Interference with Tissue Factor Prolongs Intrahepatic Islet Allograft Survival in a Nonhuman Primate Marginal Mass Model

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Background. Tissue factor (TF) expression on islets can result in an instant blood-mediated inflammatory reaction (IBMIR) that contributes to early islet loss. We tested whether peritransplant protection of islets from IBMIR with a monoclonal anti-TF antibody (CNT0859) would enhance engraftment in our nonhuman primate marginal mass model.

Methods. Each of six pairs of cynomolgus monkeys (CM) with streptozotocin-induced diabetes was closely matched for metabolic control and was transplanted with 5,000 IEQ/kg allogeneic, ABO-compatible islets from the same donor under the cover of steroid-free immunosuppression. For each pair, experimental animals received islets cultured with 20 μg/mL anti-TF and were dosed with 6 mg/kg anti-TF intravenously, 10–25 min before islet infusion; control monkeys received an equal number of islets from the same preparation cultured without anti-TF and no in vivo treatment.

Results. Early fasting C-peptide (CP) values were different between (P<0.01), but not within, pairs and correlated with in vitro functional capacity of islets as assessed by perfusion (r = 0.60; P = 0.022). Compared to their matched controls, experimental animals had decreased posttransplant markers of coagulation, higher fasting CP levels (1 month post-plant and end of study) and prolonged graft function.

Conclusions. These data suggest that pretreatment of islets and the recipient with anti-TF may limit the effects of IBMIR, thereby enhancing islet engraftment and survival.

Keywords: Islet transplantation, Islet engraftment, Graft survival, Coagulation.

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Utilizing steroid-free immune suppression (SFIS), several centers have reported extended pancreatic islet allograft survival with restoration of glycemic control and insulin independence in patients with type 1 diabetes (1–4). It has been estimated that 50–60% of islets are lost in the early posttransplant period (5), thereby contributing to the observed need for transplantation of islets from multiple donors to achieve insulin independence. Islets have been reported to express tissue factor (TF), which is abundantly present in cells surrounding the vascular bed. TF is a 47-kDa transmembrane glycoprotein that is the point of initiation of the extrinsic coagulation system and is pivotal for activation of the intrinsic pathway. Vascular injury and exposure of TF to soluble coagulation proteins in blood triggers clotting (6). TF is also a member of the class 2 cytokine receptor superfamily (7), and its binding to factor VIIa activates a number of intracellular signals that culminate in cell proliferation, diapedesis and inflammation (8). Infusion of TF expressing islets into the portal vein triggers an instant blood-mediated inflammatory reaction (IBMIR) that activates nonspecific inflammatory events and coagulation pathways (9–13). Using an in vitro system, it has been demonstrated that IBMIR results in the physical entrapment of islets in a macroscopic blood clot, which may enhance the release of cytokines, chemokines, and reactive oxygen species that are toxic to β cells (14–17), thereby delaying revascularization and engraftment in vivo (18). Evidence for IBMIR has been reported in pigs after intraportal islet transplantation (19) and in the clinical setting of human islet allotransplantation (9, 19, 20). Specific blockade of TF activity using either inactivated factor VIIa or antibodies to TF can abrogate IBMIR in vitro. Marginal mass models of islet transplantation allow for testing of agents designed to prevent or limit early islet loss in rodents (21–24). We developed a cynomolgus monkey (CM) marginal mass model and evaluated the effect of an anti-TF specific monoclonal antibody (CNT0859) on islet engraftment and survival in the liver. CNT0859 is a humanized monoclonal
antibody derived from the well-characterized murine antibody, TF8 5G9, that binds to TF alone and to the TF-FVIIa complex (25).

**MATERIALS AND METHODS**

**Anti-TF Antibody CNTO859**

Purified CNTO859, a humanized immunoglobulin G4 from Centocor (Radnor, PA), was provided in phosphate buffer with endotoxin <0.05 EU/mg. CNTO859 was quantified in the plasma of treated animals using a bead-based, sandwich electrochemiluminescence immunoassay.

**Effects of LPS Challenge in a CM Model**

Macaca fascicularis (3–4 kg, 5/group) were treated with doses of 0.006, 0.06, 0.6, 6 and 60 mg/kg CNTO859 (4.56 mg/ml, 2.4 EU/mg) 5 min prior to a challenge with 10 μg/kg lipopolysaccharide (LPS; *Escherichia coli* 0111:B4, List Biological Labs, Inc.). Physiological parameters, and markers of coagulation and fibrinolysis, were measured before and after LPS challenge at −0.2, 0, 1, 4, 8, 24, 48, 72 and 96 hr.

**CM for Islet Allotransplantation**

Donor and recipient CM were obtained from Charles River BRF (Houston, TX) or The Mannheimer Foundation, Inc. (Homestead, FL) and were screened negative for tuberculosis, herpes B virus, simian retrovirus, simian immunodeficiency virus, and simian T-cell lymphotropic virus-1. Animals >4 and >2 years of age were used as donors and recipients, respectively. Pair-housed monkeys were supplied with water ad libitum and fed twice daily. The University of Miami complies with the Animal Welfare Act of 1966 (PL89–544) as amended by the Welfare Act of 1970 (PL91–279), adheres to the principals stated in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85–23 revised) and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Donor-recipient pairs were ABO compatible (26). Pairs of recipients for the anti-TF studies had similar duration of diabetes and exogenous insulin requirements within 20% of each other.

**Diabetes Induction, Metabolic Monitoring, and Insulin Administration**

The monkey was NPO (nothing by mouth) the night before diabetes induction; streptozotocin (STZ, 1,250 mg/m² i.v.) was infused over an 8-minute period (27). Diabetes in this model was defined as fasting CP levels <0.2 mg/mL, and a negative CP response (stimulated CP <0.3 ng/mL) to a glucose challenge undertaken 4 weeks after diabetes induction.

After diabetes induction, as well as postislet cell transplant, blood glucose levels were monitored 2–3 times daily via heel stick using a OneTouch Ultra Glucometer (LifeScan, Milpitas, CA). Subcutaneous insulin was administered (Humulin N or Humulin N + Lantus) as needed and based on an individualized sliding scale, aiming for fasting and postprandial plasma glucose levels of 150–250 mg/dL postSTZ and prior to transplantation.

A double-antibody radioimmunoassay method (Diagnostics Products, Los Angeles, CA) was used to assess plasma insulin and c-peptide levels. For intravenous glucose tolerance test (IVGTT) results, the incremental area under the curve (AUC) was calculated using the trapezium rule (28) and adjusted for the fasting values. Fasting CP values were normalized in relation to fasting blood glucose values using the following ratio: (CP/fasting blood glucose [FBG]) × 100 (ng/mg).

**Nonhuman Primate Blood Coagulation Assays**

Blood for coagulation assays was collected in Vacutainer tubes containing 3.2% sodium citrate and plasma was collected and stored at −80 °C. Thrombin-antithrombin complex (TAT), D-dimer and fibrinogen 1 + 2 were measured per manufacturer’s instructions using enzyme-linked immunosorbent assay kits (Dade Behring).

**Donor Pancreatectomy, Islet Preparation, Culture, and Islet Quality Control**

The donor pancreas was recovered as previously described (29) and nonhuman primate (NHP) islet isolation performed using modifications (30) of the automated method for human islet isolation (31). Discontinuous Eurocoll/gradient densities (1.132; 1.108; 1.096; 1.037) were used for purification of islets from the pancreatic digest (31, 32). The tissue was bottom-loaded with stock Ficoll and centrifuged in a COBE 2991 blood cell processor (Lakewood, CO). Islet purity was >90%. With the exception of one experiment in which islets for both recipients were cultured in vitro with 20 μg/mL of anti-TF (pair 1), the islet preparation was divided into two batches on the day of isolation, and only one batch was cultured with 20 μg/mL of anti-TF. After culture for 16–42 hr, both batches of islets were collected from the tissue culture flasks, washed and counted to determine IEQ (30), and 70 IU heparin/kg recipient body weight was added to the transplant preparation. Islet purity was estimated based on the percentage of dithizone (DTZ) positive particles present in the preparation (33, 34), and viability was estimated based on fluorescein diacetate/propidium iodide staining (35). In vitro functional capacity was determined via assessment of glucose-stimulated insulin secretion using a column-perfusion assay (36). The stimulation index (SI) was calculated as the ratio of insulin released under high (11 mM) over insulin released under low (3 mM) glucose concentrations.

**Intrahepatic Islet Transplantation**

Under general anesthesia, the recipients underwent a minilaparotomy in order to access a mesenteric tributary of the portal vein. A small supraumbilical central midline incision was made, and the islets were infused via gravity through a 24-gauge intravenous catheter over a period of 5 min (30).

**Postoperative Monitoring and Insulin Administration**

Clinical signs, fluid balance, blood glucose, body weight, and nutritional intake were monitored regularly, and weekly blood tests were done to monitor overall health. Blood samples for determination of rapamycin and FK506 trough levels were obtained weekly. After islet transplantation, insulin was administered as needed to maintain FBG in the 100–150 mg/dL range and postprandial glucose in the 100–200 mg/dL range. C-peptide levels were monitored weekly and
IVGTT were undertaken every 8 weeks posttransplant to assess graft function (30).

Experimental Design, Immunosuppressive Regimen, and Drug Levels
Each pair of recipients received a marginal mass of islets (5,000 IEQ/kg) from the same donor, with the exception of pair 6 (Table 1), which received 4,100 IEQ/kg. SFIS was initiated on postoperative day (POD)/H11002 and consisted of FK506 (Astellas Pharma, Deerfield, IL) at 0.02 mg/kg intramuscularly twice daily, adjusted to maintain trough levels of 4 – 6 ng/mL, and rapamycin (LC Laboratories, Woburn, MA) at 0.05 mg/kg intramuscularly twice daily on POD/H11002 and SID thereafter, adjusted to maintain trough levels of 12–20 ng/mL; Zenapax induction was administered at 1 mg/kg IV and repeated every other week for 5 doses. On POD 0, the experimental animal (E) received 6 mg/kg anti-TF IV 10–25 min before transplant, followed by transplant of islets cultured in vitro with 20 μg/mL anti-TF. The control animal (C) received islets from the same donor but incubated without anti-TF, except for one pair (pair 1), and an IV bolus of saline using the same volume and timing as for anti-TF. In pair 1, islets for both recipients (C and E) were cultured in vitro with 20 μg/mL of anti-TF. Samples to measure coagulation parameters were collected from all animals immediately before islet infusion, and at 1, 3, 8, and 24 hrs posttransplant. In addition, a 15-min sample was added to the blood collection time points for the last 3 pairs (see Results).

Statistics
All data are given as the means ± SD. Means of groups were compared using nonpaired t test (Sigmaplot for Windows v.8.0, Chicago, IL). Time course data from control and treated groups were analyzed using repeated measure analysis of variance (SPSS for Windows, V.10, Chicago, IL). Significant main effects were tested post-hoc using a nonpaired t test. P values <0.05 were considered statistically significant.

RESULTS

Evaluation of the Effects of CNTO859 on Islet Viability and Function
To determine if CNTO859 had a direct adverse effect on islet viability and function in vitro, isolated islets were incubated for 24 and 48 hr with increasing concentrations of antibody (0, 10, 20, and 50 μg/mL). No significant differences were observed in viability and IEQ at the CNTO859 concentrations tested. A concentration of 20 μg/mL was chosen for culture of NHP islets.

### TABLE 1. Summary of posttransplant graft function

<table>
<thead>
<tr>
<th>Animal Pair</th>
<th>Treatment</th>
<th>Reduction EIR (%)</th>
<th>Days CP positive</th>
<th>Max CP AUC (ng/mL×min)</th>
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<tr>
<td>1</td>
<td>Control</td>
<td>49.7</td>
<td>164</td>
<td>33.2</td>
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<td></td>
<td>Experimental</td>
<td>55.3</td>
<td>586b</td>
<td>49.0</td>
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<td>2</td>
<td>Control</td>
<td>35.9</td>
<td>101</td>
<td>15.3</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>48.2</td>
<td>94</td>
<td>22.5</td>
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<tr>
<td>3</td>
<td>Control</td>
<td>25.4</td>
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<td>3.6</td>
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<td>Experimental</td>
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<td>483b</td>
<td>10.5</td>
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<tr>
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<td>356b</td>
<td>30.4</td>
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<tr>
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<td>Control</td>
<td>20.0</td>
<td>55b</td>
<td>5.8</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>33.2</td>
<td>129</td>
<td>7.2</td>
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</table>

* Islets were cultured with anti-TF.

** Fasting C-peptide positive at necropsy.

EIR, exogenous insulin requirements in the first month posttransplant; CP, fasting c-peptide/fasting blood glucose ×100 (ng/mg); AUC, area under the curve 2 months posttransplant.

### TABLE 2. Summary of marginal mass experiments using 5,000 IEQ/kg

<table>
<thead>
<tr>
<th>Identification number</th>
<th>Days duration diabetes pretransplant</th>
<th>Days C-peptide positive</th>
<th>C-peptide/fasting blood glucose ×100 (ng/mg)</th>
<th>Pretransplant</th>
<th>Posttransplant decrease in exogenous insulin requirement (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Days 0–30</td>
<td>Days 31–60</td>
<td>Days 0–30</td>
</tr>
<tr>
<td>1</td>
<td>374</td>
<td>74</td>
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<tr>
<td>2</td>
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<td>410b</td>
<td>2.14</td>
<td>2.07</td>
<td>126</td>
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<tr>
<td>3</td>
<td>113</td>
<td>101</td>
<td>1.25</td>
<td>0.94</td>
<td>121</td>
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<tr>
<td>4</td>
<td>112</td>
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<td>0.52</td>
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<td>1.26</td>
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<td>47</td>
<td>0.87</td>
<td>1.46</td>
<td>126</td>
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</table>

* Fasting C-peptide positive at necropsy.

** Insulin independent for 20 days (postoperative days 24 to 44).
Determinant of In Vivo Dose by Analysis of the Inhibitory Effects of CNTO859 on the Activation of Coagulation Induced by LPS

LPS (endotoxin) causes an abnormal upregulation of TF on endothelium and inflammatory cells, resulting in systemic activation of coagulation in CM and other NHP (37, 38). Using CM, we evaluated the effect of different IV doses of CNTO859 (0.006, 0.06, 0.6, 6.0, and 60 mg/kg), administered 5 min prior to a 10 µg/kg IV dose of LPS, on markers of coagulation. LPS induced rapid, transient appearance of a series of markers of in vivo coagulation (fragment F1+2, TAT and fibrin D-dimer) that peaked between 4–8 hr. These markers were partially and significantly inhibited by doses of CNTO859 ≥0.6 mg/kg. Eight hours after an LPS challenge, animals receiving 6 mg/kg CNTO859 had decreased levels of F1+2 (1.6±1.1 vs. 4.0±1.6 nmoL/L, n=5, P=0.04), TAT (9.2±4.3 vs. 33.7±9.1 ng/mL, n=5, P=0.001), and D-dimer (2634.2±1859.3 vs. 4876.2±483.2, n=5, P=0.03) compared to control animals. There was no prolongation of template bleeding time, nor any evidence of spontaneous bleeding at any dose of CNTO859 tested (data not shown). A dose of 6 mg/kg was chosen to study the in vivo effect of CNTO859 on islet engraftment in the NHP marginal mass model.

Establishment of a NHP Marginal Mass Model of Islet Allotransplantation

A dose of 5,000 IEQ/kg was chosen for this model, a quantity equal to half the dose normally required to reproducibly attain insulin independence in NHP models and one that has only rarely led to transient insulin independence. We have observed subtle variability in the quality of individual CM islet preparations, even in the laboratory setting where cold ischemia time is minimal, the individuals responsible for pancreas recovery and islet isolation are consistent and, unlike the clinical situation, there is no variability associated with cause of death, length of hospitalization, and medications received by donors. Furthermore, each diabetic monkey has differing insulin requirements, ranging from 2–8 units insulin/kg/day, with varying degrees of metabolic control. The results shown in Table 2 are for eight NHP transplanted with 5,000 IEQ/kg covered under the same SFIS, which demonstrate this variability. Only one of the eight animals evaluated (animal number 5) experienced a sustained period of insulin independence (20 days). All eight animals were CP-positive posttransplant and maintained graft function ranging from 47 to >500 days. Despite targeting FBG levels for all transplant recipients at 100 mg/dL, there was a wide variability in the percent reduction of exogenous insulin requirements (EIR), ranging from 22 to 86%. This reduction in EIR did not correlate with pretransplant duration of diabetes, FBG, or EIR/kg. Taken together with the multiple pathways postulated to be involved in early islet loss (inflammation, coagulation, complement activation), we postulated that interference with a single pathway might have a subtle effect that would require testing in large numbers of individuals (whether monkey or human) in order to observe a statistically significant improvement in graft function. To address this, we chose pairs of diabetic monkeys with similar age, duration of diabetes, fasting and postprandial blood glucose, weight, and insulin requirement.

In Vivo Administration of Anti-TF Prolongs Islet Graft Function

Six pairs of CM, including an SFIS-treated control (C) and an SFIS/CNTO859-treated animal (E) in each pair, were transplanted to assess the effect of anti-TF treatment on islet engraftment and long-term survival. A summary of the results obtained for all six pairs of monkeys is given in Table 1. One E animal (in pair 2) had circulating plasma CNTO859 levels that were 10-fold lower than the other five anti-TF treated animals (data not shown); coagulation parameters were not attenuated in the E animal of this pair (data not shown). The reason for this anomaly is unknown.

Overall, there was no significant difference in the percent reduction in EIR between E and C animals during the first (47.9±26.9 vs. 44.1±21.0%, Table 1) or second (40.0±33.6 vs. 39.5±34.0%, data not shown) posttransplant month. IVGTT data were abnormal and reflective of the marginal islet mass; a trend towards higher CP AUC at 2 months posttransplant was observed for E animals but was not significantly different from the C animals (Table 1). Graft function in terms of fasting CP levels, however, was either prolonged (Fig. 1A) or greater (Fig. 1B) for E animals than for matched controls. For pair 3 (Fig. 1A, Table 1), the recipients had similar CP levels in the first months posttransplant, but the E animal maintained positive CP for more than 1 year after the matched C animal had lost function. This pattern was also observed for pair 1 (Table 1). The results for pair 5 (Fig. 1B) represent the other pattern observed, in which both animals maintained graft function for the duration of the experiment (pairs 4 and 5). CP levels were similar in the first posttransplant months but declined in the C animals with a simultaneous increase in EIR, while the E monkeys maintained function. For pair 6, the C monkey died subsequent to an episode of severe hypoglycemia; in the days surrounding the C animal’s death, the E animal had higher CP levels as compared to the C monkey (Fig. 2).

The earliest in vivo CP values measured between POD 3–5 were similar within pairs and different (P=0.0025) between pairs (n=6 pairs, Fig. 2A). We normalized the values within each pair by dividing the CP levels for the E by that of the C animal. We found no differences between E and C animals for the earliest CP values measured (POD 3–5, E/C mean±SD: 1.18±0.45, NS), but at 1 month posttransplant, CP values were higher in five of six anti-TF treated animals (2.04±0.66 vs. 1.32±0.78 ×100 ng/mg; Fig. 2B) and the mean of the ratios of E/C CP (1.79±0.68, P=0.036) revealed enhanced graft function in anti-TF treated animals. Utilizing the day that the C monkey either lost function (pairs 1–3) or the day the study was electively terminated (pairs 4–6), CP levels were higher for all E animals as compared to their matched controls (Fig. 2C) and the mean of the ratios of E/C CP (4.31±2.90, P=0.015) revealed significantly enhanced function for E monkeys.
FIGURE 1. Fasting C-peptide and rapamycin levels for two pairs of diabetic monkeys that received a marginal mass of allogeneic islets: pair 3 (A) and pair 5 (B). Control (C) and experimental (E) recipients received the same immune suppression consisting of rapamycin (trough levels 12–20 ng/mL) and FK506 (trough levels 4–6 ng/mL) daily, beginning on POD –2, plus Zenapax induction at 1 mg/kg every other week for five doses. On POD 0, both animals received an intrahepatic islet transplant of 5,000 IEQ from the same donor; islets for the E animal had been incubated for 40 hr at 37°C with 20 μg/mL anti-TF; in addition, the E animal received an IV infusion of 6 mg/kg anti-TF 10 min before the islet transplant. Vertical bars represent CP levels (normalized to fasting blood glucose [100 ng/mg]) and open circles represent trough rapamycin levels (ng/mL); horizontal lines show target trough levels for rapamycin. In pair 3, the monkeys had similar CP levels in the first months posttransplant, but the E animal maintained graft function for 379 days longer than the matched control. In pair 5, the E animal maintained higher CP values over time as compared to the C animal.

FIGURE 2. (A) Initial (POD 3–5) and 1 month posttransplant normalized CP values in pairs of metabolically matched, streptozotocin-induced diabetic monkey recipients receiving a marginal mass of allogeneic islets from the same donor under the cover of SFIS. In pair number 1, both control (C) and experimental (E) animals received islets cultured with 20 μg/mL anti-TF monoclonal antibody, CNTO859; in the remaining pairs, C animals received islets incubated with culture medium alone. All E animals received islets cultured with 20 μg/mL CNTO859 and an IV infusion of 6 mg/kg anti-TF in vivo 10–25 min before the intrahepatic islet transplant. Fasting C-peptide levels normalized to fasting blood glucose $\times 100$ (ng/mg) on postoperative days 3–5, a reflection of islet quality as opposed to islet engraftment, were significantly different ($P<0.01$) between, but not within pairs. (B) One month posttransplant, five of six E animals had higher CP values compared to matched C, so that the mean ± SD of the ratio of E/C was significant. (C) CP levels for C and E animals at the time the C animal lost function or the study was electively terminated.
Correlation of In Vitro Functional Capacity and Early Posttransplant Function

Hypothesizing that the difference in the initial in vivo CP values between pairs may reflect different qualities/potencies of the transplanted islets, the relationship between the in vitro functional capacity of the islets, represented by the stimulation index from a perifusion assay of the islets done on the day of transplant, and the earliest in vivo CP values were analyzed. To increase the number of observations and the power of this analysis, two additional control animals were included that received the same SFIS and 5,000 IEQ/kg and for which perifusion data was available. There was a positive relationship between the SI from the perifusion assay and the initial CP values (n=14, r=0.60; P=0.022; Fig. 3).

Effect of In Vivo and In Vitro Anti-TF on Activation of Coagulation and Fibrinolysis

Intrahepatic islet infusion was followed by a rapid generation of the coagulation enzyme thrombin, as indicated by increases in the biomarkers F 1+2 and TAT. Figure 4 shows the mean results from measurements in three pairs of animals that had samples taken at 15 min up to 24 hr (pairs 4–6). The levels of F 1+2 in control animals were significantly higher at 1 and 3 hr after transplant (P=0.02) as compared to baseline values for this group, while the F 1+2 levels for experimental animals did not change significantly from baseline after transplant. In addition, comparison of F 1+2 levels revealed significantly lower levels in experimental (E) animals received the anti-TF monoclonal antibody CNTO859 in vitro and in vivo. Values represent means±SD. * P<0.02 vs. before transplant.

FIGURE 3. Relationship between in vitro glucose-stimulated insulin release and in vivo CP levels on POD 3–5. Aliquots of 100 islets from the islet preparations used for the intrahepatic transplants in both control (closed circle) and experimental (opened circles) animals, as well as two additional control animals that received the same immune suppression and marginal mass of islets, were used to assess in vitro functional capacity by measuring glucose-stimulated insulin release using a column-perifusion assay. The resulting stimulation index (SI) correlated positively with the earliest in vivo CP value (normalized to fasting blood glucose (FBG) ×100 [ng/mg]) measured.

FIGURE 4. Effect of anti-TF treatment on thrombin generation and fibrinolysis. Time course for the rise in biomarkers of coagulation; fragment 1+2, thrombin-antithrombin complex (TAT), and fibrinolysis (D-dimer) after intrahepatic allogeneic islet transplant in three representative pairs of monkeys. Only experimental (E) animals received the anti-TF monoclonal antibody CNTO859 in vitro and in vivo. Values represent means±SD. * P<0.02 vs. before transplant.
DISCUSSION

Our results show that NHP receiving a marginal mass of islets under the cover of SFIS are able to maintain graft function (CP >0.2 ng/mg) for a minimum of 47 and up to greater than 500 days. Observations of variability in both recipient characteristics and potency/quality of islets isolated from different donor pancreata suggest that it would be necessary to transplant large numbers of NHP to observe significant effects of a single agent on islet engraftment. It is unlikely that an agent targeting only one component of IBMIR would block all elements of the reaction (coagulation, complement activation, production of proinflammatory mediators); therefore, the effect of a single agent on engraftment may be subtle. We developed a marginal mass model in which pairs of CM recipients with similar metabolic control were transplanted with islets from the same donor. Each recipient of the pair was treated with SFIS, but only one animal received anti-TF intervention. Using this approach, we show that five of five monkeys that achieved therapeutic levels of anti-TF specific monoclonal antibody experienced enhanced islet allograft function as compared to their matched controls. This was demonstrated by prolonged graft function and/or maintenance of higher CP levels in treated animals upon elective termination of the study. The occurrence of IBMIR in CM receiving intraportal allogeneic islet transplants is demonstrated by the observed increase in TAT and F1+2 formation shortly after islet infusion. TAT levels peaked at approximately 141.0 ng/mL 15 min after transplant and returned to baseline values one hour posttransplant. In patients that underwent intraportal islet transplantation, TAT levels also peaked 15 min posttransplant, but the values were lower (approximately 50 ng/mL) than those observed in our monkeys, and they remained elevated for a longer time (at least 1 hr vs. 15 min), reaching baseline levels 1 day after transplant (9,20). The shorter duration of thrombin generation after islet infusion in monkeys compared to humans could be at least in part due to the fact that our animals received a marginal mass of islets. Furthermore, NHP preparations are generally greater than 90% pure, whereas human islet preparations are generally less pure (50% or less).

CNTO859 only partially inhibits the activation of coagulation in CM. This is because CNTO859 binds with greatly decreased affinity to TF from CM compared to humans. We showed maximum blockade of LPS-induced coagulation markers at 6 mg/mg dose, and higher doses were without significantly increased benefit. Thus, the 6 mg/kg dose was considered to be a safe and effective level for this study.

We postulate that islets entrapped within clots in the liver might release CP for several days prior to islet death. Gradual loss of functional islet mass is supported by our observation of similar CP values between E and C monkeys on POD 3–5 and graft outcome. For the anti-TF treated recipients, those animals with early CP levels >1.5 ng/mg (pairs 1, 4, 5; Fig. 2A, C) had the longest graft survival, with end of study CP levels maintained >1.5 ng/mg. The pairs with early CP levels <0.8 ng/mg (pairs 2, 3, 6; Fig. 2A, C) experienced decreased graft survival and had end of study CP levels <1.1 ng/mg. A similar trend was observed for the matched controls, with higher fasting CP levels at 1 month posttransplant for pairs 1, 4, and 5 as compared to 2, 3, and 6.

The mechanism by which interference with TF prolonged graft survival in five of five pairs of animals that achieved in vivo antibody target levels is most likely related to: 1) decreased islet induced coagulation; 2) concomitant diminution of inflammatory reactions; including complement activation and chemokine/cytokine production; 3) resultant enhancement of islet engraftment; and 4) establishment of a milieu in which the diminished innate immune reaction does not contribute to augmentation/development of an acquired immune response to the transplanted islets. Further studies are required to completely understand the effects of anti-TF on the complex coagulation cascade surrounding intraportal islet transplantation. Additional agents will most likely be needed to fully alter inflammatory reactions and allow for attainment of insulin independence with fewer islets.

In summary, our results demonstrate that interference with TF using a humanized anti-TF antibody leads to prolonged and/or enhanced islet allograft function in an a NHP marginal mass transplant setting. Although intervention with humanized anti-TF did not lead directly to insulin independence in treated NHP animals transplanted with a marginal islet mass, it did establish a foundation on which to build a more effective peritransplant strategy to minimize islet loss and maximize islet function, thereby reducing the number of islets required to achieve significant clinical benefit. Furthermore, the activity of the humanized antibody is less optimal in the NHP and use of a species specific antibody may lead to a more dramatic clinical effect. Lastly, the NHP marginal mass model is sensitive enough to detect the effect of agents aimed at the inhibition of coagulation and other events that contribute to IBMIR, or to evaluate other means to enhance engraftment and islet survival. This model can be used as a platform to assess in vitro methods aimed at pretransplant evaluation of islet quality, a critical milestone to bring islet transplantation to the next level.

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