Overexpression of Glutathione Peroxidase With Two Isoforms of Superoxide Dismutase Protects Mouse Islets From Oxidative Injury and Improves Islet Graft Function

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Primary nonfunction of transplanted islets results in part from their sensitivity to reactive oxygen species (ROS) generated during the isolation and transplantation process. Our aim was to examine whether coexpression of antioxidant enzymes to detoxify multiple ROS increased the resistance of mouse islets to oxidative stress and improved the initial function of islet grafts. Islets from transgenic mice expressing combinations of human copper/zinc superoxide dismutase (SOD), extracellular SOD, and cellular glutathione peroxidase (Gpx-1) were subjected to oxidative stress in vitro. Relative viability after hypoxanthine/xanthine oxidase treatment was as follows: extracellular SOD + Gpx-1 + Cu/Zn SOD > extracellular SOD + Gpx-1 > extracellular SOD > wild type. Expression of all three enzymes was the only combination protective against hypoxia/reoxygenation. Islets from transgenic or control wild-type mice were then transplanted into streptozotocin-induced diabetic recipients in a syngeneic marginal islet mass model, and blood glucose levels were monitored for 7 days. In contrast to single- and double-transgenic grafts, triple-transgenic grafts significantly improved control of blood glucose compared with wild type. Our results indicate that coexpression of antioxidant enzymes has a complementary beneficial effect and may be a useful approach to reduce primary nonfunction of islet grafts. Diabetes 54:2109–2116, 2005

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Islet transplantation has the potential to replace pancreatic endocrine function in patients with type 1 diabetes (1), but the requirement for two or more cadaveric donors per recipient limits wider application of the technique. This problem has led to investigation of the causes of islet primary nonfunction, so that protective strategies may be developed. A significant number of transplanted islets are lost because of apoptosis and necrosis, mediated at least in part by oxidative injury. Native islets are richly supplied with blood but lose their vasculature during isolation (3), and full revascularization takes weeks (4). Hypoxia/reoxygenation generates reactive oxygen species (ROS), including superoxide radicals (O2·−), hydrogen peroxide (H2O2), and hydroxyl radical (·OH) (5). In addition, nitric oxide (NO) is generated by upregulation of inducible NO synthase and from nitrate residues of necrotic cells (6). Simultaneous generation of superoxide radicals and NO results in the production of peroxynitrite (ONOO−) (7), which is a potent mediator of human β-cell destruction (8). ROS directly injure cells by DNA strand breakage and peroxidation of proteins and lipids but also activate a number of signaling pathways involved in inflammation and apoptosis (9).

Islets appear to be more vulnerable to oxidative injury than other tissues because they express low levels of antioxidant enzymes, including superoxide dismutase (SOD), which converts superoxide radicals to H2O2, and catalase and cellular glutathione peroxidase (Gpx-1), which detoxify H2O2 (10). Treatment of human islets with an SOD mimic promoted islet cell survival (11). The protective effect of overexpressing antioxidant enzymes has also been examined in vitro. SOD, catalase, and Gpx-1 provided rodent β-cell lines with various degrees of protection from exogenous oxidants and inflammatory cytokines (10,12–17). However, it is difficult to extrapolate from these relatively simple models to the complex process of ROS generation in a transplant setting, and in vivo results have been mixed (18,19).

Overexpression of a combination of antioxidant enzymes in islets may provide the most effective protection from ROS-mediated injury (20). SOD is required to deal with superoxide radicals, but Gpx-1 or catalase may also
be necessary to eliminate the resulting H₂O₂, which damages mitochondria in islets and blunts insulin secretion (21). The ultimate failure of manganese SOD–expressing islet grafts in a NOD scid mouse model may have been due to low expression of Gpx-1 and catalase (18). Furthermore, it is important to consider the cellular location of increased antioxidant activity because of the likely diversity of sources of ROS during islet transplantation. Here, we report the first demonstration that coexpression of enzymes to catalyze superoxide radicals and H₂O₂ protects islet grafts. We produced transgenic mice overexpressing the intracellular enzymes copper/zinc (Cu/Zn) SOD and Gpx-1 and the extracellular enzyme extracellular SOD. Because extracellular SOD is not tightly bound to the cell surface and can be lost during cellular activation, it was modified to incorporate a glycoprophatidyl inositol (GPI) tail. Gpx-1 was chosen for its broad cellular distribution and pleiotropic actions. Gpx-1 overexpression protects against Fas-mediated apoptosis (23), increases the resistance of cultured β-cells to hypoxia/reoxygenation (24), and neutralizes peroxynitrite (25). Peroxynitrite is thought to be responsible for much of the oxidative damage previously ascribed to other radicals (26,27). We found that triple-transgenic islets showed the broadest resistance to oxidative stress in vitro and when grafted provided significantly improved early control of blood glucose compared with double-transgenic, single-transgenic, and wild-type grafts.

**RESEARCH DESIGN AND METHODS**

**Preparation of transgenic constructs and design of screening primers.** Constructs were prepared by cloning cDNAs for human antioxidant enzymes into a vector containing the mouse H-2Kb promoter (28) (Fig. 1A). The cDNAs for hemagglutinin-tagged Cu/Zn SOD and His-tagged Gpx-1 were as described previously (24) except that the latter lacked its endogenous intron. Construction of a modified extracellular SOD cDNA required several steps. First, the extracellular SOD coding region was amplified from human genomic DNA. The downstream primer incorporated a site for the restriction enzyme BspHI to allow the construction of a hybrid extracellular SOD/CD55 cDNA using a previously described strategy (29). The hybrid cDNA encoded an extracellular SOD protein in which the COOH terminus including the heparin-binding domain was replaced by the GPI anchor of human CD55. Finally, a splice-overlap PCR technique (30) was used to incorporate a FLAG epitope immediately downstream of the signal peptide. The following primers (Fig. 1A, arrowheads) were used for screening: F1, 5'-CTCACCTGTTGCTTCAGCTCTGAGCTGCTF3'; F2, 5'-CTGATCTGCTTGGCGAATTCCTGGAGTGTCTGAGCTGTCF3'; F3, 5'-TTGAATTCGCGGCGTCTAGGCAAGTTAGGTGCTGTCF3'; F4, 5'-CCATTGAGCAGCAGCAGGCTGTCF3'; R1, 5'-TTTAGTGAAGTCGATGCTGCCAGGTCAGCTGTCF3'; R2, 5'-CCATTGAGCAGCAGCAGGCTGTCF3'; R3, 5'-CTGAGGGAAGAAGCTGTCF3'; and R4, 5'-CTACTCGTCCGTTGCTGGAGCTGTCF3'.

**Generation of transgenic mice.** Constructs were microinjected separately into fertilized mouse oocytes as described previously (31). An equimolar mixture at the same total concentration was microinjected to obtain triple-transgenic mice. Transgenic animals were identified by PCR of genomic DNA using the QIAGEN system (Qiagen, Clifton Hill, Australia). The primers used and products obtained were as follows: Cu/Zn SOD: F3/R1, 915 bp; Gpx-1: F2/R3, 534 bp; and extracellular SOD: F2/R4, 298 bp. All procedures involving mice were performed with the approval of the St. Vincent’s Hospital Melbourne Animal Ethics Committee.

**Islet isolation and culture.** Islets were isolated from pancreas by collagenase digestion and density centrifugation (32). Islets were maintained in RPMI-1640 with 20% FCS, 20 mmol/l HEPES, 1%L-glutamine, and 1% penicillin/streptomycin, in 5% CO₂ in air at 37°C unless otherwise indicated. Culture reagents were from Life Technologies (Grand Island, NY), collagenase type V and Histopaque from Sigma (St. Louis, MO), and deoxyribonuclease one from Worthington Biochemical (Lakewood, NJ).

**Detection of transgenic mRNA in islets.** Total RNA was extracted from hand-picked islets using TRIzol (Life Technologies). First-strand cDNA was synthesized from total RNA using SuperScript III (Life Technologies) and oligo-dT. PCR was performed using the primer sets F1/R2 for Cu/Zn SOD, F1/R3 for Gpx-1, and F1/R4 for extracellular SOD. The common forward primer F1 is specific for transcripts from the H-2Kb promoter (Fig. 1A), and the reverse primers were designed to span the intron to distinguish mRNA-derived products (350, 358, and 122 bp, respectively) from genomic DNA-derived products (230 bp larger). As a control, murine GAPDH was amplified with primers 5'-GGTGAAGCTGTCGTTGTAACCT-3' and 5'-TAGTAGACGGCGGGAAGAGC-3'.

**Flow cytometric analysis.** Mouse splenocytes were cultured for 18 h in RPMI-1640 with 200 units/ml mouse interferon-γ (IFN-γ; Chemicon, Temecula, CA), washed, incubated sequentially with anti-FLAG monoclonal antibody M2 (Roche) and phycoerythrin-conjugated sheep anti-mouse IgG1 (Chemicon), and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

**Measurement of antioxidant enzyme activity.** Splenocytes cultured as above or hand-picked islets cultured for 48 h in CMRL medium-1066 with or without mouse IFN-γ were homogenized using a PRO200 homogenizer (PRO Scientific, Oxford, CT) in 10 volumes of 50 mmol/l phosphate buffer, pH 7.4 (SOD assay), or 100 mmol/l phosphate buffer, 5 mmol/l EDTA, and 1 mmol/l β-mercaptoethanol (Gpx-1 assay). The homogenate was incubated with phosphatidylinositol-specific phospholipase C (BIOMOL, Plymouth Meeting, PA) at 2.5 units/ml for 10 min at 30°C to release GPI-anchored extracellular SOD. All homogenates were then centrifuged and assayed for protein content using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA). Total SOD activity in islet lysates was measured using the SOD assay kit WST (Doxing Laboratories, Kumamoto, Japan). Gpx activity in splenocyte lysates was measured according to Paglia and Valentine (33).

**Glucose-stimulated insulin secretion assay.** Overnight-cultured islets were washed three times with Krebs-Ringer bicarbonate buffer containing 5.5 mmol/l glucose and 0.1% BSA. Twenty islets were counted into glass tubes containing 200 µl of the same medium and preincubated for 30 min at 37°C with shaking. The medium was replaced with 200 µl of the same medium and incubated for 30 min. The medium was removed and replaced by 200 µl Krebs-Ringer bicarbonate buffer containing 5.5 mmol/l glucose and incubated for a further 30 min. Insulin content in the medium after the 30-min incubations in 5.5 and 22 mmol/l glucose was measured using a rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO). In each experiment, at least 4 groups of 20 islets isolated from two mice were assayed.

**Exposure of islets to oxidative stress in vitro.** One-hundred overnight-cultured islets in CMRL medium-1066 were exposed to 10 mmol/l xanthine (Sigma, St. Louis, MO) and 100 mmol/l hypoxanthine (Sigma) for 1 h at 37°C. Treated and control untreated islets were then cultured for 24 h in complete medium (CMRL-1066 containing 10% FCS and 1% penicillin/streptomycin) before determining viability. In addition, islets prepared as above were subjected to 18 h of hypoxia in CMRL medium-1066 in a Perspex hypoxia chamber flushed with 95% nitrogen/5% CO₂ followed by 48 h of normoxic incubation in culture medium-1066.

**In vitro viability assay.** Islet cell viability was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Islets were incubated with 0.5 mmol/l MTT in culture medium for 2 h at 37°C. Formazan produced by viable cells was dissolved in DMSO in a 96-well microtiter plate in a volume of 100 µl and measured by absorbance at 590 nm. Results were expressed as percent viability compared with parallel untreated samples of the same islets. There was no difference in MTT activity between untreated transgenic and wild-type islets (data not shown).

**Islet transplantation.** Diabetes was induced in recipient CBA/C57BL6 F1 mice by a single intraperitoneal injection of streptozotocin in 0.9% NaCl at 0.3 mg/g body wt. Blood glucose was measured daily by tail vein prick using Accu-Chek Advantage II strips (Roche) and a Redilux II blood glucose monitor (Roche). The mice were considered diabetic if blood glucose was >15 mmol/l for at least 2 consecutive days. Islets were not picked or cultured before transplantation. Islets from each donor group were pooled, quantified by calculating islet equivalents (EQ) (34), and transplanted under the left renal capsule. Blood glucose was measured for 7 days posttransplantation. Grafts were removed by unilateral nephrectomy at day 8, and recipients were monitored for a further 2–3 days to confirm return to hyperglycemia.

**Ferric-xenolone orange assay.** Protein hydroperoxide formed in islet grafts was measured by the ferric-xenolone orange assay (FXOA) (35). Islets were removed from beneath the kidney capsule, washed in 0.9% NaCl, and resuspended in 1 ml of 1:1 (vol/vol) 0.9% NaCl and CHCl₃. The aqueous layer was removed after centrifugation and incubated in 2.5 mmol/l sulfuric acid, 75 mmol/l xenolone orange (Sigma), and 75 mmol/l ferrous ammonium sulfate for...
FIG. 1. A: Constructs used to generate transgenic mice. The cDNAs (below) were cloned separately into the EcoRI site of the vector (top). Arrowheads indicate primer binding sites. Gray shading indicates open reading frames, and black shading indicates the location of epitope tags. Elements are drawn to scale except the H-2Kb promoter. TCR, T-cell receptor. B: Detection of transgene mRNA by RT-PCR of islet total RNA. Primer sets are indicated at the top; the presence of individual transgenes is indicated below (+). Arrows indicate products of the expected size. Each set of reactions is separated by a 100-bp molecular weight ladder. C: Detection of cell surface expression of extracellular (EC) SOD-GPI by flow cytometric analysis. IFN-γ-treated wild-type and extracellular SOD-GPI transgenic splenocytes stained using an antibody to the FLAG epitope tag are indicated by the thin and thick lines, respectively. D: Total SOD and Gpx activity in islet and splenocyte lysates. □, wild type; ■, extracellular SOD-GPI; □, Cu/Zn SOD; □, triple-transgenic line C; gray bars, Gpx-1.
30 min at 25°C. The FOX complex formed was measured by absorbance at 560 nm, and the concentration of protein hydroperoxide was calculated using the extinction coefficient of 48,000 (35). The concentration of protein was measured using the Bio-Rad Protein Assay (Bio-Rad).

**Statistical analysis.** Statistical analyses were performed using SigmaStat statistical software (SPSS, Chicago, IL). Data were calculated as means ± SE. Differences between means of in vitro treatment groups were analyzed by Student’s unpaired t test. Differences in islet graft function between groups in transplantation studies were assessed by Kruskal-Wallis one-way ANOVA on ranks. P < 0.05 was considered significant.

**RESULTS**

**Transgenic mice.** Five extracellular SOD-GPI transgenics were generated from 49 mice (10%), 10 Cu/Zn SOD transgenics from 75 mice (13%), and 7 Gpx-1 transgenics from 65 mice (11%). RT-PCR screening of RNA from peripheral blood leukocytes identified transgene expression in 5 of 5 extracellular SOD-GPI, 7 of 10 Cu/Zn SOD, and 5 of 7 Gpx-1 transgenic mice (data not shown). The highest expressing lines were maintained by breeding with CBA/C57BL6 F1 mice and crossbred to generate double- and triple-transgenic mice. Ten triple-transgenic mice were also generated from 67 born (15%) by coinjection. Of these, three mice expressed all three transgenes (data not shown).

RT-PCR was used to demonstrate transgene expression at the mRNA level in islets (Fig. 1B). Triple-transgenic line C islets showed similar expression of transgene mRNA to islets from the individual transgenic lines. To confirm that extracellular SOD-GPI was expressed on the cell surface, splenocytes were isolated and treated with interferon-γ (which upregulates the H-2Kb promoter used to drive transgene expression), stained using an antibody for the FLAG epitope tag, and analyzed by flow cytometry. Cell surface expression was evident on extracellular SOD-GPI transgenic splenocytes (Fig. 1C). Treatment with phosphatidylinositol-specific phospholipase C caused a reduction in FLAG expression (data not shown), confirming that the protein was attached by a GPI linkage.

Total SOD activity was modestly increased in extracellular SOD-GPI, Cu/Zn SOD, and triple-transgenic islets compared with wild-type islets (Fig. 1D). A more significant increase (2.3-, 3.8-, and 4.6-fold, respectively) was observed after IFN-γ treatment (Fig. 1D). Because it proved impractical to obtain sufficient protein from hand-picked islets to measure Gpx activity, we assayed Gpx activity in splenocytes. In the IFN-γ treatment group, Gpx-1 and triple-transgenic splenocytes showed 2.9- and 2.0-fold higher Gpx activity than wild-type splenocytes (Fig. 1D).

**Overexpression of antioxidant enzymes in islets does not affect glucose-stimulated insulin secretion.** Islet function was assessed in vitro by measuring insulin secretion after incubation of islets in medium containing low (5.5 mmol/l) followed by high (22 mmol/l) glucose. Wild-type islets showed an 11-fold increase in secreted insulin in response to high glucose (Fig. 2). None of the transgenic islets showed a significantly different response to that of wild-type islets, indicating that transgene expression was not detrimental to this aspect of islet function.

**Gpx-1 enhances extracellular SOD-mediated protection of islets from extracellular superoxide radicals.** Treatment with hypoxanthine/xanthine oxidase (HX/XO), which generates constant production of superoxide radicals close to the cell membrane (36), caused a 68% reduction in viability of wild-type islets (Fig. 3). Resistance to HX/XO toxicity was significantly increased by expression of extracellular SOD-GPI and further enhanced by coexpression of Gpx-1, despite the latter having no effect on its own (Fig. 3). Interestingly, Cu/Zn SOD islets were more sensitive than wild-type islets (P < 0.02), although this sensitivity was abolished by coexpression of Gpx-1 (Fig. 3). Maximum viability (88%) was observed in triple-transgenic islets, which were significantly more resistant than extracellular SOD-GPI/Gpx-1 islets (P = 0.005).

**Coexpression of Cu/Zn SOD, Gpx-1, and extracellular SOD-GPI protects islets from hypoxia/reoxygenation injury.** The resistance of islets to hypoxia/reoxygenation injury was tested in vitro by incubation in nutrient-poor medium under hypoxic conditions for 18 h, followed by...
incubation in complete medium under normoxia for 48 h. This caused a 51% reduction in viability of wild-type islets (Fig. 4). Triple-transgenic islets demonstrated a small but significant ($P < 0.01$) improvement in viability compared with wild-type islets, whereas single- and double-transgenic islets showed no protection (Fig. 4).

**Coexpression of Cu/Zn SOD, Gpx-1, and extracellular SOD-GPI improves early function of marginal islet grafts.** Islets from single transgenic, triple-transgenic (line C), and negative littermate controls were transplanted under the renal capsule of streptozotocin-induced diabetic recipients. Line C mice were used rather than the cross-bred triple-transgenic line B because islets from line C and B mice showed equivalent protection from in vitro oxidative challenge (data not shown). To eliminate the possibility of variable rejection responses due to the mixed genetic background (CBA/C57BL6) of the donors, CBA/C57BL6 F1 recipients were used. This allowed the analysis of protection from primary nonfunction in the absence of rejection.

Recipients were transplanted with a “marginal” islet mass of 1,500 IEQ. Preliminary experiments showed that 1,500 IEQ wild-type grafts conferred only partial glycemic control (blood glucose level [BGL], 10–15 mmol/l) in the 1st week posttransplantation. Overexpression of Cu/Zn SOD or Gpx-1 did not improve marginal islet graft function compared with wild type (Fig. 5A). In contrast, grafts from triple-transgenic donors performed significantly better than wild type (Fig. 5B). Surprisingly, recipients of extracellular SOD-GPI marginal grafts fared worse than those of wild-type grafts, with mean BGL consistently higher than 20 mmol/l (Fig. 5B).

Given the apparently detrimental action of extracellular SOD-GPI when expressed in isolation, its effect when combined with Gpx-1 and Cu/Zn SOD expression was determined by performing a second set of transplants...
was no statistically significant difference between these groups. Bn WT (Cu/Zn SOD transgenic) performed better (P < 0.01) than wild-type recipients, expressed as mean BGL 2.4-fold more protein hydroperoxide (11.5 ± 2.6 μmol/mg protein) than triple-transgenic grafts (4.9 ± 0.5 μmol/mg protein). Analysis of lipid peroxidation by the FOX assay is complicated by the reaction to cyclic peroxides (37) and was not performed in this study.

DISCUSSION

ROS have been implicated as important mediators of β-cell dysfunction in islet transplantation. Superoxide radicals and NO, produced in the extracellular compartment by activated recipient and intragraft macrophages, can in turn generate H₂O₂, hydroxyl radical, and peroxynitrite. Each of these molecules can damage the β-cell membrane and (with the exception of superoxide) readily cross into the cytosol and mitochondria to cause further damage to key enzymes, lipids, and DNA. This situation is worsened by intracellular generation of superoxide radicals by xanthine oxidase and NO by inducible NO synthase. Thus, the transplanted β-cell requires protection from a cocktail of ROS within and without. The complexity of ROS metabolism suggests that a multi-antioxidant enzyme strategy may be most effective (12,13,20). In this study, we used transgenic mice to demonstrate that this is the case in a transplant setting.

We first used in vitro assays to show that the level of expression of individual enzymes was functionally significant. Extracellular SOD-GPI protected islets from the action of extracellular superoxide radicals generated by HX/XO, but Cu/Zn SOD and Gpx-1 did not. Coexpression of Gpx-1 with extracellular SOD-GPI further increased resistance, suggesting that although the primary cause of cell death by HX/XO is extracellular injury by superoxide...
radicals, secondary damage can result from entry of its by-products (including H$_2$O$_2$) into the cell. The highest level of protection from HX/XO was observed for triple-transgenic islets. Expression of all three transgenes was necessary to increase the resistance of islets to hypoxia/reoxygenation injury. However, the degree of protection from hypoxia was modest, suggesting that the activity of one or more of the enzymes may be insufficient to fully cope with the volume of ROS generated. It is conceivable that substitution of catalase for Gpx-1 would increase protection in this in vitro model, because although catalase has a lower affinity for H$_2$O$_2$ it has a higher capacity for inactivation (12).

Although the in vitro models provided an indication of additive or synergistic protection, they are unlikely to accurately reflect the complex in vivo transplantation setting. We therefore used a marginal islet graft model to determine whether coexpression was also protective in vivo. Although others have reported that 500 hand-picked mouse islets were sufficient to cure diabetes and grafts of 250–300 islets were suboptimal (38,39), we found that wild-type grafts of 1,500 IEQ were marginal. This apparent discrepancy is probably due to differences in islet graft quantitation rather than the quality of our islet preparations, because in our hands, grafts of 400 hand-picked islets are sufficient to reverse hyperglycemia (data not shown). In the current study, we did not hand-pick islets before transplantation and accordingly measured IEQ, which is the standard procedure for human islet allografts. The marginal grafts of 1,500 IEQ corresponded to approximately one donor per recipient, which is also suboptimal in human islet transplantation.

Triple-transgenic islet grafts, but not Cu/Zn SOD/Gpx-1 or single-transgenic grafts, improved control of blood glucose compared with wild-type grafts. This suggests that extracellular superoxide radicals play a significant role in graft injury even when intracellular antioxidant activity is elevated. However, expression of extracellular SOD-GPI alone significantly worsened graft function, indicating that intracellular ROS also contribute to β-cell loss. Peroxynitrite is an obvious candidate because it can be produced from superoxide radicals and nitric oxide even in the presence of SOD (26). Furthermore, the activity of peroxynitrite is enhanced by a positive feedback loop: it inhibits mitochondrial enzymes and increases generation of H$_2$O$_2$, which in turn prolongs the half-life of peroxynitrite (40). The detrimental effect of extracellular SOD-GPI in isolation may be due to accumulation of H$_2$O$_2$ and its entry into cells, compounded by production of intracellular superoxide radicals and nitric oxide.

The opposite effects of extracellular SOD-GPI in vitro (HX/XO assay) and in vivo highlight the difficulty of accurately modeling the injury associated with islet transplantation. Even the hypoxia model, which may approximate some of the conditions of transplantation, does not allow for the contribution of surrounding recipient tissue and immune cells, and the kinetics of ROS generation may be quite different. We would argue that in vitro assays should be used only to demonstrate functional antioxidant gene expression and that graft function is the only reliable measure of efficacy.

To our knowledge, this is the first study of islets from mice coexpressing three transgenes, without apparent detrimental effects on islet function. Whether this strategy can be used in human islet allotransplantation is not clear. Safe and efficient ex vivo gene delivery into human islets has not yet been demonstrated, and the requirement for expression of multiple genes presents an additional challenge. It may be preferable to express a single enzyme capable of scavenging a range of ROS, such as metallothionein (41). The use of different models precludes comparison of the relative efficacy of metallothionein and the enzymes used here.

The potential value of antioxidant expression may lie in islet xenotransplantation. The use of nonhuman donor animals such as pigs circumvents the problems of ex vivo gene delivery because it allows direct genetic modification of the donor. We have shown that it is possible to coexpress at least three transgenes in pigs, with the genes coinherited as a single unit (28). The use of transgenic donors offers the additional advantage over postisolation gene transfer that protective genes will be expressed in islets during the damaging isolation procedure. Expression of antioxidant enzymes, perhaps in combination with other graft-protective molecules (38,42–44), is thus a promising approach to reducing primary nonfunction and rejection of islet allografts and xenografts alike.

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