Protection of Human Islets from the Effects of Interleukin-1β by Adenoviral Gene Transfer of an IκB Repressor*

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Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that inhibits β cell function and promotes Fas-triggered apoptosis. IL-1β is thought to act early in the initiation of the autoimmune destruction of pancreatic β cells in type 1 diabetes. IL-1β promotes β cell impairment, in part, by activating NF-κB transcription factor-dependent signaling pathways. We have examined whether β cells could be protected from the effects of IL-1β by overexpressing an inhibitor of NF-κB activity, IκB, by adenoviral gene transfer to intact human islets in culture. Infection of islets with an adenoviral vector encoding a non-phosphorylatable, non-degradable variant of IκBα resulted in normal insulin responses to glucose in the presence of IL-1β. Furthermore, nitric oxide production was prevented and, more importantly, Fas-triggered apoptosis was inhibited following IκBα gene transfer. These results suggest that blocking the NF-κB pathway might prevent cytokine-induced β cell impairment as a means of facilitating islet transplantation.

The processes that lead to autoimmune diabetes mellitus include an as-yet-undefined trigger within islets that activates islet-resident, antigen-presenting cells to acquire antigens expressed by β cells. It is thought that antigen acquisition is consequent to β cell apoptosis. Once activated, the antigen-presenting cells are able to migrate to the peripheral lymphoid organs where they can activate autoreactive naïve T-lymphocytes that have escaped thymic or peripheral negative selection (1). Pro-inflammatory cytokines such as interleukin-1β (IL-1β)† play important roles in this process (2).

IL-1β is released by macrophages in response to a variety of changes in a given tissue (3). The importance of IL-1β production by islet-resident macrophages in the onset of autoimmune diabetes has been suggested in several related studies. Depletion of macrophages in BB rats and in cyclophosphamide-treated non-obese diabetic (NOD) mice by silica particles has been shown to prevent diabetes (4, 5). In rodents, islet-resident macrophages are important for the initiation of β cell dysfunction and produce TNFα, resulting in the induction of nitric oxide (NO) production by up-regulation of the inducible nitric oxide synthase enzyme (iNOS) (2, 3, 6–8). The inhibition of β cell function and the promotion of NO production is an IL-1β-dependent process in rodents (6, 8, 9). Whether IL-1β is acting alone via NO in promoting β cell dysfunction and apoptosis activation in human islets is still unclear (10–12). Nitric oxide itself is toxic to β cells, because it is readily converted to peroxynitrite (13, 14). Islet-resident macrophages as well as β cells in the NOD mouse begin displaying an increase in iNOS protein at about 5 weeks of age coincident with the onset of insulitis (15). Finally, insulitis progression, defined cytologically as an increase in macrophage and T-lymphocyte infiltration in and around the islets of Langerhans, is associated with increasing levels of iNOS expression and protein in β cells of the NOD mouse (15, 16).

The promoters for a number of cytokine-sensitive genes, including iNOS, ICAM-1, Fas, and Fas ligand possess binding sites for members of the NF-κB family of transcription factors (17–22). The regulation of NF-κB activity has been implicated in β cell impairment. NF-κB activity is increased in rat insulinoma cells (RIN) in culture in response to IL-1β (23), whereas cytokines were also shown to promote NF-κB activity in human and rodent islets in vitro (24). Moreover, iNOS expression in RINm5F insulinoma cells is dependent on NF-κB transactivation of the promoter in response to IL-1β (24). Additionally, this requirement for NF-κB activation for iNOS expression was observed in human β cells as well as in rat islets in culture in response to IL-1β (24). Another gene that is activated in response to IL-1β and very likely involved in β cell dysfunction is cyclooxygenase 2 (COX-2), the enzyme that catalyzes the formation of the pro-inflammatory prostaglandin E2, as well as primary islets is paralleled by an activation of NF-κB (26).

NF-κB activity is regulated by a group of naturally occurring repressors termed IκB (27). IκB is normally bound to NF-κB, resulting in retention of the complex in the cytoplasm. Following inflammatory stimulation, such as exposure to certain cytokines like TNFα and IL-1β, IκB is rapidly phosphorylated and targeted for ubiquitin-mediated degradation. The release of IκB allows NF-κB to translocate to the nucleus, where it binds its cognate enhancer elements upstream of pro-inflammatory genes (27, 28). IκB has been shown to inhibit iNOS gene expression by associating with NF-κB and preventing its translocation to the nucleus (29). Recently, a mutant version of IκBα...
has been engineered that is non-phosphorylatable and unable to be degraded (30). Expression of the mutant IκBα in cells from human arthritic joints, including macrophages, in vitro, suppressed TNFα production (31). Adenoviral gene transfer of the mutant into human intestinal epithelial cells in vitro, blocked IL-1β and TNFα-induced iNOS expression as well as IL-1β and IL-8 production (30).

In this report, we have examined the ability of a non-phosphorylatable, non-degradable variant of IκBα, delivered by adenoviral gene transfer, to inhibit IL-1β-mediated β cell destruction and apoptosis. We demonstrate that adenoviral gene transfer of the IκBα repressor to human islets in vitro can prevent IL-1β-dependent suppression of glucose-stimulated insulin release and can inhibit nitric oxide production following exposure of the islets to IL-β. Furthermore, we show that IL-1β-mediated, Fas-triggered apoptosis induction can be prevented in islets by IκB gene transfer. These results suggest that inhibition of the NF-κB signaling pathway in islets may be one means of facilitating islet transplantation as a potential therapy for autoimmune diabetes.

**EXPERIMENTAL PROCEDURES**

**Adenoviral Infection of Intact Human Islets in Vitro**—The IκBα repressor cDNA was used to generate an E1-deleted recombinant adenoviral vector, kindly provided by Dr. David Geller (University of Pittsburgh). The virus was propagated and purified as described (32). The E1/E3-deleted eGFP and LacZ adenoviral vectors were constructed by subcloning the cDNAs into the pAdLox shuttle plasmid followed by Cre-Lox recombination (33). In all instances, transgene expression was driven by the cytomegalovirus immediate-early promoter.

Pancreata from multiorgan cadaveric donors were provided by the National Disease Research Interchange (Philadelphia, PA) and local Organ Procurement Organizations with the appropriate consent for research use. Islets were obtained from the Diabetes Research Institute of the University of Miami (Dr. C. Ricordi) and from the University of Minnesota (Dr. Bernhard Hering). These sources participate in the Juvenile Diabetes Foundation International Islet Distribution Program. Pancreata were obtained from at least five cadaveric donors of different ages and sex and subjected to the digestion, isolation, and purification as described (34). The purity of islets was usually greater than 85% as assessed by vital dye exclusion, insulin staining, and morphology. The experiments described below used islets from the pancreata of five different donors.

Islets were washed twice in serum-free RPMI 1640 (Life Technologies, Inc.) prior to infection. 200–300 islets were infected with adenoviral vectors encoding IκBα (Ad-IκBα, β-galactosidase (Ad-LacZ), or enhanced green fluorescence protein (Ad-eGFP) at 1 × 10⁶ plaque forming units (pfu) per 200–300 islets for 2 h at 37 °C. Following infection, islets were washed twice in serum-free medium and then cultured for 48 h in medium with 10% heat-inactivated fetal calf serum (Life Technologies, Inc.) containing a solution of 1% penicillin/streptomycin (Life Technologies, Inc.).

**Glucose-stimulated Insulin Production and NO Output—**To determine the effects of IL-1β on β cell function of islets infected with Ad-IκBα or Ad-LacZ/Ad-eGFP as controls, glucose-stimulated insulin secretion was used as a functional assay. Islets were first treated with 50 units of recombinant human IL-1β (Sigma) for a period between 18 and 24 h immediately following a perfusion in fresh media between 16 and 24 h. The IL-1β-containing medium was removed, and the islets were washed twice with Krebs-Ringer-HEPES buffer (KRH buffer; 25 mM HEPES, pH 7.4, 115 mM NaCl, 24 mM NaHCO₃, 5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂). Incubation was carried out at 37 °C in KRH buffer for 30 min followed by an additional incubation for 30 min in the presence of 5 and 18 mM glucose (final concentration). The buffer was subsequently removed, and its insulin content was determined by a commercially available enzyme-linked immunosorbent assay kit (Dako Chemicals), which specifically recognizes processed human insulin.

To evaluate NO production, the islet culture supernatants were collected between 18 and 24 h following the addition of IL-1β, and an aliquot was subjected to the Griess reaction. The islet culture supernatants were collected between 18 and 24 h following the addition of IL-1β, and an aliquot was subjected to the Griess reaction.

**Determination of Apoptosis Activation in Vitro**—Uninfected islets, as well as those infected with Ad-eGFP as control or Ad-IκBα, were treated with 50 units of IL-1β for 24 h. Furthermore, a subset of islets untreated or pretreated with IL-1β were challenged with the agonistic human Fas antibody (clone CH-11, Upstate Biotechnology Inc.) for 1 h at 37 °C. Following incubation with antibody, islets were lysed and processed for the detection of caspase-3 (CPP32) activity using a commercially available kit (ApoAlert, CLONTECH, Palo Alto, CA). As an indirect means of correcting for cell number, the CPP32 activity was corrected by the number of nanograms of DNA in the lysate assayed, using the PhiCoGreen reagent, an intercalating DNA fluorogenic compound (Molecular Probes Inc.).

**NF-κB Reporter Gene Assay in Mouse Insulinoma Cells in Vitro**—To examine the effects of IκBα gene transfer on NF-κB activity in vitro, subconfluent NIT-1 insulinoma cells (derived from the non-obese diabetic mouse; ATCC CRL-2055) were infected with Ad-IκBα at a multiplicity of infection of between 40 and 80 in serum-free RPMI 1640 for 1 h at 37 °C. The medium was then replaced with medium containing 10% fetal calf serum. After 24 h, the cells were transfected with a luciferase reporter gene fused to five tandemly arrayed NF-κB consensus binding sites (Stratagene, La Jolla, CA) using the LipofectAMINE reagent as suggested by the manufacturer (Life Technologies, Inc., Gaithersburg, MD). 18–24 h later, the cells were treated with 50 units of IL-1β. Uninfected, transfected cells treated with or without IL-1β were used as controls. All cells were lysed 24 h later for luciferase assay using a commercially available kit (Promega, Madison, WI).

**Statistics**—Statistics were performed using the SPSS for Windows v. 8.0 package, and a p value of less than 0.05 by ANOVA was taken to indicate statistically significant differences.

**RESULTS**

**Adenoviral IκBα Gene Transfer to Islets Prevents IL-1β-induced β Cell Impairment and NO Production in Vitro**—Previous studies have demonstrated efficient adenoviral gene transfer to human and murine islets in culture with maintenance of normal β cell function as assessed by glucose-stimulated insulin release assays and glucose perfusions (35–37). In particular, we have demonstrated the ability to infect up to 70% of islet
cells and 50% of the \( \beta \) cells with an adenovector encoding the green fluorescence protein (38, 39). To determine if adenoviral gene transfer of the I\( \kappa \)B repressor was able to prevent the IL-1\( \beta \)-induced impairment of glucose-stimulated insulin release by cultured human islets, we exposed uninfected islets as well as those infected with Ad-eGFP and Ad-I\( \kappa \)B to 50 units of recombinant IL-1\( \beta \). This amount, over a 24-h period, was sufficient to impair the ability of islets to respond to a high glucose challenge (18 mM) in all islet cultures examined (Fig. 1). However, the islets infected with Ad-I\( \kappa \)B responded similarly to uninfected control islets to increasing glucose (Fig. 1 and Table 1). Why the level of insulin release at 5 mM of glucose is higher in Ad-I\( \kappa \)B-infected islets compared with the Ad-LacZ control is unclear. However, it is possible that there is low level activation of NF-k\( \beta \) during the islet isolation procedure, which is inhibited by Ad-I\( \kappa \)B gene transfer.

NO has been suggested to be the mediator of IL-1\( \beta \)-induced suppression of insulin production in a number of instances in rodent islets (2, 6, 9, 40, 41); however, its role as a mediator in human islets is not fully clear (10–12, 42, 43). We determined the level of nitrite in the media of uninfected, Ad-LacZ-infected, and Ad-I\( \kappa \)B-infected islets in the presence or absence of 50 units of IL-1\( \beta \) by the Griess reaction. Nitrite accumulation was detectable in uninfected islets as well as Ad-LacZ-infected islets in the absence of IL-1\( \beta \). No significant accumulation above control values occurred when islets were infected with Ad-LacZ alone (Fig. 2). However, exposure of uninfected islets to IL-1\( \beta \) resulted in a significant accumulation (Fig. 2). The largest accumulation was seen in cultures infected by Ad-LacZ followed by IL-1\( \beta \) exposure (375 ± 40% versus control, \( p < 0.05 \), Fig. 2). This result is similar to previous observations demonstrating that adenoviral infection is able to partially increase the expression of NF-k\( \beta \)-dependent genes like iNOS (44). However, gene transfer of I\( \kappa \)B was able to block both IL-1\( \beta \) and adenoviral-mediated induction of NO. More importantly, nitrite accumulation in the medium of Ad-I\( \kappa \)B-infected islets was no greater than control levels (100 ± 5% versus control, \( p = NS \), Fig. 2).

Ad-I\( \kappa \)B-infected Islets in Culture Are Protected from IL-1\( \beta \)-induced, Fas-mediated Apoptosis Activation—Cross-linking of the Fas antigen on a number of cell types using an IgM antibody has been shown to activate caspase-3 activity and apoptosis (45–48). For these experiments, we used Ad-eGFP as a control vector, because we have previously demonstrated that Ad-eGFP infection, like Ad-LacZ, of human islets in vitro does not affect their function or apoptosis activation (38, 39). Uninfected, Ad-eGFP-infected, and Ad-I\( \kappa \)B-infected islets were first exposed to 50 units of IL-1\( \beta \) for 18–24 h and then treated with the agonistic Fas antibody for 1 h. Caspase-3 activity was significantly suppressed in islets infected with Ad-I\( \kappa \)B compared with uninfected and Ad-eGFP-infected controls (Fig. 3). Ad-I\( \kappa \)B also was able to reduce apoptosis following IL-1\( \beta \) treatment but had no effect following the addition of Fas antibody. However, gene transfer of I\( \kappa \)B significantly suppressed the increase in apoptosis following treatment with IL-1\( \beta \) and subsequent treatment with the Fas antibody (Fig. 3). To rule out the possibility that the protection conferred to islets against Fas-triggered apoptosis activation was due to adenovirus-mediated down-regulation of Fas, islets were infected with a replication-defective herpes simplex-1 vector encoding I\( \kappa \)B (HSV-I\( \kappa \)B) or LacZ as control (HSV-LacZ). Following exposure of the islets to IL-1\( \beta \) and the agonistic Fas antibody, a decrease in caspase-3 activity was observed in islets infected with HSV-I\( \kappa \)B compared with the uninfected control (data not shown).

Ad-I\( \kappa \)B Gene Transfer to Insulinoma Cells In Vitro Prevents IL-1\( \beta \)-stimulated NF-\( \kappa \)B Activity—To demonstrate that I\( \kappa \)B gene transfer inhibited NF-\( \kappa \)B activity, we performed transient transfection assays in Ad-I\( \kappa \)B-infected NIT-1 insulinoma cells with an NF-\( \kappa \)B-luciferase construct. In Fig. 4 we show that NF-\( \kappa \)B-luciferase reporter activity is significantly increased in uninfected cells treated with IL-1\( \beta \) (159 ± 10% versus control, \( p < 0.05 \)). Ad-I\( \kappa \)B infection followed by transient transfection with the reporter resulted in reporter gene activity at levels lower than transfected control cells (57 ± 6% versus control, \( p < 0.05 \)). More importantly, exposure of Ad-I\( \kappa \)B-infected, reporter gene-transfected cells to IL-1\( \beta \) did not lead to reporter gene activity at levels higher than those seen in uninfected, transfected control cells (65 ± 13% versus control, \( p < 0.05 \)).

**DISCUSSION**

Previous studies have demonstrated that adenoviral infection of intact human islets in culture does not change their viability or functional characteristics (36–39). Therefore, we have used adenoviral gene transfer to intact human islets in vitro to demonstrate that a human I\( \kappa \)B repressor can suppress IL-1\( \beta \)-induced impairment of glucose-stimulated insulin secretion. There was no increase in nitrite accumulation in the media of Ad-I\( \kappa \)B-infected islets exposed to IL-1\( \beta \) compared with
and NF-κB in response to the viral proteins. Nitrite, detected in the Ad-LacZ-infected islets, was derived from endothelial cells in addition to endocrine cells. Islets contain a heterogeneous cell population including alpha, beta, and delta cells. We have used islets isolated by a procedure that offers minimal cell damage. The islets we have used have been isolated by a procedure that offers minimal cell damage. We routinely used islet preparations of 80% or greater as assessed by microscopic morphology and insulin staining of intact islets and single cell cultures in addition to dithizone staining following a new and improved isolation protocol (34). Although there is strong support for IL-1β alone inducing NO production and β cell dysfunction in rat islets, this does not always appear to be the case for human β cells (2, 8, 10–12, 51). Although it is unclear why there is such variability of islet viability, some factors that could have influenced our findings includes: 1) the degree of islet contamination by non-endocrine, exocrine, or other pancreatic cells is minimal. We routinely used islet preparations of 80% or greater as assessed by microscopic morphology and insulin staining of intact islets and single cell cultures in addition to dithizone staining following a new and improved isolation protocol (34); 2) Variability of islet viability and function. We have used islets isolated from pancreata obtained from multiple donors of different ages and sex; and 3) Isolation-associated cell damage. The islets we have used have been isolated by a procedure that offers minimal cell trauma/damage (34). One important difference between our procedures and those in some other studies, where IL-1β alone could not promote NO production or β cell impairment, involves the culture conditions of the islets following isolation. It is possible that different culture conditions may promote β cell resistance to the effects of IL-1β by inducing the down-regulation (internalization) of the type I IL-1 receptor. Consequently, the relocation of the type I IL-1 receptor to the cell surface would require stimulation by cytokines like TNFα or interferon γ.

Ad-IκB infection was able to prevent the IL-1β-induced, Fas-triggered activation of caspase-3 activity. It thus appears that NF-κB activity is important for Fas-mediated apoptosis in β cells. Alternatively, NF-κB activity could be required for expression of Fas. Basal caspase-3 activity in islets was also significantly reduced following Ad-IκB infection, suggesting that NF-κB is activated in islets during the isolation procedure. Thus, IκB gene transfer could be useful to preserve islet function prior to, and following islet transplantation.

Apoptosis is a process involving the activity of caspases. To date, caspase-3 activation commits all cells examined, including β cells to apoptosis, and is an early step in mediating Fas signaling (52–56). In response to Fas ligation by the CH-11 monoclonal antibody, caspase-3 activation is seen as early as 5 min in a fibrosarcoma cell line (45). Although we have not formally ruled out the possibility that caspase-3 activity in our cultures partly derives from non-β cells, that caspase-3 activity is at control levels in islets expressing IκB demonstrates its utility to protect islet cells from the apoptotic Fas trigger.

NF-κB is a transcriptional complex composed of homo- or heterodimers of proteins belonging to the Rel family of transcription factors. Activation of NF-κB-dependent transcriptional processes usually occurs in response to inflammatory signals such as cytokines, however, it may also be associated with stress responses in a manner analogous to heat-shock proteins (57, 58). Although very little is known about non-inflammatory effects of NF-κB-dependent gene expression as well as its targets in β cells under non-pathologic situations, this is an important area that requires further understanding to appropriately modulate repression of NF-κB in gene transfer strategies. For example, levels of IκB expression may have to be regulated to promote protection against pro-inflammatory cytokines, yet preserving normal NF-κB responses to stress (57, 58). This could be achieved using gene transfer strategies using vectors with regulatable promoters like tetracycline or mifepristone (RU-486) (59–63). To prevent potential interference with normal cell function, other complementary strategies like cytokine blockade can be used. We have demonstrated such an approach by interfering with the effects of IL-1β, an upstream activator of NF-κB, using an adenoviral vector the interleukin-1 receptor antagonist protein in human islets in vitro (39).

In this report, we have demonstrated the feasibility of using an adenoviral vector encoding an IκBα repressor to infect human islets as a means of preventing IL-1β-induced impairment of β cell function. Moreover, we have demonstrated that IκBα transduction of islets can suppress NO production in the presence of IL-1β as well as Fas-triggered caspase-3 activation, an early marker of apoptosis induction. We suggest that IκBα gene transfer to islets may be a means of preserving their integrity and promoting their survival and function prior to and following transplantation into diabetic hosts as a potential therapy for type I diabetes.

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