Allotransplantation of pancreatic islets represents a promising approach to treat type 1 diabetes. Destruction of β-cells in islet allografts involves multiple immune mechanisms that lead to activation of caspases and apoptotic cell death. The X-linked inhibitor of apoptosis (XIAP) inhibits apoptosis induced by a variety of triggers, primarily by preventing the activation of caspases. To determine whether XIAP would protect β-cells from apoptosis, we used a recombinant adenovirus to overexpress XIAP in transformed murine β-cells and in freshly isolated islets. In vitro cytokine-induced β-cell death was decreased to baseline levels in XIAP-transduced MIN-6 and NIT-1 cell lines compared with controls. To evaluate the potential of XIAP overexpression to prevent in vivo allogeneic graft rejection, we transduced Balb/c islets ex vivo with XIAP before transplantation into CBA mice with streptozotocin-induced diabetes. We observed that almost all mice receiving allografts of XIAP-expressing islets maintained normoglycemia until the experiment was terminated (45–72 days posttransplant), whereas control mice receiving islets transduced with adenovirus expressing LacZ were hyperglycemic by ~17 days posttransplantation due to graft rejection. Immunohistochemistry revealed preservation of β-cells and clearance of infiltrating immune cells in the XIAP-expressing islet grafts. The in vitro allogeneic response of splenocytes isolated from recipients of XIAP-expressing grafts 8 weeks posttransplant was similar to that seen in nonprimed allogeneic mice, suggesting that XIAP overexpression may lead to the acceptance of islet allografts in diabetic recipients. Long-term protection of islet allografts by XIAP overexpression may enhance the survival of islet transplants in diabetes. Diabetes 54:2533–2540, 2005

Beta-cell replacement by allotransplantation of pancreatic islets represents a promising approach to treat type 1 diabetes. Early attempts at clinical islet transplantation used corticosteroids in combination with other immunosuppressive drugs and resulted in <5% of patients remaining insulin independent at 1 year posttransplantation (1). Recent reports of enhanced success in clinical islet transplantation have been attributed to omission of steroids, newer immunosuppressive drugs, and engraftment of a larger number of high-quality islets (2). Although many of these patients have remained insulin independent, all required at least two islet transplants and indefinite immunosuppression (3), and the potential for long-term survival of these grafts remains unknown. Moreover, clinical islet transplantation is still limited by the availability of donor tissue (2,3).

The destruction of transplanted islet cells involves a panoply of immune cells including dendritic cells, macrophages, and CD4+ and CD8+ T-cells. The production of cytotoxic mediators by these cells, including proinflammatory cytokines as well as Fas-FasL engagement (4), is thought to lead to the activation of intracellular death pathways within transplanted islet cells. However, it has also been shown that the Fas and perforin pathways alone or in combination are not required for islet allograft rejection, suggesting that other pathways may play more crucial roles (5). Cell death is likely mediated largely by caspase activation (6). In islet transplants, the death of β-cells is caused not only by immune rejection of the allograft, but also by trauma to β-cells during islet isolation, culture, and graft revascularization (7,8).

The X-linked inhibitor of apoptosis (XIAP) is the most potent caspase inhibitor in the IAP family and inhibits apoptotic cell death predominantly by preventing activation of initiator caspase 9 as well as effector caspases 3 and 7 (9,10). Notably, overexpression of XIAP is capable of preventing apoptosis induced by a variety of triggers in vitro and has been shown to inhibit cell death in several models of neurodegeneration in vivo, including neuronal ischemia (11), axotomy (12,13), glaucoma (14), and MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine)-induced dopaminergic cell death (15,16). We wished to determine whether XIAP might similarly confer protection to β-cells from apoptotic cell death. To this end, we used a recombinant adenovirus to overexpress XIAP in β-cells to evaluate whether XIAP could prevent in vitro cytokine-
induced β-cell death and in islets to determine whether XIAP could protect transplanted islets from in vivo allo- genic graft rejection.

RESEARCH DESIGN AND METHODS

In vitro cell viability assay. Transformed NIT-1 and MIN-6 β-cells were seeded at 1 × 10^6 cells/well in 24-well plates and incubated overnight in Dulbecco’s modified Eagle’s medium with 25 mmol/l glucose (Gibco, Burlington, Canada) supplemented with 10% FCS (Gibco), 100 units/ml penicillin (Sigma, Oakville, Canada), 100 μg/ml streptomycin (Sigma), and 1% Glutamax (Gibco). On the following day, cells were transduced for 1 h with 0, 5, 10, or 20 pfu (plaque forming units) of recombinant adenovirus using the chicken β-actin promoter to drive expression of either a human XIAP cDNA (Ad-XIAP at 2.1 × 10^12 pfu/ml, 1.72 × 10^12 viral particles [VP]/ml) (11) or LacZ cDNA (Ad-LacZ at 1.0 × 10^12 pfu/ml, 4.66 × 10^12 VP/ml) as control. Two days following infection with Ad-XIAP or Ad-LacZ, new medium was added containing either a cytokine mix (1 μg/ml interleukin [IL]-1β, 0.1 μg/ml interferon [IFN]-γ, and 6 mg/ml tumor necrosis factor [TNF]-α, 100 μmol/l etoposide (topoisomerase II inhibitor; Sigma), or medium alone. After 2 days of incubation, cell viability was analyzed in triplicate in three individual experiments by trypan blue exclusion (Sigma) and by phase contrast microscopy.

Transduction efficiency and expression. Transfection efficiency was assayed by staining Balb/c islets for β-galactosidase (LacZ [17]) activity following transduction with 0, 5, or 10 pfu Ad-LacZ. XIAP protein expression was determined by immunoblot of lysates of NIT-1 or MIN-6 β-cells or freshly isolated Balb/c mouse islets 48 h following transduction with Ad-XIAP (0–20 pfu/cell). Ten micrograms of extracted protein were boiled with a reducing sample buffer and resolved on a 10% SDS-polyacrylamide gel at 100 V. The separated proteins were electrotransferred onto Immobilon-P membranes (Millipore, Nepean, Canada) at 100 V for 60 min using the Trans-Blot System (BioRad, Mississauga, Canada). Immunoblot analysis was performed using polyclonal hI LP anti-XIAP antibody (1:250; Transduction Labs, San Diego, CA) and immunoreactive XIAP detected by enhanced Chemiluminescence (Amersham, Piscataway, NJ).

Islet isolation and transplantation. Donor islets were isolated from 8- to 16-week-old female Balb/c (H-2b) mice by ductal collagenase injection, oscillating digestion, and purification on a dextran gradient (18). Islets were incubated in complete Ham’s F-10 media (Gibco) and transduced overnight with 10 pfu/cell (assuming 1,000 cells per islet) of Ad-XIAP (n = 10) or Ad-LacZ (n = 10). The islets were washed, hand-counted into aliquots of 550 islets ≥100 μm, and transplanted into the left renal subcapsular space of age-matched female CBA (H-2b) mice under isoflurane anesthesia. Recipient mice were previously rendered hyperglycemic (blood glucose ≥20 mmol/l) by a single intraperitoneal injection of reconstituted streptozotocin in citrate buffer. Recipient mice were transplanted 3–5 days after streptozotocin injection, when blood glucose levels were between 14 and 26 mmol/l. Nephrectomy of the graft-bearing kidney was performed on all recipients, and survival nephrectomy was performed on recipients of Ad-XIAP–transduced islets to ensure that normoglycemia was graft dependent. Mice (Taconics, Germantown, NY, or Jackson Laboratories, Bar Harbor, ME) were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Graft survival analysis. Nonfasting, morning blood glucose concentrations were measured every 2nd day posttransplant on tail vein blood samples using a strip glucometer (OneTouch Ultra; LifeScan, Burnaby, Canada). Mice that turned normoglycemic within the first 5 days following transplantation were included in the study. The time of graft failure was determined as the day of diabetes recurrence, defined as the first of 2 consecutive days where blood glucose was ≥11 mmol/l.

Islet function. In vitro function of adenovirus-transduced and nontransduced islets was assessed by glucose-stimulated insulin release 48 h postincubation in two independent experiments. Islets were preincubated in triplicate for 1 h in Krebs-Ringer bicarbonate buffer (25 mmol/l HEPES, 115 mmol/l NaCl, 24 mmol/l NaHCO3, 5 mmol/l KCl, 1 mmol/l MgCl2, and 2.5 mmol/l CaCl2; pH 7.4 [19]) with 0.1% BSA and 1.67 mmol/l glucose, followed by a 30-min incubation in media containing either 1.67 or 67 mmol/l glucose. Immunoreactive insulin was measured in the incubation media and in islet lysates by enzyme-linked immunosorbent assay (ALPCO Diagnostics, Windham, NH). In vivo islet function was assessed by an intraperitoneal glucose tolerance test performed 4 weeks posttransplantation. Mice were fasted 5 h and injected with 2 g/kg body wt 20% glucose (Baxter Healthcare, Deerfield, IL) and tail vein blood glucose was measured at 0, 15, 30, 60, 90, and 120 min. At 0 and 15 min, 50 μl blood was taken to measure serum insulin immunoreactivity using a sensitive insulin enzyme-linked immunosorbent assay (Crystal Chem, Chicago, IL).

Total RNA isolation and real-time RT-PCR analysis. TRIzol mini spin columns (Qiagen, Mississauga, Canada) were used to extract total RNA from islets grafts at day 10 (baseline, 100%) to compare with XIAP expression at day 63 posttransplantation (both n = 2). Day 10 was chosen as an early time point posttransplantation in which XIAP expression was likely to be maximal, so as to determine the time for assessment of the XIAP expression in transplanted tissue over time. Real-time quantitative RT-PCR of 25 ng DNase I-treated total RNA using human XIAP primers and probes spanning exons 3 and 4 was used to determine levels of XIAP RNA (20). GAPDH primers and probe were used to control for RNA content. The change in relative expression between day 10 and day 63 posttransplantation is defined as 2ΔACT, where CT designates cycle threshold and dd represents the threshold cycle normalized to an endogenous control and calibrated to a reference sample.

Histological analysis of islet grafts. All islet grafts were excised, fixed in 4% paraformaldehyde, and processed for histology. Paraffin sections (5 μm) were immunostained using guinea-pig anti-insulin (1:100; Dako, Carpinteria, CA) or a rabbit anti-glucagon (1:75; Dako) primary antibody and rabbit anti–guinea-pig Alexa 488 (1:100; Molecular Probes, Eugene, OR) or donkey anti-rabbit Texas Red (1:100; Jackson Labs, Mississauga, Canada) secondary antibody, respectively. Immunostaining for leukocytes was performed following antigen retrieval by steaming for 30 min in Dako Target Retrieval Solution. Sections were stained using rat anti-mouse CD45 (1:25; BD Pharmingen, Mississauga, Canada) or guinea pig anti-insulin (1:100; Dako) primary antibody and anti-rat Alexa 488 (1:100) or goat anti–guinea pig Texas Red (1:250; Jackson Labs) secondary antibody. Immunostained sections were viewed in bright field or light on a Zeiss Axioplan2 microscope equipped for epifluorescence. Images captured and the appropriate filters were pseudo-colored and merged using Smart Capture VP software (Digital Scientific, Cambridge, U.K.) to produce the fluorescent micrographs.

Mixed leukocyte assay. Responder splenocytes were isolated from recipients of XIAP-transduced islets at 7 (n = 3) or 60 (n = 4) days posttransplantation and were compared with nontransplanted control CBA mice (n = 5). Responder cells were seeded at 2 × 10^6 cells/well in triplicate and stimulated with titrated numbers (2 × 10^4, 1 × 10^5, 2 × 10^5, 4 × 10^5, and 2 × 10^6 cells/well) of 30-Gy-irradiated Balb/c splenocytes in a final volume of 200 μl RPMI media (Gibco) supplemented with 10% FCS (Gibco), 100 units/ml penicillin (Sigma), 100 μg/ml streptomycin (Sigma), and 1% Glutamax (Gibco) in 96-well round-bottom plates. After 4 days of culture, 1 μCi/well of [3H]methyl thymidine (Amersham Life Science, Buckinghamshire, U.K.) was added for the last 16–18 h. Cells were collected onto a Filtermat A (Wallac, Turku, Finland) with a multiple Harvester 96 (Tomtec, Hamden, CT), and thymidine incorporation was measured in a 1450 Microbeta Trilux liquid scintillation counter (Wallac). Results are expressed as mean counts per minute (cpm) ± SE.

Statistics. Both trypan blue exclusion data (Fig. 1B) and glucose-stimulated insulin release data (Fig. 2C) were analyzed by two-tailed Student’s t test. Differences were considered significant at P < 0.05.

RESULTS

We overexpressed XIAP in transformed murine (MIN-6 and NIT-1) β-cells using a recombinant adenovirus containing a human XIAP cDNA (Ad-XIAP), driven by a constitutively active promoter. Overexpression of human XIAP in MIN-6 cells and in NIT-1 cells was confirmed by Western blot demonstrating increased XIAP protein expression with increasing Ad-XIAP multiplicity of infection (MOI) (Fig. 1A). We allowed 2 days following adenoviral transduction (MOI = 10 pfu/cell) for XIAP protein expression before induction of apoptosis by a mixture of the proinflammatory cytokines IL-1β, IFN-γ, and TNF-α or etoposide. XIAP overexpression reduced the degree of cytokine-induced (Fig. 1B and C) (P < 0.05) or etoposide-induced (data not shown) cell death to baseline levels in both β-cell lines. These data indicate that XIAP provides marked protection of β-cells from apoptosis in vitro.

The protective effect of XIAP on cytokine-induced β-cell death observed in vitro lead us to test whether the transplanted body of mice islet ex vivo with Ad-XIAP before transplantation into diabetic recipients would prolong islet
allograft survival. Initially, we demonstrated that a transduction efficiency of >90% of islet cells could be achieved by infecting intact islets with a recombinant adenovirus expressing LacZ (MOI = 10 pfu/cell) (Fig. 2A). Transduction efficiency was highest when islets were incubated overnight with virus, commencing immediately after isolation. XIAP overexpression by adenoviral transduction of intact islets was confirmed by immunoblot (MOI = 0, 5, and 10 pfu/cell). We observed a marked increase in XIAP protein expression that peaked 2 days following overnight transduction with Ad-XIAP (Fig. 2B). We next showed that adenoviral overexpression of XIAP in islets does not have any deleterious effect on β-cell function, as assessed by in vitro glucose-stimulated secretion of insulin. Ad-XIAP-transduced and nontransduced islets responded similarly to incubation in 16.7 mmol/l glucose with an approximate threefold increase in insulin secretion over basal 1.67 mmol/l glucose concentrations (Fig. 2C, NS). In addition, adenoviral transduction per se did not appear to compromise islet function as demonstrated by indefinite (followed up to 100 days) survival of syngeneic (Balb/c to Balb/c) transplants into diabetic recipients using either transduced (Ad-XIAP or Ad-LacZ; both n = 5) or nontransduced (n = 10) islets (data not shown).

To investigate the effect of XIAP overexpression on islet survival in an allogeneic transplantation model, we first rendered recipient CBA mice diabetic using streptozotocin. Donor Balb/c islets were transduced with either Ad-XIAP or Ad-LacZ and transplanted under the kidney capsule of CBA recipients. Remarkably, only the XIAP-expressing islet transplants were found to persist beyond the normal range for islet allograft rejection of 10–24 days in this model (21). In fact, 9 of 10 recipients of XIAP-expressing islet allografts maintained normoglycemia until the animals were killed between days 45 and 72 posttransplant (Fig. 3A and B). In contrast, all control mice receiv-
XIAP ENHANCES SURVIVAL OF ISLET ALLOGRAFTS

FIG. 2. XIAP expression in freshly isolated Balb/c islets after overnight transduction with Ad-XIAP or Ad-LacZ. A: Determination of transduction efficiency in intact islets using increasing MOI (MOI = 0–10 pfu/cell) by β-galactosidase (LacZ) histochemical staining (original magnification ×20). B: XIAP protein expression assessed by Western blot analysis 48 h following transduction of islets with Ad-XIAP. Control lane is a lysate from XIAP-transduced HeLa cells. C: Glucose-stimulated insulin secretion of nontransduced and Ad-XIAP transduced islets after 48 h in culture. Data are means ± SD of triplicate determinations of a representative experiment with groups compared by two-tailed t test (NS).

ing Ad-LacZ-transduced islets were hyperglycemic by day 24, with the majority failing by day 17 (P < 0.0001). Assessment of in vivo graft function 4 weeks postransplantation by intraperitoneal glucose tolerance test revealed that glucose tolerance was not impaired in recipients of XIAP-expressing islet allografts when compared with non-diabetic, nontransplanted controls (Fig. 3C). Serum insulin levels were 0.24 ± 0.11 ng/ml (n = 5) in recipients of XIAP-expressing islets 1 week postransplantation, similar to those seen in nontreated control CBA mice (0.25 ± 0.06 ng/ml; n = 6), indicating that the allografts were producing insulin. In agreement with this, glucose-stimulated insulin secretion, calculated as the fold increase in serum insulin 15 min following intraperitoneal glucose administration, was preserved (albeit somewhat reduced) in the recipients of Ad-XIAP–transduced islets. The fold increase in glucose-stimulated insulin secretion was 1.5 ± 0.5 (n = 5) at 1 week and 1.7 ± 0.5 (n = 4) at 6 weeks postransplantation compared with 2.5 ± 0.4 (n = 6) in untreated control mice. When recipients of XIAP-expressing islet allografts had their grafts removed by nephrectomy, diabetes returned, indicating that the islet graft was responsible for the maintenance of their normoglycemic state. When Ad-XIAP–transduced islet allografts were analyzed for XIAP mRNA by RT-PCR, the expression of XIAP observed at 63 days postransplantation was found to be decreased to 7% compared with grafts at day 10 postransplantation (Fig. 4). These data suggest that sustained high levels of XIAP expression may not be required for survival of the islet allograft.

Immunohistochemical analysis of excised islet grafts revealed that insulin– (Fig. 5) and glucagon–positive (data not shown) cells were still present in both Ad-XIAP– and Ad-LacZ–transduced islet grafts at 60 days postransplantation. At this time point, both XIAP- and LacZ–expressing grafts were surrounded by an intense CD45–positive leukocytic infiltration (Fig. 5). Despite this apparent immune attack on the graft, β-cells were still clearly preserved in the XIAP-expressing grafts even up to ~60 days postransplantation, as indicated by numerous insulin–immunopositive cells. Remarkably, by 60 days, immune cell infiltration of these grafts had almost completely disappeared (Fig. 5F). In contrast, control mice that received LacZ–expressing islet allografts had a near total loss of insulin immunopositive cells in their islet grafts at the time of failure, accompanied by the continued presence of intense leukocyte infiltration. Because these findings suggest that the recipients of Ad-XIAP–transduced islets had accepted their allografts, we next assessed allogeneic T-cell responsiveness by mixed lymphocyte assay. We isolated splenocytes from recipients of XIAP-transduced islets at 1 week or >8 weeks postransplantation, by which time the immune cell infiltration has largely dissipated. We found a marked reduction in responsiveness of allogeneic splenocytes isolated from the recipients of XIAP-expressing grafts at 60 days postransplant, comparable with that found when naïve nontransplanted control CBA splenocytes were used as responder cells (Fig. 6).

DISCUSSION

The potent anti-apoptotic protein XIAP has substantial therapeutic potential in diseases where apoptosis plays a central role (9,10). Overexpression of XIAP has been shown to inhibit cell death in models of neurodegeneration including neuronal ischemia (11) and glaucoma (14), and inhibition of XIAP expression by an antisense approach to induce apoptosis has proven beneficial in preventing oncogenesis (22–24). Because apoptosis of islet β-cells is central to the pathogenesis of both autoimmune diabetes and failure of islet transplants, we hypothesized that XIAP, as an endogenous repressor of the terminal caspase pathway, may similarly confer protection to transplanted islet cells from allograft rejection. Using a simple model of adenoviral overexpression of XIAP, we found a marked protective effect of XIAP on cytokine-induced killing of β-cells in vitro and demonstrated that XIAP overexpression provides prolonged protection of islets transplanted into diabetic allogeneic recipients.

An additional finding of importance in the present study is that XIAP overexpression does not seem to have any deleterious effect on β-cells as indicated by normal glucose-stimulated insulin secretion compared with nontransduced controls (Fig. 2C). The ability of XIAP to protect from islet loss while maintaining normal islet function may have important therapeutic advantages, since glucose-con-
trol might be achieved with fewer transplanted islets as compared with other gene therapy approaches discussed below. In addition, we found no evidence of transformation or tumorigenesis induced by overexpression of this anti-apoptotic protein.

Our finding that XIAP overexpression provides marked protection from cytokine-induced apoptosis in β-cells, coupled with its ability to prevent islet allograft rejection, suggests that XIAP is capable of preventing β-cell death induced by multiple initiator pathways. Indeed, it was recently shown that XIAP can prevent the negative effects of immuno suppressive drugs on islet insulin secretion and cell viability (25). The intracellular signaling pathways by which the proinflammatory cytokines IL-1β, IFN-γ, and TNF-α are thought to induce apoptosis include activation of mitogen-activated protein kinases or nuclear factor κB (26) or TNF-α activation of caspase 8 (27). The mechanism by which alloreactive T-cells kill islet cell allografts is not completely understood but may involve the action of IFN-γ as well as Fas-FasL engagement (4). By inhibiting apoptotic cell death at a downstream converging point of these pathways, namely the activation of the effector

FIG. 3. Stable reversal to normoglycemia in streptozotocin-induced diabetic CBA mice following transplantation of Balb/c islets transduced ex vivo with Ad-XIAP. A: Blood glucose levels of diabetic CBA mice transplanted with Ad-XIAP–transduced islets (n = 10) or Ad-LacZ (n = 10) as control. B: Kaplan-Meier graft survival curve derived from blood glucose data. Note that censored normoglycemic recipients of Ad-XIAP–transduced grafts killed for graft histology on day 47 (n = 1), 52 (n = 1), and 56 (n = 2) are indicated with a tick mark. Survival curves were compared using the log-rank test (P < 0.0001). C: Intraperitoneal glucose tolerance test of CBA mice transplanted with Ad-XIAP–transduced islets (n = 4) or of nontransplanted, nondiabetic control mice (n = 6) 4 weeks posttransplantation. Data are means ± SE.
caspases 3 and 7, XIAP may provide a unique tool to halt the immunological cascade leading to β-cell death.

Our finding that infiltrating immune cells have largely disappeared from the XIAP-expressing grafts by ~60 days posttransplant raises the intriguing possibility that ignorance to or tolerance of the allograft may have been induced. We speculate that ex vivo XIAP transduction of islets may result in islet cells that are less prone to apoptosis after transplantation. Grafting more robust islet cells may diminish subsequent phagocytosis by antigen-presenting cells, thereby reducing the production of inflammatory signals required for allogeneic T-cell priming and thus decreasing the overall alloimmune response (7). Danger signals induced by cell death have been proposed to be essential for priming and maintaining immune responses (28). However, this apparent acceptance of the islet allograft occurred despite an initial immune response to the transplanted tissue, as demonstrated by the extensive immune infiltration of both the XIAP-expressing and control grafts observed at 10 days posttransplantation. Additional studies are currently underway to determine XIAP’s mechanism of action to prevent allograft rejection, but it is conceivable that there is less activation of leukocytes in the infiltrate of the XIAP-expressing grafts.

Whether continued XIAP expression is necessary for indefinite protection from allograft rejection remains unclear from our data; however, our finding that XIAP expression is greatly decreased at day ~60 posttransplant suggests that continued expression of XIAP may not be essential. Splenocytes isolated from recipients of XIAP-expressing islet grafts at 8–9 weeks posttransplantation show an allogeneic hyporesponsiveness that is indistinguishable from naive unprimed splenocytes, further supporting the possibility that XIAP expression in the early posttransplantation period may lead to acceptance of the islet allograft. Whether the return to baseline responder levels is the result of T-cell populations rendered anergic or the production of alternative regulatory cytokines remains to be determined.

A number of other approaches have been used to prolong the survival of islet allografts in animal models of diabetes (29), including antibody blockade of costimulatory or adhesion molecules to prevent lymphocyte activation (21,30–33), microencapsulation of islet cells (34), transplantation into immunoprivileged sites (35), cytoprotective gene therapy (36,37), and transplantation of transformed β-cells (38). Despite showing considerable promise, these approaches have limitations including uncertainty regarding their ability to provide long-term allograft protection (32,33,37), the poorly differentiated...
function of transformed β-cells (38), the need for greater numbers of islets to be transplanted (31,32), and the need for pan-immunosuppression to maintain allograft survival (21,30–33). Ex vivo transfer of other cytoprotective genes such as superoxide dismutase (37) or the nuclear factor-κB inhibitor A20 (39) has also been shown to provide modest protection of transplanted islets from autoimmune destruction or to decrease the number of islets needed for transplantation in syngeneic recipients, respectively. These strategies show great potential but also indicate that it is highly likely that a combinatorial gene therapy approach may be necessary in order to ensure long-term allograft protection in the presence of immunosuppression, while maintaining a low donor-to-recipient islet ratio. Our findings suggest that short-term overexpression of XIAP, administered ex vivo, may be sufficient to confer prolonged protection to islet allografts. This approach may have considerable therapeutic advantages, since it may obviate the need for long-term expression and regulation of virally transferred cytoprotective genes. Whether this approach can achieve permanent islet allograft engraftment and will confer protection in more stringent islet allograft models will require further study.

In summary, we have observed that overexpression of the anti-apoptotic gene XIAP in islet cells by ex vivo adenoviral transduction of isolated islets confers protection of these cells from allograft rejection, without impairing β-cell function. Moreover, we have found that protection of XIAP-expressing allotransplanted islet cells appears to continue even after adenoviral expression has largely waned, suggesting that protection from apoptosis in the days and weeks immediately posttransplantation may be of considerable importance in ensuring the longer-term survival of islet grafts. If indeed inhibition of apoptosis in transplanted islet cells in the initial posttransplant period can diminish the allogeneic response, our findings may also have implications for allografts of other tissues. Inhibition of apoptosis by proteins such as XIAP may be a novel approach to provide prolonged protection to transplanted cells.

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