Perspectives in Diabetes

Interventional Strategies to Prevent β-Cell Apoptosis in Islet Transplantation

Juliet A. Emamaullee[1] and A.M. James Shapiro[1,2]

A substantial proportion of the transplanted islet mass fails to engraft due to death by apoptosis, and a number of strategies have been explored to inhibit β-cell loss. Inhibition of extrinsic signals of apoptosis (i.e., cFLIP or A20) have been explored in experimental islet transplantation but have only shown limited impact. Similarly, strategies targeted at intrinsic signal inhibition (i.e., BCL-2) have not yet provided substantial improvement in islet engraftment. Recently, investigation of downstream apoptosis inhibitors that block the final common pathway (i.e., X-linked inhibitor of apoptosis protein [XIAP]) have demonstrated promise in both human and rodent models of engraftment. In addition, XIAP has enhanced long-term murine islet allograft survival. The complexities of both intrinsic and extrinsic apoptotic pathway inhibition are discussed in depth.
***Diabetes* 55:1907–1914, 2006**

Recent results of clinical islet transplantation have clearly demonstrated that islet transplantation can provide a population of type 1 diabetic patients with sustained and improved glycemic control and a period of insulin independence, but the current necessity for >10,000 islet equivalents (IE)/kg recipient body wt and the need for life-long immunosuppression limit the availability and applicability of this procedure (1). Investigation into the pathogenesis of type 1 diabetes and the relatively low incidence of diabetes following partial pancreatectomy has suggested that only a portion, perhaps as little as 20% of the islets within a pancreas, are necessary to maintain euglycemia (2,3). However, long-term follow-up of surgical patients after hemipancreatectomy suggests that obesity and/or insulin resistance may impair glucose tolerance (4). Since many more islets must be transplanted to reverse diabetes, a significant portion of the transplanted islets fail to engraft and become functional. It has been estimated that up to 70% of the transplanted β-cell mass may be destroyed in the early posttransplant period (5–7). Since this profound loss has been observed in both immunodeficient and syngeneic islet transplantation models, the biggest factor that negatively influences islet survival likely reflects a nonimmune-mediated physiological stress, namely prolonged hypoxia during the revascularization process, which can take up to 2 weeks (8–11). Tissue factor expression and release in isolated islets may also negatively influence the engraftment of transplanted islets through the instant blood-mediated inflammatory reaction (IBMIR) and subsequent platelet activation, clot formation, and lymphocyte recruitment (12,13). Also, local secretion of proinflammatory cytokines by infiltrating lymphocytes further contributes to islet demise. For all of these reasons, a significant fraction of the islet graft becomes injured rapidly after portal infusion and is lost due to apoptosis, which begins during the isolation process, peaks 2–3 days' posttransplant, and continues for approximately 2 weeks, until what remains of the graft has stabilized and become revascularized (5,6,9,11,14–16). During this engraftment period, the islets are continuously exposed to immunosuppressive drugs, including tacrolimus and sirolimus, which are known to adversely impact β-cell survival and function (17). These negative effects are likely compounded by the proximity of the transplanted islets and high concentrations of these drugs in the hepatoportal circulation, further contributing to loss in β-cell mass over time (18,19).

Given the limited supply of cadaveric donor pancreata and the prevalence of type 1 diabetes, considerable efforts have been made to prevent the loss of islet mass in the immediate posttransplant period. Many studies targeted at enhancing islet survival during the early posttransplant period have been published, and a variety of different strategies have been tested. For example, overexpression of molecules known to enhance revascularization, such as vascular endothelial growth factor, have been attempted, but these have yet to exhibit a significant impact on islet graft survival (20). This is likely related to the fact that although vascular endothelial growth factor expression will hasten the revascularization process, it cannot provide an immediate benefit to the transplanted tissue. Other groups have tested several different molecules that inhibit the generation of and/or damage mediated by reactive oxygen species (ROS), including glutathione peroxidase, superoxide dismutase, and heme oxygenase-1 (21–24). Although these molecules can individually protect islets during controlled in vitro challenges where ROS are specifically produced, the in vivo benefit of such an approach has only been demonstrated when glutathione peroxidase and superoxide dismutase were coexpressed in transgenic islet grafts (25). Likewise, anticoagulation strategies aimed at inhibiting the IBMIR, using injection of activated protein C or inhibition of thrombin, have shown

From the [1]Department of Surgery, University of Alberta, Edmonton, Alberta, Canada; and the [2]Clinical Islet Transplant Program, University of Alberta, Edmonton, Alberta, Canada.

Address correspondence and reprint requests to Juliet Emamaullee, PhD, 1074 Dentistry-Pharmacy Centre, Surgical Medical Research Institute, University of Alberta, Edmonton, AB T6G 2N8. E-mail: juliete@ualberta.ca.

Received for publication 26 September 2005 and accepted in revised form 29 March 2006.

cFLIP, cellular FLICE-inhibitory protein; DISC, death-inducing signaling complex; FADD, Fas-associated death domain; IBMIR, instant blood-mediated inflammatory reaction; IL-1β, interleukin-1β; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; TNF, tumor necrosis factor; XIAP, X-linked inhibitor of apoptosis protein.

DOI: 10.2337/db05-1254

only a modest benefit in a limited series of in vivo studies in animal models, and early results of clinical interventional strategies are pending (13,26). The failure of each of these approaches to significantly preserve islet mass posttransplant is most likely related to the fact that the islets are already quite stressed due to the isolation process. As such, they are past the point of gaining overall benefit from molecules that block or ameliorate specific steps along the path to cell death, such as during the production of ROS, the IBMIR, or the later stages of chronic hypoxia.

Since the processes described above involve both extracellular (i.e., IBMIR) and intracellular (i.e., hypoxia and ROS) stimuli leading to β-cell apoptosis, another approach to preserve β-cell mass in the early posttransplant period has been to directly inhibit the apoptotic cascade. Widespread apoptosis in the implanted tissue may also have long-term deleterious consequences in islet transplantation, since the recipient's immune system is challenged with a large amount of apoptotic tissue, possessing both allo- and autoantigens from two or more donors. This suggests that preservation of islet graft mass and function with long-term immunosuppression or tolerance induction is a major obstacle in the clinical setting.

PATHWAYS TO CASPASE ACTIVATION AND APOPTOSIS

Higher-order organisms must regulate both the quality and quantity of each cell type they possess in order to prevent the deleterious effects associated with overgrowth, mutation, infection, or damage. This evolutionarily conserved mechanism is termed apoptosis, or "programmed cell death," and there are multiple triggers and pathways that control its initiation and progress within the cell. At the heart of this mechanism lies the caspases, a set of highly conserved cysteine proteases that are activated by a variety of proapoptotic stimuli. Caspases are activated in a hierarchical order, where initiator caspases (i.e., caspases-8, and -10) function to cleave effector caspases (i.e., caspases-3 and -7), which in turn degrade a number of intracellular protein substrates. This leads to the classical morphological changes associated with apoptosis, including chromatin condensation, nuclear degeneration, and cellular dehydration (27).

Extracellular events present during the inflammatory response, such as the release of cytokines, including tumor necrosis factor (TNF)-α, interleukin-1β (IL-1β), and interferon-γ by infiltrating leukocytes or direct cytotoxic T-cell engagement, can initiate apoptosis. These "extrinsic" cues function via surface molecules in the death receptor pathway, where specific ligand-receptor binding (such as TNF–TNF receptor I binding, Fas [CD95]–Fas ligand [CD178] binding, etc.) leads to receptor clustering, adapter molecule recruitment (i.e., Fas-associated death domain [FADD]), and formation of the death-inducing signaling complex (DISC) (28). Caspase-8 associates with the DISC complex, where it is activated and released, leading to effector caspase activation (notably caspase-3) (Fig. 1) (27,28).

Intracellular or "intrinsic" cues, such as DNA damage, hypoxia, nutrient deprivation, or ROS, function via the mitochondrial pathway, which is tightly modulated by the BCL-2 proteins. In a healthy cell, proapoptotic BCL-2 proteins (Bim, Bid, Bad, Bax, and Bak) are present in their inactive form, while antiapoptotic BCL-2 proteins (BCL-2 and BCL-XL) are constitutively active and reside in the outer membrane of the mitochondria (29). Following an intrinsic cue, proapoptotic BCL-2 proteins become activated and translocate to the mitochondria, where they either bind to and inactivate antiapoptotic BCL-2 proteins or form pores in the mitochondrial membrane, which facilitates the release of cytochrome c into the cytosol. Once cytochrome c accumulates in the cytosol, it complexes with procaspase-9 and Apaf-1 to form the "apoptosome," which in turn activates caspase-3 (Fig. 1). Thus, the default BCL-2 signal is to preserve mitochondrial membrane integrity and prevent apoptosis, and as such, apoptosis can only occur when the concentration of proapoptotic BCL-2 proteins exceeds that of the antiapoptotic BCL-2 proteins at the mitochondrial membrane. It should be noted that the mitochondrial pathway can be indirectly triggered through less obvious extracellular signals. For example, following death receptor ligation, activated caspase-8 can cleave and activate Bid (29). Both intrinsic and extrinsic signaling cascades converge at the point of caspase-3 activation, which is often considered the "point of no return" in apoptosis (rev. in 27,30).

GENETIC MANIPULATIONS TO PREVENT B-CELL APOPTOSIS

Inhibition of extrinsic signals. Type 1 diabetes occurs following the selective loss of pancreatic β-cells, which is characterized by mononuclear cell infiltration around and within the islets, a histological feature termed "insulitis." This peri-islet inflammation is associated with proinflammatory cytokine (i.e., IL-1β, TNF-α, and interferon-γ) release by monocytes and Fas ligation by autoreactive T-cells, leading to the destruction of the β-cells and the onset of hyperglycemia (31). Since the sensitivity of islets to cytokine- and Fas-mediated apoptosis during the process of autoimmunity is compelling, and since the alloimmune response exerts a similar effect on transplanted tissue, some of the earliest efforts in prevention of β-cell death following transplantation have involved the inhibition of these extrinsic signals (32–37).

Apoptosis via Fas/FasL interactions has been proposed to be a major T-cell–mediated effector mechanism in the pathogenesis of type 1 diabetes. Examination of Fas expression in transplanted syngeneic islets from nonobese diabetic (NOD) mice demonstrated that autoimmune infiltration of islet grafts leads to high Fas levels in β-cells (37). In addition, it has been shown that proinflammatory cytokine exposure leads to enhanced Fas expression in both murine and human islets (32,37). As mentioned previously, Fas signaling leads to FADD recruitment, DISC formation, and caspase-8 activation. Similarly, proinflammatory cytokines bind to their receptors on β-cells, leading to apoptosis via FADD recruitment and caspase-8 activation. In the mid-1990s, an endogenous inhibitor of caspase-8 activation was identified, termed the cellular FLICE-inhibitory protein (cFLIP) (38). (FLICE is another name for caspase-8.) cFLIP is the same length as and structurally similar to caspase-8, but its caspase domain is altered, rendering it enzymatically inactive. Thus, cFLIP functions as a dominant-negative inhibitor of caspase-8 at the DISC, preventing the activation of caspase-8 and its ability to activate downstream effector caspases (Fig. 2).

Given the attractive antiapoptotic function of cFLIP, the protective effect of cFLIP overexpression in β-cells during islet transplantation has been investigated. By stably transfecting a growth-regulatable murine β-cell line (βTC-Tet) with cFLIP, Cottet et al. (39) demonstrated that cFLIP

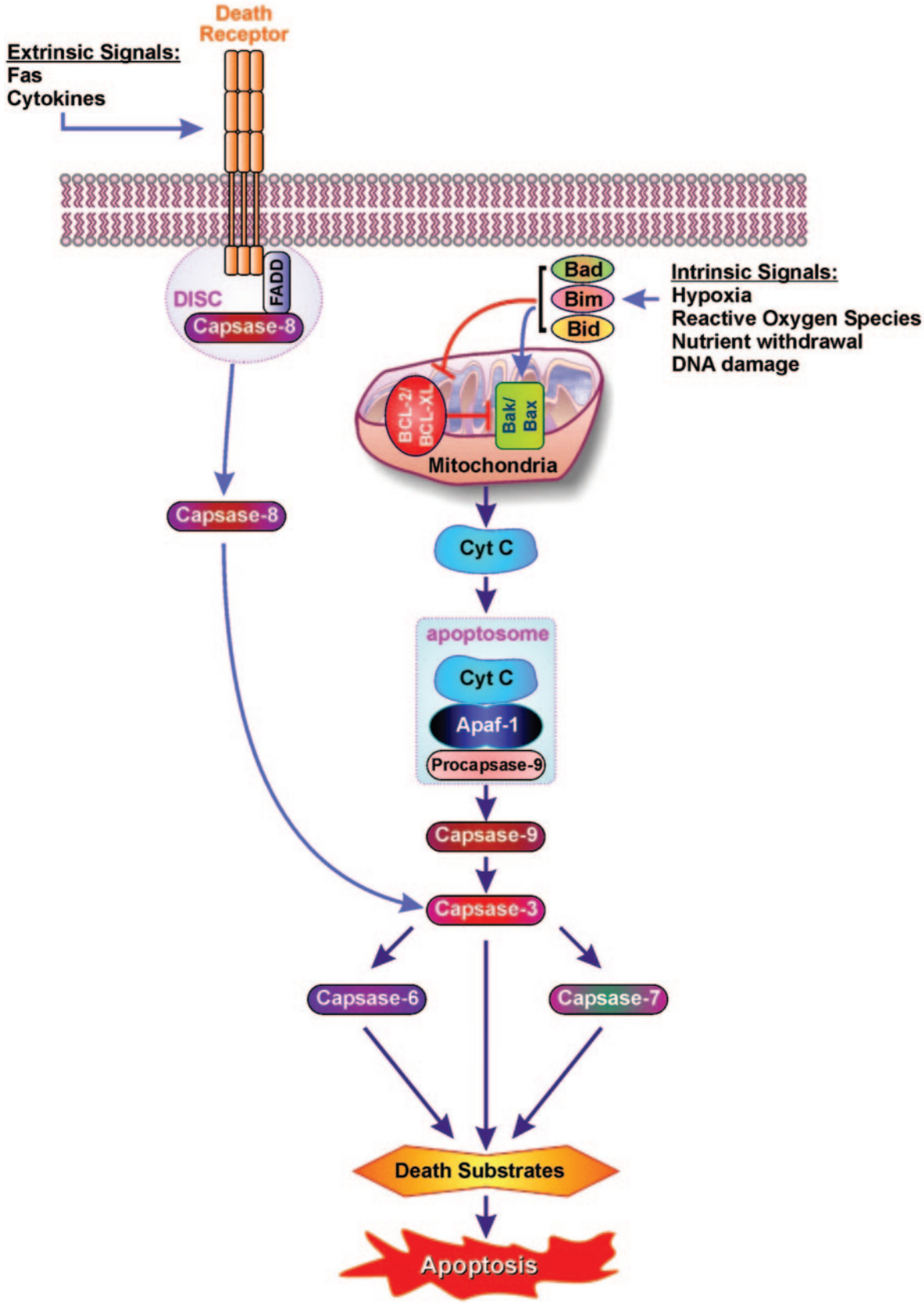

FIG. 1. Intrinsic and extrinsic pathways leading to apoptosis. Cyt C, cytochrome C.

overexpression prevented cytokine-mediated apoptosis in vitro, although it did not preserve glucose-stimulated insulin release during this insult. Lentiviral-mediated cFLIP overexpression in another murine β-cell line (NIT-1) has also been shown to prevent cytokine-mediated β-cell death in vitro (40). However, the in vitro benefit of cFLIP

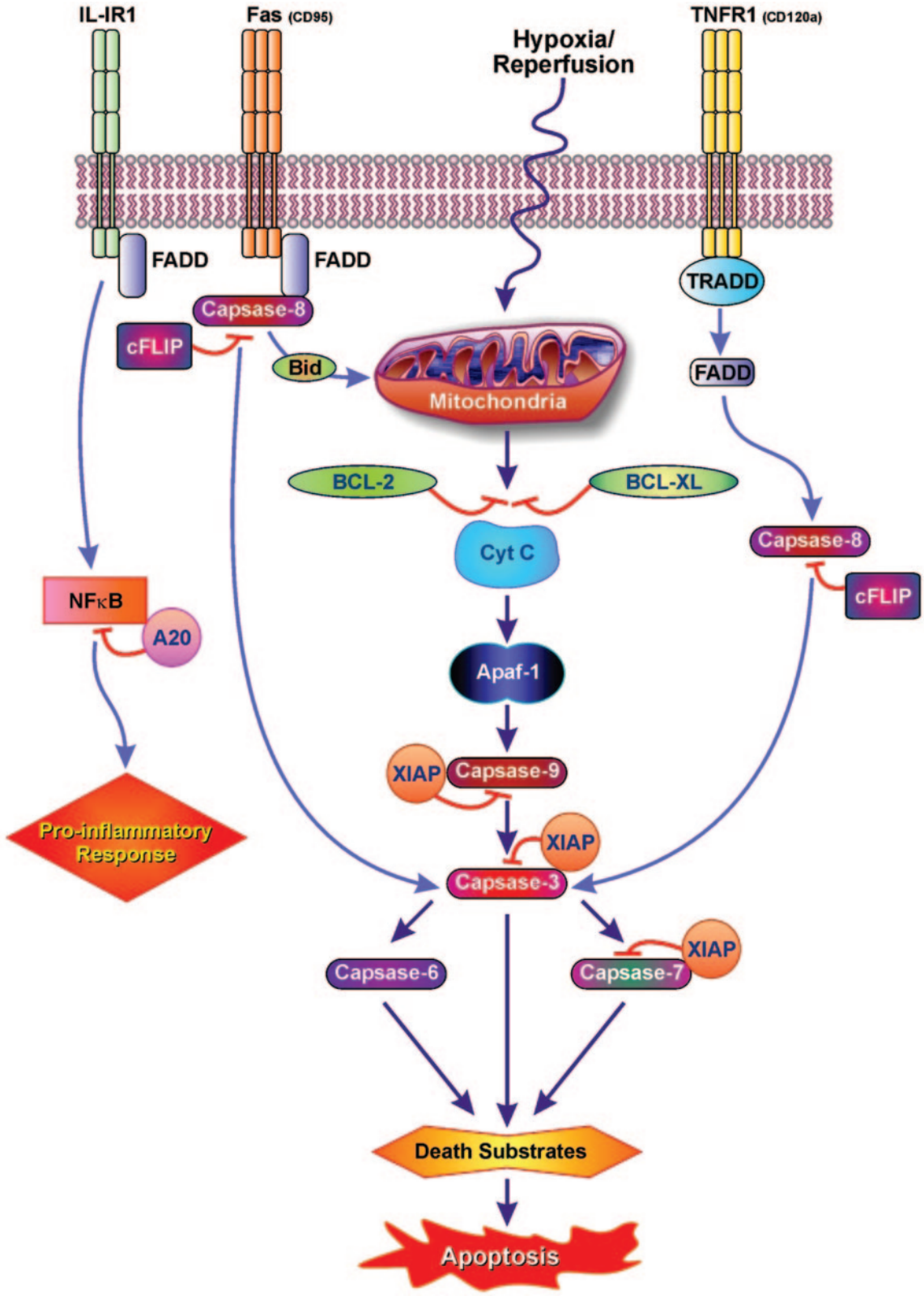

FIG. 2. Summary of antiapoptotic gene manipulations in islet transplantation. Genetic manipulations targeted to block initiation of apoptotic pathways (i.e., cFLIP, BCL-2, and BCL-XL) have proven effective in preventing apoptosis induced by their respective targets in vitro but have failed to show any significant protective effect in vivo during islet engraftment. XIAP has potential to be a potent inhibitor of islet death in vitro and in vivo, most likely due to its ability to prevent activation of effector caspases that function late in apoptosis, beyond the convergence point of many different stimuli. TRADD, TNF receptor–associated death domain.

overexpression in islet β-cells has not been observed in vivo (41). This disappointing observation can most likely be attributed to the recent finding that cFLIP has a dual functionality that depends on expression levels and can, under some circumstances, actually promote apoptosis. Initially, it was determined that cFLIP-deficient cells were

more sensitive to death receptor–induced apoptosis, which is consistent with the role of cFLIP as a dominant-negative inhibitor of caspase-8 (38). However, recent data have shown that at high expression levels, cFLIP can activate procaspase-8 via heterodimerization (38). Since caspase-8 is also involved in cell cycle progression and proliferation, complete blockade of caspase-8 may be undesirable, so it makes sense that at high levels of expression, cFLIP no longer inhibits its activation. In the context of islet transplantation, it is quite difficult, if not impossible, to consistently transduce all of the cells within an islet and establish uniform expression levels of a transgene in each of the islet β-cells using a viral vector (the most efficient way to deliver genes to intact islets). It is therefore not surprising that no in vivo experiments have been published that demonstrate a protective benefit of cFLIP overexpression in islet grafts. Also, considering that cFLIP only prevents the extrinsic pathway of apoptosis and as such has no effect on intrinsic signals, such as hypoxia or ROS, it is unlikely that cFLIP alone could adequately protect islet grafts.

In addition to transmitting apoptotic signals through receptor ligation, cytokine exposure can exert additional negative effects on islets by upregulating proteins, including inducible nitric oxide synthase, that lead to the generation of ROS within β-cells, activating the intrinsic pathway to apoptosis via the mitochondria. This secondary signal occurs via activation of the transcription factor nuclear factor-κB (NF-κB). In healthy cells, NF-κB is sequestered in the cytoplasm by the protein inhibitor of κB kinase (IκK), and following inflammatory stimuli (i.e., cytokine or lipopolysaccaride exposure, ROS generation following reperfusion injury), IκK is phosphorylated, which liberates NF-κB and allows its translocation to the nucleus (42). Depending on the cell type and stimulus, NF-κB can promote transcription of proapoptotic genes or antiapoptotic genes. NF-κB activation in β-cells has been associated with the upregulation of proinflammatory genes, including IL-1β, intracellular adhesion molecule-1, and inducible nitric oxide synthase (42). An endogenous inhibitor of NF-κB that prevents its activation has been identified, termed A20 (Fig. 2). A20 is a zinc–ring finger protein not normally expressed in healthy cells and is instead upregulated by NF-κB to provide feedback inhibition following its activation (43). The exact interaction between A20 and NF-κB has been poorly understood until recently, when it was determined that A20 inhibits IκK phosphorylation indirectly by ubiquitin modification of the upstream kinases that act upon it, rendering them inactive (43). Since inhibition of NF-κB with A20 could potentially inhibit extrinsic apoptotic signals, as well as some intrinsic signals (i.e., reoxygenation injury), the protective benefit of its overexpression in islets has been studied. Adenovirally mediated overexpression of A20 in rodent and human islets reduced cytokine-induced apoptosis and subsequent ROS production in vitro (44). A20 overexpression has also been associated with improved islet survival in vivo, using a syngeneic marginal islet mass transplantation model. Gray et al. (45) showed that grafts containing 250 islets overexpressing A20 reversed diabetes in 75% of the recipients, compared with control grafts of 250 islets, which only reversed diabetes in 20% of recipients. However, there was still evidence of caspase-3 in islet grafts from both cohorts, confirming that A20 does not inhibit all pathways of posttransplant apoptosis (45).

Despite its apparent promise, the long-term prospects of A20 in clinical islet transplantation remain unclear. NF-κB is not activated during hypoxia, a major determinant of posttransplant islet loss, so A20 overexpression cannot overcome this early trigger of β-cell death. It has also recently been determined that following oxidative stress, A20 actually enhanced ROS-induced apoptosis (46). Although NF-κB activation is most often assumed to upregulate proinflammatory genes, a series of experiments by Fan et al. (47) clearly demonstrated that the timing of NF-κB activation significantly influences apoptotic outcomes following various stimuli. For example, inhibiting NF-κB activation during reperfusion injury lead to increased apoptosis, while inhibiting NF-κB activation before reperfusion injury prevented apoptosis (47). Thus, the timing of A20 expression may be critical in determining islet survival, which provides an additional layer of complexity to the clinical implementation of A20 therapy.

Inhibition of intrinsic signals. Given the mixed success of extrinsic apoptotic signal inhibition in experimental islet transplantation and the major contribution of intrinsic signals to islet death posttransplant (i.e., hypoxia, ROS formation during reperfusion, nutrient deprivation, etc.), strategies targeted at preventing mitochondria-mediated apoptosis have been extensively investigated. Many experiments have examined the impact of BCL-2 overexpression in β-cell lines and islets and have shown that it can inhibit apoptosis due to hypoxia, proinflammatory cytokines, staurosporine, serum withdrawal, and xenoreactive antibodies in vitro (48–51). However, these promising results have been met with mixed outcomes in animal models of transplantation. Adenovirally mediated BCL-2 overexpression in macaque islets reduced the number of islets necessary to reverse diabetes in chemically diabetic nude mice, while βTC-Tet cells stably transfected with BCL-2 exhibited no survival advantage in vivo, despite enhanced proliferative capacity (41,48,51). Another antiapoptotic BCL-2 family member, BCL-XL, has proven to be effective in preventing apoptosis triggered by IL-1β, staurosporine, and serum withdrawal in vitro (52). While transgenic overexpression of BCL-XL in islets prevented ROS-induced apoptosis in vitro, the transgenic animals were severely glucose intolerant due to a defect in mitochondrial nutrient metabolism and signaling for insulin secretion (53). Also, consistent with the inability of BCL-2 family members to inhibit extrinsic signals of apoptosis, transgenic expression of BCL-2 in islets had no effect on the T-cell–mediated destruction of islets or diabetes onset in NOD mice (53). Thus, it remains unclear how much of an impact BCL-2 or BCL-XL overexpression might have in clinical islet transplantation.

EFFECTOR CASPASE INHIBITION TO PREVENT BOTH EXTRINSIC AND INTRINSIC SIGNALS

Based on the studies cited above, it has become clear that inhibition of either the extrinsic or intrinsic pathway of apoptosis alone is insufficient to profoundly promote β-cell survival posttransplantation. Combining inhibitors of both pathways might enhance islet protection, but the difficulties in efficiently and reproducibly delivering and regulating multiple genes presents a significant challenge in islet transplantation. It would therefore be most attractive to inhibit apoptosis at a point beyond the convergence point of both pathways with a single gene product, in effect, preventing all β-cell apoptosis, regardless of the specific stimuli. Over the past decade, a new family of

endogenous apoptosis modulatory proteins has been identified, the inhibitors of apoptosis proteins. These proteins share common structural motifs with unique functions that work in unison to block, and possibly degrade, multiple effector caspases late in the cascade of apoptotic triggers, thus preventing cell death at one common point for a variety of different activation pathways.

In other models of apoptotic disease, overexpression of X-linked inhibitor of apoptosis protein (XIAP), the most potent inhibitor of apoptosis protein, has produced impressive data. XIAP, which binds to the active site of all of the main effector caspases (caspase-3, -7, and -9), is known to prevent apoptosis triggered by a number of stimuli in vitro, including Fas ligation, cytokine exposure, hypoxia, reoxygenation injury, nutrient withdrawal, and chemotherapeutic agents (54–57). Brain injury due to transient forebrain ischemia and retinal degeneration in an animal model of retinitis pigmentosa were markedly reduced by overexpressing XIAP in the target tissue using an adenoviral vector (55,58,59). Thus, XIAP has shown promise as a potential gene product that could provide a significant survival advantage to transplanted islets in the face of the diverse and plentiful apoptotic stimuli present following portal vein infusion and during the prolonged engraftment period.

Several recent studies examining the effect of adenovirally delivered XIAP overexpression have shown promise in experimental islet transplant models. XIAP overexpression in βTC-Tet cells, a growth-regulatable murine β-cell line, significantly enhanced β-cell survival in vitro during periods of stress due to hypoxia, hypoxia followed by reoxygenation, and cytokine insult (60). XIAP overexpression preserved both β-cell viability and glucose responsiveness, the latter being a critical function that ordinarily disappears very early on during hypoxic stress. These results were confirmed in vivo by the finding that the mean time to achieve normoglycemia in recipient mice was decreased nearly sevenfold when XIAP was overexpressed within β-cell grafts (60). The protective benefit of XIAP overexpression has also been documented in isolated human islets (61,62). XIAP overexpression has been shown to prevent the diabetogenicity of the immunosuppressive drugs tacrolimus and sirolimus in vitro (62). It has also been demonstrated that XIAP-transduced human islets were significantly less apoptotic in an in vitro system that mimics hypoxia and hypoxia/reoxygenation injury (61). In the same study, a series of marginal mass islet graft transplants were performed in streptozotocin-induced diabetic NOD-RAG$^{-/-}$ mice, and 89% of animals became normoglycemic with only 600 XIAP-transduced human islets (61). In terms of allogeneic islet transplantation, a recent study has shown that 90% of streptozotocin-induced diabetic CBA/J (H2-k) animals receiving XIAP-transduced Balb/C (H-2d) islet allografts survived up to 72 days (an end point established to collect tissue for histology), compared with a mean allograft survival of 17 days in LACZ-transduced control grafts (63). Remarkably, despite the presence of a CD45+ immune infiltrate in XIAP grafts at day 10 posttransplant, this immune infiltration was completely absent by day 60 posttransplant, suggesting that the apoptosis-resistant XIAP grafts failed to adequately prime the recipient's immune system, resulting in a state of immune ignorance or tolerance in the absence of immunosuppressive therapy (63).

CONCLUSION

Before clinical islet transplantation can be made accessible to a broader population of patients with type 1 diabetes, the current necessity for large amounts of donor islet tissue must be overcome. Since it has been determined that ≥60% of the islet tissue is lost to apoptosis soon after portal infusion, even in the absence of immune destruction, preventing this early posttransplant death would have an immediate impact on islet transplantation. The processes that negatively influence the survival of transplanted islets are diverse and complex, but they all ultimately lead to apoptosis. Significant efforts have been made to inhibit specific apoptotic triggers, either extrinsic (cFLIP and A20) or intrinsic (BCL-2 and BCL-XL). These proteins have proven quite effective in enhancing β-cell survival in vitro, following exposure to the particular stimulus each protein is known to block. However, reproducing the protective effect using transplanted islets has been difficult and largely unfruitful. Recent studies using XIAP strongly support the concept that inhibition of apoptosis at the level of effector caspases promotes β-cell survival in islet transplantation, effectively preventing cell death triggered by extrinsic and intrinsic pathways at the same time. Since XIAP overexpression has been shown to reduce the amount of tissue required to restore euglycemia, delivery of the XIAP gene to clinical islet grafts should broaden the availability of cadaveric islet transplantation and possibly living donor islet transplantation. Enhancing islet survival posttransplant should prolong graft longevity resulting in a more quiescent immunological state and thereby enhancing long-term rates of insulin independence, a hypothesis supported by the finding that XIAP-transduced murine allografts survived long term (63). In the clinical setting, inhibition of islet apoptosis in the immediate posttransplant period may reduce the amount and intensity of antirejection therapy, accelerating "accommodation" and drug minimization. If these outcomes could be achieved, islet transplantation would be potentially safer and therefore more available to a broader spectrum of patients with type 1 diabetes, including children.

ACKNOWLEDGMENTS

J.A.E. is supported by a postdoctoral fellowship from the Alberta Heritage Foundation for Medical Research (AHFMR). A.M.J.S. is supported by a research scientist award from the AHFMR and is a recipient of a Canadian Institutes of Health Research/Wyeth Research Chair in Transplantation.

REFERENCES

1. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
2. Slezak LA, Andersen DK: Pancreatic resection: effects on glucose metabolism. *World J Surg* 25:452–460, 2001
3. Weir GC, Bonner-Weir S: Five stages of evolving β-cell dysfunction during progression to diabetes. *Diabetes* 53 (Suppl. 3):S16–S21, 2004
4. Robertson RP, Lanz KJ, Sutherland DE, Seaquist ER: Relationship between diabetes and obesity 9 to 18 years after hemipancreatectomy and transplantation in donors and recipients. *Transplantation* 73:736–741, 2002
5. Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E: β-Cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 51:66–72, 2002
6. Davalli AM, Ogawa Y, Ricordi C, Scharp DW, Bonner-Weir S, Weir GC: A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation* 59:817–820, 1995

7. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM: Five-year follow-up after clinical islet transplantation. *Diabetes* 54:2060–2069, 2005
8. Carlsson PO, Palm F, Andersson A, Liss P: Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 50:489–495, 2001
9. Carlsson PO, Palm F, Mattsson G: Low revascularization of experimentally transplanted human pancreatic islets. *J Clin Endocrinol Metab* 87:5418–5423, 2002
10. Dionne KE, Colton CK, Yarmush ML: Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans. *Diabetes* 42:12–21, 1993
11. Giuliani M, Moritz W, Bodmer E, Dindo D, Kugelmeier P, Lehmann R, Gassmann M, Groscurth P, Weber M: Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. *Cell Transplant* 14:67–76, 2005
12. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, Ostraat O, Salmela K, Tibell A, Tufveson G, Elgue G, Nilsson Ekdahl K, Korsgren O, Nilsson B: Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 360:2039–2045, 2002
13. Ozmen L, Ekdahl KN, Elgue G, Larsson R, Korsgren O, Nilsson B: Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes* 51:1779–1784, 2002
14. Paraskevas S, Maysinger D, Wang R, Duguid TP, Rosenberg L: Cell loss in isolated human islets occurs by apoptosis. *Pancreas* 20:270–276, 2000
15. Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir S, Weir GC: Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function. *Diabetes* 45:1161–1167, 1996
16. Moritz W, Meier F, Stroka DM, Giuliani M, Kugelmeier P, Nett PC, Lehmann R, Candinas D, Gassmann M, Weber M: Apoptosis in hypoxic human pancreatic islets correlates with HIF-1alpha expression. *FASEB J* 16:745–747, 2002
17. Hyder A, Laue C, Schrezenmeir J: Effect of the immunosuppressive regime of Edmonton protocol on the long-term in vitro insulin secretion from islets of two different species and age categories. *Toxicol In Vitro* 19:541–546, 2005
18. Desai NM, Goss JA, Deng S, Wolf BA, Markmann E, Palanjian M, Shock AP, Feliciano S, Brunicardi FC, Barker CF, Naji A, Markmann JF: Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? *Transplantation* 76:1623–1625, 2003
19. Shapiro AM, Gallant HL, Hao EG, Lakey JR, McCready T, Rajotte RV, Yatscoff RW, Kneteman NM: The portal immunosuppressive storm: relevance to islet transplantation? *Ther Drug Monit* 27:35–37, 2005
20. Narang AS, Cheng K, Henry J, Zhang C, Sabek O, Fraga D, Kotb M, Gaber AO, Mahato RI: Vascular endothelial growth factor gene delivery for revascularization in transplanted human islets. *Pharm Res* 21:15–25, 2004
21. Moriscot C, Pattou F, Kerr-Conte J, Richard MJ, Lemarchand P, Benhamou PY: Contribution of adenoviral-mediated superoxide dismutase gene transfer to the reduction in nitric oxide-induced cytotoxicity on human islets and INS-1 insulin-secreting cells. *Diabetologia* 43:625–631, 2000
22. Tobiasch E, Gunther L, Bach FH: Heme oxygenase-1 protects pancreatic beta cells from apoptosis caused by various stimuli. *J Invest Med* 49:566–571, 2001
23. Pileggi A, Molano RD, Berney T, Cattan P, Vizzardelli C, Oliver R, Fraker C, Ricordi C, Pastori RL, Bach FH, Inverardi L: Heme oxygenase-1 induction in islet cells results in protection from apoptosis and improved in vivo function after transplantation. *Diabetes* 50:1983–1991, 2001
24. Lepore DA, Shinkel TA, Fisicaro N, Mysore TB, Johnson LE, d'Apice AJ, Cowan PJ: Enhanced expression of glutathione peroxidase protects islet beta cells from hypoxia-reoxygenation. *Xenotransplantation* 11:53–59, 2004
25. Mysore TB, Shinkel TA, Collins J, Salvaris EJ, Fisicaro N, Murray-Segal LJ, Johnson LE, Lepore DA, Walters SN, Stokes R, Chandra AP, O'Connell PJ, d'Apice AJ, Cowan PJ: Overexpression of glutathione peroxidase with two isoforms of superoxide dismutase protects mouse islets from oxidative injury and improves islet graft function. *Diabetes* 54:2109–2116, 2005
26. Contreras JL, Eckstein C, Smyth CA, Bilbao G, Vilatoba M, Ringland SE, Young C, Thompson JA, Fernandez JA, Griffin JH, Eckhoff DE: Activated protein C preserves functional islet mass after intraportal transplantation: a novel link between endothelial cell activation, thrombosis, inflammation, and islet cell death. *Diabetes* 53:2804–2814, 2004
27. Hengartner MO: The biochemistry of apoptosis. *Nature* 407:770–776, 2000
28. Boatright KM, Salvesen GS: Mechanisms of caspase activation. *Curr Opin Cell Biol* 15:725–731, 2003
29. Chan SL, Yu VC: Proteins of the bcl-2 family in apoptosis signalling: from mechanistic insights to therapeutic opportunities. *Clin Exp Pharmacol Physiol* 31:119–128, 2004
30. Green DR: Apoptotic pathways: paper wraps stone blunts scissors. *Cell* 102:1–4, 2000
31. Hui H, Dotta F, Di Mario U, Perfetti R: Role of caspases in the regulation of apoptotic pancreatic islet beta-cells death. *J Cell Physiol* 200:177–200, 2004
32. Loweth AC, Williams GT, James RF, Scarpello JH, Morgan NG: Human islets of Langerhans express Fas ligand and undergo apoptosis in response to interleukin-1β and Fas ligation. *Diabetes* 47:727–732, 1998
33. Savinov AY, Tcherepanov A, Green EA, Flavell RA, Chervonsky AV: Contribution of Fas to diabetes development. *Proc Natl Acad Sci U S A* 100:628–632, 2003
34. Silva DG, Petrovsky N, Socha L, Slattery R, Gatenby P, Charlton B: Mechanisms of accelerated immune-mediated diabetes resulting from islet beta cell expression of a Fas ligand transgene. *J Immunol* 170:4996–5002, 2003
35. Su X, Hu Q, Kristan JM, Costa C, Shen Y, Gero D, Matis LA, Wang Y: Significant role for Fas in the pathogenesis of autoimmune diabetes. *J Immunol* 164:2523–2532, 2000
36. Chervonsky AV, Wang Y, Wong FS, Visintin I, Flavell RA, Janeway CA Jr, Matis LA: The role of Fas in autoimmune diabetes. *Cell* 89:17–24, 1997
37. Suarez-Pinzon W, Sorensen O, Bleackley RC, Elliott JF, Rajotte RV, Rabinovitch A: β-Cell destruction in NOD mice correlates with Fas (CD95) expression on β-cells and proinflammatory cytokine expression in islets. *Diabetes* 48:21–28, 1999
38. Peter ME: The flip side of FLIP. *Biochem J* 382:e1–e3, 2004
39. Cottet S, Dupraz P, Hamburger F, Dolci W, Jaquet M, Thorens B: cFLIP protein prevents tumor necrosis factor-α–mediated induction of caspase-8–dependent apoptosis in insulin-secreting βTc-Tet cells. *Diabetes* 51:1805–1814, 2002
40. Fenjves ES, Ochoa MS, Gay-Rabinstein C, Ricordi C, Curran MA: Retrovirally transferred genes inhibit apoptosis in an insulin-secreting cell line: implications for islet transplantation. *Cell Transplant* 13:489–496, 2004
41. Cattan P, Rottembourg D, Cottet S, Tardivel I, Dupraz P, Thorens B, Boitard C, Carel JC: Destruction of conditional insulinoma cell lines in NOD mice: a role for autoimmunity. *Diabetologia* 46:504–510, 2003
42. Ho E, Bray TM: Antioxidants, NFkappaB activation, and diabetogenesis. *Proc Soc Exp Biol Med* 222:205–213, 1999
43. Heyninck K, Beyaert R: A20 inhibits NF-kappaB activation by dual ubiquitin-editing functions. *Trends Biochem Sci* 30:1–4, 2005
44. Grey ST, Arvelo MB, Hasenkamp W, Bach FH, Ferran C: A20 inhibits cytokine-induced apoptosis and nuclear factor kappaB-dependent gene activation in islets. *J Exp Med* 190:1135–1146, 1999
45. Grey ST, Longo C, Shukri T, Patel VI, Csizmadia E, Daniel S, Arvelo MB, Tchipashvili V, Ferran C: Genetic engineering of a suboptimal islet graft with A20 preserves beta cell mass and function. *J Immunol* 170:6250–6256, 2003
46. Storz P, Doppler H, Ferran C, Grey ST, Toker A: Functional dichotomy of A20 in apoptotic and necrotic cell death. *Biochem J* 387:47–55, 2005
47. Fan C, Yang J, Engelhardt JF: Temporal pattern of NFkappaB activation influences apoptotic cell fate in a stimuli-dependent fashion. *J Cell Sci* 115:4843–4853, 2002
48. Dupraz P, Rinsch C, Pralong WF, Rolland E, Zufferey R, Trono D, Thorens B: Lentivirus-mediated Bcl-2 expression in betaTC-tet cells improves resistance to hypoxia and cytokine-induced apoptosis while preserving in vitro and in vivo control of insulin secretion. *Gene Ther* 6:1160–1169, 1999
49. Allison J, Thomas H, Beck D, Brady JL, Lew AM, Elefanty A, Kosaka H, Kay TW, Huang DC, Strasser A: Transgenic overexpression of human Bcl-2 in islet beta cells inhibits apoptosis but does not prevent autoimmune destruction. *Int Immunol* 12:9–17, 2000
50. Contreras JL, Bilbao G, Smyth C, Eckhoff DE, Xiang XL, Jenkins S, Cartner S, Curiel DT, Thomas FT, Thomas JM: Gene transfer of the Bcl-2 gene confers cytoprotection to isolated adult porcine pancreatic islets exposed to xenoreactive antibodies and complement. *Surgery* 130:166–174, 2001
51. Contreras JL, Bilbao G, Smyth CA, Jiang XL, Eckhoff DE, Jenkins SM, Thomas FT, Curiel DT, Thomas JM: Cytoprotection of pancreatic islets before and soon after transplantation by gene transfer of the anti-apoptotic Bcl-2 gene. *Transplantation* 71:1015–1023, 2001
52. Klein D, Ribeiro MM, Mendoza V, Jayaraman S, Kenyon NS, Pileggi A, Molano RD, Inverardi L, Ricordi C, Pastori RL: Delivery of Bcl-XL or its BH4 domain by protein transduction inhibits apoptosis in human islets. *Biochem Biophys Res Commun* 323:473–478, 2004
53. Zhou YP, Pena JC, Roe MW, Mittal A, Levisetti M, Baldwin AC, Pugh W, Ostrega D, Ahmed N, Bindokas VP, Philipson LH, Hanahan D, Thompson CB, Polonsky KS: Overexpression of Bcl-x(L) in beta-cells prevents cell

death but impairs mitochondrial signal for insulin secretion. *Am J Physiol Endocrinol Metab* 278:E340–E351, 2000
54. Straszewski-Chavez SL, Abrahams VM, Funai EF, Mor G: X-linked inhibitor of apoptosis (XIAP) confers human trophoblast cell resistance to Fas-mediated apoptosis. *Mol Hum Reprod* 10:33–41, 2004
55. Xu D, Bureau Y, McIntyre DC, Nicholson DW, Liston P, Zhu Y, Fong WG, Crocker SJ, Korneluk RG, Robertson GS: Attenuation of ischemia-induced cellular and behavioral deficits by X chromosome-linked inhibitor of apoptosis protein overexpression in the rat hippocampus. *J Neurosci* 19:5026–5033, 1999
56. Schimmer AD: Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. *Cancer Res* 64:7183–7190, 2004
57. Simons M, Beinroth S, Gleichmann M, Liston P, Korneluk RG, MacKenzie AE, Bahr M, Klockgether T, Robertson GS, Weller M, Schulz JB: Adenovirus-mediated gene transfer of inhibitors of apoptosis protein delays apoptosis in cerebellar granule neurons. *J Neurochem* 72:292–301, 1999
58. Xu DG, Crocker SJ, Doucet JP, St. Jean M, Tamai K, Hakim AM, Ikeda JE, Liston P, Thompson CS, Korneluk RG, MacKenzie A, Robertson GS: Elevation of neuronal expression of NAIP reduces ischemic damage in the rat hippocampus. *Nat Med* 3:997–1004, 1997
59. Petrin D, Baker A, Coupland SG, Liston P, Narang M, Damji K, Leonard B, Chiodo VA, Timmers A, Hauswirth W, Korneluk RG, Tsilfidis C: Structural and functional protection of photoreceptors from MNU-induced retinal degeneration by the X-linked inhibitor of apoptosis. *Invest Ophthalmol Vis Sci* 44:2757–2763, 2003
60. Emamaullee JA, Liston P, Korneluk RG, Shapiro AMJ, Elliott J: XIAP overexpression in islet beta-cells enhances engraftment and minimizes hypoxia-reperfusion injury. *Am J Transplant* 5:1297–1305, 2005
61. Emamaullee JA, Rajotte RV, Lakey JRT, Liston P, Korneluk RG, Shapiro AMJ, Elliott JF: XIAP overexpression in human islets prevents early posttransplant apoptosis and reduces the islet mass needed to treat diabetes. *Diabetes* 54:2541–2548, 2005
62. Hui H, Khoury N, Zhao X, Balkir L, D'Amico E, Bullotta A, Nguyen ED, Gambotto A, Perfetti R: Adenovirus-mediated XIAP gene transfer reverses the negative effects of immunosuppressive drugs on insulin secretion and cell viability of isolated human islets. *Diabetes* 54:424–433, 2005
63. Plesner A, Liston P, Tan R, Korneluk RG, Verchere CB: The X-linked inhibitor of apoptosis protein enhances survival of murine islet allografts. *Diabetes* 54:2533–2540, 2005