

Original article

Prevention of beta cell dysfunction and apoptosis by adenoviral gene transfer of rat insulin-like growth factor 1

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Keywords: *insulin-like growth factor 1; adenovirus; islet β -cells; nitric oxide; apoptosis*

Background Islet β -cells are almost completely destroyed when patients with type 1 diabetes are diagnosed. To date, insulin substitute therapy is still one of the main treatments. The cure of type 1 diabetes requires β -cell regeneration from islet cell precursors and prevention of recurring autoimmunity. Therefore, β -cell regeneration and proliferation emerge as a new research focus on therapy for type 1 diabetes. Islet β -cell regeneration and development are controlled by many growth factors, especially insulin-like growth factor-1 (IGF-1).

Methods Recombinant adenovirus encoding rat IGF-1 (rIGF-1) was constructed and transduced into rat β -cells, RINm5F cells. Western blotting analysis and ELISA were used to detect rIGF-1 protein. Streptozotocin (STZ) was used to induce RINm5F cell destruction. The level of nitric oxide (NO) was detected in cell culture supernatants by the Griess reaction. Islet cell function was evaluated by glucose-stimulated insulin production. Flow cytometry analysis was further used to investigate the apoptosis of RINm5F cells. Thiazolyl blue viability assay was applied to determine cell viability.

Results The recombinant adenovirus-rIGF-1 was successfully constructed and the titer was 4.0×10^8 pfu/ml. The rIGF-1 protein was effectively expressed in the RINm5F cells and cell culture supernatants. rIGF-1 expression remarkably inhibited STZ-induced islet cell apoptosis and significantly decreased the level of NO. Furthermore, IGF-1 expression also significantly protected insulin secretion and cell proliferation in a time-dependent manner.

Conclusions Our study suggests that locally produced rIGF-1 from RINm5F cells may be beneficial in maintaining β -cell function, protecting β -cells from the destruction of apoptosis factors and promoting β -cell survival and proliferation. IGF-1 might be considered as a candidate gene in gene therapy for type 1 diabetes. In addition, it appears that the apoptosis induced by STZ may be NO-dependent.

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Type 1 diabetes is characterized by progressive destruction of pancreatic β -cells, resulting in insulin deficiency and hyperglycemia. The onset of spontaneous type 1 diabetes in humans is preceded by progressive leukocyte infiltration into islets (insulinitis), which persists for a relatively long period of time before massive islet β -cell destruction.¹ Type 1 diabetes is diagnosed when β -cell destruction is almost complete and patients need insulin replacement therapy to survive. However, glycemia is not always properly regulated, and chronic hyperglycemia leads to severe microvascular, macrovascular and neurological complications. These devastating complications can be prevented by normalization of blood glucose levels. This can be achieved by pancreas and islet transplantation to restore endogenous insulin secretion.² However, the shortage of donors and potential elimination of transplanted islets by autoimmune reactions are serious limitations.¹ Thus, the restoration of functional insulin-producing β -cells is important as a curative therapy for type 1 diabetes.

Islet β -cell regeneration and development are controlled by the availability of growth factors, and especially insulin-like growth factors (IGFs). IGF-1 has insulin-like metabolic effects and stimulates cell proliferation and differentiation.³ Several *in vitro* studies confirm that IGF-1 protects islets against cytokine-mediated inhibition

of insulin secretion, stimulation of nitric oxide (NO) formation, and apoptosis.^{4,5} Other studies indicate the contrary, that IGF-1 had a tendency to induce diabetes.^{6,7} In those studies, however, isolated rat and human islet β cells were used and there was interference by endocrine, exocrine or other pancreatic cells and isolation-associated cell damage. And human IGF-1 was usually used. Therefore we examined a method for the “*in vitro*” sensitization and type of the rat islet β -cell RINm5F, by using recombinant adenovirus encoded rat IGF-1 (rIGF-1)

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for treating streptozotocin (STZ)-induced impairment. We found that rIGF-1 expressed from the adenoviral vector can counteract STZ-induced impairment of islets and β -cell death by apoptosis, and increase cell viability.

METHODS

Cell culture

The glucose-sensitive pancreatic cell line RINm5F was purchased from the American Type Culture Collection (ATCC, USA). The cells were maintained in Rosewell Park Memorial Institute (RPMI) 1640 medium (Gibco BRL, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL), 100 U/ml penicillin, and 100 mg/ml streptomycin, and incubated at 37 °C in an atmosphere of 5% CO₂ and 95% O₂ air. Two hundred and ninety-three cells were kindly provided by Prof. LUO Bing (Qingdao University Medical College, Qingdao, China) and cultured in DMEM (Gibco BRL) supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin. For assays, the cells were detached by a brief incubation with trypsin/EDTA (Gibco BRL).

Preparation of RNA and rIGF-1 cDNA

Rat livers were cut into small pieces. RNA was extracted using Trizol (Invitrogen, USA) following the manufacturer's instructions. Total RNA was reverse-transcribed with random hexamer primers and Superscript II reverse transcriptase (Invitrogen). First-strand cDNA was used as the template. Specific primers for rat IGF-1 were designed based on the sequence in GenBank (NM 178866.2). The forward primer was 5'-ggCAgATCTATgTCgTCTTCACATCTC-3' and the reverse primer was 5'-gcgATATCCCTCCTACATTCTgTAggTC-3', and these contained Bgl II and EcoR V sites (underlined), respectively. The thermal profile for PCR amplification was as follows: denaturation at 95°C for 5 minutes followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 58°C and 45 seconds at 72°C, and the end of the extension step at 72°C for 5 minutes.

Generation of recombinant adenoviral vectors

RT-PCR products were excised from 2% agarose gel and purified with a Qiaquick gel extraction kit (Qiagen, Germany). The plasmid pAdTrack-CMV and BJ5183 bacteria with pAdEasy-1 (containing the viral backbone) were also kindly provided by Prof. LUO Bing. The purified RT-PCR products and pAdTrack-CMV were digested using Bgl II and EcoR V (Takara, Japan) and religated, to give pAd-CMV-rIGF-1. This plasmid pAd-CMV-IGF-1 was linearized by PmeI (New England Biolabs, USA) and co-transformed into electro-competent BJ5183 bacteria with pAdEasy-1 and selected on Kanamycin LB plates. The plasmid pAd-rIGF-1 in bacteria was amplified and purified using a standard alkaline lysis procedure and sequenced (Shanghai Sangon, China). The complete adenovector was linearized with PacI (New England Biolabs) and used for transduction of 293 cells.

Production of adenoviruses in mammalian cells

Approximately 5×10^6 293 cells were plated in 25-cm² flasks 24 hours before transduction, when they reached about 80% confluency. Transduction was mediated by Lipofectamine 2000 and OptiMEM (Invitrogen), as described in the manufacturer's instructions. Transduced cells were monitored for green fluorescent protein (GFP) expression after 16 hours of transduction and collected 7–10 days after transduction. After three cycles of freezing in a methanol-dry ice bath and rapid thawing at 37°C, 1 ml of viral lysate was used to transduce 5×10^6 cells in a 25-cm² flask. Three to four days later, virus were harvested as described above. The recombinant adenovirus encoding rIGF-1 was named Ad-rIGF-1, and the viral particles were further amplified, purified, and titered according to GFP-positive units. Ad-eGFP was used as a control virus that does not affect the function or apoptosis of the islet *in vivo*, similar to Ad-LacZ. Therefore, Ad-eGFP was generated as described above.

Gene transfer of GFP and rIGF-1 to RINm5F cells in culture using adenoviral vectors

Approximately 5×10^6 RINm5F cells were plated in 25-cm² flasks for 48 hours before transduction. They were then transduced with Ad-eGFP or Ad-rIGF-1 at 5×10^6 plaque-forming units (pfu) in a minimal volume of serum-free RPMI 1640 for 4 hours at 37°C. After transduction, RINm5F cells were washed twice in serum-free medium and once with medium containing 10% FBS, and then incubated at 37°C in complete medium for 2 days, after which all assays were carried out. All the functional assays described below were performed in triplicate on at least three different occasions unless otherwise indicated.

Detection of secreted transgene products

(1) RT-PCR: RNA was isolated from each group (control, Ad-rIGF-1, and Ad-eGFP). Total cellular RNA was extracted using Trizol reagent and then treated with DNase I (Promega, USA) to eliminate potential contamination with residual genomic DNA. RT-PCR was then carried out to detect the transfer genes. (2) ELISA: Secreted rIGF-1 protein was detected in culture supernatants 2 days after transduction using an ELISA kit (Santa Cruz, USA). (3) Western blotting analysis: At the end of incubation, cells were harvested in lysis buffer and, after quantification by Lowry's method, 15 μ g of cell protein was loaded onto 10% SDS-acrylamide gels. At the end of the run, proteins were transferred to a nitrocellulose membrane and incubated overnight with polyclonal antibodies against IGF-1 (1:1000, Santa Cruz), and then with suitable peroxidase-conjugated secondary antibodies for 1 hour. Proteins were detected by chemiluminescence. Loading control was performed using actin immunodetection.

Evaluation of RINm5F cells function and NO levels after treatment with STZ

To assess the effects of STZ on RINm5F cells, glucose-

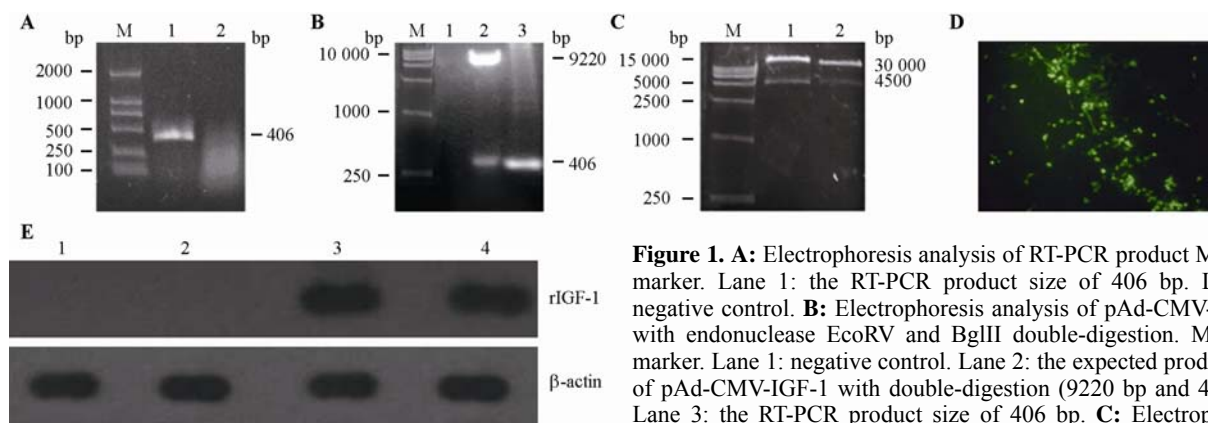


Figure 1. A: Electrophoresis analysis of RT-PCR product M: DNA marker. Lane 1: the RT-PCR product size of 406 bp. Lane 2: negative control. **B:** Electrophoresis analysis of pAd-CMV-rIGF-1 with endonuclease EcoRV and BglII double-digestion. M: DNA marker. Lane 1: negative control. Lane 2: the expected product size of pAd-CMV-IGF-1 with double-digestion (9220 bp and 406 bp). Lane 3: the RT-PCR product size of 406 bp. **C:** Electrophoresis analysis of pAd-rIGF-1 with endonuclease PacI digestion. M:

DNA marker. Lanes 1 and 2: pAd-rIGF-1 with endonuclease PacI digestion. **D:** GFP reporter expression in adenoviral transduced 293 cells (Original magnification \times 200). **E:** Western blotting analysis of the rIGF-1 in adenoviral transduced RINm5F cells. Lane 1: untransduced RINm5F cells. Lane 2: RINm5F cells transduced with Ad-eGFP. Lanes 3 and 4: RINm5F cells transduced with Ad-rIGF-1.

stimulated insulin secretion was used to detect cell function. Three groups were divided into two subgroups respectively (control, STZ, Ad-rIGF-1, Ad-rIGF-1+STZ, Ad-eGFP, Ad-eGFP+STZ) and each subgroup consisted of 8 flasks. Each group of cells were first treated with medium containing 10% FBS and/or 1.5 mmol/L STZ (final concentration) (Sigma, USA) respectively for 24 hours after preincubation in fresh medium for 16–24 hours. The medium was removed to detect NO products using the Griess reaction, and the cells were washed twice with cold RPMI 1640 medium. Incubation was carried out at 37°C in RPMI 1640 containing 0.2% BSA (Sigma) and 16.7 mmol/L (final concentration) glucose for 1 hour. The supernatant was subsequently removed, and its insulin content was determined by an ELISA kit (Santa Cruz).

Assessment of apoptosis activation by flow cytometry

After the cells in the six groups were treated by transduction and impairment by STZ as previously described, each group cells were harvested. The pellets were mixed with a 1:1 (v/v) mixture of PBS and 0.2 mol/L Na₂HPO₄, 0.1 mol/L citric acid (pH 7.5), and fixed with ice-cold ethanol at 4°C for 1 hour. The cells were washed twice with PBS and resuspended in 1 ml of a staining solution containing 10 mg/ml propidium iodide (Sigma). The cell suspensions were incubated at room temperature for 1 hour and 50 000 cells were analyzed on a FACSCalibur flow cytometer. The percentage of apoptotic cells was determined using multicycle software.

Cell viability and proliferation measurement

Cell viability was determined by colorimetric measurement of the reduction product of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma). Approximately 1×10^4 RINm5F cells were plated in 96-well plates. The treatment procedures were performed as described above (not including glucose-stimulated insulin secretion), then were continued to culture for 12, 24, 36, 48 and 60 hours. The original medium was removed from the 96-well plates, and the cells were washed in PBS. The cells were

incubated for 4 hours at 37°C in the presence of RPMI 1640 medium with 10% FBS containing 5 mg/ml MTT. The incubation medium was then removed and resulting formazan crystals were dissolved in 20 μ l dimethyl sulfoxide (DMSO) by shaking for 10 minutes. The absorbance at 490 nm was measured with a spectrophotometer. Cell viability and proliferation was defined as the ratio (expressed as a percentage) of the absorbance of treated cells to that of untreated cells on the first day.

Statistical analysis

Data are expressed as mean \pm standard deviation (SD). Statistical analysis was performed using the Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered remarkably statistically significance.

RESULTS

Generating recombinant adenovirus with rat IGF-1

rIGF-1 DNA was amplified by RT-PCR. Electrophoretic analysis of the RT-PCR products showed a band of the expected size (406 bp, Figure 1A). Figure 1A also shows the results of an electrophoretic analysis of pAd-CMV-rIGF-1 using double-digestion with Bgl II and EcoR V. The sequencing results were identical to the GenBank sequences (NM 178866.2) (data not shown). The positive recombinant vector pAd-rIGF-1 was digested with Pac I (Figure 1B). The products of pAd-rIGF-1 digested by Pac I were transduced into 293 cells. After transduction, the GFP reporter was observed by fluoroscopy (Figure 1C). The recombined Ad-rIGF-1 was constructed successfully and its titer was about 4.0×10^8 pfu/ml. The titer of Ad-eGFP was approximately 8.0×10^8 pfu/ml.

Detecting the products of rIGF-1

To detect the product of the transgene, total cellular RNA was extracted and subjected to RT-PCR. A band of the expected size (406 bp) was seen in Ad-rIGF-1-transduced RINm5F cells, but not in Ad-eGFP-

transduced RINm5F cells or untransduced RINm5F cells (data not shown). Secreted rIGF-1 was detected using the ELISA method at levels as high as (91.60±16.80) ng/ml with no detectable rIGF-1 in the media of untransduced RINm5F cells or RINm5F cells transduced with Ad-eGFP. Figure 1D shows that rIGF-1 was present in Ad-rIGF-1-transduced RINm5F cells as measured by Western blotting. rIGF-1 was not found in untransduced RINm5F cells and RINm5F cells transduced with Ad-eGFP. These results demonstrate that RINm5F cells can be efficiently transduced with adenoviral vectors and that they can synthesize and secrete significant levels of rIGF-1 protein.

rIGF-1 protects against STZ-induced impairment of insulin secretion

Previous findings had indicated that the cytotoxic effect of low doses of STZ on β -cell involves activation of the apoptotic pathway, whereas at high doses, β -cell death occurs predominantly through necrosis. Thus, a STZ concentration of 1.5 mmol/L was selected, which is sufficient to impair the ability of RINm5F cells to respond to a high glucose concentration in both untransduced RINm5F cells and in those transduced with the Ad-eGFP controls. RINm5F cells that were transduced with Ad-rIGF-1 maintained a normal insulin secretory response to glucose after STZ treatment, and this response was almost identical to that of unmodified RINm5F cells that were not treated with STZ (Figure 2A). The adenovirus itself did not affect the response of RINm5F cells to high glucose levels in the absence of STZ, as demonstrated using Ad-eGFP.

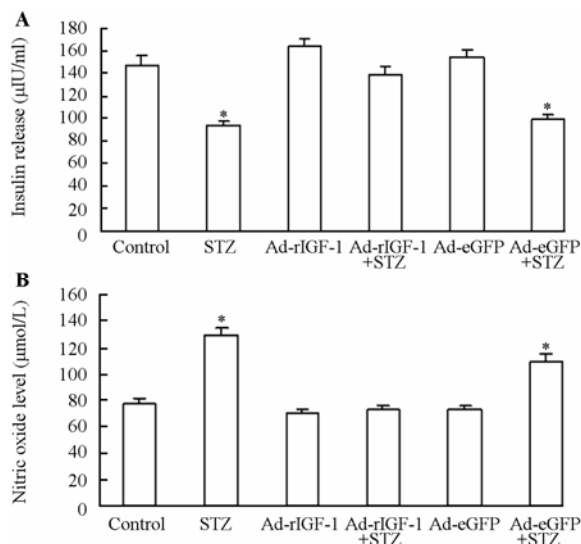


Figure 2. The levels of glucose-stimulated insulin (A) and NO production (B) from RINm5F cells transduced with Ad-rIGF-1, Ad-eGFP, or untransduced cells followed by incubation with or without STZ. * $P < 0.01$ compared with control.

rIGF-1 expression suppresses NO production

Untransduced RINm5F cells and those transduced with Ad-eGFP produced detectable levels of NO in the absence of STZ in all experiments. Untransduced

RINm5F cells exposed to STZ produced the most NO ($P < 0.01$), whereas exposure of Ad-rIGF-1-transduced RINm5F cells to STZ had no effect on the stimulation of NO production relative to mock transduced RINm5F cells (Figure 2B). Finally, RINm5F cells that had been transduced with Ad-rIGF-1 appeared to have reduced basal NO production.

rIGF-1 expression inhibits apoptosis

The percentage of apoptotic RINm5F cells among cells that had been treated with STZ was significantly higher than that among intact RINm5F cells ($P < 0.01$). No significant difference was observed in the percentage of apoptotic cells between cells transduced Ad-rIGF-1 in the presence of STZ and intact RINm5F cells. In contrast, Ad-eGFP did not protect RINm5F cells from apoptosis induced by STZ, and the percentage of apoptotic cells was similar to that in RINm5F cells after STZ treatment and significantly higher than that in intact RINm5F cells (Figure 3A).

rIGF-1 plays an important role in RINm5F cell survival

Using the MTT assay we determined that the percentage of surviving cells among untransduced cells and those transduced by Ad-eGFP treated with STZ was significantly decreased at all time points (12, 24, 36, 48 and 60 hours) compared to the controls ($P < 0.01$). IGF-1 increased the viability of RINm5F cells in a time-dependent manner. At all points between 12 hours and 60 hours (Figure 3C), the cell viability was higher by about 22% and 32% in the control and Ad-rIGF-1 group, respectively. There was no significant difference in the percentage of surviving cells between cells transduced with Ad-rIGF-1 after STZ treatment and the control (Figure 3B). These results show that IGF-1 has a very important influence on survival and proliferation of RINm5F cells.

DISCUSSION

Pancreatic β -cells are the only cells of the body that produce and secrete insulin in a manner finely controlled by blood glucose concentrations. Recent studies have confirmed that pancreatic β -cells have the capability to regenerate.⁸ Growth of β -cells can take place by at least two pathways: replication of differentiated β -cells or neogenesis.⁹ Both processes occur during neonatal development of the pancreas. However, in adults, β -cells expanding via mitogenesis make up only 1.5%–6% of the total β -cells population, which is primarily the result of replication of differentiated pre-existing β -cells. Circulating IGF-1 is mainly synthesized by the liver, but is also produced locally in most tissues where it acts in an autocrine/paracrine manner and it is of interest that serum concentrations of IGF-1 in type 1 diabetes patients are significantly decreased. The pancreatic microenvironment can play an important function in β -cell death, making these cells more susceptible or resistant to damage.¹⁰ This

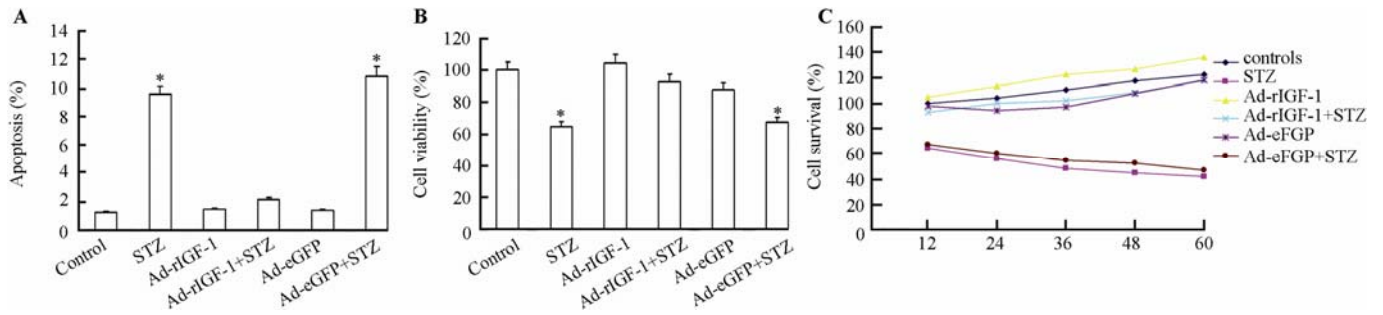


Figure 3. The percentages of apoptosis (A) and cell viability after 24 hours (B) from RINm5F cells transduced with Ad-rIGF-1, Ad-eGFP, or untransduced cells followed by incubation with or without STZ. **P* <0.01 compared to control. The proliferation rate curves of viability for RINm5F cells after 12, 24, 36, 48 and 60 hours from RINm5F cells transduced with Ad-rIGF-1, Ad-eGFP, or untransduced cells followed by incubation with or without STZ. This proliferation was in a time-dependent manner (C).

is an important theoretical concept; that a good microenvironment can ensure β -cell regeneration.

In recent years, it has become a routine procedure that a gene can be expressed in a target cells by use of recombinant technology and transgenic technology. Recombinant adenoviruses are currently used for this purpose, including gene transfer *in vitro*, vaccination *in vivo*, and gene therapy.^{11,12} Several features of adenovirus biology have made them the vectors of choice for some of these applications. For example, adenoviruses transfer genes to a broad spectrum of cell types, and gene transfer is not dependent on active cell division. Additionally, high titers of viruses and high levels of transgene expression generally can be obtained. In 1998, He et al¹³ introduced a simplified system for generating recombinant adenovirus and we used their method in this study. We successfully generated high titers of recombinant adenovirus containing rIGF-1. Ad-rIGF-1 was confirmed using restriction endonuclease digestion and sequencing. We also measured rIGF-1 that was present in Ad-rIGF-1-transduced RINm5F cells by Western blotting and the factor that had been secreted into the culture supernatant by ELISA.

Although several reports have suggested that IGF-1 inhibits insulin secretion *in vivo* and in certain instances *in vitro*,¹⁴ in our study, transduction of RINm5F cells with adenovirus containing rIGF-1 in culture did not change their viability or functional characteristics. At a high glucose concentration (16.7 mmol/L), RINm5F cells that had been transduced with Ad-rIGF-1 produced more insulin than untransduced RINm5F cells or those that had been transduced with Ad-eGFP. Our results also demonstrate that adenoviral gene transfer of reporter genes and the rIGF-1 gene to RINm5F cells *in vitro* does not impair the insulin secretory response to exogenous glucose and can suppress the STZ-induced impairment of glucose-stimulated insulin secretion.

In this study, we have demonstrated that transduction of RINm5F cells with a replication-defective adenoviral vector expressing rIGF-1 can protect them against STZ-induced NO formation. Some studies have

suggested that adenoviral transduction can lead to the stimulation of NO production and this can impede the efficiency of adenoviral-mediated gene transfer.^{15,16} We have observed that treatment of untransduced RINm5F cells with STZ yields the highest nitrite levels in all islet cells examined, whereas transduction with Ad-eGFP or Ad-rIGF-1 alone does not give nitrite levels any higher than those in the control. This could be due to a lack of resident macrophages which become activated around RINm5F cells.

In addition to blocking STZ-mediated dysfunction and NO production in RINm5F cells, rIGF-1 expression by RINm5F cells also clearly protects against STZ-stimulated apoptosis and increases cell viability in a time-dependent manner. The anti-apoptotic effects of IGF-1 have been documented in transformed cell lines, neurons, and hematopoietic progenitor cells.^{17,18} Although it is unclear exactly how IGF-1 expression in islet cells regulates apoptosis, IGF-1 has been implicated in blocking apoptosis through several different pathways. The main signaling pathway for IGF-1 receptor-mediated protection against apoptosis originates in the interaction of the IGF-1 receptor with IRS-1, which leads to the activation of PI 3'-kinase and Akt/protein kinase B^{19,20} and the phosphorylation and the activation of BAD, a member of the Bcl-2 family of proteins.²¹ The IGF-1 receptor also activates alternative pathways for protection: one through the activation of mitogen-activated protein kinase (MAPK) and another through the activation of Raf-1 and its translocation to the mitochondria.²⁰ However, the high percentages of apoptotic RINm5F cells parallels the increased NO concentration in the culture supernatant, which indicates that the apoptosis induced by STZ in RINm5F cells results from NO.

In summary, our results demonstrate the feasibility of preventing STZ-induced impairment of rat β -cell function after gene transfer of rat IGF-1. Additionally, our results demonstrate that local rIGF-1 production from genetically modified rat β -cells can suppress NO production in the presence of STZ, protect against STZ-induced apoptosis and increase cell viability in a time-dependent manner. The dysfunction and apoptosis induced by STZ in

RINm5F cells result from NO. Our present results suggest that IGF-I might be considered a candidate gene to be transferred to pancreatic islets as a new approach in gene therapy for type 1 diabetes. These results may also suggest the RINm5F cell line is a suitable model for study of β -cell growth and function.

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REFERENCES

- Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001; 358: 221-229.
- Shapiro AM, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; 343: 230-238.
- Le Roith D. Seminars in medicine of the Beth Israel Deaconess Medical Center: insulin-like growth factors. *N Engl J Med* 1997; 336: 633-640.
- Castrillo A, Bodelon OG, Bosca L. Inhibitory effect of IGF-I on type 2 nitric oxide synthase expression in Ins-1 cells and protection against activation dependent apoptosis: involvement of phosphatidylinositol 3-kinase. *Diabetes* 2000; 49: 209-217.
- Storling J, Binzer J, Andersson AK, Zullig RA, Tonnesen M, Lehmann R, et al. Nitric oxide contributes to cytokine-induced apoptosis in pancreatic beta cells via potentiation of JNK activity and inhibition of Akt. *Diabetologia* 2005; 48: 2039-2050.
- Lu Y, Herrera PL, Guo Y, Sun D, Tang Z, LeRoith D, et al. Pancreatic-specific inactivation of IGF-I gene causes enlarged pancreatic islets and significant resistance to diabetes. *Diabetes* 2004; 53: 3131-3141.
- Lu Y, Ponton A, Okamoto H, Takasawa S, Herrera PL, Liu JL. Activation of the Reg family genes by pancreatic-specific IGF-I gene deficiency and after streptozotocin-induced diabetes in mouse pancreas. *Am J Physiol Endocrinol Metab* 2006; 291: 50-58.
- Yamaoka T. Regeneration therapy of pancreatic beta cells: towards a cure for diabetes? *Biochem Biophys Res Commun* 2002; 296: 1039-1043.
- Giannoukakis N, Mi Z, Gambotto A, Eramo A, Ricordi C, Trucco M, et al. Infection of intact human islets by a lentiviral vector. *Gene Therapy* 1999; 6: 1545-1551.
- Saini KS, Thompson C, Winterford CM, Walker NI, Cameron DP. Streptozotocin at low doses induced apoptosis and at high doses cause necrosis in a murine pancreatic beta cell line, INS-1. *Biochem Mol Biol Int* 1996; 39: 1229-1237.
- Bonner-Weir S. Life and death of the pancreatic beta cells. *Trends Endocrinol Metab* 2000; 11: 375-378.
- Mellado-Gil JM, Aguilar-Diosdado M. Assay for high glucose-mediated islet cell sensitization to apoptosis induced by streptozotocin and cytokines. *Biol Proced Online* 2005; 7: 162-171.
- He TC, Zhou S, da Costa LT, Yu J, Kinzler KW, Vogelstein B. A simplified system for generation recombinant adenoviruses. *Proc Natl Acad Sci USA* 1998; 95: 2509-2514.
- Zhao AZ, Zhao H, Teaque J, Fujimoto W, Beavo JA. Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. *Proc Natl Acad Sci USA* 1997; 94: 3223-3228.
- Haddad IY, Sorscher EJ, Garver RI Jr, Hong J, Tzeng E, Mrtalon S. Modulation of adenovirus-mediated gene transfer by nitric oxide. *Am J Respir Cell Mol Biol* 1997; 16: 501-509.
- Beckman JS, Crapo JD. The role of nitric oxide in limiting gene transfer: parallels to viral host defenses. *Am J Respir Cell Mol Biol* 1997; 16: 495-496.
- Ratajczak J, Majka M, Kijowski J, Baj M, Pan ZK, Marquez LA, et al. Biological significance of MAPK, AKT and JAK-STAT protein activation by various erythropoietic factors in normal human early erythroid cells. *Br J Haematol* 2001; 115: 195-204.
- Okajima K, Harada N. Promotion of insulin-like growth factor-1 production by sensory neuron stimulation; molecular mechanism(s) and therapeutic implications. *Curr Med Chem* 2008; 15: 3095-3112.
- Friedrichs N, Kuchler J, Endl E, Koch A, Czerwitzki J, Wurst P, et al. Insulin-like growth factor-1 receptor acts as a growth regulator in synovial sarcoma. *J Pathol* 2008; 216: 428-439.
- Weinstein D, Simon M, Yehezkel E, Laron Z, Werner H. Insulin analogues display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. *Diabetes Metab Res Rev* 2009; 25: 41-49.
- Pang Y, Zheng B, Fan LW, Rhodes PG, Cai Z. IGF-1 protects oligodendrocyte progenitors against TNF α -induced damage by activation of PI3K/Akt and interruption of the mitochondrial apoptotic pathway. *Glia* 2007; 55: 1099-1107.

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