Elevated Vascular Endothelial Growth Factor Production in Islets Improves Islet Graft Vascularization

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Successful islet transplantation depends on the infusion of sufficiently large quantities of islets, of which only ~30% become stably engrafted. Rapid and adequate revascularization of transplanted islets is important for islet survival and function. Delayed and insufficient revascularization can deprive islets of oxygen and nutrients, resulting in islet cell death and early graft failure. To improve islet revascularization, we delivered human vascular endothelial growth factor (VEGF) cDNA to murine islets, followed by transplantation under the renal capsule in diabetic mice. Diabetic animals receiving a marginal mass of 300 islets that were pretransduced with a VEGF vector exhibited near normoglycemia. In contrast, diabetic mice receiving an equivalent number of islets that were transduced with a control vector remained hyperglycemic. Immunohistochemistry with anti-insulin and anti-CD31 antibodies revealed a relatively higher insulin content and greater degree of microvasculature in the VEGF vector–transduced islet grafts, which correlated with significantly improved blood glucose profiles and enhanced insulin secretion in response to glucose challenge in this group of diabetic recipient mice. These results demonstrate that VEGF production in islets stimulates graft angiogenesis and enhances islet revascularization. This mechanism might be explored as a novel strategy to accelerate islet revascularization and improve long-term survival of functional islet mass posttransplantation. *Diabetes* 53:963–970, 2004

Islet transplantation is considered a potentially curative treatment for type 1 diabetes (1,2). However, this protocol depends on the infusion of sufficiently large amounts of islets (>11,000 islet equivalent/kg body wt), requiring two to four cadaveric pancreata (3,4). Apart from immune rejection, another critical limitation to islet transplantation is the rate and extent of islet revascularization. Native islets in the pancreas have a rich glomerular-like microvasculature that consists of fine capillaries supplied by one to five feeding arterioles and drained by coalescing into an efferent plexus exiting the islet via one to five venules. Such a rich microvasculature in islets is thought to provide efficient delivery of oxygen and nutrients to islet cells and ensure rapid dispersal of pancreatic hormones to the circulation (5,6). In contrast, isolated islets are severed from their native vascular network such that after transplantation, the survival and function of islet grafts must depend on the reestablishment of new vessels within the grafts to derive blood flow from the host vascular system (5,6). Rapid and adequate islet revascularization may be crucial for the survival and function of transplanted islets (7). It is estimated that <30% of islet mass becomes stably engrafted, despite the administration of a large quantity of islets per diabetic recipient (8). This impairment in islet revascularization appears to ensue irrespective of whether islets are transplanted intraportally in the liver, retrogradely into the spleen, or under the kidney capsule (9).

Although a number of factors have been implicated to play important roles in islet revascularization (10–14), the molecular mechanism underlying islet revascularization remains elusive. Among those factors involved in islet revascularization is vascular endothelial growth factor (VEGF), a key angiogenic molecule that acts to stimulate new vessel formation (15,16). VEGF is also expressed in the pancreatic islets, but its expression in transplanted islets is significantly reduced 2–3 days posttransplantation (14). This impairment is further pronounced in the presence of prevailing hyperglycemia, which coincides with delayed and insufficient islet revascularization in diabetic mice (14,17–19).

To test the hypothesis that elevated VEGF expression in islet cells before islet transplantation would enhance islet revascularization and improve the functional performance of transplanted islets, we used an adenoviral-mediated gene delivery system to transfer human VEGF165 cDNA to murine islets, followed by transplantation under the kidney capsule in streptozotocin (STZ)-induced diabetic mice. Islet grafts with elevated VEGF production exhibited significantly increased microvasculature and insulin content, contributing to the reversal of hyperglycemia in diabetic recipient mice. In contrast, diabetic animals transplanted with control vector–transduced islets remained moderately hyperglycemic, which coincided with the lack

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EF-1α, elongation factor-1α; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; MOI, multiplicity of infection; pfu, plaque-forming unit; STZ, streptozotocin; VEGF, vascular endothelial growth factor.

of sufficient revascularization of transplanted islets. These results provide proof-of-principle that local expression of angiogenic molecules in islet grafts can stimulate graft angiogenesis and enhance islet revascularization, thereby improving the outcome of marginal islet transplantation with better glycemic control in diabetic mice.

RESEARCH DESIGN AND METHODS

Vector. The recombinant adenoviral vector Ad-EF1α-VEGF expresses the human VEGF165 protein under the control of the elongation factor-1α (EF-1α) promoter (20). To prepare adenoviruses, Ad-EF1-VEGF was propagated in HEK293 cells and purified by CsCl density centrifugation as previously described (21). The titer of Ad-EF1-VEGF was 1.1×10^{11} plaque-forming unit (pfu)/ml. The Ad-RSV-LacZ vector, with a titer of 1.9×10^{11} pfu/ml, was used as control (21).

Islet isolation and transplantation. BALB/c mice at 8 weeks of age were purchased from Charles River Laboratory (Wilmington, MA). Athymic NIH nude mice at 8 weeks of age were obtained from the National Cancer Institute (National Institutes of Health, Bethesda, MD). Animals were fed with standard rodent chow and water ad libitum in sterile cages (five mice per cage) in a barrier animal facility with a 12-h light/dark cycle. Islets were isolated as previously reported (22). Briefly, BALB/c mice were anesthetized with ketamine chloride (100 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). After intraductal infusion of 3-ml cold Hanks' balanced salt solution containing 1.5 mg/ml of collagenase P (Roche Diagnostics, Indianapolis, IN), pancreata were surgically procured and digested at 37°C for 20 min. Islets were washed with Hanks' balanced salt solution, followed by discontinuous Ficoll density-gradient centrifugation as previously described (23). Islets were resuspended in serum-free CMRL-1066 medium (Sigma-Aldrich, St. Louis, MO) and hand-picked under an inverted microscope under sterile conditions.

For islet transduction by adenoviral vectors, aliquots of islets were incubated with the VEGF or LacZ vector at a defined multiplicity of infection (MOI) in 2 ml of serum-free CMRL-1066 medium at 37°C for 2 h. After washing with Hanks' balanced salt solution, transduced islets were used for transplantation. To determine the efficiency of transduction in islets, islets pretransduced with the LacZ vector were harvested and dispersed to single islet cells by incubating islets in trypsin-EDTA solution (Invitrogen, Grant Island, NY) for 10 min at 37°C, followed by staining with X-gal. The transduction efficiency, defined as the number of β-gal–positively stained cells out of total islet cells, was determined.

For islet transplantation, athymic NIH nude mice were rendered diabetic by intraperitoneal injection of 180 mg/kg STZ as previously described (21). We chose immunodeficient NIH nude mice as diabetic recipients to avoid potential immune response caused by human VEGF production and leaky expression of residual viral proteins in the adenoviral vector. At 1 week after STZ treatment, diabetic mice (blood glucose levels ~400 mg/dl) were randomly assigned to three groups ($n = 10$ per group) and were transplanted with a marginal islet mass (300 islets) that was pretransduced with VEGF or control vector or was mock transduced. Diabetic mice were anesthetized by ketamine chloride, and the right kidney was exposed through a lumbar incision. A breach was made in the kidney capsule, and a polyethylene catheter was introduced through the breach and advanced beneath the kidney capsule to generate a subcapsular space. Islets ($n = 300$, ~50 μl in volume) were slowly injected through the catheter into the subcapsular space. After removing the catheter, the opening was cauterized, and the kidney was repositioned, followed by suturing of muscle and skin. Before and after transplantation, blood was sampled daily from tail vein for the determination of blood glucose levels, using a Glucometer Elite (Bayer, Mishawaka, IN). All procedures were approved by the institutional animal care and usage committee of the Mount Sinai School of Medicine (protocol no. 02-0816).

Glucose tolerance test. Animals were fasted for 5 h and injected intraperitoneally with 50% dextrose solution (Abbott Laboratories, Chicago) at a dose of 3 g/kg body wt, as previously described (20). Blood glucose levels were measured before and at different times after glucose infusion.

Immunoprecipitation and Western blot analysis. To verify the production of VEGF from Ad-EF1α-VEGF, aliquots ($n = 100$) of isolated mouse islets were transduced with the VEGF or control vector at an MOI of 100 pfu/cell. One islet contains an average of 1,000 cells. In addition, one aliquot of 100 islets was mock-treated as a negative control. After 16 h of transduction in 2 ml serum-free CMRL-1066 medium, the conditioned media were collected and subjected to immunoprecipitation with anti-VEGF165 antibody (RDI Research Diagnostics, Flanders, NJ), using the Catch and Release immunoprecipitation system (Upstate Biotechnology, Lake Placid, NY). The immunoprecipitates were resolved on 15% SDS-polyacrylamide gels, and proteins were blotted onto a piece of nitrocellulose membrane, which was subsequently probed with rabbit anti-VEGF165 antibody (1:1,000 dilution), followed by incubation with anti-rabbit IgG conjugated with horseradish peroxidase (HRP; 1:200 dilution). Protein bands were detected by enhanced chemiluminescence Western blotting detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ).

Immunohistochemistry and morphometric analysis. Immunohistochemistry of islet grafts was performed as follows: animals were killed by CO_2 inhalation 16 days posttransplantation, and islet grafts were retrieved from individual animals. After fixing in 10% phosphate-buffered formalin overnight, islet grafts were embedded in paraffin. Consecutive sections (4 μm thick) of paraffin-embedded islet grafts were cut, and consecutive sections were immunostained with guinea pig anti-insulin (1:200 dilution; DAKO, Carpinteria, CA), rat anti-CD31 (1:50 dilution; eBioscience, San Diego, CA), and rabbit anti-VEGF165 (1:50 dilution; Abcam, Cambridge, U.K.) antibodies, respectively. The immunoreactivity was detected using the multilink-HRP ultrasensitive system (BioGenex, San Ramon, CA), as previously described (24). After immunostaining, the sections were examined at ×200 magnification in a microscope that was linked to a computerized charge coupled device camera. Microscopic views covering engrafted islets under the kidney capsule that were immunostained by anti-insulin or anti-CD31 antibodies were captured as digitized micrographic pictures using Adobe Photoshop software (Adobe Systems, San Jose, CA). Using the color range section option of Adobe Photoshop, insulin- or CD31-positively immunostained color (brown in each case) was selected for quantification of the relative intensity per islet graft by densitometry using NIH Image 1.62 software (National Institutes of Health, Bethesda, MD). Using this technique, the relative intensity of insulin or CD31 immunostaining from three sections, on average, per islet graft was evaluated for the determination of the mean values, which were subsequently compared between different groups of diabetic recipient mice.

Human VEGF enzyme-linked immunosorbent assay. The VEGF vector used in this study encodes the human VEGF165 cDNA so that only human VEGF protein is produced from the vector-transduced islets, which is readily distinguishable from endogenous VEGF using the human VEGF-specific enzyme-linked immunosorbent assay (ELISA) kit (Alpco, Windham, NH). This ELISA kit has a lower detection limit of 20 pg/ml in 50–100 μl of serum. Insulin concentrations in conditioned media were determined using the ultrasensitive murine insulin ELISA kit (Alpco).

Statistics. Statistical analyses of data were performed by ANOVA using StatView software (Abacus Concepts, Berkeley, CA). Unpaired ANOVA t test was used to study the significance between two different treatment groups. Data were expressed as the means ± SE. P values <0.05 are statistically significant.

RESULTS

Adenoviral-mediated VEGF production in islets. To characterize the Ad-EF1α-VEGF vector, freshly isolated murine islets were equally divided into three aliquots, each containing 100 handpicked islets that were transduced with the VEGF vector at an MOI of 100 pfu/cell or control vector at the same MOI, or that were mock-transduced with vehicle buffer. After 24 h of transduction, conditioned media were collected for the determination of VEGF concentrations using the human VEGF ELISA. In addition, islets transduced with the LacZ vector were harvested and dispersed to single islet cells, followed by X-gal staining to determine the frequency of islet cell transduction. Under these experimental conditions, we detected a transduction efficiency of 72 ± 12% by adenovirus in freshly isolated murine islets. At this level of transduction, ~20 pg islet/ml of immunoreactive VEGF165 protein was detected in the conditioned media of the VEGF vector–transduced islets (Fig. 1*A*). In contrast, VEGF165 was undetectable in the conditioned media of control vector–transduced islets.

To confirm the production of human VEGF165 protein from the Ad.EF1α-VEGF vector, the conditioned media of control and VEGF vector–transduced islets were subjected to immunoprecipitation using anti-human VEGF165 antibody, followed by Western blot analysis. As shown in Fig. 1*B*, transduction of murine islets by the VEGF vector

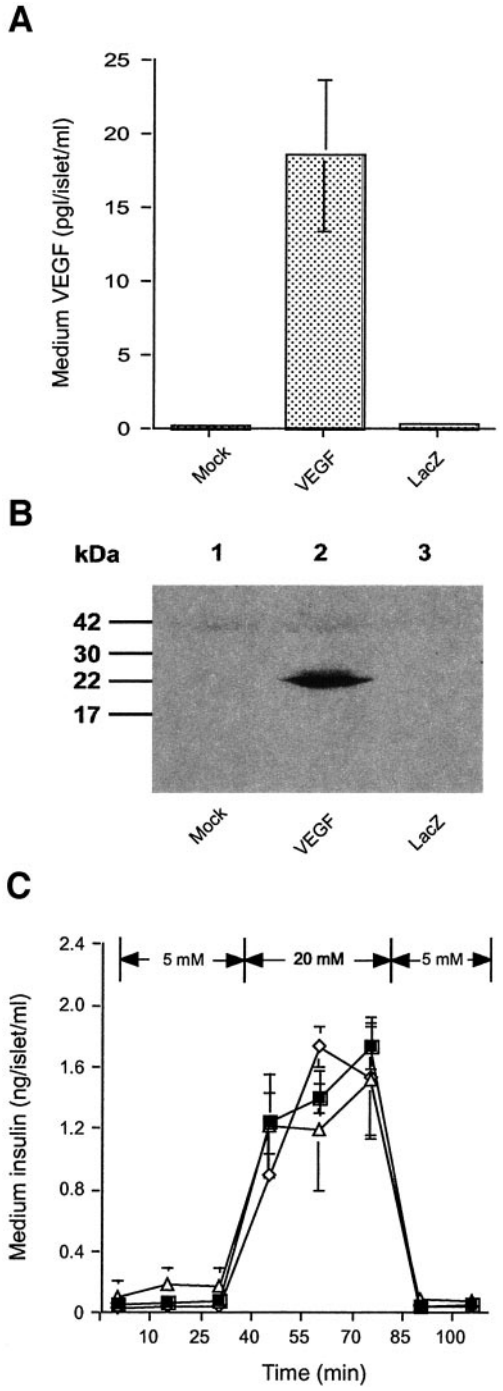

FIG. 1. Characterization of the VEGF vector. *A*: Adenoviral-mediated production of VEGF in islet cells. Freshly isolated islets (n = 100) from BALB/c mice were mock-treated or transduced with the VEGF vector at an MOI of 100 pfu/cell or LacZ vector at the same dose. After 24 h of transduction, the conditioned media were collected for measuring VEGF concentrations using human VEGF ELISA. *B*: Western blot analysis of VEGF. The conditioned media from mock-treated (*lane 1*), VEGF vector–transduced (*lane 2*), and control vector– transduced (*lane 3*) islets in the experiment described in panel *A* were immunoprecipitated using anti-human VEGF antibody. The immunoprecipitates were subjected to Western blot analysis by anti-human VEGF antibody. A specific protein band corresponding to VEGF165 (predicted mol wt 22 kDa) was detected from the conditioned media of the VEGF vector–transduced islet cells. *C*: Effects of adenoviral-mediated transduction on the functional integrity of β-cells. Islets (n = 100) were mock-treated (△) or transduced with the VEGF (■), LacZ (◇) vectors at an MOI of 100 pfu/cell. Then, 24 h later, islets were challenged by shifting the culture media from 5 to 20 mmol/l, followed by shifting back to 5 mmol/l glucose concentrations. Aliquots (50 μl) of culture media were collected at different times for determination of insulin concentrations.

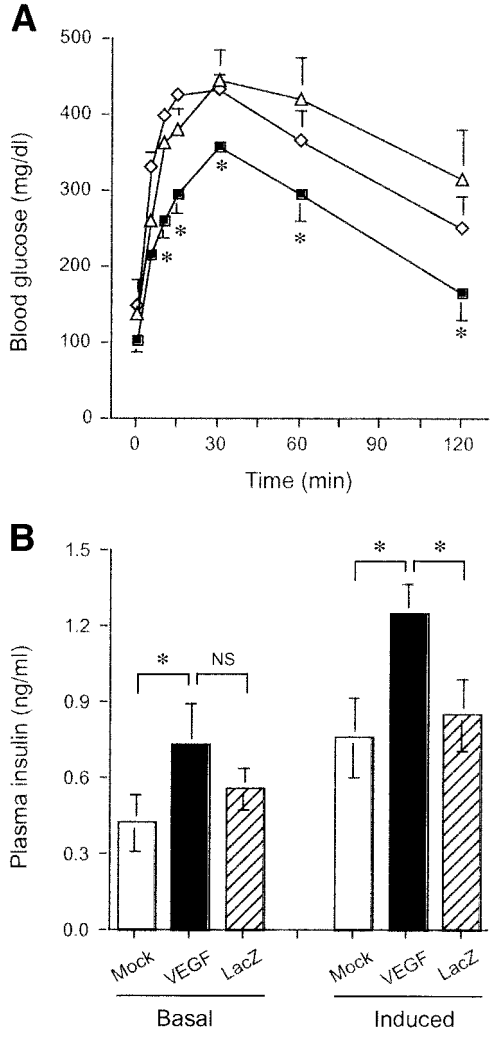

FIG. 2. Effects of VEGF production in islet grafts on glycemic control in diabetic mice. *A*: Glucose tolerance test. Diabetic recipient mice were fasted for 5 h, followed by injecting intraperitoneally 3 g/kg glucose solution. Blood glucose levels in the VEGF vector (■), LacZ vector (◇), and mock (△) treatment groups were measured before and after glucose infusion. *B*: Glucose-stimulated insulin release. During the glucose tolerance test, aliquots of blood (25 μl) were collected from the tail vein before (basal) and 5 min after (induced) glucose infusion, and plasma insulin levels were determined. Data were obtained 15 days posttransplantation. *P < 0.05 vs. control groups by ANOVA.

resulted in the production of VEGF165 protein because a specific protein band corresponding to VEGF165 (mol wt 22 kDa) was detected in the conditioned media of islets that were pretransduced with the VEGF vector but not with control vector.

To examine the potential influence of adenoviral-mediated transduction on islet function, we performed glucose perfusion experiments on islets (n = 100) that were pretransduced with the VEGF and control vectors by shifting the culture media from low to higher concentrations of glucose after 24 h of transduction. As shown in Fig. 1*C*, in response to a rise in ambient glucose concentrations (up to 20 mmol/l), ~15-fold increases in insulin concentrations were detected in the conditioned media of mock-treated or vector-transduced islets. Furthermore, no significant differences in the amplitude and kinetics of glucose-stimulated insulin release were discernible among groups. Thus, neither adenoviral-mediated transduction

nor VEGF production in islet cells significantly affects the ability of β-cells to secrete insulin in response to glucose challenge. Consistent with these results are the previous observations that recombinant adenoviruses are highly efficient in transferring genes into isolated mouse islets without perturbing the function of islet cells (25–27).

Effects of VEGF expression in islet grafts on glycemic control in diabetic mice. To examine the effect of elevated VEGF production in islet cells on glycemic control, STZ-induced diabetic nude mice were stratified by blood glucose levels and randomly assigned to three different groups ($n = 10$) to ensure a similar mean blood glucose level in each group. Aliquots ($n = 300$) of freshly isolated murine islets were transduced by the VEGF or control vector at a fixed MOI (100 pfu/cell) or were mock-treated with vehicle buffer, and then the islets were transplanted under the renal capsule in STZ-induced diabetic mice. Blood glucose levels in individual animals were measured daily posttransplantation. Diabetic mice transplanted with the VEGF vector–transduced islets were restored to normal (blood glucose levels 88 ± 6 mg/dl) 2 days posttransplantation. In contrast, diabetic animals receiving the same number of islets that were mock-treated or transduced with control vector displayed moderate hyperglycemia (190 ± 12 and 142 ± 22 mg/dl in control vector and mock-transduced groups, respectively) during the course of the study.

To study the beneficial effect of elevated VEGF production in islet cells on the whole-body glucose disposal rate, glucose tolerance tests were performed on all islet recipient diabetic mice and compared between the VEGF and LacZ treatment groups 15 days posttransplantation. As shown in Fig. 2*A*, significant differences in both the amplitude of blood glucose induction and the kinetics of blood glucose decline were detected between different treatment groups. In response to intraperitoneal injection of a high dose of glucose (3 g/kg), elevated blood glucose levels in the VEGF treatment group were restored to a normal range within 2 h, whereas blood glucose levels in control groups of diabetic recipient mice were raised to a significantly higher amplitude after glucose infusion and remained at a hyperglycemic level (blood glucose levels 249 ± 43 and 315 ± 61 mg/dl in control vector and mock-treated groups, respectively) 2-h postglucose infusion. Similar results were also produced on day 8 after islet transplantation. Together, these data indicate that local VEGF production in islet grafts conferred a beneficial effect on glycemic control in diabetic recipient mice.

To examine the effect of local VEGF production in islet grafts on the functional performance of β-cells, plasma insulin levels were determined under basal and glucose-inducible conditions 2 weeks post–islet transplantation. Diabetic recipient mice were injected intraperitoneally with 3 g/kg of glucose. Before and 5 min after glucose infusion, aliquots (25 μl) of blood were collected from the tail vein and used for the determination of plasma insulin levels. As shown in Fig. 2*B*, under basal conditions, relatively higher levels of plasma insulin were detected in diabetic mice receiving the VEGF vector–transduced islets (0.73 ± 0.15 vs. 0.42 ± 0.11 ng/ml in diabetic mice receiving mock-transduced islets, $P < 0.05$), but the difference in basal plasma insulin levels between VEGF and control vector treatment groups (0.73 ± 0.15 vs. 0.55 ± 0.08 ng/ml, $P > 0.05$ by ANOVA) did not reach a statistically significant level. However, in response to glucose challenge, significantly higher levels of plasma insulin were detected in diabetic mice receiving the VEGF vector–transduced islets (1.25 ± 0.12 vs. 0.76 ± 0.15 and 0.84 ± 0.14 ng/ml in mock-treated and control vector–transduced groups, respectively; $P < 0.05$). Thus, in keeping with their enhanced abilities to tolerate intraperitoneal glucose challenge (Fig. 2*A*), diabetic mice receiving the VEGF vector–transduced islets exhibited significantly higher insulin release in response to glucose infusion compared with control diabetic recipient mice (Fig. 2*B*).

Effects of VEGF production in islet cells on islet revascularization. To study the effects of VEGF expression in islet cells on graft revascularization, diabetic recipient mice were killed 16 days posttransplantation, and islet grafts were retrieved for studying the extent of islet revascularization after VEGF production in islet grafts. The degree of islet revascularization of transplanted islets was determined by immunohistochemistry for the endothelial marker CD31, followed by morphometric analysis. As shown in Fig. 3*A–C*, a significant difference in the relative intensity of immunostaining by anti-CD31 antibody was detected between the VEGF vector–treated and control groups. Islet grafts that were pretransduced with the VEGF vector exhibited more than twofold higher CD31-immunostaining intensity than control islet grafts that were mock-treated or transduced with the LacZ vector (Fig. 4*B*). To correlate the degree of islet revascularization with the islet mass in the kidney capsule, islet grafts retrieved from killed animals were immunostained for insulin (Fig. 3*D–E*). Consistent with the data of anti-CD31 immunohistochemistry, the renal capsule containing the VEGF vector–transduced islets displayed significantly higher levels of insulin content than control renal capsules (Fig. 4*A*). This quantitative difference in insulin content in the kidney capsule between different groups of diabetic recipient mice correlated closely with their blood glucose profiles in glucose tolerance testing (Fig. 2*A*) and glucose-stimulated insulin secretion (Fig. 2*B*).

Finally, to study VEGF165 production, islet grafts from individual diabetic recipient mice were examined after immunostaining with monoclonal anti-VEGF165 antibody. As shown in Fig. 3*G–I*, positive immunostaining for VEGF165 was detected in the VEGF vector–transduced islet grafts. In contrast, there was no detectable immunostaining in islet grafts that were mock-treated or transduced with control vector. These results confirmed the production of VEGF in the VEGF vector–transduced islet grafts, suggesting that the observed improvement in islet revascularization and glucose-inducible insulin secretion is attributable to the specific effect of elevated VEGF production in islet grafts. To study whether local production of VEGF from engrafted islet cells elevates VEGF concentrations in the circulation, plasma VEGF165 concentrations were determined by human VEGF165 ELISA after 8 and 16 days of VEGF165 transgene expression in transplanted islets. Using a specific human VEGF165 ELISA with a lower detection limit of 20 pg/ml, the amounts of plasma VEGF165 in the group of diabetic mice transplanted with the VEGF vector–transduced islets were

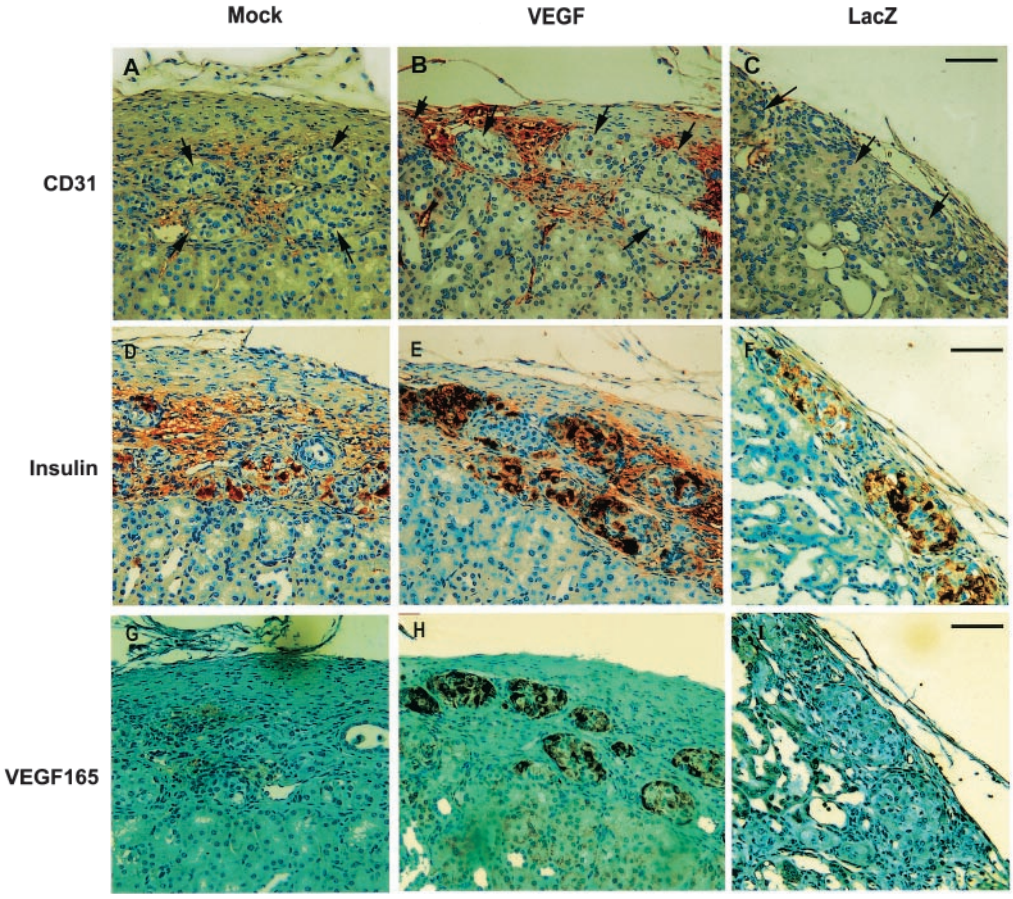

FIG. 3. Immunohistochemistry of islet grafts. Islet grafts were retrieved from recipient diabetic mice transplanted with mock-treated (*A*, *D*, and *G*), VEGF vector–transduced (*B*, *E*, and *H*), and LacZ vector–transduced (*C*, *F*, and *I*) islets and embedded in paraffin. Consecutive sections of paraffin-embedded islet grafts were cut and immunostained with anti-CD31 (*A*, *B*, and *C*), anti-insulin (*D*, *E*, and *F*), and anti-human VEGF (*G*, *H*, and *I*) antibodies, respectively. Arrows in panels *A*–*C* indicate islet grafts. Bar = 100 μm.

undetectable. These results indicate that local VEGF production in the marginal islet mass did not result in a significant elevation in systemic VEGF levels.

DISCUSSION

We have used a novel gene transfer approach to investigate the role of VEGF and its functional contribution to islet revascularization posttransplantation. Previous studies indicated that VEGF expression in transplanted islets is significantly attenuated, particularly in the presence of hyperglycemia. This impaired VEGF expression in newly transplanted islets is thought to be a contributing factor for impaired revascularization of transplanted islets in diabetic animals (10,14). In the present study, we asked *1*) whether a restoration of VEGF production in islet grafts would enhance islet revascularization and *2*) whether enhanced islet revascularization would improve glycemic control in diabetic recipient mice. To address these two fundamental questions in islet transplantation, we delivered the human VEGF gene by adenoviral-mediated gene transfer into isolated murine islet cells, followed by transplantation under the kidney capsule of STZ-induced diabetic mice. We showed that elevated VEGF expression in transplanted islet cells enhanced islet revascularization, as reflected in the significantly increased CD31 immunostaining under the kidney capsule that was grafted with the VEGF vector–transduced islets. To study the effect of increased islet revascularization on glycemic control, we evaluated the overall glucose disposal rate by performing intraperitoneal glucose tolerance tests on diabetic mice that were transplanted with the VEGF vector–transduced islets. We showed that diabetic mice receiving the VEGF vector–transduced islets exhibited significantly improved blood glucose profiles in response to intraperitoneal glucose infusion, compared with diabetic mice that were transplanted with mock-treated or control vector–transduced islets. Furthermore, we showed that all islet grafts containing the VEGF vector–transduced islets exhibited significantly increased insulin content, which correlated with the reversal of hyperglycemia in diabetic mice that were transplanted with the VEGF vector–transduced islets. In contrast, diabetic animals receiving mock-treated or control vector–transduced islets exhibited moderate hyperglycemia, which coincided with the lack of adequate islet revascularization and relative lower insulin content in their respective islet grafts, as determined 16 days posttransplantation. To correlate these findings with the specific expression of VEGF in transplanted islets, we showed that VEGF165 was expressed in the VEGF-transduced but not in control vector–transduced islet grafts, as detected by immunohistochemistry using anti-human VEGF antibody. Together, these results demonstrate that adenoviral-mediated VEGF production in transplanted islets

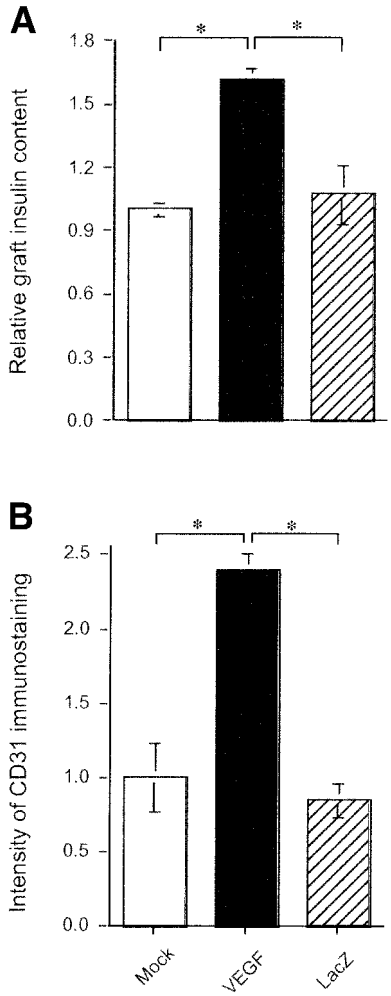

FIG. 4. Graft insulin content and anti-CD31 immunostaining intensity. The relative intensity of immunostaining with anti-insulin or anti-CD31 antibodies, respectively, was compared between different groups, as determined by morphometric analysis. *$P < 0.001$.

significantly enhanced islet revascularization and improved glycemic control in STZ-induced diabetic mice.

Rapid reestablishment of an appropriate microvascular system in newly transplanted islets is thought to be crucial for long-term survival and function of islet grafts (5–7). Islet revascularization initiates as early as 2–3 days posttransplantation and concludes within ~2 weeks (5). However, recent studies by Carlsson et al. (28) showed that the degree of vascularization in transplanted islets is considerably lower than that of the native microvasculature of pancreas islets, even after 9 months of engraftment. Insufficient islet revascularization tends to reduce intragraft blood perfusion and oxygen tension (28). This may adversely affect the functional performance of engrafted islet cells, contributing to suboptimal insulin secretion in response to intravenous glucose tolerance in posttransplant subjects (4,8). More recently, sirolimus and tacrolimus have been shown to inhibit angiogenesis in a dose-dependent manner in both in vitro and in vivo angiogenesis assays (29). Although the molecular basis underlying the inhibitory effect of these two commonly used immunosuppressants on angiogenesis is currently unknown, their application in clinical islet transplantation can potentially impair revascularization of newly transplanted islets. This may partially account for the need for sufficiently large quantities of islet mass for normalization of hyperglycemia in type 1 diabetic subjects because a vast majority (~70% of islet mass) of newly transplanted islets may be lost before revascularization posttransplantation (5).

To date, the molecular mechanism underlying islet revascularization remains unknown, but it is clear that adequate microvascular perfusion to islet cells does not resume immediately after islet transplantation. Instead, it can take up to 5 days for the formation of intragraft microvessels to occur post–islet transplantation, and the reestablishment of intragraft blood perfusion can take even longer (>15 days) (6,14). This delay in the reestablishment of functional microvasculature in newly engrafted islets can deprive islet cells of oxygen and nutrients. Indeed, several studies have shown that newly transplanted islets are hypoxic, causing islet cells to undergo apoptosis and/or necrosis, which contributes to the loss of functional β-cell mass posttransplantation (6,14). In addition, there is evidence that islet cells undergo apoptosis because of ischemia-reperfusion injury or oxidative stress in islet isolation, contributing to the loss of functional islet mass during the peri-transplant period (30,31). In this context, attempts have been made to protect islet cells from undergoing apoptosis by introducing a cytoprotective mechanism or antiapoptotic functions into islet cells to preserve functional islet mass post–islet transplantation (32–36).

Unlike previous studies, our results validated the concept that elevated production of VEGF locally in islet grafts help facilitate islet revascularization, contributing to increased islet mass and improved glycemic control in diabetic recipient mice. Using a different approach by which murine islets were immobilized with collagen in the presence of VEGF protein and encapsulated before transplantation into the peritoneal cavity, Sigrist et al. (37) showed that local release of VEGF within the grafts significantly increased the viability of transplanted islets, resulting in extended physiological control of glycemia in STZ-induced diabetic mice. Although therapeutic angiogenesis has been used for treating coronary and peripheral artery diseases by facilitating new vessel formation using plasmid or adenoviral vector–mediated VEGF gene delivery in a number of clinical trials (38–44), our present results together with others suggest that a similar strategy should be explored to allow local production/release of angiogenic molecules in islet grafts to accelerate islet revascularization.

This notion seems at variance with the concept of cell encapsulation. Here, islets are encapsulated in semipermeable membranes with pores of a size allowing passage of nutrients and metabolites (including oxygen, glucose, and insulin) but excluding much larger IgG molecules and lymphocytes, which is meant to protect encapsulated islet cells from autoimmune attack after transplantation into the peritoneal cavity (45,46). However, the long-term survival and function of encapsulated islets is significantly compromised by a number of factors (including the porosity, size, and biocompatibility of the capsule) as well as by insufficient oxygen and nutrient supplies to encapsulated islets caused by the lack of microvascular perfusion (46). Although shielded from cell-mediated immune attack, en-

capsulated islets are still subject to oxidative stress and damage caused by free radicals that are generated from activated immune cells such as macrophages, contributing to islet cell apoptosis and early graft failure (47,48). In addition, intraperitoneally transplanted microencapsulated islets are associated with significantly delayed and diminished responsiveness in releasing insulin after an oral or intravenous glucose challenge (46). Thus, transplantation of encapsulated islets has its own limitations.

Finally, we would like to point out that in the present study, islets are implanted in the renal subcapsular space, which is different from the clinical setting, in which islets are transplanted intraportally into the liver. We are currently investigating whether elevated VEGF production/release in islets transplanted in the liver also improves intrahepatic graft vascularization and provides better glycemic control in posttransplant diabetic subjects.

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