Clinical islet cell transplantation has resulted in insulin independence in a limited number of cases. Rejection, recurrence of autoimmunity, and impairment of normal islet function by conventional immunosuppressive drugs, e.g., steroids, tacrolimus, and cyclosporin A, may all contribute to islet allograft loss. Furthermore, intraportal infusion of allogeneic islets results in the activation of intrahepatic macrophages and endothelial cells, followed by production of proinflammatory mediators that can contribute to islet primary nonfunction. We reasoned that the beneficial effects of anti-CD154 treatment on autoimmunity, alloreactivity, and proinflammatory events mediated by macrophages and endothelial cells made it an ideal agent for the prevention of islet allograft failure. In this study, a nonhuman primate model (Papio hamadryas) was used to assess the effect of humanized anti-CD154 (hu5c8) on allogeneic islet engraftment and function. Nonimmunosuppressed and tacrolimus-treated recipients were insulin independent posttransplant, but rejected their islet allografts in 8 days. Engraftment and insulin independence were achieved in seven of seven baboon recipients of anti-CD154 induction therapy administered on days –1, 3, and 10 relative to the islet transplant. Three of three baboons treated with 20 mg/kg anti-CD154 induction therapy experienced delayed rejection episodes, first detected by elevations in postprandial glucose levels, on postoperative day (POD) 31 for one and on POD 58 for the other two. Re-treatment with three doses of anti-CD154 resulted in reversal of rejection in all three animals and in a return to normoglycemia and insulin independence in two of three baboons. It was possible to reverse multiple episodes of rejection with this approach. A loss of functional islet mass, as detected by reduced first-phase insulin release in response to intravenous glucose tolerance testing, was observed after each episode of rejection. One of two baboons treated with 10 mg/kg induction therapy became insulin independent posttransplant but rejected the islet graft on POD 10; the other animal experienced a reversible rejection episode on POD 58 and remained insulin independent and normoglycemic until POD 264. Two additional baboon recipients of allogeneic islets and donor bone marrow (infused on PODs 5 and 11) were treated with induction therapy (PODs –1, 3, 10), followed by initiation of monthly maintenance therapy (for a period of 6 months) on POD 28. Rejection-free graft survival and insulin independence was maintained for 114 and 238 days, with preservation of functional islet mass observed in the absence of rejection. Prevention and reversal of rejection, in the absence of the deleterious effects associated with the use of conventional immunosuppressive drugs, make anti-CD154 a unique agent for further study in islet cell transplantation.

Diabetes 48:XXX–XXX, 1999

The requirement for and incomplete success with chronic generalized immunosuppression has limited the application of curative islet cell transplantation for type 1 diabetes. Moreover, conventional immunosuppressive drugs, such as steroids, cyclosporin A, and tacrolimus, are known to impair normal islet function (1), increase susceptibility to infection and malignancy, stunt normal growth and development, and result in direct organ toxicity (2). Nevertheless, human islet cell allotransplantation in patients with type 1 diabetes has resulted in normoglycemia and normalization of other metabolic parameters, in the absence of hypoglycemia, in a limited number of patients who have received standard immunosuppression (3–9). Graft function of >6 years’ duration resulting in improved glycemic control has now been documented for two diabetic recipients of allogeneic islets (9).
The limited success in human islet allotransplantation is probably multifactorial. Factors limiting success include the diabetogenic effect of immunosuppressive drugs, recurrent anti-β-cell autoimmunity, and/or nonspecific inflammatory events that occur when islets are transplanted into the hepatic microenvironment. Over the years, while several approaches have proven successful for engraftment and long-term graft survival in rodent islet cell transplantation models, these approaches have not translated uniformly or consistently to either canine or nonhuman primate preclinical models or to humans.

Recently, modulation of the immune response, via blockade of the CD40-CD154 costimulatory pathway (10–12), has been shown to prevent the rejection of allografts (19–24), ameliorate autoimmune disease (25–34), and prevent the diabetogenic effect of immunosuppressive drugs, recurrent events that occur when islets are transplanted into the hepatic microenvironment. This study was designed to assess the efficacy of human-anti-CD154 (hu5c8) in a rhesus monkey model (13), achieved via phages, and endothelial cells. CD154, also known as CD40 (Homestead, FL). Donors ranged from 4 to 10 years of age and recipients from nonhuman primates (13). CD40 is expressed on antigen presenting cells (APC), including B-cells, dendritic cells, macrophages, and endothelial cells. CD154, also known as CD40 ligand (CD40L), is rapidly and transiently upregulated on CD4+ T-cells upon interaction of the T-cell receptor with the antigen/major histocompatibility complex on APC. The subsequent interaction of CD40 on APC with CD154 on the T-cell has multiple effects, including upregulation of the CD80 and CD86 costimulatory molecules and activation of the signal cascade that leads to generation of an allospecific immune response. Blockade of this interaction prevents the generation of cytotoxic effector cells and T-dependent antibodies (35–37). Even more striking is the aforementioned demonstration of engraftment and long-term survival of allogeneic kidneys in a rhesus monkey model (13), achieved via monotherapy with a humanized anti-CD154 specific monoclonal antibody 5C8 (Biogen, Cambridge, MA) (38).

This study was designed to assess the efficacy of humanized anti-CD154 (hu5c8) in a nonhuman primate model of islet allotransplantation. The results obtained clearly demonstrate allogeneic islet engraftment and insulin independence, with long-term function (up to 300 days) for all baboons treated with adequate doses of anti-CD154 (hu5c8). Furthermore, we have demonstrated that it is possible to halt the progression of islet allograft rejection and return the animals to normoglycemia and insulin independence by treating the recipients with additional doses of anti-CD154 (hu5c8).

RESEARCH DESIGN AND METHODS

Baboons. Baboons (Papio hamadryas) donors were obtained from the Southwest Foundation (Alice, Texas) and recipients from the Mannheimer Foundation (Homestead, FL). Donors ranged from 4 to 10 years of age and recipients from 0.5 to 2 years of age; both males and females were used. Recipients were pair-housed and fed twice daily. The experiments described in this study were conducted according to the principles set forth by the Institute of Laboratory Animal Resources, National Research Council (38a).

Identification of donor-recipient pairs. Alloreactive donor-recipient pairs were chosen based on positive mixed leukocyte culture (MLC) reactivity, with stimulation indices of ≥11.3. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood via density gradient centrifugation on ficoll-paque (Pharmacia, Piscataway, NJ). In a one-way MLC, 105 recipient PBMC were used as responders against 106 γ-irradiated (3,000 rad) donor PBMC. Cultures were established in 96-well U-bottom tissue culture clusters. The medium consisted of RPMI-1640 (Gibco BRL, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine, 100 µM β-mercaptoethanol, 1% vitamins, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and 10 mM sodium 4-HEPES buffer (all Gibco BRL). Cultures were incubated for 5 days in an atmosphere of 5% CO2 in air at 37°C, pulsed with 1 µCi of tritiated thymidine ([3H]thymidine), incubated overnight, and harvested. Counts per minute of incorporated [3H]thymidine were determined, and stimulation indices were calculated as counts per minute of stimulation divided by counts per minute from experimental cultures of recipient PBMC versus irradiated donor cells divided by counts/min from control cultures of recipient PBMC versus irradiated autologous cells). Results of preliminary in vitro experiments revealed that use of MLC medium supplemented with human serum (Sigma, St. Louis, MO) resulted in low background and high specific reactivity (compared with medium containing calf or normal baboon serum). The stimulation indices for donor-recipient pairs used in this study was 11.3–40.8, reflecting a high degree of donor-recipient alloreactivity.

Donor pancreactectomy and islet isolation. The technique used for donor pancreactectomy is as follows. First, the splenocolic and splenorenal ligaments were divided so that the spleen, together with the tail of the pancreas, was mobilized. After the performance of a Kocher maneuver, the head of the pancreas was dissected from the second portion of the duodenum. The common bile duct, the main (Wirsung), and occasionally, the secondary (Santorini) pancreatic ducts were ligated and divided. A 14-gauge catheter was placed in the infrarenal aorta, and the animal was exsanguinated (the blood was collected to obtain donor serum and peripheral blood leukocytes). After exsanguination, the gastrosplenic ligament was divided and sharp dissection was performed between the sharp margin and the pancreas. The splenic and pancreaticoduodenal vessels were divided, and the pancreas was taken out, en bloc, with the spleen. The mesenteric lymph nodes were also collected. The pancreas, spleen, and lymph nodes were placed in Hank’s balanced salt solution for transportation to the lab. The spleen and nodes were processed and cryopreserved to serve as a source of donor cells posttransplant.

Recipient pancreactectomy and intrahepatic islet cell transplantation. The anesthesia and mechanical cleansing of the recipient was similar to that undertaken for the donor. Hemodynamic parameters, respiration, temperature, and arterial hemoglobin oxygen saturation (noninvasive measurement via pulse oximetry) were monitored. A 24-gauge intravenous catheter was placed for administration of fluids and medications. The technique used for total pancreactectomy has been previously described by Ericzon et al. (44) for a cynomolgous monkey model. Cultured islets were washed and resuspended in 20 ml of medium containing 10% fetal calf serum and 20 mg of bacitracin. The final islet preparation was suspended in 250 ml RPMI 1640 solution, and 100-µl samples were stained with dithizone (42) and then counted to assess total islet yield. These data were mathematically converted (43) to the total number of islets with an average diameter of 150 µm (islet equivalent IEQ). Islets were cultured overnight at 25°C, 5% CO2.

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Postoperative care and diet. On the day of islet transplantation, baboons were given intravenous fluids. Buprenorphine hydrochloride (Buprenex, 0.05 mg/kg s.c.; Reckitt & Colman Pharmaceuticals, Richmond, VA) was administered for pain on the day of surgery and on postoperative day (POD) 1. Baytril antibiotic (enrofloxacin; Bayer, Agriculture Division, Animal Health, Shawnee Mission, KS) was given at a dose of 5 mg/kg i.m. on PODs 0–4. On POD 1, the animals were given water ad libitum (Quaker Oats Company, Water Mill, NY). Fruit was given on POD 2, and banana mixed with biscuit crumbs on POD 3. The animals were subsequently fed a morning and an afternoon meal of High Protein Monkey Chow (product code #5045; Purina Mills, Richmond, IN) and fruit (total daily intake: 2–4% body wt, including 47.9% carbohydrates, 5% fat, 25% protein, and 6% fibers). Pancreatic exocrine insufficiency, resulting from the pancreactectomy procedure, was compensated with Viokase-V (Fort Dodge Animal Health, Fort Dodge, IA). Except for the animals that rejected islets early (in the first 2 weeks posttransplant), all baboons gained weight (up to 1 kg); none experienced steatorrhea.
**Insulin.** Diabetic baboons with no endogenous insulin secretion capacity required 4–6 U of insulin · kg·day⁻¹ to maintain blood glucose levels in the 100–200 mg/dl range and had an absence of stimulated C-peptide production. When necessary, baboons were treated with Humulin R (Eli Lilly, Indianapolis, IN) to maintain blood glucose levels in the 100–200 mg/dl range. In some cases, reduced dosages of exogenous insulin (≤1.0 U · kg⁻¹ · day⁻¹) were administered for 6–12 U · kg⁻¹ · day⁻¹ for diabetic baboons that were completely deficient in insulin/C-peptide secretion) were administered for 7–10 days posttransplant or for variable periods of time after a rejection episode to optimize the conditions necessary for successful engraftment or recovery from rejection, respectively. Subsequent to a rejection episode, insulin was gradually discontinued as follows. The total daily insulin dose was decreased, and the baboon was observed for stability of metabolic control for 1–3 days, followed by a further dose decrease and continued observation. This process was continued until the animal no longer required insulin to sustain euglycemia. Considering the absence of a pancreas, we then considered the islet allografts to be functionally competent, and the recipients were labeled “insulin independent.” For animals that had experienced multiple or severe rejection episodes, the functional islet mass was no longer adequate to allow for insulin independence; many of these baboons, however, had clinically relevant graft function. Animals were considered to have partial graft function if fasting C-peptide was >0.5 ng/ml, response to intravenous glucose uncovered augmented insulin and C-peptide secretory responses, and reduced dosages of exogenous insulin (≤1.0 U · kg⁻¹ · day⁻¹) for animals with no endogenous insulin secretion capacity) were required to maintain blood glucose levels between 100 and 200 mg/dl. The ease of maintaining normoglycemia was related to the functional islet mass i.e., it was possible to control glycemia with very small doses of insulin in all but two baboons (BA-04 and -06).

**Bone marrow preparation and administration.** Vertebral bodies were harvested from the donor and preprocessed to obtain donor bone marrow cells (DBMC) via modification of methods for processing of human vertebral bodies (45). DBMC were cryopreserved and were thawed immediately before intravenous infusion on PODs 5 and 11. A total of 10⁶ nucleated DBMC were administered per kilogram of recipient body weight.

**Immunosuppression.** Tacrolimus was administered at a dose of 0.1 mg · kg⁻¹ · day⁻¹ IM starting on POD 7. Drug levels were monitored daily, and dosage was adjusted to maintain trough levels of 7–15 ng/ml. Animals were observed for signs of toxicity (neurotoxicity, hyperkalemia), and dosage was adjusted accordingly.

**hu5c8 monotherapy.** Humanized anti-CD154 (hu5c8) was obtained from Biogen. The production and characterization of the 5c8 clone has been described (38). Induction therapy consisted of intravenous administration of 10 or 20 mg/kg hu5c8 on PODs –1, 3, and 10. For treatment of rejection, 20 mg/kg hu5c8 was administered on the day rejection was detected, as well as 4 and 11 days thereafter. For BA-05 and -06, 20 mg/kg maintenance therapy was given every 28 days for 6 months, beginning on POD 28. BA-03 was switched to monthly maintenance therapy after POD 200. Blood samples were drawn periodically to assess levels of hu5c8 and anti-5c8 by enzyme-linked immunosorbent assay (Biogen).

**Glucose monitoring and definition of rejection.** In most cases, fasting and postprandial plasma glucose (FG and PPG, respectively) were monitored via heel stick and blood testing with test strips and a Glucometer Elite (Bayer, Elkhart, IN). PPG was defined as elevations in PPG (followed by elevated FG on POD 59), occurred on POD 58 for two of three animals (BA-01 and -02) and on POD 31 for the other baboon (BA-03).

**RESULTS**

Diabetes was induced in all recipients by total pancreatectomy (see METHODS). As shown in Fig. 1A, intrahepatic transplantation of allogeneic islets into a nonimmunosuppressed baboon, or into a baboon treated with tacrolimus, successfully but transiently restored normoglycemia and insulin independence. Within 8 days, however, both baboons acutely developed fasting hyperglycemia, reflecting allograft failure. In contrast, three of three baboons treated with 20 mg/kg humanized anti-CD154 (hu5c8) on PODs –1, 3, and 10 were insulin independent and normoglycemic for the first 30 days posttransplant (BA-01, -02, and -03; Fig. 1). In the absence of additional immune intervention, rejection episodes, first detected as elevations in PPG (followed by elevated FG on POD 59), occurred on POD 58 for two of three animals (BA-01 and -02) and on POD 31 for the other baboon (BA-03).

Two additional baboons were treated with reduced doses of 10 mg/kg hu5c8 induction therapy (BA-04 and -05). One of these baboons (BA-04) did not experience rejection until POD 58, while the other baboon (BA-05) rejected the islets on POD 10 (Fig. 1A). There was no correlation between the day of rejection and the number of IEQ/kg.

**FIG. 1.** FG levels in baboon recipients of allogeneic islets. Shown are the results obtained for the first 30 (A) and the subsequent 200 (B) days after islet cell transplant.
When hyperglycemia developed in BA-01, -02, -03, and -04, they were given anti-rejection therapy consisting of three additional doses of anti-CD154 (hu5c8) and small doses of exogenous regular insulin (Humulin R, <1 U·kg\(^{-1}\)·day\(^{-1}\)) for several days to stabilize metabolic control during recovery. Insulin was gradually tapered as follows: the dose of insulin (per day) was reduced, the animal was observed for stable metabolic control, and the dose was reduced again. The process was continued until the baboon no longer required insulin to sustain euglycemia, and the recipients were considered to be insulin independent. For comparison, pancreatectomized baboons that do not have functioning islet allografts require insulin doses of 4–6 U·kg\(^{-1}\)·day\(^{-1}\) to maintain blood glucose values in the 100–200 mg/dl range.

Anti-CD154 appeared to effectively halt rejection in all four baboons. One of the animals (BA-01; Fig. 1) was euthanatized on POD 79 to obtain tissues for immunohistological analysis. Several intrahepatic islets with intact insulin-positive cells were detected (Fig. 2A). Some islets were surrounded by noninfiltrating CD4\(^+\) (Fig. 2B) and CD8\(^+\) lymphocytes, and some areas of apparent islet loss were also evident (data not shown). Before the baboon was euthanatized, and to test whether euglycemia resulted from functioning intrahepatic islets, an IVGTT was performed on BA-01 with simultaneous sampling from both the portal vein and the suprahepatic vena cava. As shown in Fig. 3, we observed a reversal of the physiological portal to systemic gradient of insulin release, thus confirming the intrahepatic source of insulin.

When hyperglycemia developed in BA-01, -02, -03, and -04, they were given anti-rejection therapy consisting of three additional doses of anti-CD154 (hu5c8) and small doses of exogenous regular insulin (Humulin R, <1 U·kg\(^{-1}\)·day\(^{-1}\) for several days to stabilize metabolic control during recovery. Insulin was gradually tapered as follows: the dose of insulin (per day) was reduced, the animal was observed for stable metabolic control, and the dose was reduced again. The process was continued until the baboon no longer required insulin to sustain euglycemia, and the recipients were considered to be insulin independent. For comparison, pancreatectomized baboons that do not have functioning islet allografts require insulin doses of 4–6 U·kg\(^{-1}\)·day\(^{-1}\) to maintain blood glucose values in the 100–200 mg/dl range.

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Subsequent to the resolution of rejection on POD 31, BA-03 experienced additional reversible rejection episodes on PODs 112 and 172 (Fig. 1B), regained insulin independence, and was subsequently placed on monthly maintenance doses of 20 mg/kg anti-CD154 (hu5c8, given on PODs 200, 228, and 256). The baboon remained insulin independent, but experienced a rejection episode on POD 284 that appeared to be successfully reversed with anti-CD154 but resulted in a continued requirement for low-dose insulin to maintain normoglycemia. The animal was euthanatized on POD 301. As shown in Fig. 4, postmortem histological analysis of the intrahepatic islets revealed well-preserved insulin-positive cells, with rare inflammatory leukocytes.

The remaining baboon (BA-04) experienced a rejection episode on POD 58, was retreated with anti-CD154 (hu5c8) and transient low-dose insulin, and did not experience an additional rejection episode until POD 264. In this case, rejection was rapid, acute, and not reversible (Fig. 5). At the time of rejection, and unlike the other three baboons, this recipient had not received any anti-CD154 therapy for 206 days. During the course of these studies, we observed that elevated PPG preceded the onset of fasting hyperglycemia; this is clearly reflected in Fig. 5. The PPG allowed us to detect acute rejection episodes on PODs 58 and 264, while the FG remained normal.

IVGTTs were performed on BA-03 and BA-04, both pretransplant and at intervals posttransplant. The results revealed that glucose response curves were very similar at all time points in animals with a functioning islet allograft. The first-phase insulin release (FPIR), however, decreased over time for both animals, strongly suggesting a loss of functional islet mass with each rejection episode (47). Representative results for glucose and insulin release after intravenous glucose challenge of BA-03 are given in Fig. 6. FPIR on POD 35 was lower than that observed 12 days before pancreatectomy and islet cell transplant and may reflect incomplete engraftment (e.g., due to incomplete revascularization) and a loss of functional islet mass subsequent to the rejection episode detected on POD 31. The results obtained at 149 days posttransplant revealed a decrease in FPIR (rejection episode detected at POD 112). At PODs 168 and 279, further reductions in FPIR were observed, coinciding temporally with clinically relevant rejection episodes on PODs 172 and 284, respectively.

Subsequent to these initial experiments and the observation of a loss of functional islet mass temporally related to each episode of rejection, two baboons (BA-06 and -07) were treated with 20 mg/kg anti-CD154 (hu5c8) induction therapy on PODs −1, 3, 10, and 28, followed by monthly anti-CD154 (hu5c8) maintenance therapy at a dose of 20 mg/kg, given every 28 days. In addition to 5c8 therapy, these baboons received infusions of whole donor vertebral body marrow on PODs 5 and 11 (total dose of 10^9 nucleated cells/kg). These animals remained normoglycemic and insulin independent for 114 and 238 days.

Results of sequential IVGTT revealed that in the absence of rejection, FPIR was maintained in BA-06 and -07. Representative results are given in Fig. 7 for BA-06. The pretransplant and POD 66 insulin response curves were very similar (Fig. 7), reflecting maintenance of functional islet mass in the absence of rejection. Performance of random IVGTT on POD 113 revealed a slight decrease in insulin release. Immediately thereafter, we determined that the baboon was undergoing graft rejection. Our definition of graft rejection was FG >100 mg/dl and PPG >150 mg/dl. The PPG was >150 mg/dl on POD 114, and the FG increased to >100 mg/dl on POD 116. In this case, rejection was not reversible with additional anti-CD154 (hu5c8) therapy: i.e., the baboon was initially treated with reduced dosages of exogenous insulin (<1 U·kg^-1·day^-1), but the glucose levels and insulin requirement continued to increase (to >6 U·kg^-1·day^-1) in association with progression of rejection. Insulin secretion in response to IVGTT on POD 154 (Fig. 7A) was undetectable, thus confirming that the intrahepatic islets had

**FIG. 5.** FG and PPG levels for BA-04. This baboon experienced a rejection episode on POD 58, was treated with anti-CD154 (hu5c8), and did not experience rejection again until POD 264. Note that rejection is first detectable as an elevation in PPG.

**FIG. 6.** Glucose (A) and insulin (B) responses to intravenous glucose challenge before pancreatectomy and after islet transplantation in a baboon (BA-03) treated with anti-CD154 induction and anti-rejection therapy. BA-03 experienced rejection episodes on PODs 31, 112, 172, and 284.
been rejected. In contrast, BA-07 experienced excellent metabolic control for over 6 months, and no rejection was detected via monitoring of FG and PPG. After the seventh maintenance dose on POD 198, the baboon was no longer given anti-CD154 (hu5c8) and subsequently experienced rejection on POD 239. The animal was euthanatized with clinically relevant graft function on POD 253.

The overall results for duration of insulin independence for all transplanted baboons are summarized in Table 1. Two nontransplanted control baboons were treated with hu5c8 induction therapy. Before, and periodically after, treatment with anti-CD154, the animals underwent IVGTT. The administration of hu5c8 did not influence the results of IVGTT (data not shown).

While we did not observe any physical or behavioral evidence of drug-induced toxicity, blood samples were obtained pretransplant, and at various times posttransplant, for complete blood counts, flow cytometric analysis of peripheral blood leukocytes, and chemistries. All values were normal except for a marked decrease in the circulating CD4+ lymphocyte cell numbers in all animals treated with anti-CD154 (hu5c8). The percentage of CD4+ T-cells ranged from 2 to 7% (whole blood lysis method, analysis of total leukocyte gate) in the 5c8 treated baboons, compared with a mean value of 11% in untreated animals. Similar, in animals given anti-CD154 (hu5c8), 16–18% of lymph node cells were CD2/CD4 dual positive, compared with 44% in control animals. The decrease in CD4+ cell numbers occurred ~3 weeks after islet cell transplantation. Since total lymphocyte counts did not decrease, an increase in the percentage of CD8+ T-cells was observed that was coincident with the decline in CD4+ cell counts. We observed the same phenomenon in two normal nontransplanted baboons treated with anti-CD154 (hu5c8) alone; after discontinuation of hu5c8 treatment, the CD4+ T-cell counts gradually recovered. Interestingly, we detected CD8/CD69 dual positive cells in the peripheral blood of transplanted animals at ~2 weeks posttransplant. We were unable to measure any CD154 on peripheral blood T-cells harvested from these baboons at any time point, although in vitro activation with phorbol myristate acetate and ionomycin led to rapid (6-h) and transient (gone by 24 h) upregulation of CD154 on baboon CD4+ T-cells (data not shown). As mentioned previously, CD4+ lymphocytes were detected in biopsies taken from an animal that had recently undergone a rejection episode (BA-01), and this baboon had the lowest CD4 counts of all the animals tested. No adverse side effects, such as increased incidence of infection, were observed in association with lowered peripheral CD4 counts. In addition, no alteration in leukocyte subsets has been detected in rhesus monkey recipients of allogeneic kidneys (13) or islets (N.S.K., M.C., M.M., A.R., M. Oliveira, J.L.W., A.D.K., D.M.H., L.C.B., C.R., unpublished observations) or in cynomolgous monkeys treated with anti-CD154 (hu5c8) (Biogen).

All recipients were MLC reactive against their donors pretransplant. For two animals tested within the first month posttransplant, anti-donor-specific MLC reactivity was maintained (BA-01 and BA-02). As shown in Table 2, periodic MLC screening at various intervals posttransplant revealed decreased reactivity to donor and third-party cells, as well as decreased proliferative responses to phytohemagglutinin, for all recipients after the first month posttransplant. Anti-donor-specific MLC reactivity was decreased to background levels for all but one animal (BA-06), which experienced irre-

![FIG. 7. Glucose (A) and insulin (B) responses to intravenous glucose challenge before pancreatectomy and after islet transplantation in a baboon (BA-06) treated with anti-CD154 induction plus maintenance therapy. BA-06 experienced irreversible rejection on POD 114.](image-url)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Duration of insulin independence (day of death)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1</td>
<td>8 (21)</td>
</tr>
<tr>
<td>Anti-CD154induction/anti-rejection</td>
<td>5</td>
<td>10 (13)<em>, 59 (79), 229 (303), 264 (300)</em>, 284 (301)</td>
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<tr>
<td>Anti-CD154 induction/maintenance + donor marrow</td>
<td>2</td>
<td>114 (162), 238 (253)</td>
</tr>
</tbody>
</table>

Animals were euthanatized with varying degrees of graft function. *10 mg/kg anti-CD154; all other baboons received 20 mg/kg therapy.
versatile rejection within 12 days of the MLC test. Anti–third-party reactivity was maintained above background, as was proliferative responsiveness to PHA (Table 2).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Anti-self</th>
<th>Anti-donor</th>
<th>Anti-third</th>
<th>PHA</th>
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<tbody>
<tr>
<td>BA-01</td>
<td>Pretransplant</td>
<td>3,404</td>
<td>42,262</td>
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<td></td>
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<td></td>
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<td>1,258</td>
<td>1,439</td>
<td>8,217</td>
</tr>
<tr>
<td>BA-03</td>
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<td>38,653</td>
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<td></td>
<td>POD 119</td>
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<td>Pretransplant</td>
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<tr>
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Data are counts per minute of incorporated [3H]thymidine. ND, not determined.

DISCUSSION

Costimulatory blockade, with agents that block the B7:CD28 and/or the CD40:CD154 pathways, has proven to be an effective means to promote allograft acceptance in various rodent models, and has more recently been shown to promote acceptance and long-term survival of renal allografts in rhesus monkeys (13). Blockade of the B7:CD28 pathway via treatment with CTLA4-Ig has been shown to prolong the survival of allogeneic islets, compared with control animals, in a cynomolgus monkey model (48). In murine models, islet allograft acceptance via therapy with anti-CD154 has required infusion of donor-derived hematopoietic cells (21,22).

Our data demonstrate a uniform effect of CD40:CD154 costimulatory blockade on engraftment and long-term survival (up to 300 days) of allogeneic islets in a baboon model. In contrast to the murine data, infusion of donor-derived cells was not necessary. In fact, infusion of donor vertebral body marrow on PODs 5 and 11 did not add to the graft-promoting effect of anti-CD154 (hu5c8) alone. Alteration of the timing of marrow infusion may, however, yield different results and is currently under study.

The observation that elevations in PPG preceded increases in FG and were correlated with rejection enabled us to detect and reverse islet allograft rejection in most cases. The results of this study demonstrate that intrahepatic islet allografts can successfully engraft and survive long term and that preventing rejection allows for preservation of functional islet mass. We can think of several possible explanations for our success, and all may contribute. First, in the presence of costimulatory receptor blockade with anti-CD154, rejection is prevented in this model. Several groups have recently suggested that T-cell help is delivered to CD8+ cells via dendritic cells and that CD40+ dendritic cells are activated by CD154+ CD4 cells (49–51). Thus, anti-CD154 could function, at least in part, by impairing the functional maturation of CD8+ T-cells. The detection of CD8+CD69-positive T-cells in the initial posttransplant period suggests that early activation events had occurred in the CD8 subset, yet islets were not rejected, consistent with the notion that CD8 functional maturation is impaired.

A second possible reason for these findings is the reported ability of anti-CD154 to prevent macrophage- and endothelial cell–mediated production of nitric oxide and proinflammatory cytokines (14–18). Costimulatory blockade with anti-CD154 (hu5c8) may, therefore, play a key role in preventing the early islet loss thought to occur subsequent to intrahepatic transplantation (52,53). To capitalize on this possibility, islets were transplanted in medium containing 20 mg of antiCD154 (hu5c8).

Third, our in vivo studies have demonstrated that hu5c8 does not have any direct adverse effect on islet cell function. Fourth, the deletion of CD4+ cells we observed could have contributed to islet engraftment and survival. While this possibility cannot be completely refuted, we observed rejection episodes in baboons with low peripheral CD4 cell counts, and these episodes, when detected early, were reversed with anti-CD154 (hu5c8) monoclonal antibody readministration and did not result in a further decrease in CD4+ cell counts. Furthermore, CD4+ cells were detected in the infiltrate surrounding intrahepatic islets subsequent to a rejection episode.

In summary, costimulatory blockade with anti-CD154 monotherapy allows for islet engraftment, insulin independence, and long-term function in baboon recipients of allogeneic islets. Reversal of rejection can be achieved with repeated administration of anti-CD154, and prevention of rejection allows for preservation of functional islet mass and insulin independence. These observations on costimulatory blockade in nonhuman primates with islet transplants are sufficiently unique to stimulate further study of this and other approaches, alone or in combination, to intervention in the activation signals responsible for allograft rejection.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grants ROI DK51703 01A1 and ROI DK25802 17, by the Diabetes Research Institute Foundation, Hollywood, FL, and by Biogen, Cambridge, MA.

The authors gratefully acknowledge the expert technical assistance of Dr. Maria Oliveira, Xi-Min Xu, John Knapp, Elina Linetsky, Jorge Montelongo, and James McMannis, as well as the participation of Drs. Hisham Al-Khayat and Haitham Al-Khayat. Special thanks are extended to Michael Disbrow of the Mannheimer Foundation for his assistance in this project.
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