

Adenoviral Gene Transfer of the Interleukin-1 Receptor Antagonist Protein to Human Islets Prevents IL-1 β -Induced β -Cell Impairment and Activation of Islet Cell Apoptosis In Vitro

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The β -cells in the pancreatic islets of Langerhans are the targets of autoreactive T-cells and are destroyed in type 1 diabetes. Macrophage-derived interleukin-1 β (IL-1 β) is important in eliciting β -cell dysfunction and initiating β -cell damage in response to microenvironmental changes within islets. In particular, IL-1 β can impair glucose-stimulated insulin production in β -cells in vitro and can sensitize them to Fas (CD95)/FasL-triggered apoptosis. In this report, we have examined the ability to block the detrimental effects of IL-1 β by genetically modifying islets by adenoviral gene transfer to express the IL-1 receptor antagonist protein. We demonstrate that adenoviral gene delivery of the cDNA encoding the interleukin-1 receptor antagonist protein (IL-1Ra) to cultured islets results in protection of human islets in vitro against IL-1 β -induced nitric oxide formation, impairment in glucose-stimulated insulin production, and Fas-triggered apoptosis activation. Our results further support the hypothesis that IL-1 β antagonism in situ may prevent intra-islet proinsulinitic inflammatory events and may allow for an in vivo gene therapy strategy to prevent insulinitis and the consequent pathogenesis of diabetes. *Diabetes* 48:1730–1736, 1999

Type 1 diabetes is an autoimmune disorder culminating in the destruction of the insulin-producing β -cells of the pancreas. In both humans and the nonobese diabetic (NOD) mice, the immunopathology is characterized by an early-onset insulinitis with a significant proportion of the invading cells consisting of

T-cells (1,2). There is very strong evidence that activated T-cells can directly damage β -cells by secreting proinflammatory interleukins (1,3). Recent observations indicate that a substantial amount of the β -cell death may also be due to Fas-mediated apoptosis. The Fas antigen (CD95/apoptosis antigen-1) is a transmembrane molecule that belongs to the tumor necrosis factor (TNF) family of receptor proteins and is involved in the induction of apoptosis of a wide variety of cells (4). Several different lines of evidence suggest that apoptosis of β -cells in NOD mice occurs as early as 3 weeks of age and is mediated through Fas-FasL interaction. In particular, T-cells are able to induce apoptosis of β -cells through direct interaction of FasL on T-cells with Fas expressed specifically on β -cells.

In addition to dendritic cells, macrophages can efficiently present antigen and act as important initiators of inflammation. Immunohistochemistry and morphology have demonstrated the increase in infiltrating macrophages just before and at onset of insulinitis in the NOD mouse (5). The earliest event after macrophage activation is the synthesis and secretion of interleukin-1 β (IL-1 β), a proinflammatory cytokine with pleiotropic actions on many cell types including β -cells. IL-1 β induces the expression and enhances the activity of the inducible form of nitric oxide synthase (iNOS), resulting in elevated levels of nitric oxide (NO) (6–10). IL-1 β has been shown to be the initiating cytokine that is directly responsible for the impairment of glucose-stimulated insulin production in mouse, rat, and human islets in vitro (8–10). IL-1 β also stimulates the cell surface expression in murine and human islets of Fas, and evidence is accumulating that it may be NO-dependent (11,12). That nitric oxide is a direct mediator of IL-1 β -stimulated impairment of glucose-stimulated insulin secretion by islets and single dispersed islet cells in vitro derives from a series of studies using L-N^G-monomethyl arginine, an inhibitor of iNOS, to prevent IL-1 β -induced impairment of glucose-stimulated insulin production (6,7,9,10,13,14). The IL-1 β -stimulated decrease of cell viability in vivo in rats is inhibited after the administration of chemical inhibitors of iNOS (15). Earlier studies showed that cytokines such as TNF- α and interferon (IFN)- γ could also impair β -cell function, but more recent data strongly indicate that these cytokines act on resident macrophages to produce IL-1 β , which can then bind the signaling type 1 IL-1 receptor that is expressed on β -cells and mediates the IL-1 β signal (6,7,16–18). These observations strongly suggest that inhibition

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Ad-eGFP, adenoviral vector encoding enhanced green fluorescent protein; Ad-LacZ, adenovirus encoding β -galactosidase; CPP32, caspase-3; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist protein; iNOS, inducible nitric oxide synthase; pfu, plaque-forming unit; TNF, tumor necrosis factor.

of IL-1 β binding to the type 1 IL-1 receptor or inhibition of nitric oxide production within islets may prevent the impairment and destruction of β -cells and could, perhaps, prevent insulinitis. In fact, blocking IL-1 β using an antibody prevented the induction of insulinitis and diabetes in cyclophosphamide-induced diabetic NOD mice (19).

A naturally occurring inhibitor of IL-1 β action is the interleukin-1 receptor antagonist protein (IL-1Ra). It binds to the type 1 IL-1 receptor, which transmits the biological actions of IL-1 in a competitive manner and does not bind the type 2 receptor believed to act as a scavenger of IL-1 β (20–26). IL-1Ra was shown to be effective in recombinant form in suppressing NO production and the IL-1 β -stimulated and NO-mediated suppression of insulin production after a glucose challenge to islets and purified β -cells in vitro (7,18). In vivo, recombinant IL-1Ra given as a continuous infusion into NOD mice prevented the loss of syngeneic islet transplants, suggesting that IL-1 β blockade can interfere not only with insulinitis onset but also with existing autoreactivity (27). The high molar excess required for IL-1 β competitive binding, however, indicates that in order for IL-1Ra, or any other IL-1 β antagonists for that matter, to be effective locally, they must be expressed at high levels in a continuous manner.

In this report, we have examined the ability to block the detrimental effects of IL-1 β by genetically modifying human islets to express IL-1Ra. We demonstrate that replication-defective adenovirus is an efficient delivery vehicle for the cDNA encoding IL-1Ra to human islets in vitro. We also show that the gene product is secreted at significant levels. More importantly, we confirm that IL-1Ra expressed from the adenoviral vector can suppress NO production and can prevent IL-1 β -induced impairment of β -cell function. Finally, human islets genetically engineered to express IL-1Ra can be protected from IL-1 β -induced and Fas-triggered apoptosis activation. Taken together, these results suggest that local gene transfer of inhibitors of IL-1 β action may be an effective means of preventing insulinitis onset and perhaps diabetes. The modification of allograft islets to express IL-1Ra may also improve islet function after transplantation.

RESEARCH DESIGN AND METHODS

Generation of recombinant adenoviruses. E1- and E3-deleted adenoviral vector encoding enhanced green fluorescent protein (Ad-eGFP) was constructed as described (28,29) by Cre-lox recombination with reagents generously provided by S. Hardy (Somatix, Alameda, CA). Briefly, a *SnaBI-HpaI* fragment containing part of the cytomegalovirus promoter, the eGFP cDNA, and part of the SV40 poly(A) sequence was inserted in the pAdlox shuttle plasmid. E1-substituted recombinant adenovirus was generated by cotransfection of *SfiI*-digested pAdlox-transgene and ψ 5 helper virus DNA into the adenoviral packaging cell line CRE8, propagated, and purified as described (28). The E1- and E3-deleted adenovirus encoding β -galactosidase (Ad-LacZ) was provided by I. Kovesdi (Genvec, Rockville, MD) whereas the Ad-IL-1Ra virus was provided by Amgen (Boulder, CO). The transgene is driven by the CMV promoter in both the Ad-LacZ and Ad-IL-1Ra vectors.

Isolation of human islets. Pancreata from multiorgan cadaver donors were provided by the National Disease Research Interchange (Philadelphia, PA) and local organ procurement organizations with the appropriate consent for research use. Human islets were obtained using the automated method and Liberase-HI enzyme blend (Roche/Boehringer Mannheim, Indianapolis, IN) for tissue dissociation (30). The islets were purified by centrifugation on discontinuous density gradients using a COBE-2991 Cell Processor (Cobe, Lakewood, CO) (30). We routinely obtained islets with a purity >80%. We have used islets from at least 10 different pancreata. The time between pancreas procurement and islet isolation was routinely <2 days. All the experiments were performed on the islets derived from a single donor in triplicate and repeated on at least four occasions on islets from subsequent donors.

Gene transfer of β -galactosidase, eGFP, and human IL-1Ra to islets in culture using E1- and E3-deleted adenoviral vectors. Islets were washed twice in serum-free RPMI 1640 (Gibco-BRL, Gaithersburg, MD) supplemented with

a 1% penicillin/streptomycin solution (Gibco-BRL) before infection. There were 200–300 islets infected with Ad-LacZ, Ad-eGFP, or Ad-IL-1Ra at a plaque-forming unit (pfu) of 1×10^6 in a minimal volume of serum-free RPMI 1640 for 2–4 h at 37°C. After the infection, the islets were washed twice in serum-free medium and then once with medium containing 10% heat-inactivated fetal calf serum (Gibco-BRL). Islets were then incubated at 37°C in medium with serum for 2 days, after which all the 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 and evaluation of β -cell function after IL-1 β treatment. β -Galactosidase was detected by X-gal staining, and eGFP fluorescence was visualized microscopically under standard excitation/emission parameters. Secreted IL-1Ra protein was detected in the culture supernatants 2 days after infection using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN). This also coincided with the day of IL-1 β treatment in the designated cultures. To assess the effects of IL-1 β on β -cell function of genetically modified and unmodified islets, we used glucose-stimulated insulin secretion as a functional assay. Islets were first treated with 50 U of recombinant human IL-1 β (Sigma) for a period between 18 and 24 h immediately after a preincubation in fresh media for 16–24 h. The IL-1 β -containing medium was removed, and the islets were washed twice with Krebs-Ringer HEPES buffer (25 mmol/l HEPES, pH 7.4, 115 mmol/l NaCl, 24 mmol/l NaHCO₃, 5 mmol/l KCl, 2.5 mmol/l CaCl₂, 1 mmol/l MgCl₂). Incubation was carried out at 37°C in Krebs-Ringer HEPES buffer for 30 min followed by an additional incubation for 30 min in the presence of 5 or 18 mmol/l glucose (final concentration). The buffer was subsequently removed, and its insulin content was determined by a commercially available ELISA kit (Dako Chemicals, Carpinteria, CA) that specifically recognizes processed insulin.

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

Assessment of apoptosis activation in vitro. Uninfected islets, as well as those infected with Ad-eGFP or Ad-IL-1Ra, were treated with 50 U IL-1 β for 24 h as described above. Furthermore, a subset of islets (pretreated or not with IL-1 β) were challenged with the agonistic human Fas antibody (clone CH-11; Upstate Biotechnology, Waltham, MA) for 1 h at 37°C. At the end of the incubation, islets were lysed and processed for the detection of caspase-3 (CPP32) activity using a commercially available kit (ApoAlert, Clontech, 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 PicoGreen reagent, an intercalating DNA fluorogenic compound (Molecular Probes, Eugene, OR).

Statistical analysis. Statistics were performed using the SPSS for Windows v. 8.0 package, and differences among experimental groups at a *P* value <0.05 using the two-tailed Student's *t* test were considered statistically significant.

RESULTS

IL-1Ra is produced at high levels from genetically modified human islets in vitro. Recent results from a number of laboratories have demonstrated that adenoviruses are very efficient at infecting human and rodent islets in vitro (31). We have examined the ability to infect human islets with adenoviral vectors at low multiplicities of infection using Ad-eGFP and Ad-IL-1Ra vectors. We routinely cultured 200–300 human islets for no more than 10 days after removing them from the cadaveric donor. Within this period, the islets were infected in serum-free medium. Representative results of human islets infected with 1×10^6 pfu of Ad-eGFP (Fig. 1A) and Ad-LacZ (Fig. 1B) or mock-infected islets (Fig. 1C) under fluorescence microscopy are shown in Fig. 1. Approximately 70% of the cells within the islet were infected with the eGFP virus as determined by dispersing the cells followed by FACS analysis (data not shown; N.G., Z. Mi, A.G., A. Eramo, C.R., M.T., P.D.R., unpublished observations). The secretion of IL-1Ra into the culture supernatant of Ad-IL-1Ra-infected islets using commercially available ELISA kits was also examined. Table 1 shows that IL-1Ra was secreted to levels as high as 31 ng/48 h with no detectable IL-1Ra in the media of uninfected islets or in those of islets infected with Ad-LacZ or Ad-eGFP. These results demonstrate that human islets can be infected efficiently with adenoviral vectors and that they can secrete significant levels of IL-1Ra.

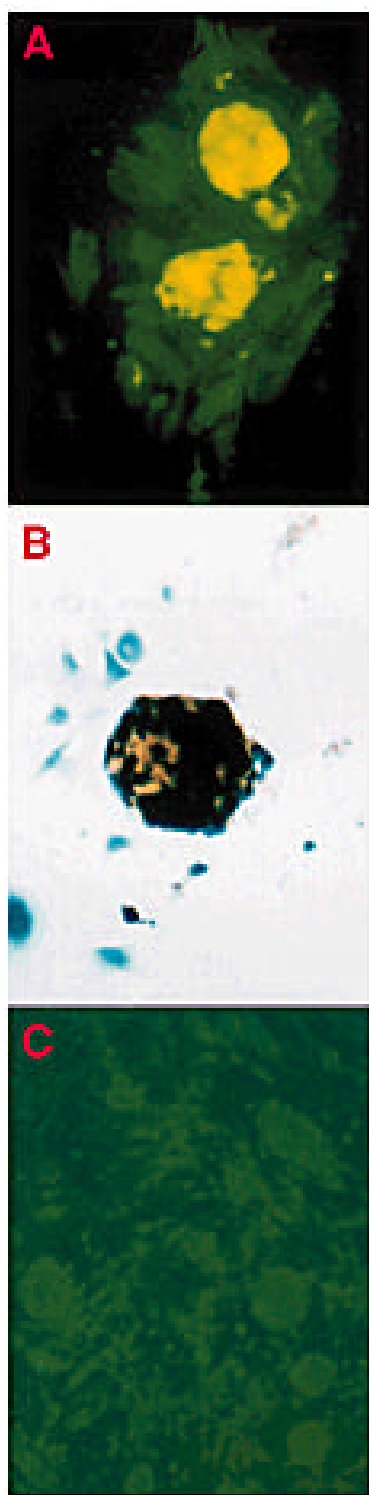


FIG. 1. Reporter gene expression in adenovirally infected human islets in vitro. The efficiency of E1-deleted adenoviral vectors to infect human islets in vitro was tested using Ad-eGFP or Ad-LacZ at a pfu of 1×10^6 per 200 islets. Then, 48 h later, infected as well as mock-infected islets were stained with X-gal (Ad-LacZ) or directly visualized for eGFP fluorescence. A representative set of islets are shown. *A*: Ad-eGFP-infected islets are the intensely fluorescent spherical cellular aggregates (original magnification $\times 200$); *B*: Ad-LacZ-infected islet is the X-gal-positive spherical cell aggregate in the middle of the panel (original magnification $\times 200$); *C*: mock-infected islets visualized under fluorescence microscopy (original magnification $\times 20$). Besides intact islets, single islet cells and fibroblasts are also infected by adenoviral vectors (*B*).

TABLE 1
IL-1Ra production (ng/ml)

Control	Ad-LacZ/Ad-eGFP	Ad-IL-1Ra
Undetectable	Undetectable	31 ± 0.76

Production of IL-1Ra by human islets infected with Ad-IL-1Ra in vitro. Groups of 200 islets were infected with 1×10^6 pfu of Ad-LacZ, Ad-IL-1Ra, or mock-infected as described in METHODS. The culture media were collected 48 h after infection, and IL-1Ra was measured by ELISA, performed in triplicate.

Adenoviral gene transfer of IL-1Ra leads to protection from IL-1 β -induced impairment of insulin secretion after a glucose challenge.

The ability of genetic modification of islets by Ad-IL-1Ra to inhibit or suppress the IL-1 β -induced impairment of insulin secretion in vitro in response to a glucose stimulation (9,14) was examined. In parallel, the IL-1 β -induced production of NO by infected and uninfected islets was also examined. The percent changes in insulin secretion are shown in Fig. 2 with the actual insulin secretion profile for each treatment group shown in Table 2. The production of insulin by untreated uninfected islets in the presence of 5 mmol/l glucose is taken as control (100%). In uninfected islets, insulin secretion in response to high glucose concentration (18 mmol/l final) was impaired in islets exposed to IL-1 β . In contrast, the islets that were infected with adenovirus encoding IL-1Ra maintained a normal insulin secretory response to glucose after IL-1 β treatment. The response was almost identical to unmodified islets that were not treated with IL-1 β , although the amount of insulin secreted in the presence of 5 mmol/l glucose in Ad-IL-1Ra-infected islets was greater than that from uninfected islets exposed to the same concentration of glucose. The dif-

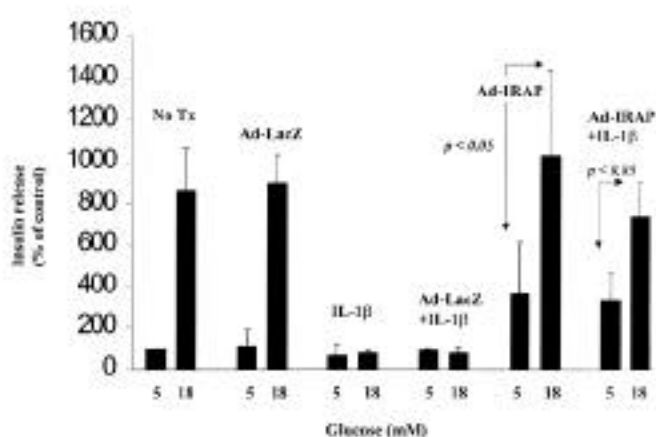


FIG. 2. Glucose-stimulated insulin release from human islets infected with Ad-LacZ, Ad-IL-1Ra, or mock-infected followed by an incubation with or without IL-1 β . There were 200 islets infected with the indicated viruses as described in METHODS. IL-1 β (50 U) was added 48 h after the infection, immediately after the incubation of islets in fresh media for 5–7 h. The incubation in the presence of IL-1 β was continued for another 18–24 h. Glucose-stimulated insulin release was performed as described in METHODS, and insulin was detected by ELISA in the buffer. All experiments were performed in triplicate and represent the percent above control, where the production of insulin by untreated uninfected islets in the presence of 5 mmol/l glucose is taken as control (100%). The error bars indicate the SE.

TABLE 2
Glucose-stimulated insulin release assay

Islet status	Insulin release	
	5 mmol/l glucose	18 mmol/l glucose
Control	100	866 ± 200
Ad-LacZ	113 ± 80	900 ± 133
IL-1 β	66 ± 53	80 ± 13
Ad-LacZ + IL-1 β	93 ± 6	80 ± 26
Ad-IL-1Ra	364 ± 250	1,029 ± 400
Ad-IL-1Ra + IL-1 β	333 ± 133	733 ± 166

Data are % ± SE.

ferences in the means were statistically significant ($364 \pm 250\%$ at 5 mmol/l vs. $1,029 \pm 400\%$ at 18 mmol/l, $P < 0.05$). The adenovirus itself does not affect islet response to high glucose levels in the absence of IL-1 β as demonstrated using an adenovirus encoding the β -galactosidase transgene (Ad-LacZ). Furthermore, Ad-LacZ-infected islets respond to IL-1 β in an identical manner as do the mock-infected islets.

IL-1Ra expression suppresses NO production. The ability of genetic modification of islets by Ad-IL-1Ra to inhibit or suppress the IL-1 β -induced NO production was examined next. Total NO production in the culture supernatants of IL-1 β -treated unmodified and modified islets was determined using the Griess reaction to measure nitrite in the culture supernatant. At 72 h after the infection with Ad-IL-1Ra or Ad-LacZ, the media of the cultures were replaced with fresh media, and then after an additional 16–24 h, 50 U of IL-1 β were added for a further 18–24 h of incubation. Normal islets and those infected with Ad-LacZ produced detectable levels of NO in the absence of any IL-1 β in all experiments. This level increased significantly after IL-1 β treatment. In cultures that were infected with Ad-IL-1Ra, NO production was not statistically different from that detected in islets not subjected to IL-1 β . Ad-LacZ-infected islets exposed to IL-1 β produced the most NO, whereas exposure of Ad-IL-1Ra-infected islets

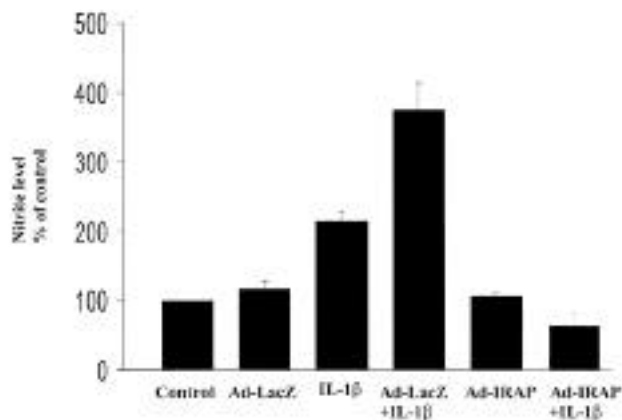


FIG. 3. NO production by human islets infected with Ad-IL-1Ra in vitro. Nitrite levels in the culture media of islets (groups of 200) infected with Ad-LacZ or Ad-IL-1Ra at a pfu of 1×10^6 , or mock-infected, as detected by the Griess reaction, were used as a marker of NO production in the presence and absence of IL-1 β . Bars indicate the means of three separate experiments, each performed in triplicate (expressed as the percent of control where the nitrate level in uninfected control islet is taken as 100%), and the error bars indicate the SE.

to IL-1 β had no effect on stimulating NO production relative to mock-infected islets. Finally, islets infected with Ad-IL-1Ra appeared to have a reduction in the amount of basal NO produced (Fig. 3).

Adenoviral delivery of IL-1Ra suppresses IL- β -induced Fas-triggered apoptosis activation of islets in vitro. Fas expression at the cell surface of islets is increased after IL-1 β treatment, but recent reports indicate that IL-1 β itself may also induce apoptosis in a Fas-independent manner (11,12). To examine whether islet-derived IL-1Ra could prevent IL-1 β -induced Fas-mediated apoptosis activation after adenoviral gene transfer, CPP32 activity was used a marker. CPP32 is specifically stimulated after ligation of the Fas antigen, and its activity is therefore a reliable marker of apoptosis induction (32–34). CPP32 activity was examined 1 h after treating islet cultures with the agonistic Fas antibody (CH-11) with activity corrected for cell number by determining the total amount of DNA in the cell lysate using PicoGreen. As shown in Fig. 4, CH-11 treatment of control or Ad-eGFP-infected islets after IL-1 β treatment induces significant CPP32 activity relative to untreated islets. In contrast, infection with Ad-IL-1Ra completely protected the islets from CPP32 activation after IL-1 β and Fas antibody treatment. Interestingly, the level of CPP32 activity in untreated or CH-11-treated control islets also was significantly reduced by infection with Ad-IL-1Ra. Why CPP32 levels were somewhat elevated in Ad-IL-1Ra-infected islets after IL-1 β exposure is unclear, but is currently under investigation. However, taken together, these results demonstrate that expression of IL-1Ra by islets is able to suppress Fas-mediated apoptosis after IL-1 β treatment.

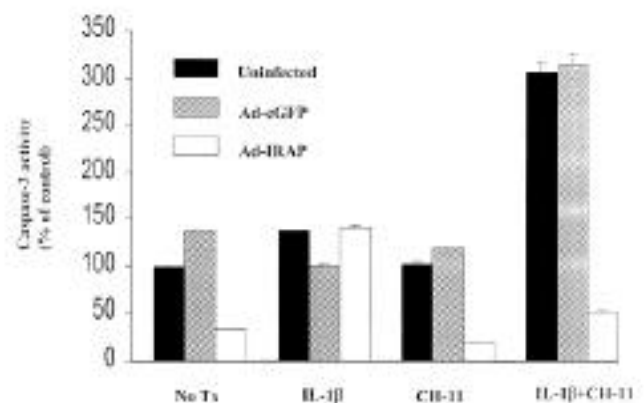


FIG. 4. CPP32 activity in human islets infected with Ad-IL-1Ra in vitro. IL-1 β -stimulated Fas-triggered apoptosis induction was assessed in groups of 200 islets infected with 1×10^6 pfu of Ad-eGFP, Ad-IL-1Ra, or mock-infected islets. At 48 h after infection and after an incubation in fresh medium for an additional 5–7 h, islets were exposed to 50 U of IL-1 β for a period between 18 and 25 h. The incubation was continued for an additional hour in the presence of the agonistic Fas antibody (CH-11). Islets were collected, lysed, and assayed for CPP32 activity using the ApoAlert CPP32 kit (indicated in METHODS). Enzyme activity was corrected by DNA content in the lysate using a fluorescent dye that intercalates in DNA quantitatively (picomoles of CPP32 per hour divided by nanograms of DNA detected in the lysate; this value was normalized by the appropriate dilution factors). Bars indicate the means of three separate experiments, each performed in triplicate. The values are expressed as percent of control, where the mean of the CPP32 activity to DNA content ratio in uninfected untreated control islets is taken as 100%.

DISCUSSION

An important role for IL-1 β -induced activation of antigen-presenting cells that can in turn stimulate circulating autoreactive β -cell-specific lymphocytes that provoke β -cell destruction has been suggested based on a number of direct and indirect observations. First, the main producer of IL-1 β is the macrophage, an immune cell that is resident, albeit at very low numbers, within islets and that constitutes part of the first wave of insulinitis (5,6,17,35–37). Second, depletion of islet-resident macrophages in diabetes-prone BB rats and in mice of the CD-1 strain rendered diabetic with streptozotocin (with silica particle injection) completely prevented diabetes (38,39). Third, inhibitors of IL-1 β binding to its signaling receptor prevent downstream effectors of β -cell dysfunction and apoptosis. Prevention of insulinitis and diabetes has been achieved with an antibody against IL-1 β in cyclophosphamide-treated NOD mice, recombinant IL-1Ra, and a soluble type 1 IL-1 receptor in NOD mice (19,27,40).

IL-1 β promotes the expression and enhances the activity of iNOS. This enzyme generates NO, which is involved in the suppression of glucose-stimulated insulin release by islets in vitro as well as the direct and indirect induction of apoptosis—the latter after the upregulation of Fas on β -cells (11,12). IL-1 β may also be important for the initiation of disease. Although the agent(s) responsible for triggering islet inflammation remains unknown, recent evidence points to the role of viruses like Coxsackie B, which can activate resident antigen-presenting cells (41–43). Viral infection of islets can result in local nonspecific destruction of cells that can, in a bystander-type of β -cell destruction with ensuing release of β -cell proteins, promote the uptake of these antigens by resident macrophages and dendritic cells. These cells in response could secrete proinflammatory cytokines including IL-1 β , which can further amplify opposing β -cell dysfunction and possibly induce apoptosis. In fact, recent observations have been made that support this hypothesis (44,45).

Given the importance of IL-1 β in type 1 diabetes, we have examined the feasibility of inhibiting IL-1 β effects on islet function by genetically engineering human islets in vitro to express an IL-1 β antagonist. We have demonstrated that infection of human islets with a replication-defective adenoviral vector expressing IL-1Ra can protect against IL-1 β -induced NO formation, impairment of glucose-stimulated insulin production, and Fas-triggered apoptosis activation. Thus, local production of IL-1Ra by genetically modified islets is highly effective in blocking multiple detrimental effects of IL-1 β on islet function. Interestingly, we observed a consistent potentiation of insulin secretion in the presence of 5 mmol/l glucose in islets expressing IL-1Ra. It is possible that the IL-1Ra-mediated suppression of basal NO production could lower the threshold for glucose sensing by β -cells, although we are unaware of any effects of NO on the expression or activity of the glucose-sensing apparatus such as glucokinase or GLUT2. Additionally, it is possible that the lower levels of NO may affect intracellular second messengers downstream of the glucose-sensing apparatus that affect insulin secretion. Our results also demonstrate that adenoviral gene transfer to islets of reporter genes (LacZ and eGFP) and of an antagonist of IL-1 β action (IL-1Ra) does not impair the in vitro insulin secretory response of β -cells to exogenously added glucose.

IL-1Ra is not normally secreted by islets, but it can be produced by islet resident macrophages as well as by fibroblasts in response to an inflammatory signal. Based on previous quantitation of IL-1Ra production from cultured human peripheral blood monocyte-derived macrophages, we find it highly unlikely that the IL-1Ra we detect from intact islets could be due to endogenous production from islet-resident macrophages instead of from the vector. Janson et al. (46) have demonstrated that cultured macrophages secrete 3.65×10^{-7} ng \cdot cell $^{-1}$ \cdot h $^{-1}$ IL-1Ra. We calculate that IL-1Ra is being secreted at a rate of 3.1×10^{-6} ng \cdot cell $^{-1}$ \cdot h $^{-1}$ from intact islets (assuming 1,000 cells per islet and an infection efficiency of 70%). There are at most 15 resident macrophages per islet (16,17), and based on our calculations, it is highly unlikely that the secreted IL-1Ra could have been derived from these cells. Although we have not extensively tested islet function by assays other than glucose-stimulated insulin secretion, we do not believe that high levels of IL-1Ra have negative effects on islets. Continuous infusion of IL-1Ra into NOD mice prevents insulinitis and diabetes onset and prolongs syngeneic islet graft survival (27). Moreover, in human clinical trials, systemic administration of IL-1Ra has proven beneficial for rheumatoid arthritis without any noticeable side effects (47).

In addition to blocking IL-1 β -mediated islet dysfunction, our results also demonstrate that IL-1Ra expression by islets clearly protected against IL-1 β -stimulated Fas-triggered apoptosis induction as detected by CPP32 activity. A few observations are worth mentioning at this point. First, it appears that the CPP32 activity in uninfected islets in culture can be reduced in the presence of IL-1Ra. Although the CPP32 activity in normal islets may reflect a natural response to the trauma, possibly involving IL-1 β , that the islets experienced at the time of pancreas procurement and/or during the isolation procedure, it could be that β -cells are highly susceptible to apoptosis and that the continuous presence of local trophic/survival factors in the islet may prevent the activation of apoptosis. We also observed a higher level of CPP32 activity in Ad-eGFP-infected islets that is paralleled by an increase in NO levels relative to normal uninfected islets. This suggests that adenoviral infection may trigger intrinsic antiviral defense mechanisms that might include increased NO production with concomitant apoptosis activation. This may most likely occur because of the activation of islet-resident macrophages. In fact, activated mouse macrophages in response to flavivirus infection in vitro do produce NO (48). Chronic hepatitis C virus infection in humans results in an augmentation of inducible NO synthase expression and activity in patient liver tissue (49). The replication of Coxsackie B3 virus leading to myocarditis was attenuated in a NO-dependent manner consequent to infection-induced upregulation of iNOS expression in mice (50). More importantly, adenoviral infection can lead to stimulation of NO production and this can impede the efficiency of adenoviral-mediated gene transfer (51,52). We have observed that IL-1 β treatment of Ad-LacZ-infected islets yields the highest nitrite levels among all islets examined, whereas Ad-LacZ infection alone does not lead to nitrite levels any higher than control. This could be due to the low number of resident macrophages that become activated, yet require IL-1 β to produce NO and to affect NO production from islet cells.

Ad-IL-1Ra-infected islets appear still to be sensitive to the low level of IL-1 β -mediated, but not CH-11-mediated, CPP32

activation. The reason for this effect is currently unknown. Nonetheless, our results demonstrate the feasibility of preventing IL-1 β -induced impairment of human islet β -cell function after gene transfer of an IL-1 β antagonist. Additionally, our results demonstrate that IL-1Ra expressed from an adenoviral vector can protect against IL-1 β -induced Fas-triggered apoptosis. That we are able to achieve protection against IL-1 β doses 10 times higher than those already shown to impair dynamic insulin secretion concomitant with increased NO production lends further support to the therapeutic potential of IL-1Ra gene transfer to islets. It is interesting to note that although 5 U of IL-1 β alone can impair glucose-stimulated insulin release (6,9,14,35), 50 U are required to fully sensitize human islets to Fas-triggered apoptosis (12). Thus, it could be possible that divergent pathways exist for IL-1 β -stimulated impairment of glucose-stimulated insulin production and sensitization to Fas signaling. Regardless of the mechanism, these experiments pave the way for testing IL-1Ra gene transfer to islets in preventing insulinitis and diabetes onset in genetically diabetic murine models as well as for application in allogeneic islet transplantation.

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