Elimination of Negative Feedback Control Mechanisms Along the Insulin Signaling Pathway Improves β-Cell Function Under Stress

Diana Gurevitch,[1] Sigalit Boura-Halfon,[1] Roi Isaac,[1] Galit Shahaf,[2] Moti Alberstein,[1] Denise Ronen,[1] Eli C. Lewis,[2] and Yehiel Zick[1]

OBJECTIVE—Cellular stress and proinflammatory cytokines induce phosphorylation of insulin receptor substrate (IRS) proteins at Ser sites that inhibit insulin and IGF-1 signaling. Here, we examined the role of Ser phosphorylation of IRS-2 in mediating the inhibitory effects of proinflammatory cytokines and cellular stress on β-cell function.

RESEARCH DESIGN AND METHODS—Five potential inhibitory Ser sites located proximally to the P-Tyr binding domain of IRS-2 were mutated to Ala. These IRS-2 mutants, denoted IRS-2^{5A}, and their wild-type controls (IRS-2^{WT}) were introduced into adenoviral constructs that were infected into Min6 cells or into cultured murine islets.

RESULTS—When expressed in cultured mouse islets, IRS-2^{5A} was better than IRS-2^{WT} in protecting β-cells from apoptosis induced by a combination of IL-1β, IFN-γ, TNF-α, and Fas ligand. Cytokine-treated islets expressing IRS2^{5A} secreted significantly more insulin in response to glucose than did islets expressing IRS-2^{WT}. This could be attributed to the higher transcription of *Pdx1* in cytokine-treated islets that expressed IRS-2^{5A}. Accordingly, transplantation of 200 islets expressing IRS2^{5A} into STZ-induced diabetic mice restored their ability to respond to a glucose load similar to naïve mice. In contrast, mice transplanted with islets expressing IRS2^{WT} maintained sustained hyperglycemia 3 days after transplantation.

CONCLUSIONS—Elimination of a physiological negative feedback control mechanism along the insulin-signaling pathway that involves Ser/Thr phosphorylation of IRS-2 affords protection against the adverse effects of proinflammatory cytokines and improves β-cell function under stress. Genetic approaches that promote IRS2^{5A} expression in pancreatic β-cells, therefore, could be considered a rational treatment against β-cell failure after islet transplantation. ***Diabetes*** **59:2188–2197, 2010**

Islet transplantation is the only treatment of type-1 diabetes that achieves insulin-independence (1). Still, islet allografts lose function over time with an increasing proportion of subjects returning to insulin dependence after each year of transplantation (1). This outcome is mainly attributed to inflammatory reactions capable of inflicting severe β-cell damage and impaired β-cell function through the release of cytokines and free radicals (2). IGF-1, a mediator of cell growth and differentiation (3), has been implicated in the regulation of β-cell function (4–6). It stimulates angiogenesis and promotes re-epithelialization of transplants (7), prevents cytokine-mediated β-cell death (8), and increases insulin secretion (9). Conversely, β-cell–specific deletion of the IGF-1 receptors leads to hyperinsulinemia, glucose intolerance (10), and defective insulin secretion (11). These activities can be attributed to the antiapoptotic functions of IGF-1 (3,12).

IGF-1 action is mediated by the IGF-1 receptor (IGF-1R) and its homologue, the insulin receptor (IR), that function as receptor Tyr-kinases. Key substrates for these receptors are the insulin receptor substrate (IRS) proteins, IRS-1 and IRS-2, which integrate many of the pleiotropic effects of insulin and IGF-1 on cellular functions. IRS proteins, mainly IRS-2, play a critical role in β-cells (13). Decreased IRS-2 expression causes β-cell apoptosis (13,14), and mice lacking IRS-2 develop diabetes 8–10 weeks after birth due to reduced β-cell mass and impaired β-cell function (13). Conversely, increased IRS-2 expression promotes β-cell survival (15) and prevents diabetes in $Irs2^{-/-}$ mice (16).

Both IRS-1 and -2 have a pleckstrin homology domain flanked by a P-Tyr binding (PTB) domain that mediates the interactions of IRS proteins with the juxtamembrane domains of insulin receptor and IGF-1R (17,18). IRS proteins undergo phosphorylation on multiple Tyr residues at their COOH-terminal region, which serves as a docking site for SH2-containing proteins that further propagate the insulin and IGF-I signals (19).

IRS proteins contain >70 potential Ser/Thr phosphorylation sites for kinases such as PKA, PKC, Akt, S6K, JNK, IKKβ, MAPK, and AMPK {reviewed in refs (20,21)}. Insulin-induced Ser/Thr phosphorylation of IRS proteins dissociates them from the IR, prevents their Tyr phosphorylation, and inhibits their interactions with downstream effectors (22). This serves as part of a physiological negative feedback control mechanism, used by insulin and IGF-1 to turn off their own signaling cascades. However, proinflammatory cytokines and other inducers of insulin/IGF-1 resistance take advantage of this mechanism. By activating a number of IRS kinases, they uncouple the IRS proteins from insulin receptor or IGF-1R and inhibit their biological activities (23). Accordingly, mutation of selected inhibitory Ser sites of IRS-1, located in close proximity to its PTB domain, renders this mutant less prone to the action of IRS kinases. As a result, the mutated IRS-1 can better propagate insulin and IGF-1 actions (24,25).

In the present study, we sought to determine whether an IRS-2 protein, mutated at five potential inhibitory Ser sites

From the [1]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel; and the [2]Department of Clinical Biochemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
Corresponding author: Yehiel Zick, yehiel.zick@weizmann.ac.il.
Received 16 June 2009 and accepted 3 June 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 14 June 2010. DOI: 10.2337/db09-0890.
D.G. and S.B.-H. contributed equally to this study.
© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

(IRS-2^{5A}), improves β-cell survival and function. Our results indicate that IRS-2^{5A} confers upon β-cells protection from the adverse effects of proinflammatory cytokines and other stress responses both in culture and in vivo. Thus, elimination of negative feedback control mechanisms along the insulin/IGF-1 signaling pathway improves β-cell function under stress. This suggests that IRS-2^{5A} expression could be a new modality for treatment of β-cells before transplantation.

RESEARCH DESIGN AND METHODS

Supplementary material is also available in the online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0890/DC1.

Mice. Male C57BL/6J (age 9–10 weeks) mice were housed under standard light/dark conditions and were given access to food and water ad libitum. Experiments were approved by the Animal Care and Use Committee of the Weizmann institute of Science.

Cell culture. Chinese hamster ovary (CHO) cells that overexpress the insulin receptor (CHO-T cells) were grown in F-12 medium. Min6 insulin-secreting cells (26) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mmol/l glutamine. Both cell lines were supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Cytokines. Cytokine mixture (1X Cytomix) consisted of 3 nmol/l TNF-α, 3 nmol/l INF-γ, 1.5 nmol/l IL-1β, and FasL (1.25% v/v). Their biological activities were 10 units/ng (TNF-α, INF-γ) and 200 units/ng (IL-1β).

Isolation of murine islets. Murine islets were isolated as described (27). In brief, digested pancreata were filtered through 1,000-μm and 500-μm sieves, and islets >75 and <250 μm were handpicked under a stereoscope. Islets were cultured in suspension in RPMI 1,640 medium, 5 mmol/l glucose, 10% fetal calf serum (FCS), 50 units/ml penicillin, 50 μg/ml streptomycin, and 40 μg/ml Gentamicin. For Western blot analysis, islets (50–60 islets per treatment) were cultured in 35 mm Extracellular Matrix Protein (ECM) coated plates (Novamed, Jerusalem).

Construction of Myc-tagged IRS-2^{WT} and IRS-2^{5A} and transfection of CHO-T cells. Myc-IRS-2^{WT} and Myc-IRS-2^{5A} were generated as we described (24). Site-directed mutagenesis of S303, 343, 362, 381, and S480 of IRS-2 into Ala was performed using a Quick-Change site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. To generate stable clones, CHO-T cells were transfected with the above constructs, together with pBabe-Puro, encoding puromycin resistance. After 24 h, the transfected cells were subjected to selection with 10 μg/ml puromycin.

Generation of adenoviruses. Adenoviral constructs were generated as we described (24) according to the protocol provided by AdEasy vector system (Quantum). Briefly, cDNA encoding Myc-IRS-2 (WT or 5A) was ligated into the shuttle plasmid pAdTrack-CMV, which contains a green fluorescent protein (GFP) cassette driven by an independent promoter that serves as a tracing marker. The recombinant pAdTrack-CMV was cotransformed with pAdEasy-1 containing the adenovirus genome, into *Escherichia coli* strain BJ5183, where homologous recombination took place. Positive colonies were identified by restriction analysis. The recombinant pAdEasy-1-CMV-IRS-2 plasmids (WT or 5A) were transfected into HEK293 cells and viruses were amplified. Viruses were stored at −80°C at a viral titer of ~10^{10} PFU/ml.

Infection with adenoviral constructs. Murine islets were infected 24 h after isolation with adenoviral constructs (MOI 600) for the indicated times. Min6 cells were infected at MOI of 200 for 1.5 h in serum-free medium. Treatments were applied up to 72 h after infection.

Western blot analysis. CHO-T cells or murine islets were washed and harvested in buffer A (25 mmol/l Tris-HCl [pH 7.4], 10 mmol/l sodium orthovanadate, 10 mmol/l sodium pyrophosphate, 100 mmol/l sodium fluoride, 10 mmol/l EDTA, 10 mmol/l EGTA, and 1 mmol/l phenylmethylsulfonyl fluoride). Supernatants (12,000 g) of cell extracts (50–150 μg CHO-T cells; 15–30 μg murine islets) were resolved by SDS-PAGE and Western blotted with the indicated antibodies.

Islets immunohistochemistry. Approximately 100 islets embedded in 1% agarose gel were fixed for 16 h in 4% paraformaldehyde and were then transferred to PBS until being embedded in paraffin. Graft-bearing kidneys were formalin-fixed, and serial sections (5 μm each) were immunostained with the indicated antibodies as described (28).

Caspase activity. Apoptosis of Min6 cells (25,000 cells per well) and mouse islets (10 islets per well) was determined by Caspase-3/7 activity kit (Enzolyte-Caspase-3–RH110, AnaSpec Ltd.) according to the manufacturer instructions using fluorescent microplate reader Ex/Em = 496 nm/520 nm.

Glucose-stimulated insulin secretion. Islets were isolated and infected with adenoviral constructs as indicated. Groups of five islets were incubated for 1 h in Krebs-Ringer bicarbonate HEPES buffer (KRBH) at 37°C with 2.5 mmol/l glucose followed by incubation for 1 h in KRBH with 2.5 mmol/l or 20 mmol/l glucose. Insulin concentration in the culture mediums was determined using Mercodia mouse insulin ELISA kit.

Islet transplantation. Mice were made diabetic by an intraperitoneal injection of streptozotocin (STZ; 175 mg/kg) and were transplanted 5 days later when their fasting blood glucose levels were >400 mg/dl. Islets used for transplantation were isolated from naive mice. Islets either remained uninfected or were infected with adenoviral constructs expressing IRS2^{WT} or IRS2^{5A} (and GFP). Twenty-four h thereafter, 200 islets were washed, mounted on a 0.2-ml tip, and released into the renal subcapsular space of the kidneys of recipient mice through a puncture in the capsule, which was immediately sealed with 1-mm^3 absorbable gelatin sponge (Surgifoam, Ethicon; Somerville, NJ).

Glucose tolerance test. Mice were fasted overnight with water access ad libitum. Mice were then injected intraperitoneally with glucose (1 mg/g body wt). Glucose levels were monitored using MediSense Optium Blood Glucose test strips (Abbott Laboratories, IL) on blood drawn at timed intervals from a tail vein.

Quantitative real-time PCR. Islet RNA was extracted using the PerfectPure RNA kit (5Prime, MD), and first-strand cDNA was generated by cDNA Reverse Transcription kit (Applied Biosystems, CA). Quantitative detection of specific mRNA transcripts was carried out by real-time PCR using ABI-PRISM 7900HT instrument (Applied Biosystems, CA). Data were normalized for the content of actin mRNA.

Statistical analysis. Data are presented as mean ± SEM. Data were analyzed by Student t test within a three-way ANOVA [factors were cytokine, treatment, and date of experiment (random factor)] at a minimum $P < 0.05$ threshold.

RESULTS

Generation of IRS-2^{5A}. Mutations to Ala of inhibitory Ser sites of IRS-1 that conform to consensus PKC phosphorylation sites **RXXS/T**Ψ (Ψ, mostly hydrophobic), located proximal to the PTB domain of IRS-1, improve insulin signaling in rat hepatoma Fao cells (24,25). To study whether similar mutations improve the function of IRS-2, five potential inhibitory serines (S303, S343, S362, S381, and S480) were mutated to Ala. To determine whether these sites are subjected to phosphorylation, tandem mass spectrometric analysis (MS/MS) was carried out. We found that S303 and S343 are subjected to phosphorylation in response to insulin treatment of Min6 β-cell line (not shown). Phosphorylation of the other three sites (S362, S381, S480) could not be determined due to low yields of peptides containing these residues.

Tyr phosphorylation of IRS-2^{5A} and activation of its downstream effectors. The effects of mutation of inhibitory Ser sites on IRS-2 function were initially studied in CHO cells that coexpress the insulin receptor and either IRS-2^{WT} or IRS-2^{5A}. Both IRS-2^{WT} and IRS-2^{5A} underwent rapid insulin-induced Tyr-phosphorylation (Fig. 1*A*, B), indicating that the 5A mutations did not affect the conformation of IRS-2^{5A}, leaving it as equipotent substrate to the insulin receptor. Still, IRS-2^{5A} better maintained its Tyr phosphorylated active conformation after 60 min of insulin treatment, compared with IRS-2^{WT}. These results suggest that mutation of potential inhibitory Ser sites protects IRS-2^{5A} from the action of insulin-stimulated IRS-2 Ser/Thr-kinases that otherwise induce the dissociation of IRS-2 from insulin receptor (22). Indeed, IRS-2^{5A} maintained stronger coupling with the insulin receptor, as evidenced by the higher amounts of insulin receptor associated with IRS-2^{5A} immunoprecipitates, compared with IRS-2^{WT} (Fig. 1*B*).

The phosphorylation of downstream effectors of IRS-2 was studied as well. We found that, in response to insulin, p85α, the regulatory subunit of PI3K, better couples to IRS-2^{5A} compared with IRS-2^{WT} (Fig. 1*B*).

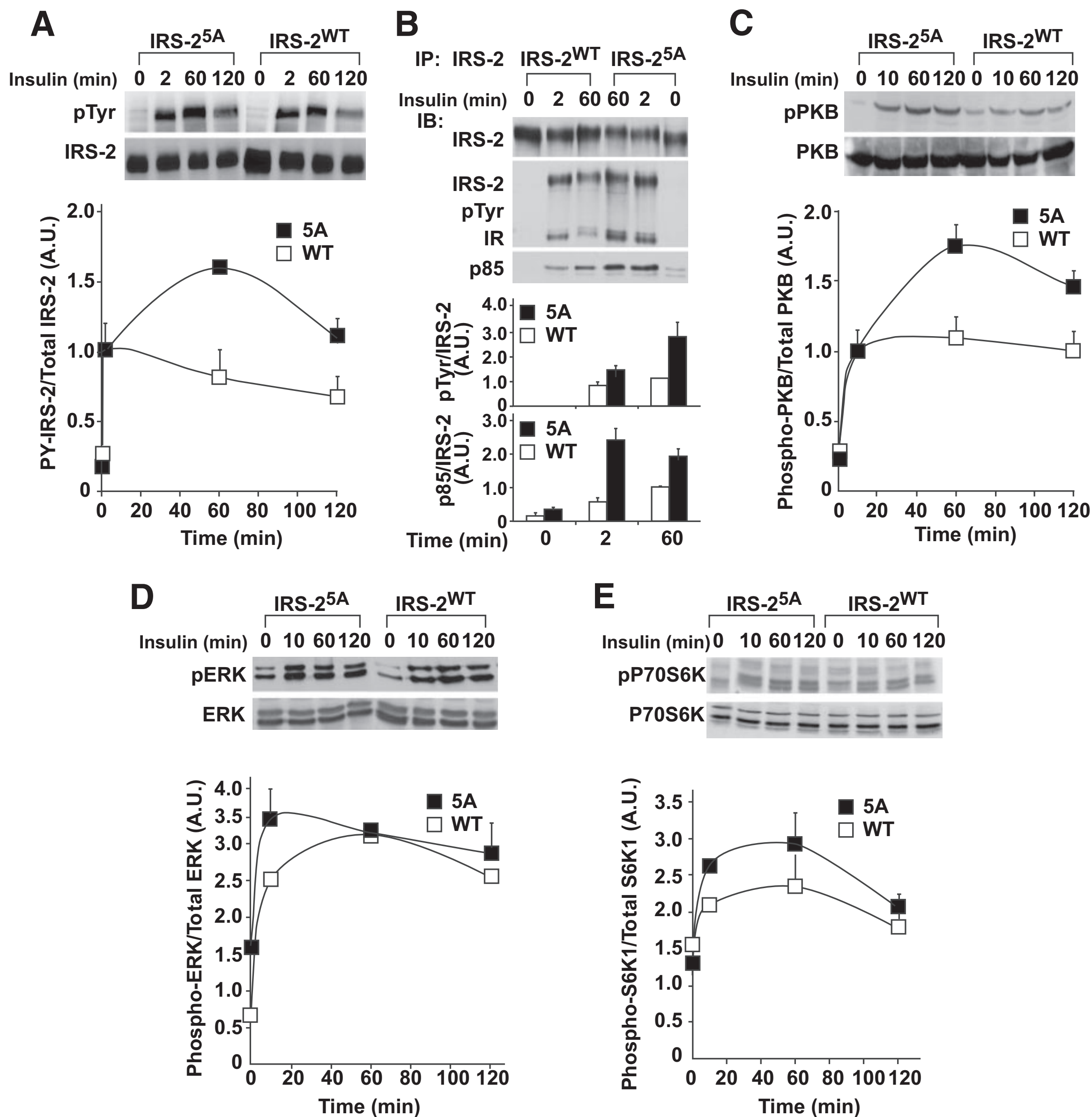

FIG. 1. IRS-2^{5A} is better protected than IRS-2^{WT} from a reduction in its P-Tyr content and can better activate its downstream effectors after insulin treatment. CHO-T cells overexpressing IRS-2^{WT} or IRS-2^{5A} by a stable transfection were deprived of serum for 16 h. The cells were then incubated with 1 nmol/l insulin for the indicated times at 37°C. Total cell extracts (100 μg) (*A*), or protein samples (500 μg) subjected to immunoprecipitation (IP) with IRS-2 antibodies (*B*), were resolved by SDS-PAGE and were immunoblotted (IB) with anti-pTyr or anti-IRS-2 antibodies (*A*) or with anti pTyr anti-IRS-2 or anti-p85α antibodies (*B*). Similarly, total cell extracts (100 μg) were immunoblotted with antibodies to phosphorylated- and total extracellular signal–related kinase (*C*); phosphorylated- and total PKB (*D*); and phosphorylated- and total p70S6K (S6K1) (*E*). Band densities were quantified by densitometry. Results normalized to protein content represent mean ± SEM of three independent experiments. Western blots of one representative experiment are displayed.

Accordingly, protein kinase B (PKB) was activated to a greater extent (Fig. 1*C*), while ERK1/2 and S6K1 were also better activated, though to a lesser extent (Fig. 1*D*,*E*).

Infection of murine islets with adenoviral constructs expressing Myc-IRS-2^{WT} or Myc-IRS-2^{5A}. Next, Min6 cells and murine islets were infected with adenoviral constructs expressing Myc-IRS-2^{WT} or Myc-IRS-2^{5A}. Expression of the IRS-2 proteins in Min6 cells results in ~1.5–2.0-fold increase in the protein level compared with the endogenous IRS-2 protein (Fig. 2*A*). Maximal expression of the IRS-2 proteins occurred 48 h after infection (Fig. 2*B* and *D*); therefore, this time period was selected for all subsequent experiments. Calibration experiments were carried out throughout this study to ensure that IRS-2^{5A} and IRS-2^{WT} are expressed to a similar level in Min6 cells or isolated islets (Figs. 2*C*–*E*).

To assess the yield of infected β-cells, we took advantage of the fact that the adenoviral constructs also express GFP driven by an independent promoter. As shown in Fig. 2*F*, a significant fraction of β-cells, mainly those localized to the periphery of the islets, were stained for GFP. Approximately 80% of the total cells within representative islets sections were insulin-containing β-cells (Fig. 2*G*). Of these, ~50% were adenovirus-infected cells (GFP positive). The relatively high infection yield enabled us to detect the effects of the IRS-2 proteins at the level of whole islets. Still, the effects of both IRS-2^{WT} and IRS-2^{5A} were underestimated because not all β-cells incorporated these constructs.

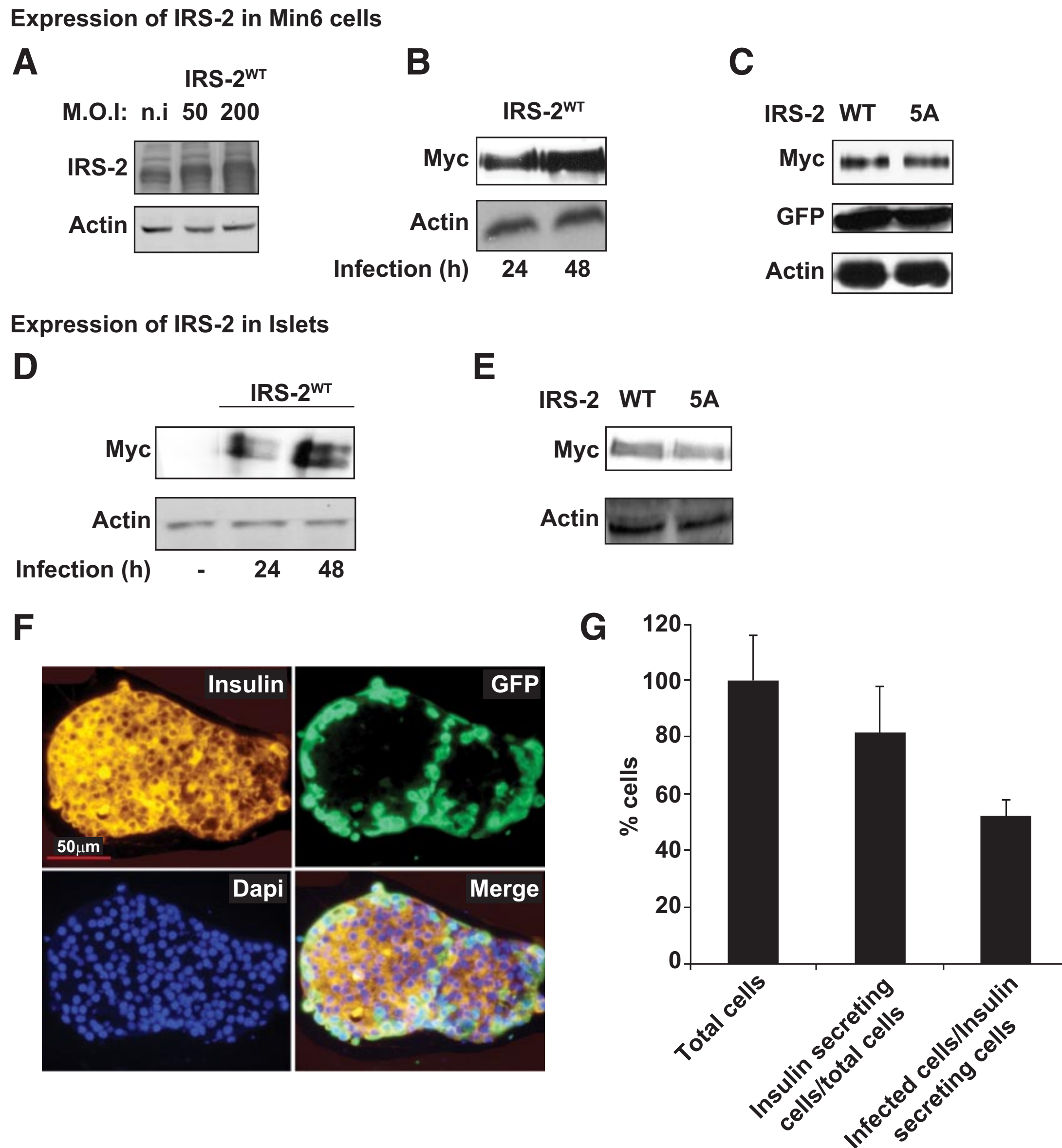

FIG. 2. Infection of Min6 cells and pancreatic islets with adenoviral constructs coexpressing GFP and Myc-IRS-2. *A*: Min-6 cells were infected with Adv-IRS-2^{WT} at MOI of 50 or 200 or were left uninfected. Forty-eight h after infection, cell extracts (40 μg) were resolved by SDS-PAGE and were immunoblotted with anti-IRS-2 antibodies. Min6 cells (*B* and *C*), or murine islets (40 islets/group) (*D* and *E*), were infected with adenoviral constructs expressing Myc-IRS-2^{WT} or Myc-IRS-2^{5A} at MOI of 200 or 600, respectively. At the indicated times after infection, Min6 cells and isolated islets were collected; samples (15–30 μg) were resolved by means of 7.5% SDS-PAGE and were subjected to Western immunoblotting using anti-Myc, anti-GFP, or anti-actin antibodies (*B–E*). For immunostaining (*F*), islets were infected with adenoviral constructs expressing GFP and Myc-IRS-2^{WT} driven by two independent promoters. Forty-eight h after infection, the islets were collected and fixed and serial sections (5 μm each) were immunostained for insulin using guinea-pig anti-insulin as a first antibody and Rhodamine-conjugated Rabbit-anti-guinea-pig as a secondary antibody. The yield of infection of β-cells was assessed by their GFP fluorescence. Nuclear staining with di aminido phenyl indol served to calculate the total number of cells in each islet. Quantization of the percent of infected islets (mean ± SD) represents counting of ten islets (*G*). The Western blots shown in *A–E* are representatives of three similar experiments. (A high-quality color representation of this figure is available in the online issue.)

Effects of IRS-2^{5A} on cytokine-induced apoptosis. To study the ability of IRS-2^{5A} to confer resistance from apoptosis induced by proinflammatory cytokines (29,30), Min6 cells were incubated for 24 h with a "Cytomix (1X)" composed of 3 nmol/l TNFα, 3 nmol/l INF-γ, 1.5 nmol/l IL-1β, and FasL (1.25% v/v). This treatment induced activation of Caspase-3 (Fig. 3*A*). Introduction of either IRS-2^{WT} or IRS-2^{5A} into Min6 cells reduced apoptosis to a similar extent (~30–40%). However, when the experiments were carried out in cultured murine islets, the differences between IRS-2^{WT} and IRS-2^{5A} became apparent (Fig. 3*B*). Whereas IRS-2^{WT} failed to exert any protective effects, IRS-2^{5A} reduced ~40% cytokine-induced apoptosis, compared with islets infected with an empty vector control. Somewhat lower protection (25%) was obtained in islets treated with cytokines without FasL (Fig. 3*C*). Total Caspase activity in cytokine-treated noninfected (control) islets was ~2-fold higher in the presence of FasL (Fig. 3*B* versus 3*C*). This indicates that FasL is an important constituent in the induction of apoptosis. Also, cytokine-induced FasL secretion cannot be ruled out. Of note, infection per se of murine islets with adenoviral constructs might impede islet function to a certain extent (31). Therefore, Caspase-3 activity is higher in islets infected

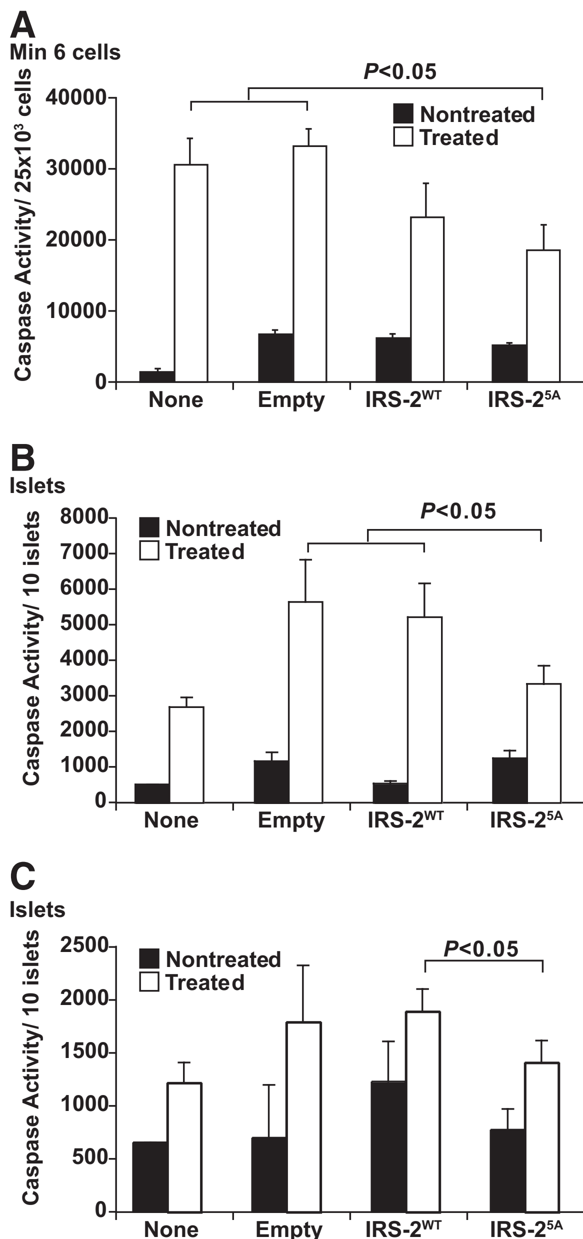

FIG. 3. Overexpression of IRS-2^{5A} protects Min6 cells and cultured murine islets from cytokine-induced apoptosis. *A*: Min6 cells, seeded in 96-well plates, were infected with Adv-IRS-2^{WT}, Adv-IRS-2^{5A}, or Adv-GFP at MOI of 200, or were left untreated. The cells were then stimulated with "1X Cytomix" for 16 h at 37°C. Apoptosis was assayed using Caspase-3 activity. Results are mean ± SEM of five independent experiments. *B*: Groups of 40 islets were left untreated or were infected with Adv-IRS-2^{WT}, Adv-IRS-2^{5A}, Adv-GFP at MOI of 600, for 48 h. Thirty-two h after infection, "10X Cytomix" was added to the islets for 16 h. Islets were then divided into groups of 10 per group, and cell death was assayed using Caspase-3 activity. Results are mean ± SEM of four independent experiments. *C*: Groups of 40 islets were left untreated or were infected with Adv-IRS-2^{WT}, Adv-IRS-2^{5A}, or Adv-GFP at MOI of 600, for 48 h. Thirty-two h after infection, "10X Cytomix" without FasL was added to the islets for 16 h. Islets were then divided into groups of 10 per group, and cell death was assayed using Caspase-3 activity. Results are means ± SEM of at least four experiments. $P <$ 0.05 compared with cells/islets overexpressing IRS-2^{5A}.

with an empty virus, compared with noninfected cells (Figs. 3*B* and *C*). Still, in the absence of cytokines, islets infected with IRS-2^{5A} do not differ significantly from islets infected with an empty vector. Hence, IRS-2^{5A} does not provide protection from the adverse effects of infection.

Effects of IRS-2^{5A} on insulin secretion. The effects of IRS-2^{WT} versus IRS-2^{5A} on glucose-stimulated insulin secretion (GSIS) in isolated islets were evaluated next. Consistent with previous findings (32), overexpression of IRS-2^{WT} increased GSIS 1.5-fold in cultured islets, when compared with islets infected with an empty virus (Fig. 4). The effects of IRS-2^{5A} were ~2.8-fold greater than those of IRS-2^{WT}. The stronger effects of IRS-2^{5A} were restricted to GSIS, as IRS-2^{5A} was equipotent to IRS-2^{WT} in elevating by ~50% the glucose-independent insulin secretion, induced by a combination of L-arginine and KCl (Fig. 5). The decrease in GSIS in islets infected with empty adenoviral constructs (compared with noninfected cells) could be attributed to the adverse effects of infection per se on islet function (31).

As previously shown (33) and confirmed in Fig. 4, incubation of murine islets with a combination of cytokines inhibited (~55%) islets function assessed by GSIS (*None*). Expression of IRS-2^{5A} in cytokine-treated islets improved GSIS ~4-fold, (compared with empty vector, Fig. 4), while introduction of IRS-2^{WT} improved GSIS only 2.6-fold. In fact, GSIS in cytokine-treated islets expressing IRS-2^{5A} was 1.6-fold higher than GSIS in naïve islets. The effects of IRS-2^{5A} could not be attributed to alterations in cellular insulin content (not shown). These results support our hypothesis that IRS-2^{5A} is less prone to Ser phosphorylation by cytokine-activated IRS kinases than is IRS-2^{WT} and, as a result, can better protect pancreatic islets from their adverse effects.

Effects of IRS-2^{5A} on *Pdx1* gene transcription. IRS proteins are upstream activators of Akt that promote activation of Pdx1, a transcription factor that mediates GSIS (34,35). Therefore, we examined whether IRS-2^{5A} stimulates GSIS better than IRS-2^{WT} because of its effects on Pdx1. As shown in Fig. 6*A*, expression of IRS-2^{WT} or IRS-2^{5A} in naïve islets increased *Pdx1* expression 2- and 3.8-fold, respectively. These effects were specific as the IRS proteins did not affect actin gene transcription (not shown). Preincubation of murine islets with cytokines inhibited *Pdx1* (but not actin) transcription in a time-dependent manner, reaching 80% inhibition by 24 h (Fig. 6*B*). Still, IRS-2^{5A}, but not IRS-2^{WT}, maintained its potency to promote ~2-fold *Pdx1* transcription even in cytokine-treated islets (Fig. 6*C*). These results suggest that IRS-2^{5A} could mediate its beneficial effects through promoting the expression of Pdx1.

Effects of IRS-2^{5A} on transplanted pancreatic islets. To determine whether ex vivo introduction of IRS-2^{5A} into pancreatic islets prior to their transplantation improves the functionality of the transplanted β-cells, C57BL/6J mice were rendered diabetic by a single high-dose injection of STZ. Mice were transplanted five days later when their fasting blood glucose levels exceeded 400 mg/dl. Islets used for transplantation were isolated from healthy syngeneic mice and were infected with adenoviruses harboring either IRS2^{WT}, IRS2^{5A}, or empty GFP control. As shown in Fig. 7*A*, the transplanted islets remained as a packed mass under the kidney capsule for 20 days after transplantation. Most transplanted cells retained their insulin content (Fig. 7*A*, *middle*) and expressed the adenoviral constructs, revealed by their GFP staining (Fig. 7*A*, *left*). As expected, the grafted islets exhibited an overlap between GFP expression and insulin-positive cells (Fig. 7*A*, *right*).

Fasting blood glucose levels. The effects of islet transplantation were assessed by measuring the fasting blood

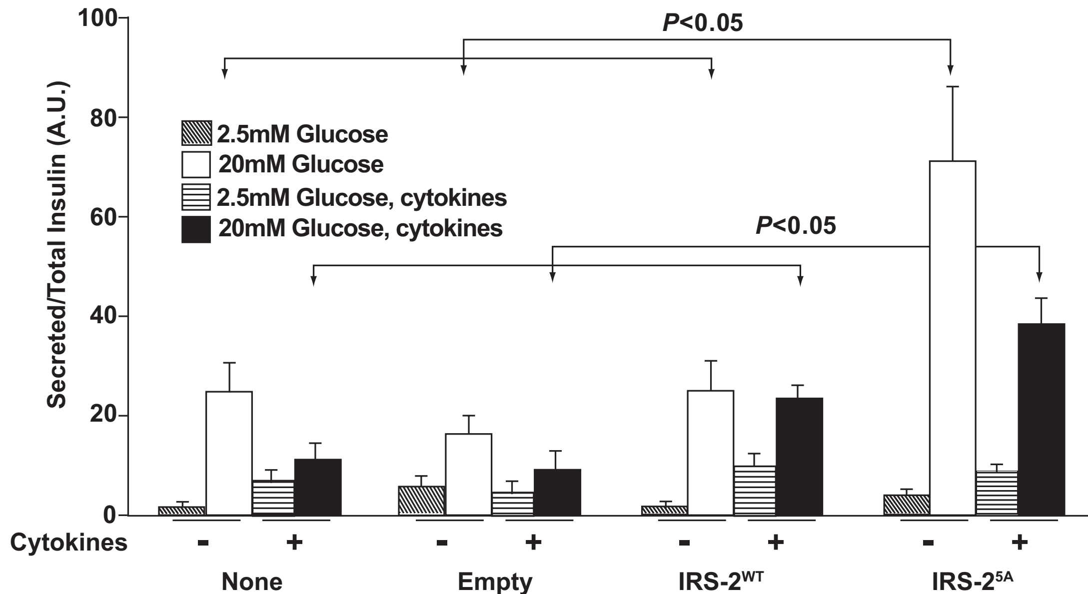

FIG. 4. Effects of IRS-2^{WT} and IRS-2^{5A} on insulin secretion from murine islets. Isolated murine islets remained uninfected (*None*) or were infected with adenoviral constructs expressing Myc-IRS-2^{WT}, Myc-IRS-2^{5A}, or GFP only (empty virus, control) at MOI of 600. Forty-eight h after infections, islets (10 per group) were treated for 18 h in the absence or presence of "0.2X Cytomix." At the end of incubation, GSIS was performed as described under RESEARCH DESIGN AND METHODS. Data represent secreted insulin per total insulin in each sample. Data are means ± SEM of at least three experiments carried out in triplicate. $P < 0.05$ compared with islets overexpressing IRS-2^{5A}.

glucose levels of the recipient diabetic mice. Blood glucose levels of healthy naïve mice were lower than 120 mg/dl (Fig. 7*B*), while those of the diabetic mice exceeded 400 mg/dl. Diabetic mice transplanted with a subtherapeutic mass of 200 islets expressing an empty/control adenoviral construct exhibited high fasting blood glucose levels, three days after transplantation, while mice transplanted with 200 islets expressing either IRS-2^{WT} or IRS-2^{5A} showed normal fasting blood glucose levels. These results suggest that islets overexpressing either IRS-2^{WT} or IRS-2^{5A} are equipotent in restoring fasting normoglycemia in STZ-treated diabetic mice.

Glucose tolerance tests. The ability of the transplanted islets to restore normoglycemia in diabetic mice after glucose injection was next evaluated. As shown in Fig. 7*C*, STZ-treated diabetic mice, transplanted with suboptimal doses of naïve islets or islets infected with GFP-control vector, exhibited a diabetic pattern of glucose tolerance (>400 mg/dl). Mice transplanted with islets infected with IRS-2^{WT} remained significantly hyperglycemic (~350 mg/dl) even 90 min after glucose injection. In contrast, mice transplanted with islets infected with IRS-2^{5A} exhibited near-normal glucose clearance rate, with blood glucose levels reaching normal level at 90 min. To evaluate the beneficial effects of IRS-2^{5A} on a longer time scale, STZ-induced diabetic mice were transplanted with a higher number (300) of islets expressing the different IRS-2 constructs, or an empty control virus. As shown in Fig. 8, islets expressing IRS-2^{5A} performed much better during the first 2–4 days posttransplantation as measured by postprandial blood glucose levels than islets expressing IRS-2^{WT} or an empty virus (control). By 8–90 days, all transplanted islets were equipotent in restoring postprandial normoglycemia. Hence, the beneficial effects of IRS-2^{5A} were most pronounced at the time when the transplanted islets faced the least vasculature. These results suggest that IRS-2 plays an important role in insulin secretion and islet function at the early stages immediately after transplantation.

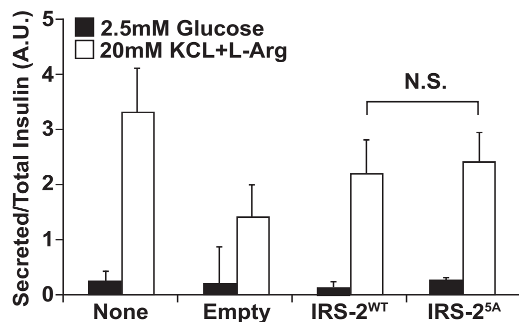

FIG. 5. Effects of IRS-2^{WT} and IRS-2^{5A} on KCl–arginine induced insulin secretion from murine islets. Isolated murine islets remained uninfected (*None*) or were infected with adenoviral constructs expressing Myc-IRS-2^{WT}, Myc-IRS-2^{5A}, or GFP only (empty virus, control) at MOI of 600. Forty-eight h after infections, the islets were divided into samples containing 10 islets per group, and insulin secretion was determined in the presence of 2.5 mmol/l glucose or a mixture containing 2.5 mmol/l glucose, 20 mmol/l L-arginine, and 20 mmol/l KCl. Data shown are means ± SEM of two experiments, each done in triplicate. Data represent secreted insulin per total insulin in each sample. $P < 0.05$ compared with islets overexpressing IRS-2^{5A}.

DISCUSSION

In the present study, we provide evidence that elimination of negative feedback-control mechanisms along the insulin signaling pathway improves β-cells function under stress. Such elimination is based upon introduction into β-cells of IRS-2 proteins harboring mutations at five potential inhibitory Ser sites, the phosphorylation of which negatively regulates IRS protein function. The mutated IRS-2 proteins (IRS-2^{5A}) are less prone than native IRS-2 proteins to phosphorylation by IRS kinases activated by proinflammatory cytokines and other stress-inducers and can, therefore, better couple with their upstream activators (the insulin and IGF-1 receptors) and their downstream effectors. As a result, β-cells expressing IRS-2^{5A} can resist more efficiently the action of proinflammatory cytokines. This is

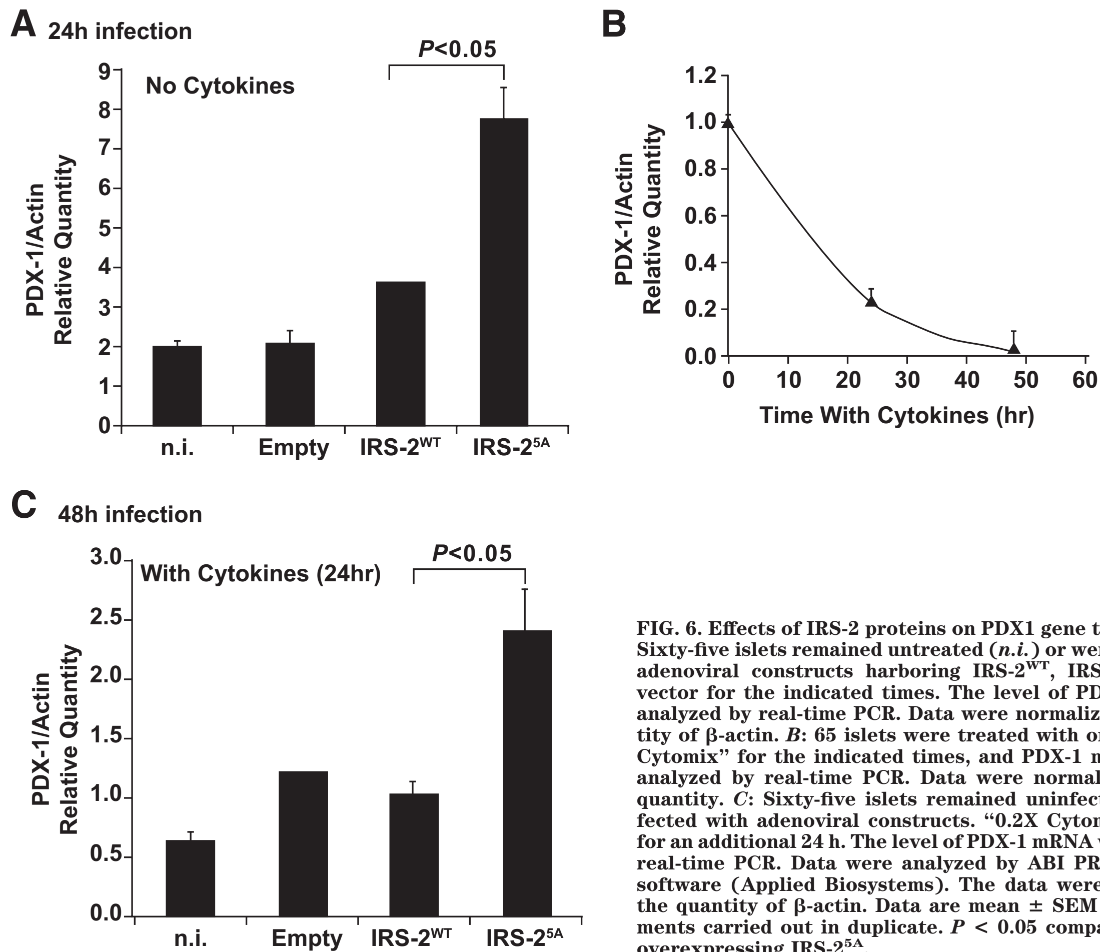

FIG. 6. Effects of IRS-2 proteins on PDX1 gene transcription. ***A*****: Sixty-five islets remained untreated (*n.i.*) or were infected with adenoviral constructs harboring IRS-2^{WT}, IRS-2^{5A}, or empty vector for the indicated times. The level of PDX-1 mRNA was analyzed by real-time PCR. Data were normalized to the quantity of β-actin.** ***B*****: 65 islets were treated with or without "0.2X Cytomix" for the indicated times, and PDX-1 mRNA level was analyzed by real-time PCR. Data were normalized to β-actin quantity.** ***C*****: Sixty-five islets remained uninfected or were infected with adenoviral constructs. "0.2X Cytomix" was added for an additional 24 h. The level of PDX-1 mRNA was analyzed by real-time PCR. Data were analyzed by ABI PRISM SDS 7,000 software (Applied Biosystems). The data were normalized to the quantity of β-actin. Data are mean ± SEM of four experiments carried out in duplicate. P < 0.05 compared with islets overexpressing IRS-2^{5A}.**

evident in cultured islets that express IRS-2^{5A}, which secrete more insulin even when challenged with proinflammatory cytokines. Moreover, glucose disposal was better in diabetic mice transplanted with islets expressing IRS-2^{5A} compared with islets expressing IRS-2^{WT} or empty vector.

Several lines of evidence support the above conclusions. First, we showed that at least two of the five Ser sites, S303 and S343, are subjected to in vivo phosphorylation in response to insulin in Min6 cells. Second, when expressed in CHO-T cells, IRS-2^{5A} better maintains its Tyr-phosphorylated active conformation compared with IRS-2^{WT}. This can be attributed to the ability of IRS-2^{5A} to efficiently couple with the insulin and IGF-1 receptors, which otherwise dissociate from the IRS proteins once they are subjected to Ser phosphorylation by IRS kinases activated after prolonged insulin treatment (23). As a consequence, IRS-2^{5A} activates to a greater extent its downstream targets PI3K, Akt, ERK, and S6K1. In this respect, IRS-2^{5A} resembles IRS-1^{7A}, an IRS-1 mutated at seven potential inhibitory Ser sites, which better couples to the insulin receptor and better propagates insulin signaling (24,25).

The superior ability of IRS-2^{5A} to prevent β-cell apoptosis, induced by a combination of cytokines, suggests that the Ser sites mutated in IRS-2^{5A} serve as targets for cytokine-activated IRS kinases. Indeed, IL-1β, TNFα, and IFNγ promote activation of PKCδ (36), IKK (37), JNK (38), p38 MAPK, and ERK (39), which phosphorylate IRS proteins at "inhibitory" Ser sites (21,40). Cytokines are also known inhibitors of glucose-stimulated insulin secretion (41). Indeed, IRS-2^{5A}, unlike IRS-2^{WT}, exerts a significant protection and could almost eliminate the inhibitory effects of cytokines on GSIS.

The capability of IRS-2^{5A} to promote GSIS better than IRS-2^{WT} could be attributed to its ability to promote transcription of *Pdx1*, a transcription factor which is activated once its repressor FOXO1 is inhibited through Akt phosphorylation and nuclear exclusion (42,43). PDX1 promotes the expression of *pro-insulin* (44), *Glut2* (45), and *glucokinase* (46), all contributing to increased insulin secretion. Indeed, we could show the greater potency of IRS-2^{5A} to increase transcription of *Pdx1* in naïve islets and in islets treated with cytokines that inhibit *Pdx1* gene transcription (34). This could be attributed to the ability of IRS-2^{5A} to couple more efficiently with p85α and activate Akt/PKB. These findings are consistent with observations that disruption of *Pdx1* in murine β-cells reduces insulin secretion and causes progressive β-cell loss (34).

The effects of IRS-2^{5A} on GSIS are specific, because it does not fare much better than IRS-2^{WT} when insulin secretion is triggered independent of glucose stimulation (by membrane depolarization induced by a combination of KCl and arginine). These results suggest that IRS-2^{5A} promotes insulin secretion mainly through its effects on PDX1. Indeed, PDX1 does not regulate glucose-independent insulin secretion, and the magnitude of insulin response to arginine stimulation is unchanged in *Pdx-1*$^{+/-}$ mice (46). IRS-2^{5A} did not modify cytokine-

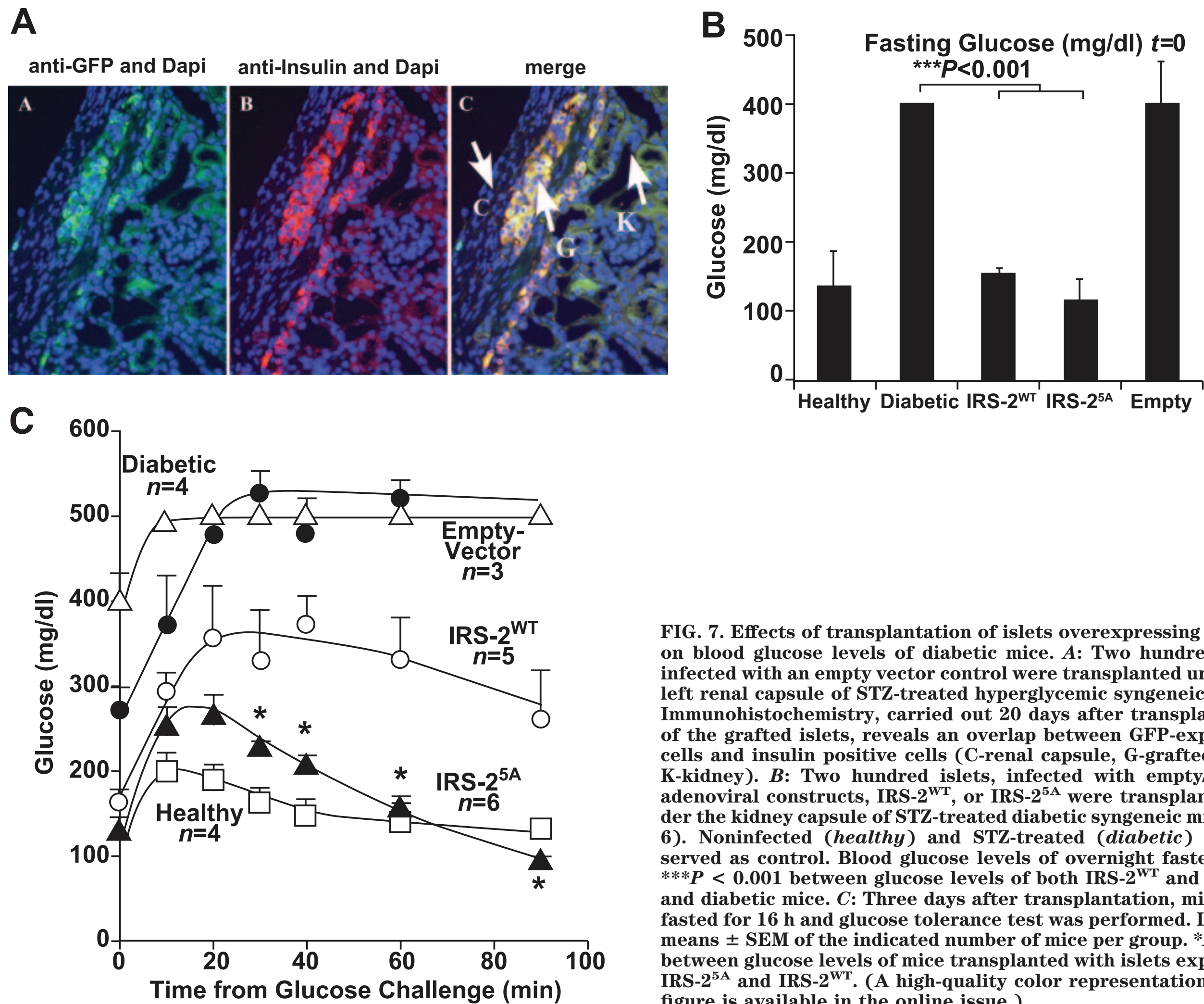

FIG. 7. Effects of transplantation of islets overexpressing IRS-2^{5A} on blood glucose levels of diabetic mice. *A*: Two hundred islets infected with an empty vector control were transplanted under the left renal capsule of STZ-treated hyperglycemic syngeneic mouse. Immunohistochemistry, carried out 20 days after transplantation of the grafted islets, reveals an overlap between GFP-expressing cells and insulin positive cells (C-renal capsule, G-grafted islets, K-kidney). *B*: Two hundred islets, infected with empty/control adenoviral constructs, IRS-2^{WT}, or IRS-2^{5A} were transplanted under the kidney capsule of STZ-treated diabetic syngeneic mice (n = 6). Noninfected (*healthy*) and STZ-treated (*diabetic*) animals served as control. Blood glucose levels of overnight fasted mice. ***P < 0.001 between glucose levels of both IRS-2^{WT} and IRS-2^{5A} and diabetic mice. *C*: Three days after transplantation, mice were fasted for 16 h and glucose tolerance test was performed. Data are means ± SEM of the indicated number of mice per group. *P <0.05 between glucose levels of mice transplanted with islets expressing IRS-2^{5A} and IRS-2^{WT}. (A high-quality color representation of this figure is available in the online issue.)

induced iNOS and Fas mRNA expression (not shown), suggesting that it presumably acts downstream of these death inducers.

The ability of IRS-2^{5A} to improve islet functionality better than IRS-2^{WT} was also demonstrated in vivo. Because overexpression of IRS-2^{WT} improves islets functionality (32), a suboptimal mass of 200 islets was transplanted to enable comparison of the effects of IRS-2^{5A} versus IRS-2^{WT}. Interestingly, even with these suboptimal doses, transplanted islets expressing either IRS-2^{WT} or IRS-2^{5A} were capable of maintaining normal fasting blood glucose levels, suggesting that both IRS-2 proteins improve islets functionality when the challenge to the islets is moderate. The differences between IRS-2^{WT} and IRS-2^{5A} became apparent only when the transplanted islets were faced with a greater challenge in the form of a glucose load. Under these conditions, only mice transplanted with islets expressing IRS-2^{5A} behaved like normal mice three days after transplantation, while mice transplanted with islets expressing IRS-2^{WT} behaved almost like diabetic animals. When diabetic mice were transplanted with a higher number of islets, the beneficial effects of IRS-2^{5A} on postprandial blood glucose levels were most pronounced during the first 2–4 days after transplantation, a critical time when the transplanted islets were poorly vascularized. These results suggest that IRS-2^{5A} can better confer upon transplanted islets protection from the stressful environment they are facing immediately after transplantation.

Factors that negatively influence islet survival after transplantation include prolonged hypoxia during the revascularization process (47). The hypoxic conditions trigger stress-activated kinases to reduce β-cell survival and functionality. Being partially resistant to the action of these kinases, IRS-2^{5A} provides the transplanted islets with two advantages: it increases their intrinsic ability to perform GSIS by promoting the activity of Pdx1 and it protects them from external insults mediated by stress-activated kinases.

Different strategies were attempted to increase islet survival after transplantation [Reviewed in refs (48,49)], so far with limited success. Therefore, IRS-2^{5A} expression in β-cells ex vivo could be considered as a new mode for promoting β-cell survival. This strategy might be even more effective in human islets, where the β-cells localize to the islet periphery and, therefore, are more prone to gene transfer by viral infection. The ability of IRS-2^{5A} to confer upon β-cells protection from immunosuppressive drugs that otherwise induce insulin resistance is another aspect of relevance in this context. Still, the potential caveats of insulin signaling pathway being hyperactivated in cells expressing IRS-2^{5A} need to be addressed because sustained hyperactivation of IRS-2 might contribute to uncontrolled cell growth (50). Consequently, further stud-

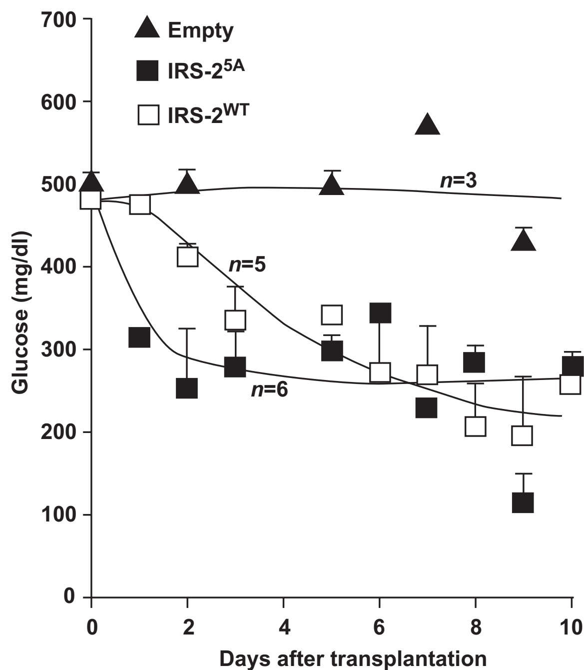

FIG. 8. Long-term effects of islets transplantation on blood glucose levels of diabetic mice. Three hundred islets, infected with empty/control adenoviral constructs, IRS-2^{WT}, or IRS-2^{5A} were transplanted under the kidney capsule of STZ-treated diabetic syngeneic mice. Blood glucose levels were determined at the indicated days after transplantation. Data are means ± SEM of the indicated number of mice per group.

ies are required before this technique becomes suitable for clinical implementation.

ACKNOWLEDGMENTS

This work was supported by research grants from the Juvenile Diabetes Research Foundation International; The Israel Science Foundation (founded by the Israel Academy of Sciences and Humanities); D-CURE Israel; The Mitchel Kaplan Fund; and the MINERVA Foundation. Y.Z. is an incumbent of the Marte R. Gomez Professorial Chair.

No potential conflicts of interest relevant to this article were reported.

D.G., R.I., G.S., M.A., and D.R. researched data. S.B.-H. and E.C.L. researched data and reviewed/edited the manuscript. R.I. researched data. Y.Z. wrote the manuscript.

We thank Michael Walker (Weizmann Institute, Rehovot, Israel) for his guidance in islet isolation; Hila Giladi (Hebrew University, Jerusalem) for her help with adenovirus generation; and Raya Eilam (Weizmann Institute, Rehovot, Israel) for her help with immunohistochemistry staining. We thank Sanford Sampson (Bar-Ilan University, Israel) for a critical review of this manuscript.

REFERENCES

1. Hogan A, Pileggi A, Ricordi C. Transplantation: current developments and future directions; the future of clinical islet transplantation as a cure for diabetes. Front Biosci 2008;13:1192–1205
2. Mathis D, Vence L, Benoist C. β-Cell death during progression to diabetes. Nature 2001;414:792–798
3. Baserga R. The IGF-I receptor in cancer research. Exp Cell Res 1999;253:1–6
4. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF. Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet 1999;23:32–40
5. Kulkarni RN. Receptors for insulin and insulin-like growth factor-1 and insulin receptor substrate-1 mediate pathways that regulate islet function. Biochem Soc Trans 2002;30:317–322
6. Lingohr MK, Dickson LM, McCuaig JF, Hugl SR, Twardzik DR, Rhodes CJ. Activation of IRS-2-mediated signal transduction by IGF-1, but not TGF-α or EGF, augments pancreatic beta-cell proliferation. Diabetes 2002;51:966–976
7. Bitar MS, Labbad ZN. Transforming growth factor-beta and insulin-like growth factor-I in relation to diabetes-induced impairment of wound healing. J Surg Res 1996;61:113–119
8. Hill DJ, Petrik J, Arany E, McDonald TJ, Delovitch TL. Insulin-like growth factors prevent cytokine-mediated cell death in isolated islets of Langerhans from pre-diabetic non-obese diabetic mice. J Endocrinol 1999;161:153–165
9. Sieradzki J, Fleck H, Chatterjee AK, Schatz H. Stimulatory effect of insulin-like growth factor-I on [3H]thymidine incorporation, DNA content and insulin biosynthesis and secretion of isolated pancreatic rat islets. J Endocrinol 1988;117:59–62
10. Kulkarni RN, Holzenberger M, Shih DQ, Ozcan U, Stoffel M, Magnuson MA, Kahn CR. β-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter β-cell mass. Nat Genet 2002;31:111–115
11. Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White MF, Herrera PL, Accili D, Efstratiadis A. Defective insulin secretion in pancreatic beta cells lacking type 1 IGF receptor. J Clin Invest 2002;110:1011–1019
12. Butler AA, Yakar S, Gewolb IH, Karas M, Okubo Y, LeRoith D. Insulin-like growth factor-I receptor signal transduction: at the interface between physiology and cell biology. Comp Biochem Physiol B Biochem Mol Biol 1998;121:19–26
13. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF. Disruption of IRS-2 causes type 2 diabetes in mice. Nature 1998;391:900–904
14. Lingohr MK, Dickson LM, Wrede CE, Briaud I, McCuaig JF, Myers MG, Jr, Rhodes CJ. Decreasing IRS-2 expression in pancreatic beta-cells (INS-1) promotes apoptosis, which can be compensated for by introduction of IRS-4 expression. Mol Cell Endocrinol 2003;209:17–31
15. Lin X, Taguchi A, Park S, Kushner JA, Li F, Li Y, White MF. Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. J Clin Invest 2004;114:908–916
16. Hennige AM, Burks DJ, Ozcan U, Kulkarni RN, Ye J, Park S, Schubert M, Fisher TL, Dow MA, Leshan R, Zakaria M, Mossa-Basha M, White MF. Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. J Clin Invest 2003;112:1521–1532
17. Eck MJ, Dhe-Paganon S, Trüb T, Nolte RT, Shoelson SE. Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. Cell 1996;85:695–705
18. Craparo A, O'Neill TJ, Gustafson, T. Non-SH2 domains within insulin receptor substrate-1 and SHC mediate their phosphotyrosine-dependent interaction with the NPEY motif of the insulin-like growth factor I receptor. J Biol Chem 1995;270:15639–15643
19. Khan AH, Pessin JE. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia 2002;45:1475–1483
20. Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? Diabetes Care 2008;31(Suppl. 2):S262–S268
21. Boura-Halfon S, Zick Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. Am J Physiol Endocrinol Metab 2009;296:E581–E591
22. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y. A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 1997;272:29911–29918
23. Zick Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. Trends Cell Biol 2001;11:437–441
24. Liu YF, Herschkovitz A, Boura-Halfon S, Ronen D, Paz K, Leroith D, Zick Y. Serine phosphorylation proximal to its phosphotyrosine binding domain inhibits insulin receptor substrate 1 function and promotes insulin resistance. Mol Cell Biol 2004;24:9668–9681
25. Herschkovitz A, Liu YF, Ilan E, Ronen D, Boura-Halfon S, Zick Y. Common inhibitory serine sites phosphorylated by IRS-1 kinases, triggered by insulin and inducers of insulin resistance. J Biol Chem 2007;282:18018–18027
26. Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K. Establishment of a pancreatic beta cell line that retains

glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 1990;127:126–132
27. Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 1999;96:329–339
28. Casellas A, Salavert A, Agudo J, Ayuso E, Jimenez V, Moya M, Muñoz S, Franckhauser S, Bosch F. Expression of IGF-I in pancreatic islets prevents lymphocytic infiltration and protects mice from type 1 diabetes. Diabetes 2006;55:3246–3255
29. Rydgren T, Bengtsson D, Sandler S. Complete protection against interleukin-1beta-induced functional suppression and cytokine-mediated cytotoxicity in rat pancreatic islets in vitro using an interleukin-1 cytokine trap. Diabetes 2006;55:1407–1412
30. Rabinovitch A. An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. Diabetes Metab Rev 1998;14:129–151
31. Weber M, Deng S, Kucher T, Shaked A, Ketchum RJ, Brayman KL. Adenoviral transfection of isolated pancreatic islets: a study of programmed cell death (apoptosis) and islet function. J Surg Res 1997;69: 23–32
32. Mohanty S, Spinas GA, Maedler K, Zuellig RA, Lehmann R, Donath MY, Trüb T, Niessen M. Overexpression of IRS2 in isolated pancreatic islets causes proliferation and protects human beta-cells from hyperglycemia-induced apoptosis. Exp Cell Res 2005;303:68–78
33. Bleich D, Chen S, Zipser B, Sun D, Funk CD, Nadler JL. Resistance to type 1 diabetes induction in 12-lipoxygenase knockout mice. J Clin Invest 1999;103:1431–1436
34. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. β-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the β-cell phenotype and maturity onset diabetes. Genes Dev 1998;12:1763–1768
35. Kushner JA, Ye J, Schubert M, Burks DJ, Dow MA, Flint CL, Dutta S, Wright CV, Montminy MR, White MF. Pdx1 restores beta cell function in Irs2 knockout mice. J Clin Invest 2002;109:1193–1201
36. Rhodes CJ. Type 2 diabetes-a matter of beta-cell life and death? Science 2005;307:380–384
37. Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A, Rao A. IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. Science 1997;278: 860–866
38. Donath MY, Størling J, Maedler K, Mandrup-Poulsen T. Inflammatory mediators and islet β-cell failure: a link between type 1 and type 2 diabetes. J Mol Med 2003;81:455–470
39. Larsen CM, Wadt KA, Juhl LF, Andersen HU, Karlsen AE, Su MS, Seedorf K, Shapiro L, Dinarello CA, Mandrup-Poulsen T. Interleukin-1beta-induced rat pancreatic islet nitric oxide synthesis requires both the p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. J Biol Chem 1998;273:15294–15300
40. Zick Y. Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. Sci STKE 2005;2005:pe4
41. Maedler K, Schulthess FT, Bielman C, Berney T, Bonny C, Prentki M, Donath MY, Roduit R. Glucose and leptin induce apoptosis in human β-cells and impair glucose-stimulated insulin secretion through activation of c-Jun N-terminal kinases. FASEB J. 2008;22:1905–1913
42. Nakae J, Biggs WH 3rd, Kitamura T, Cavenee WK, Wright CV, Arden KC, Accili D. Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat Genet 2002;32:245–253
43. Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH 3rd, Wright CV, White MF, Arden KC, Accili D. The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. J Clin Invest 2002;110:1839–1847
44. Peers B, Leonard J, Sharma S, Teitelman G, Montminy MR. Insulin expression in pancreatic islet cells relies on cooperative interactions between the helix loop helix factor E47 and the homeobox factor STF-1. Mol Endocrinol 1994;8:1798–1806
45. Yasuda K, Yamada Y, Inagaki N, Yano H, Okamoto Y, Tsuji K, Fukumoto H, Imura H, Seino S, Seino Y. Expression of GLUT1 and GLUT2 glucose transporter isoforms in rat islets of Langerhans and their regulation by glucose. Diabetes 1992;41:76–81
46. Brissova M, Shiota M, Nicholson WE, Gannon M, Knobel SM, Piston DW, Wright CV, Powers AC. Reduction in pancreatic transcription factor PDX-1 impairs glucose-stimulated insulin secretion. J Biol Chem 2002;277:11225–11232
47. Linn T, Schmitz J, Hauck-Schmalenberger I, Lai Y, Bretzel RG, Brandhorst H, Brandhorst D. Ischaemia is linked to inflammation and induction of angiogenesis in pancreatic islets. Clin Exp Immunol 2006;144:179–187
48. Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 2005;54(Suppl. 2):S97–S107
49. Emamaullee JA, Shapiro AM. Interventional strategies to prevent β-cell apoptosis in islet transplantation. Diabetes 2006;55:1907–1914
50. Mardilovich K, Shaw LM. Hypoxia regulates insulin receptor substrate-2 expression to promote breast carcinoma cell survival and invasion. Cancer Res 2009;69:8894–8901