Section 2: β-Cell Apoptosis

Role of Apoptosis in Pancreatic β-Cell Death in Diabetes

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Apoptosis is a physiological form of cell death that occurs during normal development, and critical mediators of this process include caspases, reactive oxygen species, and Ca^{2+}. Excessive apoptosis of the pancreatic β-cell has been associated with diabetes. Consequently, apoptosis research has focused on how infiltrating macrophages or cytotoxic T-cells might kill pancreatic β-cells using cytokines or death receptor triggering. Meanwhile, the intracellular events in the target β-cell have been largely ignored. Elucidation of such targets might help develop improved treatment strategies for diabetes. This article will outline recent developments in apoptosis research, with emphasis on mechanisms that may be relevant to β-cell death in type 1 and type 2 diabetes. Several of the models proposed in β-cell killing converge on Ca^{2+} signaling, indicating that the pancreatic β-cell may be an ideal system in which to carefully dissect the role of Ca^{2+} during apoptosis. *Diabetes* 50 (Suppl. 1):S44–S47, 2001

Pancreatic β-cell dysfunction is a common feature of both type 1 and type 2 diabetes. In the case of type 1 diabetes, β-cells are selectively destroyed after lymphoid infiltration of the islet. This autoimmune destruction results in insulin deficiency and hyperglycemia. Type 2 diabetes is associated with reduced insulin secretion and glucose toxicity that may contribute to β-cell death. In both cases, β-cell death is thought to occur by apoptosis, and several mediators have been put forth, including death receptor activation, oxidative stress, and Ca^{2+}.

SIGNALING DURING APOPTOSIS

Cell death can follow two distinct pathways: apoptosis or necrosis. However, the early biochemical events that dictate the mode of cell death are still unclear. Necrosis appears to be the result of acute cellular dysfunction in response to severe stress conditions or after exposure to toxic agents and is a relatively passive process associated with rapid cellular ATP depletion. Morphologically, necrosis is characterized by a dramatic increase in cell volume and rupture of the plasma membrane, with spilling of the cellular contents into the intercellular milieu (1). This release of the dying cells' contents into the extracellular space can cause further tissue damage by affecting neighboring cells or by attracting proinflammatory cells (2). Apoptosis is a form of cell death that occurs during several pathological situations in multicellular organisms and constitutes a common mechanism of cell replacement, tissue remodeling, and removal of damaged cells (3). Apoptosis is a complex process characterized by cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation, and formation of "apoptotic bodies" (4–6).

Several protease families are implicated in apoptosis, the most prominent being caspases (7). Caspases are cysteine-containing aspartic acid–specific proteases that exist as zymogens in the soluble cytoplasm, endoplasmic reticulum, mitochondrial intermembrane space, and nuclear matrix of virtually all cells (8). At least three models for caspase activation have been proposed. Apoptosis induced by ligation of cell surface receptors like the Fas or tumor necrosis factor (TNF) receptor, dubbed "death receptors," represents a pathway almost exclusively controlled by caspases. Here, ligand binding of the receptor causes the assembly of a series of proteins called the death-inducing signaling complex, which then activates an apical caspase, procaspase-8 (9). The ensuing events are the strongest evidence that caspases act in cascades, with caspase-8 causing activation of caspase-3, which can activate other caspases and ultimately cleave a variety of other cellular proteins. One of these proteins is a caspase-dependent endonuclease (CAD), which is freed from its inhibitor (ICAD) by caspase-3 and subsequently cuts DNA into oligonucleosomal (180-bp) fragments (10).

A different model for caspase activation has been proposed for the numerous agents that trigger apoptosis without involving cell surface receptors. This pathway focuses on mitochondria and contends that mitochondrial dysfunction occurs during apoptosis and causes the release of cytochrome c from mitochondria into cytosol, where it binds to apoptotic protease activating factor 1 (Apaf-1), a mammalian homolog of the proapoptotic nematode protein ced-4 (11). Apaf-1 contains binding sites for cytochrome c and dATP and oligomerizes. This complex, christened the apoptosome, recruits and binds procaspase-9 by using the caspase recruitment domain of Apaf-1 (12). Mature caspase-9 is released from the multimeric complex and activates the more distal caspase-3 and caspase-7.

Finally, a third pathway that can activate the caspase cascade is initiated by cytotoxic cells (13). Perforin and granzyme B cooperate to induce apoptosis in tumor cells and

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Apaf-1, apoptotic protease activating factor 1; CAD, caspase-dependent endonuclease; ICAD, caspase-dependent endonuclease inhibitor; IL, interleukin; $InsP_3$, inositol 1,4,5-trisphosphate; MEF2 myocyte enhancer factor-2; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; TNF, tumor necrosis factor.

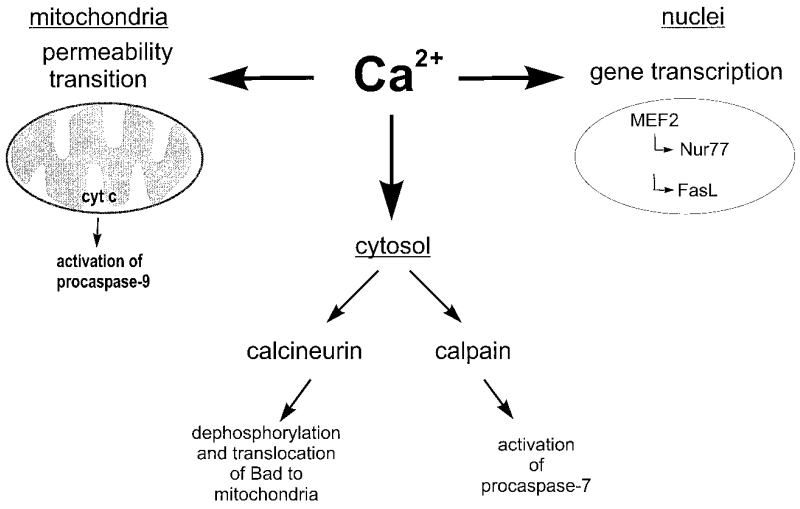

FIG. 1. Targets for Ca^{2+} during apoptosis. Although caspases dominate the field of apoptosis signaling, Ca^{2+} is still considered an important mediator; potential targets in mitochondria, cytosol, and nuclei are depicted here. Increases in intracellular Ca^{2+} may occur during apoptosis as a result of $InsP_3$-mediated spikes. Opening of mitochondrial permeability transition pores is controlled by Ca^{2+}, pH, adenine nucleotides, free radicals, and mitochondrial membrane potential. Pore opening has been cited as the mechanism by which cytochrome c (cyt c) is released. Once in the cytosol, cytochrome c binds to Apaf-1 in the presence of dATP and forms a complex (apoptosome), which recruits procaspase-9 and causes its activation. Cytosolic targets for Ca^{2+} include calcineurin and calpain. Calcineurin is a Ca^{2+}-regulated phosphatase shown to dephosphorylate Bad, a proapoptotic member of the Bcl-2 family. This allows Bad to translocate to the mitochondrial membrane, which may promote cytochrome c release. Calpains, cysteine proteases similar to caspases, are a second cytosolic target for Ca^{2+}. Both calpains and caspases have been reported to cleave fodrin and PARP, conceivably because calpain may cleave caspases. In WEHI-231 cells, calpain mediates activation of procaspase-7, a downstream effector, without the participation of caspase-8 or cytochrome c release from mitochondria. Transcriptional regulation of apoptosis-related genes such as FasL and Nur77 by Ca^{2+} has been demonstrated in T-cells. During activation-induced T-cell death, calcineurin and protein kinase C-θ synergize to stimulate FasL promoter activation (24). A separate report showed that calcineurin is also involved in transcriptional regulation of Nur77 by a more indirect mechanism. Cabin1 is an endogenous inhibitor of calcineurin that can bind to the transcription factor MEF2 and thereby inactivate it. However, during T-cell receptor engagement, increases in intracellular Ca^{2+} cause activated calmodulin to displace MEF2, which then enters the nucleus and binds to the Nur77 promoter. These investigators also showed that overexpression of Cabin1 inhibits T-cell receptor–mediated Nur77 expression and apoptosis (25).

cells infected with intracellular pathogens. Perforin permeabilizes cells, allowing granzyme into the cytosol, where it activates caspase-3 at a preferred and specific site. Regardless of the mechanism, upon activation, caspases cleave numerous cellular proteins, including poly(ADP-ribose) polymerase (PARP) and fodrin (8). In fact, close to 100 cellular proteins have now been identified as potential caspase substrates during apoptosis, and most events in apoptosis appear to require a caspase-mediated proteolytic step.

Oxidative stress has been cited as another critical mediator of cell death, and may either trigger or modulate apoptosis. A role for oxidative stress in apoptosis has been shaped by several independent observations. For many years, direct treatment of cells with oxidants such as hydrogen peroxide or redox-active quinones was thought to exclusively cause necrosis, but more recent studies have shown that lower doses of these agents can trigger apoptosis (14). In addition to this direct evidence, several groups have suggested that intracellular reactive oxygen species (ROS) generation may constitute a conserved apoptotic event and cite ROS production as a critical determinant of toxicity associated with exposure to ionizing radiation and chemotherapeutic drugs (15). Depletion of glutathione GSH pools has also been suggested to be part of the cell death effector machinery and accompanies ROS production during apoptosis in relevant systems (16). The ability of various cellular antioxidants such as catalase and *N*-acetylcysteine to block apoptosis induced by diverse agents other than oxidants also argues for a central role of oxidative stress in apoptosis (17). Reciprocally, broad-spectrum antiapoptotic proteins such as Bcl-2 and the baculovirus protein p35 have been ascribed antioxidant function (18,19), again indicating that ROS generation may be a requisite apoptotic event. Meanwhile, in contrast to the body of literature aligning oxidative stress and apoptosis, we and others have shown that some pro-oxidants can attenuate apoptosis (20).

Sporadic reports of caspase-independent routes to apoptosis exist; however, closer inspection of these routes often reveals links to caspases. This appears to be the case with Ca^{2+}-mediated apoptosis. Historically, a role for Ca^{2+} in apoptosis has focused on activation of a Ca^{2+}-dependent endonuclease; however, the discovery of CAD/ICAD and its apparent Ca^{2+}-independent action has detracted from this idea. Meanwhile, other intracellular targets for Ca^{2+} during apoptosis are emerging, and activation of several of these Ca^{2+}-dependent pathways may feed into the caspase cascade (Fig. 1). One example is the effect of Ca^{2+} on mitochondrial function. High intracellular Ca^{2+}, stemming from a direct challenge (21) or from inositol 1,4,5-trisphosphate ($InsP_3$)- mediated cytosolic Ca^{2+} spikes (22), can cause depolarization of mitochondria, induction of the mitochondrial permeability transition, and cytochrome c release (Fig. 1). This initiates apoptosome formation and subsequent caspase activation. A second target for Ca^{2+} is calcineurin, a Ca^{2+}/calmodulin-dependent protein phosphatase that has been implicated in apoptosis by a number of findings. Calcineurin may mobilize the proapoptotic Bcl-2 family member, Bad, by dephosphorylating it and allowing it to localize to mitochondria (23). Theoretically, Bad can then dimerize with other Bcl-2 family members in the

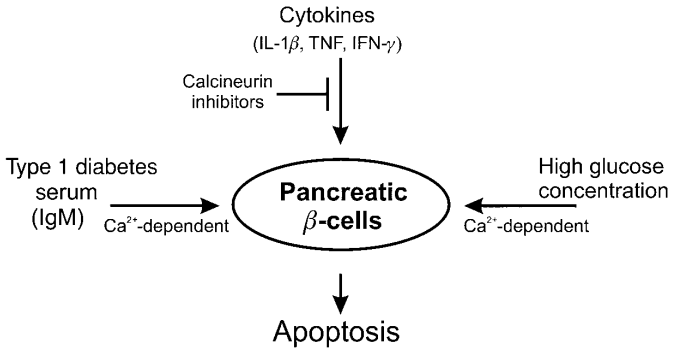

FIG. 2. Various inducers of β-cell apoptosis. Our previous work has shown that a factor present in the serum of patients with type 1 diabetes induces DNA fragmentation characteristic of apoptosis in a Ca^{2+}-dependent manner. More recently, we discovered a similar mechanism in β-cells treated with high concentrations of glucose. A well-characterized pathway of apoptosis in the β-cell involves treatment with the cytokine IL-1β, probably released from neighboring macrophages in vivo. We obtained evidence that inhibitors of calcineurin can prevent IL-1β– induced apoptosis, suggesting that Ca^{2+} is involved in this pathway as well. Thus, Ca^{2+} signaling is a common denominator within several models of β-cell apoptosis. IFN-γ, γ-interferon.

mitochondrial membrane, creating a conductance pore with ability to release cytochrome c. An alternate role for calcineurin is in controlling gene expression. Calcineurin is known to dephosphorylate nuclear factor of activated T-cells (NF-AT), permitting translocation to the nucleus where it combines with activator protein-1 (AP-1) and other transcription factors (24). FasL expression has recently been identified to be driven by such a transcriptional complex regulated by calcineurin (Fig. 1). Myocyte enhancer factor-2 (MEF2) is another transcription factor that is controlled by calcineurin. An endogenous inhibitor of calcineurin, Cabin1, binds to MEF2, blocking its activity. However, the Ca^{2+}-induced release of MEF2 mediates apoptosis in T-cells, and MEF2 activity is required for Nur77 expression (25).

Calcium-dependent proteases, such as calpains, represent another apoptotic target for Ca^{2+} action (Fig. 1). Calpains, like caspases, are also intracellular cysteine proteases but do not have a defined sequence-specific cleavage site within their target substrates. Substrates for calpain include calcineurin, protein kinase C and the cytoskeletal protein α-fodrin (also known as spectrin). Some of these proteins are also cleaved by caspases. The relationship between calpain and caspases has proven to be complex. Calpain can activate caspase-7 in a lymphoma cell line (WEHI) in the absence of cytochrome c release or caspase-3 activity (26). However, other reports assert that procaspase-3 and PARP are cleaved by calpain (27). More recently, calpain has been assigned a role of negative regulator of caspases (28). Recombinant calpain cleaves and inactivates caspase-7, -8, and -9, creating proteolytically inactive fragments. Whether this mechanism is physiologically relevant remains to be seen.

APOPTOSIS AND DIABETES

Much attention has been focused on induction of β-cell apoptosis by death receptors through studies in type 1 diabetes, which is considered to be an autoimmune disease. As a result, macrophages and cytotoxic T lymphocytes have been accused of dealing the lethal blow to β-cells with Fas/FasL, perforin, TNF, or interleukin (IL)-1β as effectors of apoptotic islet cell death. Animal models of diabetes such as the NOD mouse support this idea. Strikingly, inbred Fas-, perforin-, or TNF-deficient NOD mice display reduced incidence and delayed onset of diabetes (29–32). Furthermore, Fas-deficient NOD mice (NOD-*lpr/lpr*) fail to develop diabetes (29).

Whether death receptor activation is the primary way in which caspase activation occurs in the β-cell is not known. Less attention has been focused on mitochondrial events as an accessory mechanism of caspase activation in β-cell apoptosis. Intracellular ROS are well-established byproducts of TNF, Fas, and IL-1β signaling. Furthermore, a wealth of information has focused on nitric oxide production in diabetes. A proapoptotic role for ROS in this context is via disruption of mitochondrial function, causing cytochrome c release. In β-cells, as in several cell types, different doses of pro-oxidants can cause diverse outcomes. Treatment of insulin-secreting RINm5F cells with a redox cycling quinone stimulated proliferation at low doses (10 μmol/l), whereas slightly higher concentrations (30 μmol/l) triggered apoptosis. Necrotic cell death was evident with 100 μmol/l doses of the same compound (33).

Direct as well as indirect evidence has pointed toward Ca^{2+} as an important determinant of β-cell apoptosis. Our previous work has shown that voltage-gated L-type Ca^{2+} channels in primary β-cells and in a pancreatic β-cell line are activated by a factor present in the serum of many patients with type 1 diabetes. Activation of L-type Ca^{2+}-channels was associated with DNA fragmentation characteristic of apoptosis, and specific blockers of these channels prevented endonuclease activation (Fig. 2). The identity of the serum factor that acted on the Ca^{2+} channels has not yet been determined; however, when the serum was depleted of the IgM fraction, no effect was observed on cytoplasmic Ca^{2+} (34).

A similar mechanism involving Ca^{2+} was implicated in islet cells treated with high concentrations of glucose, comparable to levels seen in diabetic patients (Fig. 2), and caused increases in the cytosolic Ca^{2+} and oligonucleosomal DNA fragmentation (35). An endonuclease inhibitor prevented high glucose–induced DNA fragmentation, as did diazoxide, an opener of K_{ATP} channels that hyperpolarizes β-cell membranes. D-600, a blocker of voltage-gated L-type channels, had the same effect.

As described above and depicted in Fig. 2, several of the inducers of β-cell apoptosis are directly regulated by Ca^{2+}. Strikingly, even cytokine-induced apoptosis may require Ca^{2+} participation. A low voltage–activated Ca^{2+} current has been implicated in cytokine-induced pancreatic β-cell death (36). Also, we have obtained preliminary evidence that IL-1β–induced apoptosis can be blocked by inhibitors of calcineurin (37). Thus, Ca^{2+} appears to be a common denominator in β-cell apoptosis. Further detailed analysis of targets and regulators of Ca^{2+} signaling in the β-cell should reveal novel therapeutic options for the management and treatment of diabetes.

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