Protection of Islets by \textit{in Situ} Peptide-mediated Transduction of the I\(\kappa\)B Kinase Inhibitor Nemo-binding Domain Peptide*

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We have previously demonstrated that adenoviral gene transfer of the NF-\(\kappa\)B inhibitor I\(\kappa\)B to human islets results in protection from interleukin (IL)-1\(\beta\)-mediated dysfunction and apoptosis. Here we report that human and mouse islets can be efficiently transduced by a cationic peptide transduction domain (PTD-5) without impairment of islet function. PTD mediated delivery of a peptide inhibitor of the IL-1\(\beta\)-induced I\(\kappa\)B kinase (IKK), derived from I\(\kappa\)K\(\beta\) (NBD; Nemo-binding domain), and completely blocked the detrimental effects of IL-1\(\beta\) on islet function and NF-\(\kappa\)B activity, in a similar manner to Ad-I\(\kappa\)B. We also demonstrate that mouse islets can be transduced \textit{in situ} by infusion of the transduction peptide through the bile duct prior to isolation, resulting in 40\% peptide transduction of the \(\beta\)-cells. Delivery of the IKK inhibitor transduction fusion peptide (PTD-5-NBD) \textit{in situ} to mouse islets resulted in improved islet function and viability after isolation. These results demonstrate the feasibility of using PTD-mediated delivery to transiently modify islets \textit{in situ} to improve their viability and function during isolation, prior to transplantation.

Type 1 diabetes is an autoimmune disease characterized by an inflammatory response resulting in selective destruction of the insulin secreting \(\beta\)-cells of the pancreas. Proinflammatory cytokines such as IL-1\(\alpha\) and interferon-\(\gamma\) and the free radical, nitric oxide (NO), have been implicated to play key roles in the initial destruction of \(\beta\)-cells leading to the development of the disease (1). In particular, IL-1\(\beta\) is released by resident macrophages in islets in response to a variety of stimuli that can stimulate NO production by \(\beta\)-cells (1–4). Cytokine-induced NO production by rodent and human \(\beta\)-cells results in islet dysfunction and inhibition of insulin secretion. This NO-mediated damage has been shown to be attenuated by inducible nitric-oxide synthase inhibitors (5–7). IL-1\(\beta\) exposure also results in up-regulation of Fas on the surface of \(\beta\)-cells, resulting in an increase in apoptosis (1, 8, 9). Approaches to inhibit the adverse effects of IL-1\(\beta\) on islets, such as through gene transfer of the IL-1 inhibitor IL-1Ra, have been shown to improve islet function and viability (9). In addition to the adverse effects of IL-1\(\beta\), islets undergo enzymatic, osmotic, mechanical, and ischemic stresses during isolation, resulting in loss of viability, reduction of cell number, and initiation of apoptosis (10–12). The loss of viability of isolated islets has been associated with detachment from the surrounding extracellular matrix, leading to activation of caspase-3 and NF-\(\kappa\)B (15–18).

The promoters for a number of cytokine-sensitive genes, including inducible nitric-oxide synthase, intercellular adhesion molecule 1 (ICAM-1), Fas, and FasL contain binding sites for the NF-\(\kappa\)B family of transcription factors (19–22). Proinflammatory cytokines have been shown to stimulate NF-\(\kappa\)B activity in human and rodent islets \textit{in vitro}, resulting in \(\beta\)-cell impairment (23). NF-\(\kappa\)B binds to a family of naturally occurring repressors termed I\(\kappa\)B (24, 25), resulting in retention of the transcription factor complex in the cytoplasm. A variety of inflammatory agents including cytokines, endotoxin, double stranded RNA, and the viral transactivator Tax activate the NF-\(\kappa\)B by rapid phosphorylation and subsequent ubiquitin-mediated degradation of the I\(\kappa\)B repressor (24, 26–28). These agents increase the activity of the two related I\(\kappa\)B kinases, I\(\kappa\)K\(\alpha\) and I\(\kappa\)K\(\beta\), which phosphorylate I\(\kappa\)B. The release and degradation of I\(\kappa\)B following phosphorylation allows NF-\(\kappa\)B to translocate to the nucleus where it binds to its cognate enhancer/promoter elements upstream of certain proinflammatory genes (24, 29). In particular, I\(\kappa\)B has been shown to inhibit inducible nitric-oxide synthase gene expression by associating with NF-\(\kappa\)B and preventing its translocation to the nucleus (30). Intracellular expression of an I\(\kappa\)B mutant that is nonphosphorylatable and thus unable to be degraded prevented the nuclear translocation of the NF-\(\kappa\)B proteins, even in the presence of cytokines (31, 32).

In addition to IKK-\(\alpha\) and IKK-\(\beta\), a regulatory protein known as I\(\kappa\)K\(\gamma\)NEMO has been shown to be a critical component of I\(\kappa\)K complex (33, 34). I\(\kappa\)K\(\gamma\)NEMO also was identified independently in biochemical studies as an essential component of the high molecular weight I\(\kappa\)K complex (33, 34). Cells that do not express I\(\kappa\)K\(\gamma\)NEMO are unable to assemble the high molecular weight I\(\kappa\)K complex, preventing stimulation of I\(\kappa\)K activity in response to agents that stimulate the NF-\(\kappa\)B pathway (33, 34). I\(\kappa\)K\(\gamma\)NEMO preferentially associates with IKK-\(\beta\), but also binds to IKK-\(\alpha\) (34–37). Furthermore, inhibition of the IKK\(\beta\)-I\(\kappa\)K\(\gamma\)NEMO interaction and subsequent activation of NF-\(\kappa\)B \textit{in vivo} and \textit{in vitro} using a cell-permeable PTD-NEMO-binding domain (NBD) peptide fusion has been reported recently (38, 39).

In our earlier studies we have shown protection of human islets from the effect of IL-1\(\beta\) by adenoviral gene transfer of
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several different genes including IL-1Ra, soluble IL-1 receptors, and insulin-like growth factor-1 (9, 40). Moreover, we have demonstrated that adenoviral gene transfer of the nondegradable form of IκB repressor was able to block IL-1β-mediated dysfunction and apoptosis (41). These results suggest that specific inhibition of NF-κB is able to inhibit the adverse effects of IL-1β on β-cells. Although it has been shown that virus-mediated transfer of anti-apoptotic genes such as Bel-2 into islets in culture inhibits apoptosis (42–44), this class of genes is unable to block the adverse effects of IL-1β on islet function. Moreover, even though adenoviral vectors do not appear to interfere with β-cell function in vitro, the inherent immunogenicity of these viral vectors may be detrimental to islet transplantation. Although there are promising preclinical results with lentiviral and aden-associated virus-mediated gene transfer to islets, there is currently no clinically appropriate method for improving islet function and viability by gene transfer.

Recently it was shown that large protein complexes can be delivered directly to cells when they are linked to protein transduction domains (PTDs). PTDs are short, positively charged domains that can freely cross cell membranes through a receptor and energy independent process (45–48). Proteins fused to the PTD derived from HIV Tat or the Drosophila Antennapedia homeobox protein (Antp) transduce a variety of different cell types and are even able to cross the blood/brain barrier when administered to mice by intraperitoneal injection (49–51). Previously we have identified a series of cationic peptides that were able to transduce certain cell types as or more efficiently than the Tat PTD (52). Moreover, we demonstrated that a specific PTD, termed PTD-5, was able to transduce human islets efficiently in culture (53).

Given that gene transfer of IκB was effective in preventing islet dysfunction and apoptosis, we hypothesized that peptide-mediated transduction of NF-κB inhibitors into islet cells would result in improved viability and function following IL-1β exposure. Thus, we have examined the ability of PTD-5-mediated transduction of the NBD peptide to block islet dysfunction and loss of viability. Here we demonstrate that transduction of mouse islets in culture with the PTD-5-NBD fusion peptide can prevent IL-1β-dependent suppression of glucose-stimulated insulin release and NF-κB activation. In addition, we demonstrate the feasibility of transducing mouse islets in situ by infusion of the transduction peptide into the mouse pancreas via the common bile duct prior to islet isolation. Administration of the PTD-5-NBD fusion peptide into mouse islets in situ before isolation increased their viability and function following isolation. These results suggest that peptide-mediated transduction of an NF-κB inhibitor is able to protect islets from the adverse effects associated with islet isolation. Clinically, this approach could be used to improve the viability, function, and mass of human islets following isolation by in situ delivery of PTD-5-NBD to islets prior to isolation.

EXPERIMENTAL PROCEDURES

Animals—BALB/c mice were purchased from Taconic (Germantown, NY). All animals were housed at the University of Pittsburgh, Center for Biotechnology animal facility in compliance with the United States Department of Agriculture and National Institutes of Health regulations. All animal manipulations were conducted and monitored under protocols reviewed and approved by the Institution Animal Care and Use Committee.

Peptides—Peptides PTD-5 (RQRGRRLMKMRGQGTDWSSLQ) and PTD-5-NBD (RQRGRRLMKMRGQGTDWSSLQIHEL) were synthesized by the peptide synthesis facility (University of Pittsburgh) by the solid-phase procedure on an automated peptide synthesizer (PerSeptive Biosystems, Inc., Framingham, MA) using N-α-fluorenlymethoxy carbonyl (Fmoc) synthesis protocols. Peptides were N-terminal conjugated to fluorescent probe using 6-carboxyfluorescein (6CF, Molecular Probes Inc., Eugene OR) and were subsequently purified and characterized by reversed-phase high performance liquid chromatography and mass spectrometry. The construction of the peptide-eGFP fusion protein has been described previously (53).

Islet Isolation and Culture—Mouse (BALB/c) pancreatic islets were isolated by intraduodenal collagenase digestion (Type V, 1.75 mg/ml, Sigma) as described, with modifications (54, 55). For the in situ peptide transduction, prior to collagenase infusion, 200 μl of a solution of Hanks’ balanced salt solution containing 1 mM peptide or 200 μg of PTD-5-eGFP fusion protein (6.25 μM) was injected into the common bile duct followed by 2–3 ml of collagenase infusion. The isolated islets were purified by Ficol density gradient centrifugation and were handpicked under a stereomicroscope. In all the experiments, islets of 150–200 μm diameter were used. The purity of the islets was monitored by the common bile zone staining and was ~95% in all islets. Islets were maintained in CMRL-1066 supplemented with 2 mM l-glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin, and 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA; complete CMRL) in a humidified 5% CO2 incubator at 37 °C. In addition, isolated islets were maintained in media containing 200 μM peptide.

Human islets were obtained from the islet isolation core of the University of Pittsburgh. Pancreas obtained from cadaveric donors were subjected to digestion, isolation, and purification as described by Ricordi et al. (56), with modifications (57). The purity of islets was usually greater than 75% as determined by dithizone staining. Viability of the cultured islets was usually greater than 85% as assessed by Calcein AM and propidium iodide staining. The isolated islets were cultured in complete CMRL.

Recombinant Adenoviral Vector Construction and Gene Transfer to Isolated Islets—The construction and propagation of the adenoviral E1/E3-deleted vectors expressing IκB and LacZ from the cytomegalo-virus promoter has been described previously (41). For the Ad-IκB infection experiments, groups of 200 islets were washed in serum-free CMRL-1066 and subsequently infected with adenoviral vectors encoding IκB and β-galactosidase at 100 multiplicity of infection for 1 h at room temperature (55). Following infection, islets were washed 2–3 times with fresh medium and then cultured for 48 h in complete CMRL.

Stimulated Glucose-stimulated Insulin Release and NF-κB Activity Measurements—In Situ Peptide Transduction, Adenoviral Infection, and Exposure to IL-1β—To determine the protective effects of NF-κB inhibition in the presence and absence of IL-1β treatment, static glucose-stimulated insulin release was used as a functional assay. In vitro transduced, adenovirus-infected, and control islets were incubated in the presence of 50 units of recombinant mouse IL-1β (R&D Systems, Minneapolis, MN) for a period of 24–30 h prior to insulin release studies. The IL-1β containing medium was then removed and the islets were washed twice in glucose-free Krebs-Ringer bicarbonate buffer (pH 7.35) containing 10 mM HEPES, 0.5% bovine serum albumin (Sigma), and a group of 100 islets in triplicate were conditioned by preincubation for 1 h at 37 °C using low glucose concentration (2.8 mM). The preincubation buffer was discarded and replaced with Krebs-low glucose buffer for a subsequent 1-h incubation at 37 °C, the Krebs buffer was removed and stored, and the buffer was replaced with Krebs containing high glucose (20 mM) and incubated for 1 h at 37 °C. After 1 h, the high glucose buffer was collected and replaced with Krebs low glucose buffer for a final 1-h incubation. The insulin content of the buffers collected after each incubation step was determined by a commercially available enzyme-linked immunosorbent assay kit (ALPCO, Windham, NH). The islets isolated after in situ treatment, with or without PTD-5-NBD, were cultured overnight and then handpicked, washed in Krebs-Ringer bicarbonate buffer, and glucose-stimulated insulin release was determined as described above.

NF-κB p65 Transcription Factor Assay in Mouse Islets—To examine the protective effects of NBD peptide from IL-1β exposure on NF-κB activity in vitro, the isolated islets were transduced with NBD peptide for 2 h, and the islets were subsequently treated with 50 units of IL-1β for an additional 2 h. As controls, transduced and nontransduced islets were treated with medium only. All islet groups were washed twice with cold phosphate-buffered saline and stored at ~80 °C until whole cell extracts were prepared. A total of 10 μg of cellular protein from each group was analyzed for p65 binding activity according to the manufacturers instructions, using the enzyme-linked immunosorbent assay-based Trans-AM™ NF-κB p65 transcription factor assay kit (Active Motif, Carlsbad, CA). The specificity of NF-κB DNA binding activity was confirmed by competition with a wild type or mutant oligonucleotide with an immobilized oligonucleotide probe containing the NF-κB consensus site. NF-κB binding activity was measured at 450 nm and the OD reading normalized to protein content.
were incubated in the presence of the dyes for 30 min at 37 °C according to the manufacturer guidelines (Molecular Probes Inc.). Islets using MetaMorph™ software package version 4.6r9 (Universal Imaging Corp., Downingtown, PA). Percent viability was analyzed by flow cytometry of biotinylated PTD-5 coupled to streptavidin-Alexa Fluor 488 (SA-488); and F, static glucose-stimulated insulin release by peptide-transduced islets as compared with control. L1, initial low (2.8 mM) glucose; H, high glucose (20 mM); and L2, second low glucose concentrations. The data represents the mean ± S.E. of four independent experiments in triplicate. The bars are presented as percentage of insulin secretion above the control group of untreated islets exposed to the first 2.8 mM glucose treatment given a relative value of 100%.

was measured using a Bradford protein assay (Bio-Rad) with bovine serum albumin as standard.

**Islet Cell Dispersion and Fluorescence-activated Cell Sorting Analysis of β-Cells for Peptide Transduction**—Islets isolated with infusion of PTD-5–6CF peptide in situ were dispersed into individual cells by treatment with Hank’s based enzyme-free cell dissociation buffer (Invitrogen) at 37 °C for 5–10 min and incubated in complete CMRL for 2 h at 37 °C prior to cell sorting. β-Cells were analyzed based on size and endogenous FAD fluorescence as described (6, 58), using a FACStar flow cytometer (BD Biosciences), with a laser illuminated at 488 nm and with the gated cells having forward scatter monitored at 515–535 nm. Under this selection the majority of the cells gated (>70%) are β-cells (59). In addition, following uptake of biotinylated PTD-5 coupled to streptavidin-Alexa Fluor 488 (Molecular Probes Inc.) in β-cells, the human islets also were dissociated into single cells and peptide transduction was measured by flow cytometry as described above. The biotinylated PTD-5 coupled to streptavidin-Alexa Fluor 488 was prepared as previously described (52).

**Determination of Islet Viability by Simultaneous Staining of Live and Dead Cells Using a Two-color Fluorescence Assay**—Viable cells convert Calcein AM into green fluorescent products and dead cells incorporate propidium iodide in the nucleus, resulting in an intense red fluorescence (60). A working phosphate buffer solution containing 1 μg/ml Calcein AM and 10 μg/ml propidium iodide was freshly prepared according to the manufacturer guidelines (Molecular Probes Inc.). Islets were incubated in the presence of the dyes for 30 min at 37 °C prior to evaluation under a fluorescence microscope. Pictures were captured by a two-photon confocal microscope and percent viability was analyzed using MetaMorph™ software package version 4.6r9 (Universal Imaging Corp., Downingtown, PA). Percentage of viable cell aggregates over the total was determined by scoring green versus red fluorescence in at least 25–30 islet cell aggregates.

**Statistical Analysis**—All data collected were expressed as mean ± S.E. and statistics were performed using the SPSS package, and a p value of less than 0.05 by analysis of variance was used to indicate statistically significant differences.

**RESULTS**

**Transduction of PTD-5 Peptide into Intact Islets in Culture**—Earlier studies have demonstrated that PTDs are capable of mediating internalization of heterologous peptides and proteins in a receptor- and energy-independent manner into nearly 100% of a variety of cell types (45–49). In particular, we have demonstrated that human islets can be transduced with a specific PTD, PTD-5 marker protein complex in culture (53). To examine further the transduction efficiency of PTD-5 into isolated islets, mouse and human islets were incubated with fluorescently labeled PTD-5. As shown in Fig. 1, both mouse (B) and human islets (D) were efficiently transduced with PTD-5 compared with controls (A and C). Analysis of transduction of human β-cells by flow cytometry (Fig. 1E) demonstrated that the majority of the β-cells within the intact islets, as determined by size and endogenous FAD fluorescence, were transduced with biotinylated PTD-5 linked to streptavidin-Alexa Fluor 488. To determine whether transduction with PTD-5 affected islet function, the ability of the treated islets to respond to glucose was determined. As shown in Fig. 1F, both the PTD-5-treated and control islets produced similar levels of insulin in response to increasing concentrations of glucose. These experiments demonstrate that islets and, in particular, β-cells can be efficiently transduced with cationic peptides in vitro without any apparent impairment of glucose signaling and insulin production.
Inhibition of NF-κB in Mouse Islets by Peptide-mediated Transduction Prevents IL-1β-mediated Impairment—We have previously demonstrated that adenoviral-mediated gene transfer of IκB to mouse islets was able to block IL-1β-mediated islet dysfunction in cultured islets (41). This result suggests that islet integrity and function, at least in culture, can be preserved by inhibition of NF-κB activity in β-cells. To determine whether peptide-mediated transduction of an NF-κB inhibitor into islets is able to inhibit NF-κB activity, blocking IL-1β-mediated islet dysfunction, a peptide containing PTD-5 fused to the IKKβ peptide inhibitor of IKK kinase (NBD) through a diglycine spacer was synthesized (PTD-5-NBD) and used for islet transduction experiments (39). As a positive control for the effects of NF-κB inhibition, the Ad-1xIκB vector was also used for gene transfer. As shown in Fig. 2, transduction of mouse islets with the PTD-5-NBD fusion peptide was able to prevent IL-1β-mediated impairment of glucose-stimulated insulin release by cultured islets. Moreover, the inhibition of IL-1β-mediated islet dysfunction by the fusion peptide was similar to that conferred by adenoviral-mediated gene transfer of IκB. Thus, PTD-mediated delivery of an inhibitor of the IκB kinase is effective in blocking the detrimental effects of IL-1β on islet function.

Transduction of PTD-5-NBD to Mouse Islets Prevents IL-1β-stimulated NF-κB Activation—To demonstrate that transduction of the NBD peptide inhibited NF-κB activation, analysis of the NF-κB binding activity was performed using a NF-κB p65 transcription factor assay on PTD-5-NBD-transduced islets. As shown in Fig. 3, NF-κB activity was significantly increased in nontransduced islets following treatment with IL-1β. However, in the NBD peptide-treated islets exposed to IL-1β, the NF-κB level was similar to control nontransduced islets. These results demonstrate that transduction of islets with the NBD peptide blocked IL-1β-mediated induction of NF-κB binding activity.
mice were injected with a PTD-5-6CF peptide as well as with a PTD-5-eGFP fusion protein and an eGFP control protein. Islets isolated from PTD-5–6CF-treated pancreata showed extensive transduction by confocal microscopy (Fig. 4B) compared with controls (Fig. 4A). Analysis of β-cells from the isolated islets by fluorescence-activated cell sorting showed that at least 30–40% of the β-cells were transduced (Fig. 4E). Islets treated with recombinant PTD-5-eGFP fusion protein in situ (Fig. 4D) also showed evidence of transduction compared with the eGFP control (Fig. 4C), albeit at reduced levels compared with the 12-amino acid peptide, PTD-5–6CF. However, these results clearly demonstrate that islets and in particular, β-cells, can be modified by peptide-mediated transduction in situ prior to isolation.

**Transduction of PTD-5-NBD Peptide during Isolation of Mouse Islets: Effect on Cell Viability and Static Glucose-stimulated Insulin Release**—It has been reported that islets are exposed to osmotic, mechanical, and ischemic stresses during the isolation procedure, resulting in apoptosis or loss of viability (10–18). To determine whether PTD-5-NBD peptide transduction in situ was able to reduce the impairment of islet function and viability, the PTD-5-NBD fusion peptide was injected into mouse pancreata prior to collagenase infusion. To determine the viability of the islets following isolation, the isolated islets were stained with Calcein AM and propidium iodide (60). As shown in Fig. 5, the islets treated with the PTD-5-NBD peptide showed greater viability compared with control islets. Analysis of the extent of viable green cells compared with nonviable red cells demonstrated that treatment with the PTD-5-NBD fusion peptide resulted in ~97% viability as compared with 80–90% in the control.

To confirm that improved cell survival by PTD-5-NBD peptide treatment in situ resulted in improved islet function, the ability of the islets to respond to glucose challenge was examined. Glucose-stimulated static insulin release of PTD-5-NBD-treated islets was −10–30% higher compared with either untreated or PTD-5 only (Fig. 6). In addition, the enhanced secretory response under high glucose stimulation was fol...
lowed by a physiologic return to basal levels, when the ambient glucose concentration was returned to low glucose. The higher level of insulin release in PTD-NBD islets as compared with PTD-5 control and untreated islets suggests that inhibition of NF-κB during the islet isolation procedure is able to improve islet viability and therefore function in culture following isolation.

**DISCUSSION**

Transplantation of pancreatic islets promises to be the most effective approach for treating type 1 diabetes (61, 62). However, the need for large amounts of islets and the poor survival of the graft following transplantation represent significant hurdles that need to be overcome to make the islet replacement theory useful for treating diabetic patients. Islets undergo enzymatic, osmotic, mechanical, and ischemic stresses during isolation, resulting in loss of viability, reduced cell number, and initiation of apoptosis (10–12). These facts increase the number of islet donors needed to treat a single diabetic transplant recipient. Loss of viability can also be attributed in part to detachment of the islets from the surrounding extracellular matrix that is essential for β-cell viability and function (13–18). In addition, release of inflammatory cytokines such as IL-1β from the pancreatic tissue during the isolation procedure contributes to islet dysfunction and reduced mass. The reduction in islet viability and increased β-cell death prior to transplant most likely leads to stimulation of a stronger immune response toward the allogeneic islets.

To improve the viability of islets prior to and following transplantation, we have been examining gene transfer methods for delivery of agents able to protect islets from dysfunction and death in culture and to block the immune response to the transplanted islets in vivo. Previously, we have demonstrated that adenoviral-mediated gene transfer of the NF-κB inhibitor, IκB, to islets results in complete inhibition of IL-1β-mediated islet dysfunction (41). However, there currently are no clinically applicable gene transfer approaches to modify islets in culture. In addition, significant damage to islets occurs during the isolation procedure, necessitating the delivery of protective agents to islets in situ. To date, gene transfer to islets in situ has been extremely inefficient.

In this report, we demonstrate that both human and mouse islets are efficiently transduced by cationic peptide transduction domains, in particular, PTD-5, which functions similarly to the Tat PTD derived from HIV. The majority of β-cells can be transduced in culture without a significant effect on islet function. Moreover, we have demonstrated that PTD-5-mediated delivery of a peptide inhibitor of the IκB kinase (IKK) is able to block IL-1β-mediated induction of NF-κB as well as islet dysfunction in culture. The inhibitory effect of the PTD-5-NBD peptide in blocking IL-1β was similar to the protective effect conferred by adenoviral-mediated gene transfer of IκB. The use of the peptide to protect islets in culture, however, has significant advantages over gene transfer in regard to simplicity and cost as well as risk. Our results using the PTD-5-NBD peptide are similar to those previously reported for Tat PTD-mediated delivery of Bcl-2 to islets to block apoptosis (63). However, in contrast to the Bcl-2 fusion, the peptide inhibitor of NF-κB is also able to block induction of NO, and thus preserve islet dysfunction, in addition to blocking cell apoptosis. Thus the inhibition of NF-κB activation by PTD-5-NBD is able to prevent loss of islet function as well as improve islet viability.

We also have demonstrated the ability to transduce islets in situ as a way of delivering agents able to preserve islet function during isolation. Importantly, the presence of PTD-5-NBD in the collagenase solution and its intraductal delivery appears to have no impact on the efficiency of digestion at any step. The yield of islets was unaffected, demonstrating the safety and feasibility of this approach. Injection of a fluorescent transduction peptide into the pancreas prior to islet isolation resulted in almost 40% of the β-cells being transduced, with an additional percentage most likely being weakly transduced. Transduction of islets with the PTD-5-eGFP fusion protein appeared to be less efficient, however, the difference in transduction most likely is because of the 160-fold difference in the concentration used between PTD-5-6CF and PTD-5-eGFP. Furthermore, these results may be because of the differences between the eGFP and 6CF cargos with respect to efficiency of internalization and the stability and function of the markers following internalization. The delivery of the PTD-5-NBD peptide to islets in situ resulted in an increase in the percent of viable cells within the islets as well as improved islet function. Thus, this approach could be used to improve the quality of islets during isolation, resulting in recovery of greater islet mass and improved islet function. Indeed, in preliminary experiments with human islets, injection of the PTD-5 peptide into the pancreas prior to isolation also resulted in efficient islet transduction. Moreover, the injection of the PTD-5-NBD peptide into the human pancreas resulted in islet isolation with greater β-cell mass and viability. Taken together, these results suggest that the PTD-5-NBD peptide could be used to modify islets in situ prior to isolation to improve the integrity of islets for transplantation.

In the experiments described in this report, we have focused on inhibiting NF-κB by peptide-mediated transduction to improve islet function. Although we demonstrate that inhibition of NF-κB activity in response to IL-1β protects the islet from dysfunction and death, it is highly likely that additional pathways may have to be targeted. For instance, inhibiting the effects of interferon-γ through delivery of signal transducers and activators of transcription inhibitory peptides or proteins could improve islet function. Moreover, delivery of inhibitors of apoptosis such as Bcl-2, dnCaspase 9, or agents that block free radical damage such as manganese-superoxide dismutase could also be able to improve islet mass and function following isolation. Regardless of the therapeutic agent to be delivered, the use of peptide transduction has the advantage over gene transfer in that it can be applied in situ. Moreover, peptide transduction results in only transient modification of the cells, eliminating any risks associated with long term gene expression of anti-apoptotic gene products.

The use of peptide transduction of protective peptides and fusion proteins to protect tissue from damage during isolation and culture, prior to transplant, can be applied to a wide variety of organs in addition to islets. In preliminary experiments, we have demonstrated that both cardiac tissue and cartilage can be efficiently transduced, allowing for peptide-mediated transduction approaches to block ischemic damage to these and other tissues. Given that NF-κB activation plays a major role in mediating ischemic tissue damage, the PTD-5-NBD fusion peptide could be used to protect other tissues from damage during isolation. Taken together, our results demonstrate the feasibility of using peptide transduction domains to modify tissues in situ to improve their viability prior to transplantation. Moreover, we have demonstrated that inhibition of NF-κB activity is clearly protective to islets in culture and in situ.

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2 R. Bottino, K. Rehman, P. Robbins, and M. Trucco, unpublished data.
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