Immunosuppressive drugs are routinely used to provide tolerance after whole pancreas and islet cell transplantations. While they are essential in inhibiting graft rejection, little is known about their effect on islet function and β-cell viability. In this study, we report that tacrolimus, sirolimus, and mycophenolic acid, when added to cultures of freshly isolated human islets, induce a downregulation of the synthesis and secretion of insulin. These functional changes are associated with decreased islet cell viability. All three agents induce a decrease of intracellular levels of Bcl-2 and Bcl-xL, with an increased level of Smac, indicating that they are capable of promoting a downregulation of anti-apoptotic factors and an accumulation of pro-apoptotic mediators. Transduction of islet cells with the anti-apoptotic gene XIAP prevents the negative effects of these drugs on the function and viability of islets. XIAP-infected cells show a higher expression of phospho-CREB (cAMP-responsive element binding protein) and a reduced level of Smac, resulting in a significant reduction of apoptotic cells and a preservation of the glucose-dependent secretion of insulin. In conclusion, the present study demonstrates that genetically modified human islets expressing XIAP are resistant to the negative effects of immunosuppressive drugs on insulin secretion and cell viability. *Diabetes* 54:424–433, 2005

**Immunosuppressive drugs** are used for the prevention and treatment of graft rejection, graft-versus-host disease, and autoimmune disorders. Although these agents are critical in the success of organ transplantation, their use has been shown to be a major contributor toward the onset of posttransplantation diabetes. Several studies have described that 5–15% of nondiabetic renal transplant recipients experience the onset of posttransplantation diabetes (1,2). In addition, as life expectancy of individuals subjected to organ transplantation increases, secondary complications of diabetes have also been observed in posttransplantation patients (3).

Unfortunately, poor glycemic control has also been reported in subjects with type 1 diabetes who received either a whole pancreas transplant or islet cell transplantation. The survival of the graft has been shown to be dependent on the quality of the organ at the time of transplantation, as well as on the toxic effect that the immunosuppressive drugs might have on the transplanted organ. An insulin resistance effect of immunosuppression drugs has also been described (4). In the last few years, with the introduction of a glucocorticoid-free immunosuppressive regimen, as described in the Edmonton protocol, a better islet graft survival has been reported, and many patients with diabetes have been able to maintain a euglycemic state for a long period of time (5).

Because the goal of immunosuppressive drugs is to induce tolerance without promoting damage to the transplant, several studies have investigated the effect(s) of nonsteroid immunosuppressive agents on the function and survival of β-cells. Interestingly, conclusions from previous investigations have often been paradoxical. Tacrolimus (FK506), rapamycin, and cyclosporin A were reported to be able to induce rapid and significant exocytosis of cellular insulin without causing cell death when tested with cultures of β-cell lines or intact human islets (6,7). In contrast, cytoplasmic swelling, vacuolization, apoptosis, and abnormal immunostaining for insulin have been observed in biopsies from patients receiving either FK506 or cyclosporin A (8). At concentrations that inhibit the calcineurin phosphatase activity, tacrolimus and cyclosporin A have been shown to inhibit human insulin gene transcription (9). Significant inhibition of insulin secretion by mycophenolic acid, sirolimus, or tacrolimus has also been observed in rat islets and HIT-T15 cells (10).
These reports indicate that studies aimed at characterizing the effect of immunosuppressive drugs on human islets remain extremely important. In the present study, we evaluated the diabetogenic potential of a series of immunosuppressive agents (used individually or in combination) on freshly isolated human islets. We demonstrated that glucose-dependent insulin secretion and cell viability are significantly impaired by the addition of immunosuppressive agents to the culture medium. This phenomenon was associated with the downregulation of various anti-apoptotic proteins and with the accumulation of pro-apoptotic markers. Finally, we demonstrated that viral-mediate gene transfer of the anti-apoptotic gene XIAP (X-linked inhibitor of apoptosis) (11) was capable of preserving cell viability, upregulating the cellular anti-apoptotic machinery, and restoring the glucose-dependent secretion of insulin.

**RESEARCH DESIGN AND METHODS**

Dithizone, BSA, sirolimus (Rapamycin), and γ-glucose were purchased from Sigma (St. Louis, MO). Culture media along with fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco-BRL (Gaithersburg, MD). The Live/Dead Viability/Cytotoxicity Kit was obtained from Molecular Probe (Eugene, OR). The anti-Bcl-xL, anti-Bcl-2, anti-CREB (cAMP-responsive element binding protein), anti-Phospho-CREB, anti-Smac, and anti-β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Electrochemiluminescence Western blotting detection reagents and Hybond-C nitrocellulose membrane were purchased from Amersham Pharma Biotech (Denver, CO). Mycophenolic acid was purchased from Calbiochem (La Jolla, CA), and tacrolimus was purchased from Prograf (Fujisawa, Deerfield, NJ). Insulin radioimmunoassay was purchased from Linco Research (St. Charles, MO). The polystyrene beads Bio-Gel 200–400 mesh and the Bradford Protein Assay reagent were purchased from Bio-Rad Laboratories (Richmond, CA). The [3H]dCTP radioactivity was purchased from Amersham Life Science (Arlington Heights, IL). The enzyme sequence, used for probe labeling, was purchased from U.S. Biochemical (Cleveland, OH).

**Islet isolation and cell culture.** Human pancreases were recovered from cadaver donors. Islet isolation was performed in the Islet Transplantation and Autoimmunity Branch of the National Institutes of Health and kindly donated for the present study by Dr. David M. Harlan (National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD). All pancreases were processed using identical techniques of collagenase digestion and ficoll purification. Islet recovery after purification was assessed in duplicate by counts of dithizone-stained aliquots of the final suspension of tissue. Purity was assessed by comparing the relative quantity of dithizone-stained endocrine tissue with unstained exocrine tissue. Isolated islets were cultured in 75-mL flasks in the presence of M199 medium with 100 μg/ml penicillin, 50 μg/ml streptomycin, and 10% FBS at 37°C under a humidified condition of 95% air/5% CO₂. Six independent islet preparations from separate donors were used for the present study.

Mouse insulinoma (MIN6) cells were obtained from Dr. J. Miyazaki (Kumamoto University, Kumamoto, Japan). MIN6 cells were cultured in 75-mL flasks, in the presence of Dulbecco’s modified Eagle’s medium with 10% FBS, 100 μg/ml penicillin, 50 μg/ml streptomycin, and 10% FBS at 37°C under humidified conditions of 95% air/5% CO₂.

**Generation of recombinant adenovirus vectors and viral infection.** Complementary DNA encoding human XIAP and green fluorescent protein (GFP) were inserted into the cytomegalovirus promoter-containing adenovirus-based plasmid pAd5lox. They were then co-transfected with a second plasmid (pBM17), which contained an additional 4 kb of the pBR plasmid interrupting the E1 region and DNA from a replication-defective adenovirus, into HEK-293 cells using Superfect. After recombination and lysis of the cells, the medium was collected, and cells were lysed by freezing and thawing. Cell debris was pelleted, and the viral supernatant was saved for subsequent experiments. Adenovirus was purified on a CsCl-Tris (two tier CsCl solution [1.25 and 1.40 g/ml] in 10 mmol/l Tris-HCl, pH 8.1) gradient, separated into aliquots, and...
Islet

Islet-XIAP

Tacrolimus 0 50 ng 100 ng

Viability of Islets (%)

Tacrolimus (ng/ml)

FIG. 2. Detection of living/dead cells from cultures of human islets treated with tacrolimus. In the top panels, living cells are identified by the green staining, whereas dead cells are identified by the brown nuclear staining. A and D show islets cultured without tacrolimus, whereas B and C show islets treated with either 50 (B) or 100 (C) ng/ml tacrolimus for 48 h. E and F show islets infected with the Adlox-XIAP virus (Islet-XIAP); 4–6 h after the infection, islets were treated with 50 (E) or 100 (F) ng/ml tacrolimus for 48 h. All pictures were taken after the 48-h drug treatment. The lower panel shows a quantitative analysis of cell viability, as derived from three independent experiments. *P < 0.05.

stored at 80°C until use. The multiplicity of infection was determined by a spectrophotometry reading at A405.

Isolated human islets were washed with M199 medium and exposed to purified adenovirus for 4–6 h at 37°C. Islets were then washed and incubated in 3 ml M199 medium containing 10% FBS, 5 mmol/l D-glucose, 100 units/ml penicillin, and 100 μg/ml streptomycin. Four to six hours after the infection, islets were harvested and used in the experiments, as described below.

MIN6 cells were cultured, as described above. Medium was aspirated, and cells were rinsed briefly with 1 mmol/l MgCl₂ in PBS. Viral dilutions, for both pAdlox-GFP and pAdlox-XIAP viruses, were made in 5 ml with Dulbecco's modified Eagle's medium and applied to each flask. The optimal viral concentration for >90% expression was 10–50 multiplicity of infection. Noninfected cells were treated solely with complete medium. After incubation at 37°C for 12 h, the virus was aspirated, fresh medium was added, and the cells were returned to 37°C for the treatment with immunosuppressive drugs, as hereafter described.

Immunosuppressive drug treatment. Drugs were selected for this study based on current and standard use in clinical islet transplantation. Drug concentrations were selected to encompass the upper and lower plasma-drug concentrations usually targeted in clinical practice (12). The drugs included mycophenolic acid at concentrations of 10 and 25 μg/ml, tacrolimus at concentrations of 50 and 100 ng/ml, and sirolimus at concentrations of 1 and 50 ng/ml. Drug dilutions were made from fresh stock before each experiment. Tacrolimus, mycophenolic acid, and sirolimus were solubilized in sterile water, 70% ethanol, and dimethyl sulfoxide, respectively. All drugs were diluted to appropriate final concentrations in sterile water. Control cultures were grown under the same conditions as treated cells but in the absence of the drugs. The final concentrations of ethanol and dimethyl sulfoxide (used for the dilution of drugs) were identical in every culture, irrespective of the particular treatment group.

Drugs (tested individually or in association) were added to the culture medium of islets, or MIN6 cells, in what we define hereafter as low concentration (10 μg/ml mycophenolic acid, 50 ng/ml tacrolimus, and 1 ng/ml sirolimus) or high concentration (25 μg/ml mycophenolic acid, 100 ng/ml tacrolimus, and 50 ng/ml sirolimus). Treatment was performed for 48 or 72 h, as specifically described in the figure legends.

Measurement of insulin secretion by static and dynamic challenge with glucose. For static insulin secretion, islets were subjected to drug treatment for 48 h, then washed with serum-free and glucose-free medium (twice), and incubated in fresh medium for 2 h. They were then exposed to a new aliquot of medium containing 3, 5.6, or 16.7 mmol/l glucose for 1 h. Insulin released into the medium was measured by radioimmunoassay. Total insulin accumulation in the culture medium was then normalized by the total cellular protein content per each individual culture.

For dynamic assessment of insulin secretion in response to glucose, islet function was evaluated by the perifusion technique, performed after a 48-h treatment with tacrolimus. After a preincubation for 30 min in Krebs-Ringer bicarbonate buffer medium containing 5 mmol/l glucose and 2 g/l BSA, ~100 islets per treatment group were added to each of two perifusion chambers, layered between inert polystyrene beads. The Krebs-Ringer bicarbonate buffer medium was used with the flow rate of 0.2 ml/min. The perifusion protocol was started after the 30-min equilibration period with basal medium, and it was followed by five 12-min stimulation periods with medium containing in a time-dependent order the following concentrations of glucose: 3, 5.6, 3, 16.7, and 3 mmol/l. Samples were collected at 2-min intervals, ice-chilled immediately, frozen, and kept at ~20°C to be analyzed as described above.

Protein assay. Total cellular protein content was measured using the Bradford protein assay reagent. The amount of proteins measured was used as a normalization factor to determine the relative amount of insulin in the medium assayed for each culture condition.

RNA isolation and Northern blot analysis. Islet cellular RNA was extracted as routinely described. Northern blots were hybridized with the
full-length rat insulin II and the β-actin cDNA probes. Probes were labeled with [32P]dCTP by the random priming procedure using the enzyme sequence. Hybridization and washing conditions were carried out as routinely described.

Living/dead cell determination. Cell viability was evaluated using the Living/Dead Viability/Cytotoxicity Kit. Briefly, isolated islets were washed twice with PBS, and then 50-μl aliquots were placed in a 96-well plate. Then, 10 μl of solution Component A (Calcein AM) and Component B (Ethidium homodimer-1) from the detection kit were added to each well. After 30 min, cells were analyzed under a CK-2 Olympus microscope (Olympus, Melville, NY). Living cells were identified by a green staining (Calcein AM staining), whereas dead cells showed a brown-red nuclear staining (Ethidium homodimer-1 staining). Quantitative analysis was performed using a CytoFluor fluorescence microplate reader (Millipore, Bedford, MA). Fluorescence at 530 nm of the cell-free sample without dye added (cells treated with drugs) were labeled as F(530)max and F(530)sample, respectively; fluorescence at 530 nm of control sample (cultured without drugs) and of experimental samples (cells treated with drugs) were labeled as F(530)min. The relative numbers of live cells was expressed in terms of percentage and calculated from the fluorescence readings defined above as follows: % live cells = \{[F(530)sample − F(530)min] / [F(530)max − F(530)min]\} × 100%.

**RESULTS**

Immunosuppressive drugs inhibit insulin secretion of freshly isolated human islets. Insulin concentration in the culture medium of islets was measured using an insulin radioimmunoassay detection kit and normalized for the total concentration of proteins. Glucose responsiveness, determined by mean insulin secretion in the presence of either 3, 5.6, or 16.7 mmol/l glucose, demonstrated that there was 170.4 ± 23, 230.2 ± 35, and 487.5 ± 49 ng insulin/mg protein, respectively, in the culture medium collected from human islets (Fig. 1A). A significant reduction in glucose-stimulated secretion of insulin by human islets was observed after the cells were exposed to sirilimus, mycophenolic acid, and tacrolimus either separately or in combination. In the presence of 16.7 mmol/l glucose, the insulin secreted from islets exposed to control medium, or medium containing either 50 or 100 ng/ml of tacrolimus, was 490.3 ± 43.6, 342.3 ± 16.2, and 225.3 ± 12.4 ng insulin/mg protein, respectively (Fig. 1A). In the medium collected from islets cultured in 5.6 mmol/l glucose, the level of insulin was 295.3 ± 18.6 (control medium), 154.7 ± 13.4 (50 ng/ml tacrolimus), and 88.3 ± 8.2 (100 ng/ml tacrolimus) ng insulin/mg protein. The amount of insulin detected in islets cultured in the presence of 3 mmol/l glucose did not vary significantly among the different treatment groups (Fig. 1A). Experiments with
sirolimus, mycophenolic acid, and the cocktail of these three drugs showed a similar inhibition of glucose-dependent secretion of insulin (Fig. 1B–D). In Fig. 1, the amount of insulin secreted in the presence of 16.7 mmol/l glucose (in the absence of drugs) was considered as a maximal insulin secretion, equal to 100% (hereafter, termed as control); data in all other treatment groups were expressed as percentage of control. Whereas an inhibition of insulin secretion was observed with all drug combinations tested (Fig. 1A–D), notably this was greater when islets were cultured in high concentrations of glucose and exposed to the higher concentrations of immunosuppressive drugs.

**Immunosuppressive drugs induce islet cell death.**

Human islet morphology was observed in standard culture conditions, as well as in islets cultured in the presence, or absence, of tacrolimus for 48 h. Living cells were identified by the emission of a green fluorescence (Calcein AM staining), whereas dead cells were identified by a brown-red nuclear fluorescence (Ethidium homodimer-1 staining). We demonstrated that while most of the islets cultured without immunosuppressive agents had a prevalent presence of live cells, a significant number of cells from islets cultured in either a low or high concentration of tacrolimus showed a brown-red staining, signifying the presence of numerous dead cells. Cells exposed to 100 ng/ml tacrolimus showed a greater number of dead cells than the islets that were treated with 50 ng/ml (Fig. 2A–C). These findings indicate that human islets are directly susceptible to the detrimental effects of the immunosuppressive drugs currently used after transplantation.

**Adenovirus (Adlox-XIAP and Adlox-GFP) infection.**

The XIAP-expressing adenovirus (Adlox-XIAP) vector was generated to investigate whether XIAP could improve viability and restore glucose-dependent secretion of insulin of cells exposed to immunosuppressive agents. Adlox-GFP was used as a control. Cultures were infected by Ad-GFP and Ad-XIAP using 10–50 plaque-forming units/cell. Cells were observed after 48 h from the time of infection and appeared normal, with no apparent cell death observed by phase-contrast microscopy. Cells that were infected with the Adlox-GFP exhibited a uniform green staining (data not show), and the incorporation of XIAP was further confirmed by Western blot analysis.

**XIAP enhances the expression of anti-apoptotic proteins.**

Western blot analysis for Bcl-2 and Bcl-xL showed that the protective effect of XIAP was associated with an increased level of both anti-apoptotic proteins (Fig. 3). This is consistent with data derived from the live/death assay indicating that XIAP improved the survival of cells exposed to immunosuppressive agents.

**XIAP counteracts the downregulation of insulin mRNA level induced by immunosuppressive drugs.**

Northern blot analysis was conducted to investigate the putative inhibitory effect of immunosuppressive drugs on insulin gene transcription. Total RNA was prepared from islets exposed to control medium and medium containing either 50 or 100 ng/ml tacrolimus for 48 h. The signal
density of islets exposed to tacrolimus revealed a decrease in the abundance of insulin mRNA when compared with controls (Fig. 4). XIAP gene transfer prevented the downregulation of insulin mRNA induced by tacrolimus.

**XIAP improves insulin secretion of islets exposed to immunosuppressive agents.** Measurement of insulin release by in vitro perifusion assay revealed that control islets exposed to tacrolimus exhibited a decreased secretory activity in response to glucose when compared with islets cultured in the presence of vehicle alone. XIAP gene transfer performed 4–6 h before treatment with tacrolimus was capable of partially preventing the downregulation of insulin secretion observed after drug treatment (Fig. 5). Calculation of the area under curve for the glucose ramp study indicated that the induced insulin secretion for islets cultured in the presence of either 50 or 100 ng/ml tacrolimus was only 64% (50 ng/ml tacrolimus; \( P < 0.01 \) control vs. treated) and 49% (100 ng/ml tacrolimus; \( P < 0.01 \) control vs. treated) of that of control islets cultured with vehicle alone (Fig. 6). Islets infected with XIAP were partially protected from the toxic effect of tacrolimus. Indeed, there was a statistically significant greater amount of insulin being secreted from islets infected with XIAP compared with controls (\( P < 0.05 \) in the presence of 50 ng/ml tacrolimus, and \( P < 0.01 \) in the presence of 100 ng/dl tacrolimus) (Fig. 5).

**XIAP reverses the effect of immunosuppressive drugs on the activation of CREB activation and the cytosolic abundance of Smac.** Because of the limited availability of human islets for a comprehensive study, MIN6 cells were chosen for further studies. To investigate the mechanism by which tacrolimus induced cell damage, we performed Western blotting analysis for CREB, an important mediator in regulation of various cellular activities, including insulin synthesis and cell viability. Phosphorylation of CREB, an active form of CREB (hereafter termed phospho-CREB), was also investigated. While we did not detect any different expression of CREB among the various treatment groups, we observed a decrease in the phosphorylation of CREB in parental MIN6 and GFP-MIN6.
When they were exposed to tacrolimus, cells (compared with cultures in the absence of tacrolimus) (Fig. 6). Transfection of cells with XIAP restored the capability of activating CREB by means of phosphorylation.

To further clarify the role of XIAP in the protection of cells from apoptosis, the presence of Smac in the cytoplasm of MIN6 cells was investigated by Western blot analysis. In intact cells, Smac is localized primarily in the mitochondria, and its detection in the cytosol of cells is associated with cell damage. Our study indicated that tacrolimus stimulated the accumulation of Smac in the cellular cytosol (Fig. 6). A decreased level of Smac was observed in XIAP-transfected cells, indicating that mitochondrial integrity in XIAP-transfected cells was better preserved when compared with parental and MIN6GFP cells exposed to tacrolimus.

**DISCUSSION**

The present study demonstrates that tacrolimus, mycophenolic acid, and sirolimus, three of the most widely used immunosuppressive agents in the postislet transplantation clinical setting, have a negative effect on function and viability of pancreatic β-cells. Using isolated human islets, we observed that these agents (administered individually or in association) alter the glucose-dependent insulin secretion and the intracellular abundance of insulin mRNA, in addition to cell death. Gene transfer with XIAP, an anti-apoptotic protein, had a protective effect, since it was able to enhance the viability and function of cells challenged with immunosuppressive drugs. Finally, we demonstrated that the cytotoxicity of tacrolimus, mycophenolic acid, and sirolimus was mediated by a decreased activation of CREB, and it was associated with the release of the mitochondrial protein Smac.

In "graft rejections" after organ transplantation, T-cells play a critical role in immune reactions. Immunosuppressive drugs were developed for targeting and destroying T-cells. Sirolimus, tacrolimus, and mycophenolic acid inhibit lymphocyte proliferation by different mechanisms: sirolimus inhibits the activity of phosphorylation of p70(s6) kinase and the eukaryotic initiation factor-4E binding protein, PHAS-1; tacrolimus binds an immunophilin, FKBP (FK506 binding protein), whereas mycophenolic acid inhibits the pathway for the de novo synthesis of guanosine (14,15). Their inhibitory effect on immune cells have been successfully observed in diabetes models: 1)
mycophenolic acid has been shown to exert a protective effect in certain animal models of autoimmunity, including diabetes in diabetes-prone bio-breeding (BB) rats (16); 2) sirolimus, in combination with interleukin-2, has been shown to protect islet β-cells from autoimmune destruction, possibly due to deletion of autoreactive Th1 cells (17).

While few studies have investigated the direct effect of immunosuppressive drugs on pancreatic β-cells, the high incidence of posttransplantation diabetes would suggest that a diabeticogenic potential for those agents may exist. The mechanism(s) for the development of hyperglycemia in subjects treated with steroid-free immunosuppressive drugs is largely unknown.

In the present study, we report that tacrolimus, mycophenolic acid, and sirolimus inhibit insulin secretion along with insulin gene transcription. Whereas a low concentration of immunosuppressive agents primarily affected the secretion of insulin, a higher concentration of those agents had a significant negative effect on the viability of cells, inducing cell death. Culturing cells, or islets, in high glucose enhanced the cytotoxic action observed with the use of immunosuppressive drugs. This is consistent with the recent report by Bell et al. (18) that demonstrated that rapamycin had a deleterious effect on the viability of rat and human islets.

This observation, in conjunction with the previously described effect of immunosuppressive agents on insulin resistance (4), may explain the high incidence of posttransplantation diabetes observed in nondiabetic recipients of solid organ transplant (19,20). Furthermore, the decrease in insulin secretion caused by those agents may also explain the requirement of exogenous insulin administration a few months after successful islet transplantation in subjects with type 1 diabetes (21).

The diabeticogenic potential of immunosuppressive drugs is likely mediated via different mechanisms. A combination of glucocorticoids and calcineurin inhibitors has been shown to induce both insulin resistance and insulinopenia and to be responsible for the occurrence of posttransplantation diabetes (18). Cyclosporin A and FK506 have been shown to have direct toxic effects on pancreatic islets of several animal species (8). Recently, tacrolimus has been suggested to impair glucose-stimulated insulin secretion downstream of the increase in intracellular [Ca\(^{2+}\)], leading to insulin exocytosis (22). Our studies showed that immunosuppressive drugs alter islet cell function and may induce cell death, even when used in concentrations that are within the therapeutic range in humans. This observation suggests that the number of surviving β-cells, after treatment with immunosuppressive agents, may be another contributing factor to the development of hyperglycemia in the posttransplantation setting. In addition, the decreased insulin secretion (with preservation of cell viability) that we observed when using low concentrations of drugs indicates that another mechanism, involving the exocytosis of insulin, may also be involved in the development of hyperglycemia after immunosuppression.

In past few years, various studies have investigated strategies to increase, and protect, islets viability before and after islet transplantation. Rabinovitch et al. (23) have shown that human islets could be transfected with a Bcl-2–expressing viral vector and that the overexpression of the transgene led the protection of cells from cytokine-induced dysfunction and destruction. Giannoukakis et al. (24) have been able to produce an islet population capable of resistance to the cytotoxic action of interleukin-β by an adeno-viral–mediated gene transfer of an IκB repressor. Grey et al. (25) demonstrated that genetically engineering a suboptimal islet graft with the citoprotective gene A20 was capable of preserving β-cell mass and function. Lopez-Talavera et al. (26) reported that hepatocyte growth factor enhances islet function and survival, even in the setting of immunosuppressant-induced insulin resistance and β-cell toxicity induced by the Edmonton immunosuppression regimen.

In the present studies, we used an adenovirus expression vector to infect islets and MIN6 cells with the anti-apoptotic gene XIAP. We demonstrated that the expression of the foreign gene protected cells from damage induced by the exposure to immunosuppressive drugs, thus improving function and enhancing the survival of insulin-producing cells. Our finding is consistent with previous reports demonstrating that, in other cell systems, XIAP was capable of inhibiting apoptosis by binding to several partner molecules, including caspase 3, caspase 9, DIABLO/Smac, HtrA2/Omi, TAB1, the bone morphogenetic protein receptor, and a presumptive E2 ubiquitin-conjugating enzyme (27,28).

Adenovirus is a widely used vector for gene transfer (29). Our GFP control results demonstrated the efficiency of the adenovirus vector, and Western blot confirmed the high expression of XIAP in infected islets and cells. Our results demonstrated that XIAP protected islets and maintained cell viability against immunosuppressive damage and that part of this protection may be attributed to the increased expression of anti-apoptotic proteins. This report demonstrates that anti-apoptotic action of XIAP, originally demonstrated for glial-derived cell lines (30), cerebellar granule neurons (31), and LNCaP cells (32), extends to pancreatic β-cells. Our results suggested that using XIAP to infect isolated islets before transplantation would enhance cell resistance to immunosuppressive drugs, increase cell viability, and partially restore insulin secretion ability.

Previous observations reported that both tacrolimus and cyclosporin A blocked cAMP- as well as calcium-induced activity of the human insulin gene at concentrations that inhibit calcineurin phosphatase activity (33). CREB is a member of the leucine zipper family and ubiquitous trans-activator transcription factors, it binds to the cAMP response element, and regulates many inducible genes, including Bcl-2, c-fos, and junB (34). Activation of CREB occurs by serine phosphorylation mediated by protein kinase A in most cell types (35). Our results showed that tacrolimus did not inhibit the expression of CREB in cells exposed to drugs, but inhibited the phosphorylation of CREB, suggesting that tacrolimus could inhibit protein kinase A activity and that XIAP may restore cellular capability of protein kinase A activation. Indeed, XIAP-transfected pancreatic islets exposed to tacrolimus maintained an excellent response to high glucose stimuli, whereas naïve cells exhibited a subphysiological response to glucose. Our observation is consistent with previous
ADENOVIRUS-MEDIATED XIAP GENE TRANSFER

reports indicating the role of CREB activation in both cell function and survival (36,37).

Our results also showed that XIAP-transfected cells, when exposed to tacrolimus, had a decreased release of Smac compared with either parental or GFP-transfected cells. It has been shown that Smac is released from the mitochondria into the cytosol at the time of apoptosis, where it binds to inhibitors of apoptosis (including XIAP) and enables caspase 3 activation (38,39). Previous studies, using X-ray crystallography, have shown that the first four amino acids (AVPI [Ala-Val-Pro-Ile]) of mature Smac binds to a portion of the third BIR (BIR3) domain of XIAP (40).

In our experiments, drug-induced apoptosis was prevented by overexpressing XIAP. A potential explanation for this may be that the overexpression of XIAP is capable of blocking Smac-induced caspase 3 activation, or alternatively, that overexpressed XIAP is helpful in stabilizing the mitochondrial membrane, allowing it to inhibit the release of Smac. Further experiments may be required to address this question more directly.

In conclusion, our results demonstrate that tacrolimus, mycophenolic acid, and sirolimus negatively affected function and viability of insulin-producing cells. Overexpression of XIAP was capable of protecting cells from the undesired effects of immunosuppressive drugs. The protective action of XIAP involved the activation of CREB and the inhibition of the pathway leading to the release of Smac.

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