Modest Effects of Fas-Ligand and Heme Oxygenase-1 Double Transgenic Mouse Islets on Transplantation Outcomes

J.-H. Juang, C.-F. Tu, and C.-H. Kuo

ABSTRACT

Interactions of Fas with its ligand (FasL) play an important role in the maintenance of immunologic homeostasis and peripheral tolerance. Heme oxygenase-1 (HO-1) is a protein capable of cytoprotection via radical scavenging and apoptosis prevention. The aim of this study was to test whether overexpression of FasL and HO-1 in murine islets resulted in cell protection and improved functional performance after transplantation. We first generated FasL and HO-1 double transgenic mice to investigate the protective effect of transgenic islets on transplantation. Islets were isolated from FasL and HO-1 double transgenic and nontransgenic Balb/c mice, for transplantation of 300 islets under the left kidney capsule of each streptozotocin-diabetic Balb/c mouse. During 6 weeks after transplantation, the blood glucose gradually decreased in recipients of double transgenic and nontransgenic islets. However, the decrease in blood glucose was more pronounced in the former (450 ± 16 mg/dL at day 0 to 302 ± 55 mg/dL at day 42; $P = .01$) than the latter (468 ± 17 mg/dL at day 0 to 379 ± 71 mg/dL at day 42; $P = .24$). The areas under the curve of intraperitoneal glucose tolerance tests at 2, 4, and 6 weeks were not significantly different between recipients of double transgenic and nontransgenic islets. The body weight increased in recipients of double transgenic islets (21.1 ± 1.4 g at day 0 to 26.2 ± 0.8 g at day 42; $P = .0002$) and nontransgenic islets (21.0 ± 1.4 g at day 0 to 25.1 ± 0.4 g at day 42; $P = .0448$). Our data suggested modest beneficial effects of transgenic islets with FasL and HO-1 overexpression for transplantation.

SINCE 1990, islet transplantation has led to insulin-independence in humans with type 1 diabetes mellitus.[1] Although the success rate was markedly improved recently by the Edmonton Protocol, 2 or more pancreata are usually required to achieve normoglycemia.[2] Moreover, long-term function of the transplanted islets has been disappointing.[3,4] Allograft failure may be due to nonimmunologic (eg, insufficient β-cell mass and islet engraftment problems) as well as immunologic (eg, immune rejection, toxicity of immunosuppressants, and autoimmune recurrence) factors.[1] Interactions of Fas with its ligand (FasL) are believed to play a major role in the maintenance of immunologic homeostasis and peripheral tolerance.[5] Cotransplantation of myoblasts expressing FasL has been shown to protect islet allografts from immune rejection although rarely did it lead to indefinite graft survival.[6] However, we observed that human FasL transgenic islets could not prolong islet allograft survival.[7,8] Heme oxygenases (HOs), including HO-1, 2, and 3 isoforms, are ubiquitous enzymes that catalyze the initial and rate-limiting steps in the oxidative degradation of heme to bilirubin.[9] Highly induced HO-1 confers protective effects on the oxidative stress responses both in vivo and in vitro. The mechanisms have been centered on the biological effects of the reaction product(s) that potentially possess important antioxidant, anti-inflammatory, antiapoptotic, and possible

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immune modulatory functions.[10–12] Previously, Pileggi et al showed HO-1 induction in islets to protect them from apoptosis in vitro and shorten the time to normoglycemia in diabetic islet recipients.[13] However, transplanting HO-1 transgenic islets into diabetic mice, we observed higher blood glucose values than those after implantation of nontransgenic islets.[14] We hypothesized that overexpression of both FasL and HO-1 in islets might have beneficial effects for transplantation. Therefore, in this study, we investigated the effects of FasL and HO-1 double transgenic islets on transplantation outcomes.

MATERIALS AND METHODS

Animals

We generated human FasL and HO-1 double transgenic mice in which the human FasL transgene was driven by a rat insulin promoter and the human HO-1 transgene, by a chicken beta-actin promoter in Balb/c mice. Transgenic or nontransgenic Balb/c mice, aged 8–12 weeks, were used as donors. Non-transgenic Balb/c mice were rendered diabetic by intravenous injection 14 days before transplantation of alloxan (90 mg/kg; Sigma-Aldrich, St Louis, Mo, United States), which had been freshly dissolved in hydrochloric acid (1 mmol/L).[15] Diabetes was confirmed by the presence of hyperglycemia, weight loss, and polyuria. We only transplanted mice with a blood glucose >350 mg/dL. Blood was obtained from the snipped tail, and glucose measured with a portable glucometer (One Touch II, Lifescan Inc. Milpitas, Calif, United States). The animals were kept under conventional conditions with free access to tap water and standard pelleted food.

Islet Isolation

Islets were isolated from FasL and HO-1 double transgenic and from nontransgenic Balb/c mice. Under sodium amobarbital anesthesia, excised pancreata were distended with 2.5 mL of RPMI-1640 medium (GIBCO BRL, Grand Island, NY, United States) containing 1.5 mg/mL of collagenase (collagenase from *Clostridium histolyticum,* type XI, Sigma-Aldrich) during incubation in a water bath at 37°C. The islets were separated by a density gradient (Histopaque-1077; Sigma-Aldrich), with purified islets handpicked under a dissecting microscope.[16]

Islet Transplantation

Three hundred FasL and HO-1 double transgenic (n = 10) or nontransgenic (n = 7) islets were transplanted under the left kidney capsule of each Balb/c mouse. The islets were centrifuged in PE-50 tubing (Clay Adams, Parsippany, NJ, United States) connected to a 200-μL pipette tip. With the mouse under amobarbital anesthesia, the left kidney was exposed through a lumbar incision. A capsulotomy in the lower pole of the kidney allowed the tip of the tubing to be advanced under the capsule of the upper pole, the site of final injection. The capsulotomy was left unsutured.[16]

Intraperitoneal Glucose Tolerance Test

Intraperitoneal glucose tolerance test (IPGTT) was performed at 2, 4, and 6 weeks after islet transplantation. After an overnight fast, glucose was injected intraperitoneally as a 5% glucose solution (1.5 g/kg), with blood glucose measurements at 0, 30, 60, 90, and 120 minutes by tail snipping. The areas under the curves were calculated as the sum of blood glucose values during the test.[17]

Statistical Analysis

Results were expressed as mean values and standard errors (M ± SE). For comparisons between 2 groups, we used unpaired Student *t* test. Paired Student *t* test was used to compare measurements at 2 time points in 1 group. A value of $P < .05$ was considered significant.

RESULTS

Over 6 weeks after transplantation, blood glucose levels gradually decreased among recipients of either FasL/HO-1 double transgenic or nontransgenic islets. However, the decrease in blood glucose was more pronounced in the former setting (450 ± 16 mg/dL at day 0 to 302 ± 55 mg/dL at day 42; $P = .01$) versus the latter (468 ± 17 mg/dL at day 0 to 379 ± 71 mg/dL at day 42; $P = .24$). There was no significant difference between the 2 groups at any time point. The area under the curve of IPGTT at 2 (21,395 ± 11,256 vs 33,159 ± 16,200 mg/dL), 4 (27,845 ± 14,145 vs 41,314 ± 20,958 mg/dL), and 6 weeks (24,292 ± 16,197 vs 40,050 ± 22,086 mg/dL) was not significantly different between recipients of double transgenic versus nontransgenic islets. The body weight increased among recipient of double transgenic islets (21.1 ± 1.4 g at day 0 to 26.2 ± 0.8 g at day 42; $P = .0002$) versus nontransgenic islets (21.0 ± 1.4 g at day 0 to 25.1 ± 0.4 g at day 42; $P = .0448$). However, the difference between the 2 groups was not significant at any time point.

DISCUSSION

We demonstrated that human FasL and HO-1 double transgenic mice showed a more pronounced decrease in blood glucose than recipients of nontransgenic islets, suggesting beneficial effects of overexpression of both FasL and HO-1 in islets.

Although cotransplantation of FasL-transfected myoblasts prolonged islet allograft survival,[4] further studies demonstrated that islet cells transgenic for mouse FasL provoked intense neutrophil infiltration and induced insulitis in the pancreas, thus leading to the destruction of β cells.[18,19] We generated mice transgenic for human FasL, instead of mouse FasL, observing the absence of insulitis,[7] although they did not prolong islet allograft survival.[7,8]

HO-1 induced in islets by cobalt-protoporphyrin may protect them from apoptosis[13] and from the suppressive effects of interleukin (IL)-1β[20] in vitro, leading to a shorter time to normoglycemia among diabetic islet recipients[13] and enhanced islet engraftment.[21] Accordingly, we have generated transgenic mice with high HO-1 expression in heart, liver, spleen, lung, kidney, muscle, intestine, and pancreas.[14] The protective effects of HO-1 overexpression were demonstrated by the resistance of HO-1 transgenic mice to lipopolysaccharide challenge. However, at 4 weeks mice transplanted with HO-1 transgenic islets displayed higher blood glucose levels than those with nontransgenic islets.[14]

Human FasL transgenic islets and HO-1 transgenic islets may produce insufficient effects on immunoregulation and cytoprotection to prolong islet allograft survival. Therefore, in the present study, we tested whether overexpression of both FasL and HO-1 in islets could be beneficial for transplantation. We demonstrated that the rate of decrease in blood glucose was more pronounced over 6 weeks among recipients of double transgenic than nontransgenic islets. Our data suggested that overexpression of both FasL and HO-1 in islets may have potential to improve transplantation outcomes.

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