13. APOPTOTIC DEATH OF BETA-CELLS

Prof. Tihana Žanić-Grubišić, Ph.D.
University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Medical Biochemistry and Hematology, Zagreb, Croatia

Pancreatic b-cell dysfunction is a common feature of both type 1 and type 2 diabetes. In the case of type 1 diabetes, b-cell are selectively destroyed after lymphoid infiltration of the islet. This autoimmune destruction results in insulin deficiency and hyperglycaemia. Type 2 diabetes is associated with inadequate insulin secretion and glucose toxicity that may contribute to the death of b-cell. In both cases, b-cell death is thought to occur by apoptosis, and later in severe cases by necrosis. These two ways of cell death are following two distinct pathways. However, the early biochemical events that dictate the mode of cell death are still unclear.

Necrosis appears to be result of the acute cellular dysfunction in response to severe stress conditions, or occurs after exposure to toxic agents. Necrosis is a relatively passive process associated with rapid cellular ATP depletion. It is accompanied by massive tissue damage leading to rapid collapse of internal cell homeostasis, characterized by cell swelling, early loss of plasma membrane integrity, major changes of the organelles, and enlargement of the nucleus with flocculation of the chromatin. Affected cells rupture, and the cellular components spill into the surrounding tissue, producing an inflammatory response. In necrosis, DNA degradation is a later phenomenon when proteases and endonucleases have already digested the chromatin. The products of DNA digestion appears as a smear pattern on the agar electrophoresis because proteases destroy the histones and expose the entire length of DNA to nucleases. This is different from a characteristic ladder pattern that may be seen in apoptosis, where intact histones protect DNA from a random digestion.

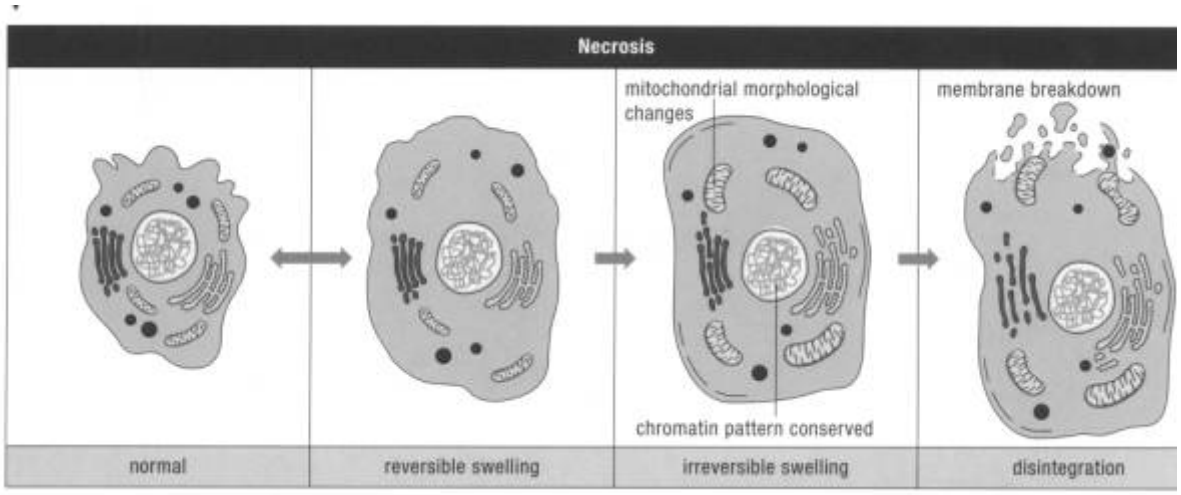

Figure 1. Necrosis

Apoptosis is an energy-requiring, gene-directed process that results in cell suicide. Apoptosis is a physiological form of cell death that occurs during normal development, it is a common mechanism of cell replacement, tissue remodelling, and remove of damaged cells. During apoptosis, cells decrease in size, the chromatin undergoes condensation and fragmentation and finally cells break apart into plasma membrane-bound vesicles that are rapidly phagocytosed, protecting the surrounding tissues from injury. The apoptotic cascade may be elicited by number of varying stimuli, including intracellular events such as metabolic imbalance, cell cycle perturbation, or DNA damage, and extracellular factors such as activation of death receptors (Fas and tumor necrosis factor receptors) and withdrawal of growth factors, metabolic factors, certain hormones and inflammatory mediators such as cytokines. However, there are two major execution programs downstream of the death signal: the caspase pathway and mitochondrial dysfunction. Upstream of irreversible cell damage reside the Bcl family members, which are proteins with both proapoptotic and antiapoptotic properties that play a pivotal role in decision whether cell lives or dies. Transcriptional dependent apoptotic events require the upregulation of death genes or the down regulation of survival genes. Intracellular signals involve ceramides, free oxygen and nitric oxide radicals; and protein kinases such as mitogen-activated protein kinase, stress-activated protein kinase and protein kinase C. The role of the NO signalling pathway has not been completely understood.

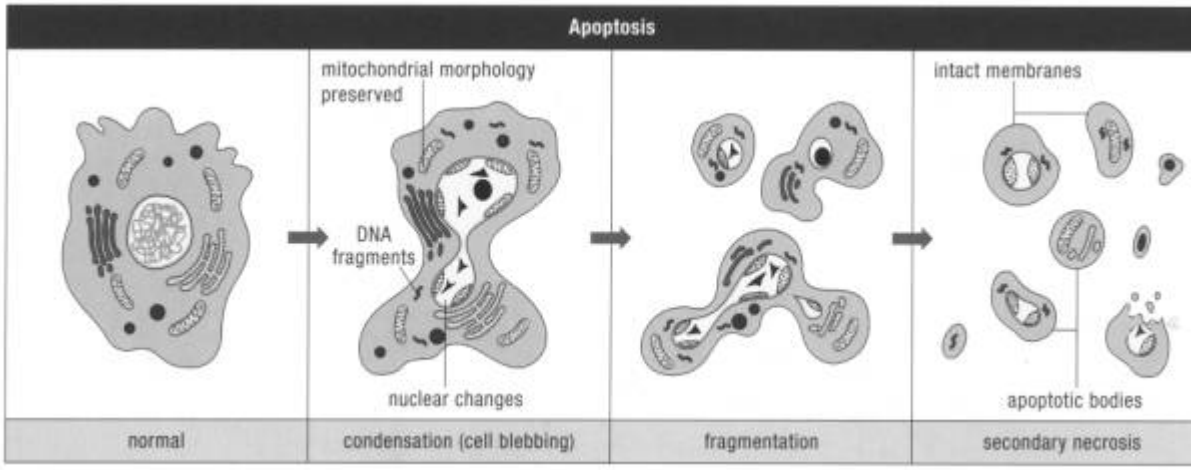

Figure 2. Apoptosis

The critical mediators responsible for activation of this complex processes include caspases, reactive oxygen species, and Ca2+.

1.1. CASPASE MEDIATED SIGNALLING

All apoptotic pathways so far described converge toward the activation of cytoplasmic cysteine proteases named caspases. Caspases are cysteine proteases that exist as proenzymes in the soluble cytoplasm, endoplasmic reticulum, mitochondrial intramembrane space, and nuclear matrix of virtually all cells. They stand out as being crucial for apoptosis in diverse

organisms. Caspases share a specific enzymatic activity, cleaving their substrate after aspartic acid residues, and procaspases themselves are similarly processed into the active form through cleavage at aspartic acid residues. This activation is performed by other caspases, or through autocatalysis. Although there are at least 14 caspases in humans, only some of them are shown to be activated by various death stimuli in different cell types. At least three modes of caspase activation have been proposed.

Apoptosis induced by activation of cell surface receptors like the Fas or tumour necrosis factor receptor, called "death receptors", represent the pathway almost exclusively controlled by caspases. Here, ligand binding to the receptor causes the assembly of a series of proteins called the "death-inducing signalling complex", which then activates an apical caspase, following by further activation of the other caspases that are forming the activation cascade. Targets of these proteins are not completely known, but their activation leads to the cleavage of particular cellular proteins that are involved in apoptotic cell death. This, apoptose promoting and executing protein group has more than 100 members.

A different mode for caspase activation has been proposed for the numerous agents that trigger apoptosis without involving cell surface receptors. This pathway focuses on mitochondrial dysfunction that occurs during apoptosis and causes the release of cytochrome c from mitochondria into cytosol. Cytochrome c binds to apoptotic protease activating factor 1 (Apaf-1) and to dATP and oligomerizes forming an apoptosome. Apoptosome than triggers apoptotic death program.

Finally, a third pathway that can activate the caspase cascade is initiated by cytotoxic cells. Perforin and granzyme B cooperate to induce apoptosis in tumour cells and cells infected with intracellular pathogens. Perforyn permeabilizes cells allowing granzyme into cytosol, where it activates caspase-3 and the whole apoptotic cascade.

1.2. SIGNALLING via REACTIVE OXIDATIVE SPECIES

Oxidative stress has been implicated as another critical mediator of cell death, and may either trigger or modulate apoptosis. Intracellular reactive oxygen species generation appears to constitute a co-served apoptotic event being critical in toxicity associated with various extracellular signals or endogenous products. Oxidative stress can provoke activation of the stress-activated protein kinases, of the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) families, and activation of caspase-3-like proteases what might induce both apoptosis and necrosis. MAPKs play a key role in transducing extracellular signals to the nucleus. The common feature for activation of all MAPK isoforms is the requirement for reversible dual threonine and tyrosine phosphorylation in the activation loop by a specific upstream protein kinase. The best characterised mammalian MAPK is extracellular signal-regulated kinase (ERK). ERK cascade is responsible for signal transduction involving cell growth and differentiation. In contrast to ERK, JNK and p38 MAPK are suggested to inhibit cellular proliferation and to induce apoptosis. The final decision whether a cell will initiate apoptosis or not may depend on the balance between anti-apoptotic signals transduced by the ERK cascade and pro-apoptotic signals transduced by the JNK/p38 cascades.

1.3. Ca++ SIGNALLING

Several models proposed in -cell killing converge on Calcium signalling, and emphasise the important role of Ca++ during apoptosis. Increases in intracellular Ca++ might result from inositol-triphosphate mediated pathways, or from other reasons. That can cause depolarisation of mitochondria and induction of mitochondrial permeability and cytochrome c release leading to apoptosome formation. A second target for increased intracellular Ca++ is calcineurin, a Ca++/calmodulin-dependent protein phosphatase that has been implicated in apoptosis. Calcineurin may mobilise the proapoptotic Bcl-2 family member, Bad, by dephosphorylating it and allowing it to localise to the mitochondrial membrane. That creates a conductance pore with ability to release cytochrom c. An alternate role for calcineurin is in controlling gene expression. Calcium-dependent proteases, such as calpains, represent another apoptotic target for Ca++ action. Calpains, like caspases, are also intracellular cysteine proteases cleaving substrates such as calcineurin, protein kinase C and the skeletal proteins.

1.4. NITRIC OXIDE SIGNALLING

The signalling pathways of apoptosis in pancreatic beta-cells mediated by increased nitric oxide production and are not fully understood. It appears that increased production of NO, due to induction of inducible nitric oxide synthase (iNOS) is the result of activation by nuclear transcription factor kB (NFkB). The gene encoding for the inducible form of nitric oxide synthase is induced also by interleukin (IL)-1beta, tumour necrosis factor-alpha (TNF-a a) and gamma-interferon. This leads to nitric oxide (NO) formation, which contributes to a major extent to b-cell necrosis and to a minor extent to the process of b-cell apoptosis. However, NO may cause b-cell toxicity via different mechanisms:

1. NO inactivates the Krebs cycle enzyme aconitase, thus inhibiting mitochondrial ATP production. However, human islets, possess antioxidant defences that are able to preserve glucose oxidation and ATP production that are needed to complete the apoptotic program after the death signal being delivered by cytokines;
2. NO damages cellular DNA by causing DNA strand breaks that could induce apoptosis through activation of tumour suppressor protein p53 and;
3. NO may function as a redox mediator in the cytokine-induced apoptotic pathway. Although it is evident that NO is capable of killing pancreatic islet cells it appears that NO-independent mechanisms are more important in b-cell destruction in vivo.

1.5. APOPTOSIS IN TYPE 1 DIABETES

Studies of the pathogenesis of type 1 diabetes have mainly focused on the role of the immune system in the destruction of the pancreatic b cells. However, lack of data on the cellular and molecular events at the beginning of the disease is caused by the inaccessibility of these cells during development of the disease. Indirect information has been collected from human and rodent islet cell preparations that were exposed to various cytotoxic conditions.

It has been established that macrophages as well as CD4+ and CD8+ cells are needed to activate beta-cell destruction. The role of CD4+ and CD8+ cells is to feedback activate macrophages upon antigen stimulation and co-stimulation. These activated macrophages facilitate

islet destruction by an NO synthesis-dependent pathway.

Apart from macrophage-dependent NO synthesis macrophages and T-cells could affect b-cell viability via the proinflammatory cytokines: interleukin beta (IL-1b), tumour necrosis factor-alpha (TNF-a a) and gamma-interferon (IFN-g). Cytokine-induced p38/Jun activation participates in beta-cell apoptosis, possibly by a nitric oxide-independent mechanism. However, the combination of IL-1beta and IFN-gamma increased both apoptosis and necrosis in rat islet cells.

It could be concluded that b-cell destruction and type 1 diabetes depend on interaction between macrophages, CD4+ and CD8+ T-cells that establish a chronic inflammatory lesion, in which soluble mediators such as NO and cytokines are involved.

1.6. APOPTOSIS AND TYPE 2 DIABETES

Type 2 diabetes manifests itself clinically when the b-cell mass cannot compensate for insulin resistance with increased insulin release. Numerous findings suggest that apoptosis is involved in beta-cell failure in type 2 diabetes. It has been shown that free fatty acids, high glucose, sulfonylurea, and amylin could cause b-cell apoptosis and thus comprise the pathogenesis of type 2 diabetes. Furthermore, there is evidence favouring a convergence in signalling pathways toward common effectors of b-cell apoptosis implicated in the pathogenesis of both type 1 and type 2 diabetes. It appears that immunological, inflammatory, metabolic, as well as signaling, pathways involving mitogen- and stress-activated protein kinases cause b-cell apoptosis. Moreover, there is the possibility that these signals converge toward a common b-cell death-signalling pathway.

Chronically elevated free fatty acid levels can cause apoptosis of pancreatic b-cells as a result of the activation of sphyngomyelinase and increased formation of its products ceramides, which induce nitric oxide (NO)-dependent cell death. This “lipotoxicity” hypothesis could explain development of type 2 diabetes in obesity. The ability of normal beta-cells to form and accumulate cytoplasmic triglycerides might serve as a cytoprotective mechanism against FFA-induced apoptosis by preventing a cellular rise in toxic free fatty acyl moieties. However, this potential may be lost or insufficient in cells with a prolonged triglyceride accumulation as may occur in vivo. FFA cytotoxicity is also followed by reduction of the anti-apoptotic factor Bcl-2.

It was also shown that long-term exposure to sulphonylurea triggers b-cell apoptosis in a Ca++ dependent manner.

It has been shown that long-time exposure to high glucose levels influences the level of expression of the Bcl family genes and may modulate the balance of pro-apoptotic and anti-apoptotic Bcl proteins towards apoptosis, thus favouring b-cell death. However, the anti-apoptotic gene Bcl-2 remains unaffected, whereas pro-apoptotic genes Bad, Bid, Bik become over-expressed.

Another important mechanism underlying induction of b-cell death involves production of amyloid deposits. Intracellular accumulation of amylin-activate specific signalling pathways

that result in apoptosis. Amylin or islet amyloid peptide (IAPP) is a 37-amino acid peptide that is co-synthesized, co-stored and co-secreted with insulin in pancreatic b-cells . Amylin is the major component of islet amyloid found in the pancreas of >90% patients with type 2 diabetes. The increase in the pancreatic amyloid deposits correlates with the gradual destruction of b-cell of individuals with type 2 diabetes. Human amylin is cytotoxic and induces apoptosis in rat and human islet cells, as well as in some other cell lines. The primary structure of amylin taken on its own cannot provide the plausible explanation for the formation of amyloid. Other products (apolipoprotein E and heparan sulphate proteoglycan perlecan) found within pancreatic amyloid deposits may also be necessary for islet amyloidogenesis. Alteration in b-cell function resulting in changed production, processing, and/or secretion of IAPP could also be important for the initial formation of islet amyloid fibrils in human diabetes. Amyloid formation follows the polymerisation mechanism and proceeds via transition of soluble hIAPP into aggregated beta-sheets. It was suggested that beta-pleated sheet conformation of human amylin may play a role in its toxicity. The central region of amylin between amino acid residues 20 and 29 is likely to be responsible for the tendency of this peptide to form amyloid fibril in some species. Moreover, it was found that human amylin induced free radical production and oxidative stress and that the mechanism underlying induction of islet b-cell death by human amylin involves activation of MAPKs family members and/or caspase machinery. It has been shown that amylin is disturbing the delicate balance between activity of ERK (involved in cell growth and differentiation) and the JNK/p38 (inducing apoptosis) towards the apoptotic program.

It has been shown that b-cells may undergo apoptosis to metabolic and immunolgical stimuli and that the numerous signalling pathways are either converging or crossing their roads leading to b-cell incompetence and death. Numerous targets have been named, but only clear elucidation of such targets might help develop improved treatment strategies for diabetes.

Recommended literature:

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