in recipients treated with conventional immunosuppressive drugs, which are known to increase the metabolic demand on islets by as much as 2.5-fold (37). In light of the lack of adverse effects of anti-CD154 (hu5c8) on islet cell function (50) our results suggest that transplantation of <6,000 IEQ/kg may allow for insulin independence in humans treated with anti-CD154 (hu5c8).

Anti-CD154 monotherapy effectively and reproducibly prevents islet allograft rejection in rhesus monkeys, as well as in baboons (50). The question that remains unresolved is whether or not this therapy will effectively prevent the recurrence of donor specific mixed lymphocyte reactions hyporesponsiveness.

**DISCUSSION**

Islet cell transplantation offers the promise of a cure for type 1 diabetes, and long-term survival of human islet allografts has now been documented (7). Islet allograft failure in recipients with type 1 diabetes is a complex, multifactorial process involving rejection, recurrence of islet directed autoimmunity, and early loss of islets because of damage mediated by cytokines produced as a result of intrahepatic transplantation (48, 49). In addition, the adverse effects of conventional immunosuppressive drugs, such as steroids, cyclosporin A, and Tacrolimus, contribute to islet loss and dysfunction (37). Blockade of the CD40–CD154 pathway can prevent allograft rejection (11–16) and graft versus host disease (17–19), can alter the course of autoimmunity (20–29), and can prevent the production of proinflammatory mediators by activated macrophages (32–34) and endothelial cells (35, 36). For these reasons, anti-CD154 (hu5c8) appeared to be an ideal agent for use in islet allotransplantation.

The results of this study clearly demonstrate that monotherapy with anti-CD154 (hu5c8) results in islet engraftment and long-term survival in a highly relevant, pre-clinical allotransplant model. Six/six monkeys treated with anti-CD154 and transplanted with an adequate mass of functional islets became insulin independent and remain so at the time of submission of this study (POD >125, >246, >266, >405, >419, >476). To date, the monkeys have been maintained on monthly doses of anti-CD154 (hu5c8) for 1 year, with three animals no longer receiving treatment (RH-01) and plans to discontinue therapy on the other monkeys at 1 year post-islet transplantation. Post-transplant, all recipients became MLC-nonreactive to donor cells, with maintenance of third-party reactivity, thus suggesting some degree of donor specificity in the maintenance of the islet graft. Continued insulin independence and maintenance of anti-donor mixed lymphocyte reaction hyporesponsiveness has been observed in three recipients that have been taken off anti-CD154 therapy. Only one recipient, of four tested, has developed anti-donor antibody.

| Table 3. Anti-donor and anti-third-party MLC reactivity, cpm |
|---------------------------------|----------------|----------------|----------------|----------------|
|                                | α self         | α donor        | α third        | Con A          |
| RH-01 pre                       | 62             | 4,920          | 6,083          | 5,671          |
| POD 136                         | 2,693          | 7,413          | ND             | 22,906         |
| POD 276                         | 231            | 765            | 4,041          | 9,724          |
| RH-02 pre                       | 404            | 11,489         | 20,367         | 36,396         |
| POD 34                          | 188            | 8,263          | ND             | 15,375         |
| POD 174                         | 94             | 243            | 12,192         | 18,538         |
| RH-03 pre                       | 359            | 4,971          | 4,113          | 160,143        |
| POD 50                          | 381            | 818            | ND             | 14,286         |
| POD 190                         | 538            | 139            | 4,681          | 16,378         |
| RH-04 pre                       | 1,493          | 21,690         | 7,759          | 60,209         |
| POD 51                          | 1,493          | 252            | 4,772          | 15,509         |
| RH-05 pre                       | 349            | 6,028          | 20,562         | 4,284          |
| POD 31                          | 683            | 145            | 12,114         | 37,708         |
| RH-07 pre-1                     | 741            | 9,109          | ND             | 71,171         |
| POD 357                         | 285            | 432            | 2,461          | 16,534         |
| RH-07 pre-2                     | 354            | 3,905          | 9,367          | 43,932         |
| POD 187                         | 229            | 301            | 2,461          | 16,534         |

ND, not determined; pre, before treatment and transplant.
autoimmunity in recipients with type 1 diabetes. Data from rodent models suggests that anti-CD154 is effective in altering the course of autoimmune disease (20–29). Although we do not have a non-human primate model of autoimmune, type 1 diabetes, CD40-CD154 interaction clearly plays a role in the development of diabetes in the non-obese diabetic mouse model (29). The consistent engraftment and insulin independence obtained in these studies, in the absence of the toxic effects of conventional immunosuppression, and the observation of long-term maintenance of islet mass support the initiation of carefully controlled clinical trials of islet allotransplantation in patients with type 1 diabetes.

The authors gratefully acknowledge the expert assistance of Dr. Nicola Cautero, Dr. Werviston DeFaria, Mr. Waldo Diaz, Dr. Domeni Han, Drs. Hisham and Haitham Al-Khayat, Ms. Elina Linetsky, Nicola Cautero, Dr. Werviston DeFaria, Mr. Waldo Diaz, Dr. Dong-plantation in patients with type 1 diabetes.

diabetes, CD40-CD154 interaction clearly plays a role in the course of autoimmune disease (20–29). Although we do not have a non-human primate model of autoimmune, type 1 diabetes, CD40-CD154 interaction clearly plays a role in the development of diabetes in the non-obese diabetic mouse model (29). The consistent engraftment and insulin independence obtained in these studies, in the absence of the toxic effects of conventional immunosuppression, and the observation of long-term maintenance of islet mass support the initiation of carefully controlled clinical trials of islet allotransplantation in patients with type 1 diabetes.

The authors gratefully acknowledge the expert assistance of Dr. Nicola Cautero, Dr. Werviston DeFaria, Mr. Waldo Diaz, Dr. Domeni Han, Drs. Hisham and Haitham Al-Khayat, Ms. Elina Linetsky, Nicola Cautero, Dr. Werviston DeFaria, Mr. Waldo Diaz, Dr. Dong-plantation in patients with type 1 diabetes.

diabetes, CD40-CD154 interaction clearly plays a role in the course of autoimmune disease (20–29). Although we do not have a non-human primate model of autoimmune, type 1 diabetes, CD40-CD154 interaction clearly plays a role in the development of diabetes in the non-obese diabetic mouse model (29). The consistent engraftment and insulin independence obtained in these studies, in the absence of the toxic effects of conventional immunosuppression, and the observation of long-term maintenance of islet mass support the initiation of carefully controlled clinical trials of islet allotransplantation in patients with type 1 diabetes.

The authors gratefully acknowledge the expert assistance of Dr. Nicola Cautero, Dr. Werviston DeFaria, Mr. Waldo Diaz, Dr. Domeni Han, Drs. Hisham and Haitham Al-Khayat, Ms. Elina Linetsky, Nicola Cautero, Dr. Werviston DeFaria, Mr. Waldo Diaz, Dr. Dong-plantation in patients with type 1 diabetes.