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# Cytokine-Induced $\beta$ -Cell Stress and Death in Type 1 Diabetes Mellitus

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## 1. Introduction

### 1.1 Pathophysiology of type I diabetes mellitus: Role of pro-inflammatory cytokines

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterised by the destruction of insulin-producing  $\beta$ -cells in the pancreatic islets of Langerhans (Fig.1), which is mediated by autoreactive T cells, macrophages and pro-inflammatory cytokines (Fig.2). This leads to an inability to produce sufficient insulin resulting in elevated blood glucose levels and pathological effects (Eizirik & Mandrup-Poulsen, 2001).

T1DM is believed to be initiated by physiological  $\beta$ -cell death or islet injury triggering the homing of macrophages and dendritic cells that in turn launch an inflammatory reaction. The infiltrating macrophages secrete pro-inflammatory cytokines, namely interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) as well as various chemokines that attract immune cells such as dendritic cells, macrophages and T lymphocytes. T cells recognising  $\beta$ -cell-specific antigens become activated, infiltrate the inflamed islets and attack the  $\beta$ -cells (Baekkeskov et al., 1990, Elias et al., 1995, Lieberman et al., 2003, Nakayama et al., 2005). In a normally functioning immune system, T cells with a high affinity for self-antigens are eliminated during their differentiation resulting in immune 'tolerance'. Autoreactive cells that have escaped these mechanisms are subject to 'peripheral immune regulation' that blocks their activation and clonal expansion, preventing development of an autoimmune disease (Mathis & Benoist, 2004). For reasons we do not fully understand, these immune regulatory mechanisms either fail to launch, or are ineffective in stopping the immune attack against the  $\beta$ -cells in T1DM, and a positive feedback cycle is established (Mathis & Benoist, 2004). This forward-feeding process of T cell- and cytokine-mediated  $\beta$ -cell killing can be ongoing for years progressively destroying the  $\beta$ -cells. When over 80 % of the  $\beta$ -cells are deleted by this continuous T lymphocyte and inflammatory cytokine-driven attack the insulin secretory capacity falls below a certain threshold and the disease manifests itself.

Activated T cells induce death of a target cell by (1) secreting perforin and granzymes, (2) releasing pro-inflammatory cytokines including interferon- $\gamma$  (IFN $\gamma$ ) and TNF $\alpha$  or (3) activation of Fas receptors on the surface of target cells. All these factors have also been described to contribute to  $\beta$ -cell killing in T1DM (Kägi et al., 1997, D. Liu et al., 2000, Petrovsky et al., 2002, Suk et al., 2001). In particular, recent evidence suggests that the

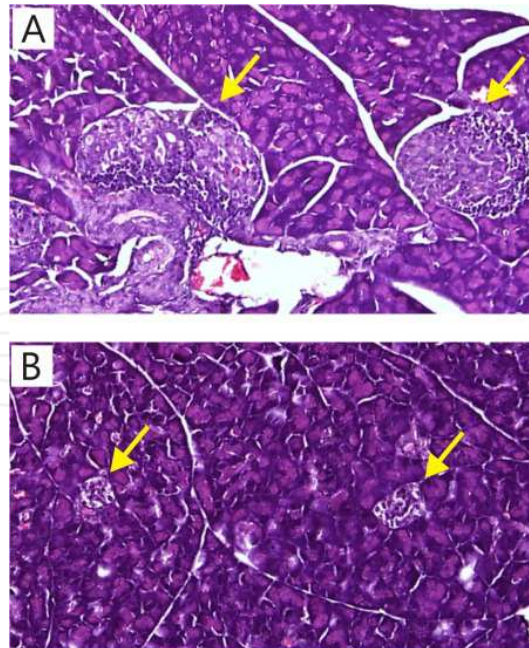


Fig. 1.  $\beta$ -cell islets in the pancreas of (A) pre-diabetic and (B) diabetic NOD mice. The yellow arrows indicate the islets in the haematoxylin-eosin stained tissue section (original magnification 200X).

cytokines IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$  that are secreted by macrophages and T cells have a broader role in the development of T1DM than previously thought. They are the main inducers of  $\beta$ -cell stress responsible for significant levels of  $\beta$ -cell death in both rodent (Iwahashi et al., 1996, Rabinovitch et al., 1994) and human (Delaney et al., 1997) experimental models of T1DM.

Underlining the importance of the cytokines, it has been shown that neutralisation of the pro-inflammatory cytokines by antibodies and/or soluble cytokine receptors against IL-1 $\beta$ , IFN $\gamma$ , IL-6 and TNF $\alpha$  can inhibit the development of T1DM in NOD mice or BB rats (Mandrup-Poulsen, 1996). Transgenic mice expressing IFN $\gamma$  in  $\beta$ -cells develop severe insulinitis (pre-diabetes) and destruction of  $\beta$ -cells. Treatment of these mice with anti-IFN $\gamma$  antibody prevents the development of T1DM. IFN $\gamma$ -deficient mice as well as mice injected with neutralising anti-IFN $\gamma$  receptor antibodies were resistant to development of experimentally-induced T1DM (Cailleau et al., 1997, Seewaldt et al., 2000, B. Wang et al., 1997). Similar to IFN $\gamma$ , genetic or pharmacological abrogation of IL-1 $\beta$  action also reduces disease development in animal models of T1DM (Mandrup-Poulsen et al., 2010).

Although many factors contribute to  $\beta$ -cell destruction during T1DM, in this book chapter we review current knowledge regarding the role of cytokines mediating  $\beta$ -cell stress and death in T1DM.

### 1.2 Signal transduction of pro-inflammatory cytokines in $\beta$ -cells

IL-1 $\beta$ , IFN $\gamma$  and TNF $\alpha$  exert a variety of effects on  $\beta$ -cells. They sensitise  $\beta$ -cells to apoptosis by increasing the expression of pro-apoptotic proteins, such as the Fas receptor (Stassi et al., 1997). They drive and stabilise the autoimmune response by triggering the secretion of chemokines (e.g. CXCL9 and CXCL10) by  $\beta$ -cells (Frigerio et al., 2002), which results in constant recruitment of autoreactive T cells. Finally, pro-inflammatory cytokines directly

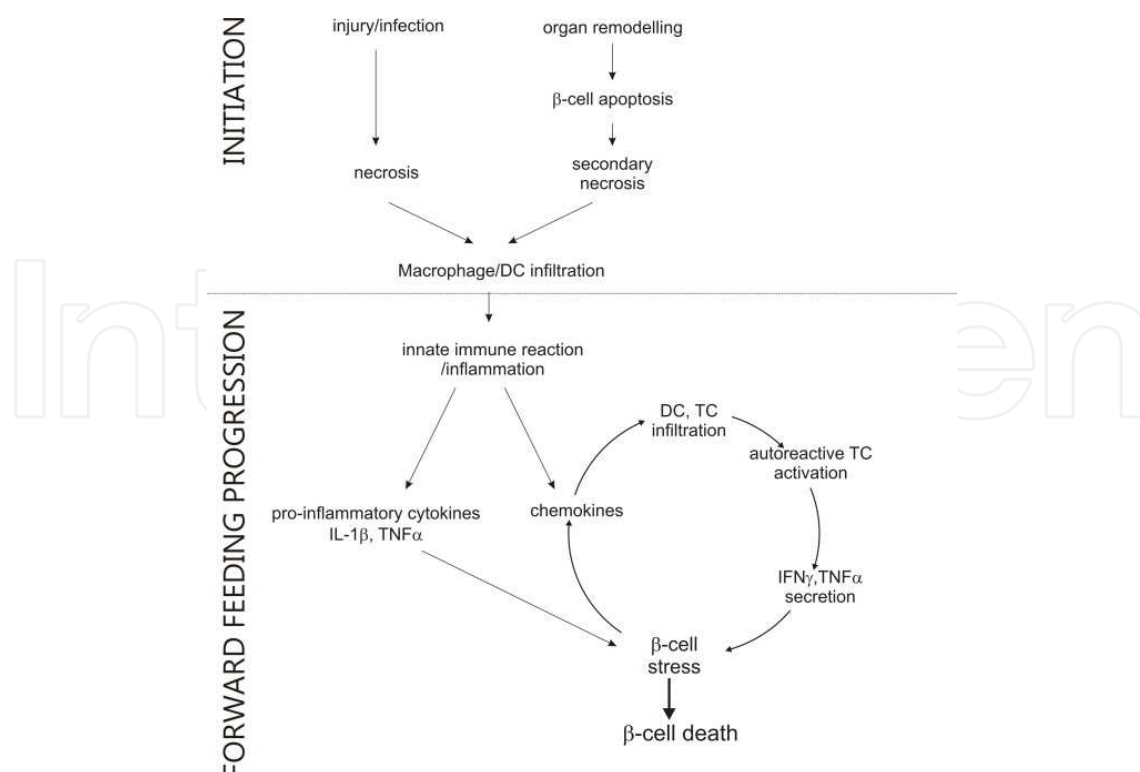


Fig. 2. Cytokine-induced  $\beta$ -cell death. Initial  $\beta$ -cell death caused by injury, infection or physiologically during development can activate an autoimmune response that leads to activation and infiltration of cytokine-secreting macrophages, dendritic cells (DC) and T cells (TC). Pro-inflammatory cytokines IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$  secreted by macrophages and TCs cause  $\beta$ -cell stress and death and secretion of chemokines that further stimulate autoimmune cell infiltration.

cause stress in  $\beta$ -cells which eventually activates the cell's death machinery. The signal transduction pathways activated by these pro-inflammatory cytokines leading to chemokine secretion,  $\beta$ -cell stress and death are detailed below (also see Fig. 3).

It is very important to note that any of the above pro-inflammatory cytokines alone has limited effects in terms of cell stress or death, on  $\beta$ -cells. However, combinations of IL-1 $\beta$ /IFN $\gamma$  or TNF $\alpha$ /IFN $\gamma$  have very strong, synergistic effects that trigger serious levels of stress culminating in cell death.

### 1.2.1 IL-1 $\beta$ signalling

The main mediator of IL-1 $\beta$  signalling is the transcription factor nuclear factor kappa B (NF- $\kappa$ B) (Flodström et al., 1996, Kwon et al., 1995). The pathway by which IL-1 $\beta$  activates NF- $\kappa$ B has been delineated in a number of cell types and experimental models (Fig.3). It is thought that the same mechanisms are involved in pancreatic  $\beta$ -cells. IL-1 $\beta$ , secreted by activated macrophages and T cells, binds to the IL-1 receptor 1 (IL-1R1) on the surface of target cells. IL-1R1 then recruits IL-1 receptor accessory protein (IL-1RAcP) (Dinarello, 1997). This allows binding of the adaptor protein myeloid differentiation factor 88 (MyD88) and recruitment of IL-1R1 activated kinase 1 (IRAK1) and/or IRAK2 (Burns et al., 1998, Muzio et al., 1997, Wesche et al., 1997). IRAK proteins are in complex with a protein named Tollip prior to recruitment to the receptor (Burns et al., 2000). Tollip associates with IL-1RAcP when the IRAK/Tollip complex is recruited to the activated receptor. TNF-receptor-associated

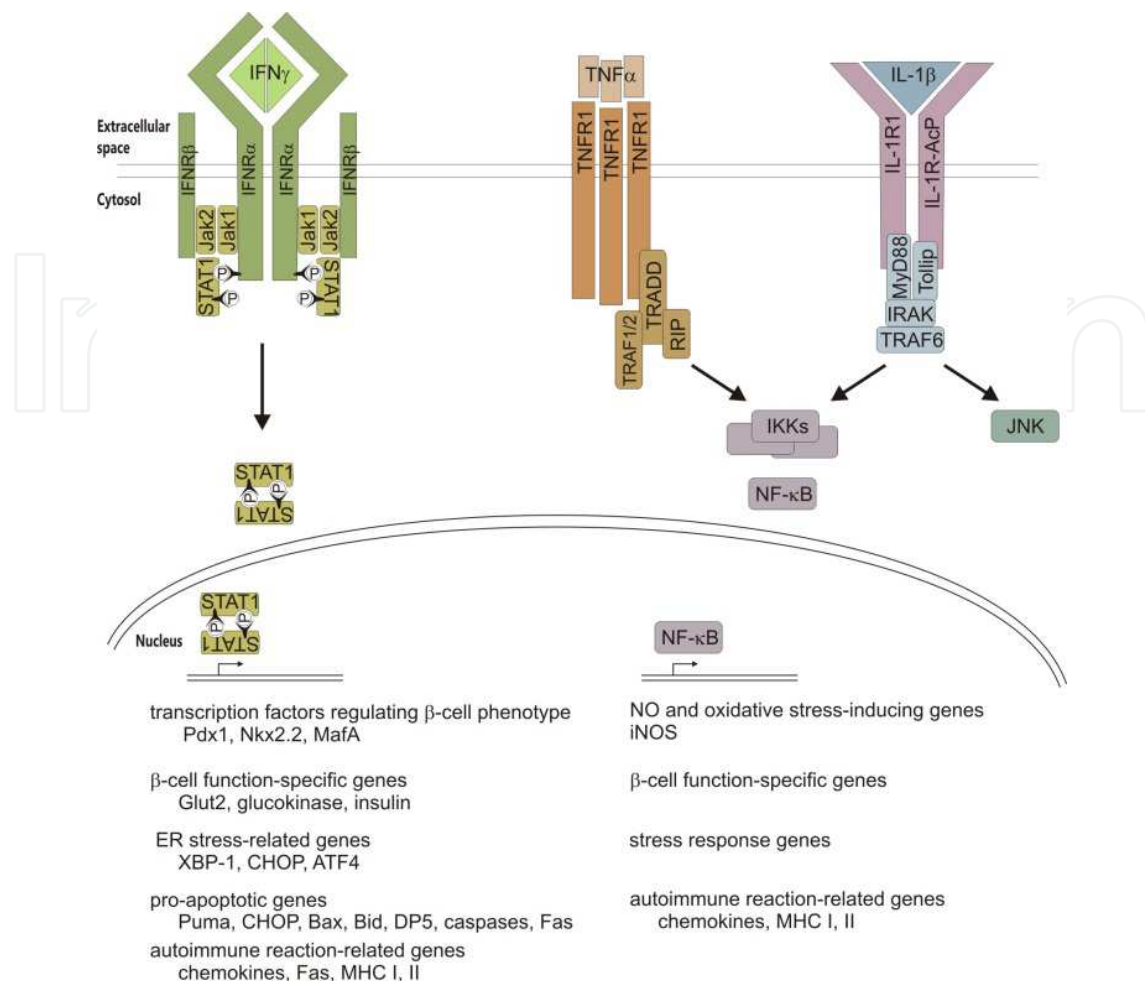


Fig. 3. Cytokine-signalling in pancreatic  $\beta$ -cells. IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$  activate receptors on the surface of  $\beta$ -cells inducing a signalling cascade leading to the activation of transcription factors STAT1 and NF- $\kappa$ B that control numerous genes involved in  $\beta$ -cell function, inflammation, stress responses and apoptosis.

factor-6 (TRAF6) is recruited to IRAK1 and IRAK2 (Muzio et al., 1997, Yamin & Miller, 1997) leading to the activation of inhibitor of NF- $\kappa$ B (I $\kappa$ B) kinase (IKK) *via* NF- $\kappa$ B inducing kinase (NIK). IKK then phosphorylates I $\kappa$ B which triggers its degradation and the release of the transcription factor NF- $\kappa$ B from the inhibitory interaction.

In addition, phosphatidylinositol-3 kinase (PI3K) is recruited to the activated IL1-R1 complex where it becomes activated (Reddy et al., 1997, Reddy et al., 2004). PI3K activity is required, but not sufficient for NF- $\kappa$ B activation (Reddy et al., 1997).

NF- $\kappa$ B can regulate the transcription of numerous target genes (for review see (Pahl, 1999)). The target genes include cytokines (e.g. IL-1 $\beta$ , TNF $\alpha$ , IFN $\gamma$ ), chemokines, immunoreceptors, proteins involved in antigen presentation, cell adhesion molecules, stress response genes, regulators of apoptosis (both pro- and anti-apoptotic), growth factors and other transcription factors. The effects of NF- $\kappa$ B signalling are highly cell type-specific. In most cell types the net effect of NF- $\kappa$ B activation is to promote cell survival. In contrast, in  $\beta$ -cells NF- $\kappa$ B activation has a pro-apoptotic effect (Eldor et al., 2006, Ortis et al., 2008). These studies demonstrate that inhibition of NF- $\kappa$ B protects rodent pancreatic  $\beta$ -cells from the damaging effects of cytokine-exposure *in vitro* and prevents streptozocin-induced diabetes *in vivo*.

A large number of NF- $\kappa$ B target genes were identified using DNA microarray technology in cytokine-treated primary rat  $\beta$ -cells (Cardozo et al., 2001a). Cytokines induced NF- $\kappa$ B-dependent up-regulation of genes involved in stress responses (including CHOP, C/EBP $\beta$  and  $\delta$ , Hsp27 and MnSOD), immune responses (e.g. MHC-II-associated invariant chain  $\gamma$  and MHC-I) and down-regulation of genes involved in  $\beta$ -cell function (glucose transporter-2 (Glut-2)), insulin production (Isl-1), insulin processing (PC-1), insulin release (PLD-1, CCKA-receptor) and Ca<sup>2+</sup> homeostasis (SERCA2, IP 3-kinase) (Cardozo et al., 2001a).

Inducible nitric oxide synthase (iNOS) is strongly induced and is the best characterised NF- $\kappa$ B target in both rat  $\beta$ -cells (Cardozo et al., 2001a, Kutlu et al., 2003) and human pancreatic islets (Flodström et al., 1996). Induction of iNOS increases nitric oxide (NO) production in  $\beta$ -cells, resulting in the generation of reactive oxygen species (ROS) and oxidative stress. The cellular stress triggered by NO in rodent and human cells will be discussed later in this chapter (under section 2.2).

In addition to NF- $\kappa$ B, IL-1 $\beta$  signalling also activates the mitogen activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK) 1/2 and induces suppressor of cytokine signalling-3 (SOCS-3) (Emanuelli et al., 2004). Signal transduction pathways induced by MAPKs and SOCS-3 are interlinked with the NF- $\kappa$ B-regulated pathways; MAPK activation potentiates IL-1 $\beta$ -dependent NF- $\kappa$ B activation and subsequent iNOS induction, and (ERK)1/2 activation was shown to contribute to cytokine-induced apoptosis in rat pancreatic  $\beta$ -cells (Pavlovic et al., 2000). While MAPKs positively affect NF- $\kappa$ B signalling and enhance  $\beta$ -cell death, SOCS-3 has a negative effect. SOCS-3 belongs to a family of proteins that provide a negative feedback for cytokine-induced signalling. It was also identified as an inhibitor of insulin signalling (Emanuelli et al., 2000) as SOCS-3 can bind to the insulin receptor and block its insulin-induced autophosphorylation and activation (Emanuelli et al., 2004). SOCS-3 inhibits IL-1 $\beta$  signalling upstream and thus negatively regulates nearly all effects of IL-1 $\beta$ . SOCS-3 suppresses the expression of several IL-1 $\beta$ -induced pro-apoptotic genes, many of them known to be NF- $\kappa$ B-dependent (Karlsen et al., 2004) and protects rat  $\beta$ -cells from IL-1 $\beta$ - and TNF $\alpha$ -induced cell death (Bruun et al., 2009).

As mentioned above, over 200 genes have been identified to be NF- $\kappa$ B-regulated in  $\beta$ -cells treated with pro-inflammatory cytokines. However, which of these genes are targets of IL-1 $\beta$  signalling, or to what extent their expression is regulated by IL-1 $\beta$  alone is currently unknown. Determining the individual targets of the cytokines would lead to a better understanding of how the cytokines synergise to cause  $\beta$ -cell stress and death.

### 1.2.2 TNF $\alpha$ signalling

TNF $\alpha$  was also shown to lead to activation of NF- $\kappa$ B in pancreatic  $\beta$ -cells (Ortis et al., 2006). TNF $\alpha$  binds to and activates the TNF receptor (TNFR1), which is present on the surface of  $\beta$ -cells (Kägi et al., 1999). TNF $\alpha$  binding to TNFR1 leads to the latter's trimerisation and activation (Fig. 3). Upon activation, the cytosolic death domain of TNFR1 recruits TNF receptor-associated death domain (TRADD) (Hsu et al., 1995), TRAF2 (Hsu et al., 1996b) and the death domain kinase receptor interacting protein (RIP) (Hsu et al., 1996a). TRAF2, in turn, recruits I $\kappa$ B kinase (IKK) and induces its activation in a RIP-dependent manner via activation of an IKK kinase (e.g. NIK) (Devin et al., 2000). Activated IKK phosphorylates I $\kappa$ B proteins leading to their proteasomal degradation and release of NF- $\kappa$ B. The activation of NF- $\kappa$ B by both TNF $\alpha$  and IL-1 $\beta$  has a pro-apoptotic effect in rat pancreatic  $\beta$ -cells (Ortis et al., 2008). This effect was more pronounced in response to IL-1 $\beta$  than TNF $\alpha$ .

TNF $\alpha$  signalling can lead to RIP-dependent activation of three MAPKs (c-Jun N-terminal kinase JNK, p38 and ERK) in a cell type-specific manner (Devin et al., 2003). In rat pancreatic  $\beta$ -cells, TNF $\alpha$  treatment induced activation of JNK and p38 which has been suggested to contribute to an inhibitory effect of TNF $\alpha$  on glucose-stimulated insulin secretion (H.-E. Kim et al., 2008) and hence  $\beta$ -cell dysfunction in response to TNF $\alpha$ .

### 1.2.3 IFN $\gamma$ signalling

IFN $\gamma$  is a homodimeric cytokine. It binds to two IFN $\gamma$  receptor  $\alpha$  (IFN $\gamma$ R $\alpha$ ) chains (Fig. 3). A third unit of IFN $\gamma$ R $\alpha$  and two molecules of IFN $\gamma$  receptor  $\beta$  (IFN $\gamma$ R $\beta$ , also termed accessory factor 1, AF-1) bind to the IFN $\gamma$ R $\alpha$  (Thiel et al., 2000). This leads to the activation and transphosphorylation of Janus tyrosine kinase 1 and 2 (JAK1 and JAK2) which are associated with IFN $\gamma$ R $\alpha$  and IFN $\gamma$ R $\beta$ , respectively, and are brought together upon receptor oligomerisation (Igarashi et al., 1994, Kotenko et al., 1995). JAK1 and JAK2 phosphorylate IFN $\gamma$ R leading to the recruitment of two molecules of the transcription factor, signal transducer and activator of transcription-1 (STAT-1). After phosphorylation and activation by JAK2, STAT-1 homodimerises and translocates to the nucleus where it stimulates the expression of target genes (Takeda & Akira, 2000). Islet cells isolated from STAT-1<sup>-/-</sup> non-obese diabetic (NOD) mice were resistant to apoptosis induced by combined treatment with IFN $\gamma$  and TNF $\alpha$  or IFN $\gamma$  and IL-1 $\beta$  (S. Kim et al., 2007). In support of this, blockade of STAT-1 protected against diabetes induced by injection of multiple low doses of streptozotocin in mice (Callewaert et al., 2007, C.A. Gysemans et al., 2005). A recent gene expression analysis showed that nearly two thousand genes are regulated by STAT-1 in response to cytokine exposure (IL-1 $\beta$  and IFN $\gamma$ ) in  $\beta$ -cells (Moore et al., 2011). STAT-1 was found to regulate the IL-1 $\beta$ /IFN $\gamma$ -mediated induction of chemokines, including CXCL9, CXCL10, CXCL11 and CCL20 (Moore et al., 2011) and islets from STAT-1<sup>-/-</sup> mice have decreased production of CXCL10 upon cytokine exposure both *in vitro* and *in vivo* (C.A. Gysemans et al., 2005).

STAT-1 also down-regulates several genes specific to  $\beta$ -cell functions, such as insulin, glucokinase, Glut2, prohormone convertases, as well as many transcription factors involved in the differentiation and maintenance of  $\beta$ -cell phenotype (e.g. Pdx1, MafA, Nkx2.2) (Moore et al., 2011, Perez-Arana et al., 2010).

Finally, STAT-1 is an important regulator of genes mediating intracellular stress and apoptotic pathways. Several apoptosis-related genes such as Puma, CHOP, Bax, Bid, caspase-3, -4, -7, DP5/Hrk and endoplasmic reticulum stress-transducing genes (XBp1, ATF4) are regulated by STAT-1 (Eizirik & Darville, 2001, Moore et al., 2011, Anastasis Stephanou et al., 2000). IFN $\gamma$  has been found to profoundly accelerate IL-1 $\beta$ -mediated iNOS induction and thus cause oxidative stress. We have demonstrated that treatment of a rat insulinoma cell line (RIN-r) with a combination of IL-1 $\beta$  and IFN $\gamma$  induces the mitochondrial apoptotic pathway in an iNOS-dependent manner (Holohan et al., 2008). This is in line with reports from other groups (Gurzov et al., 2009).

The inflammatory effects of IFN $\gamma$  are controlled by negative feedback regulation, exerted by interferon regulated factor-1 (IRF-1) (Moore et al., 2011) and SOCS-1 and -3 (Alexander, 2002). IRF-1 is likely to exert its STAT-1 regulatory role by up-regulation of SOCS-1 (Moore et al., 2011). IRF-1 expression reduces chemokine expression in  $\beta$ -cells and resulting T cell infiltration in Langerhans islets (C. Gysemans et al., 2008, Moore et al., 2011), however the effect of IRF-1 on STAT-1-mediated  $\beta$ -cell de-differentiation (loss of  $\beta$ -cell function) and  $\beta$ -cell stress is minor (Moore et al., 2011). In line with this, transgenic expression of SOCS-1 in  $\beta$ -cells reduced diabetes development in non-obese diabetic (NOD) mice (Flodström-

Tullberg et al., 2003) and protected  $\beta$ -cells against infiltrating autoreactive T cells (Chong et al., 2004). In summary, the effect of IFN $\gamma$  in  $\beta$ -cells is primarily mediated by STAT-1 through which IFN $\gamma$  controls key processes culminating in loss of  $\beta$ -cell function, stress and finally death. IFN $\gamma$  regulates a number of genes that increase the sensitivity of  $\beta$ -cells to apoptotic stimuli and intracellular stress.

## 2. Cytokine-induced $\beta$ -cell death

### 2.1 Mechanisms of cytokine-induced $\beta$ -cell death

During the development of T1DM, there are two waves of  $\beta$ -cell death. It is believed that  $\beta$ -cell death is the initial trigger for the autoimmune attack. While autoimmune attack was thought to be initiated by cytolytic activity or immune-stimulation of viruses (Jun & Yoon, 2003), it is also possible that physiological  $\beta$ -cell death might be a trigger. Instead of an exogenous impact, or environmental effect, induction of diabetes might be initiated during physiological tissue remodelling of the pancreas peaking at age 2-3 weeks in rodents. At this time, an increased level of  $\beta$ -cell death occurs in the islets and might be the primary trigger of the autoimmune attack (Turley et al., 2003). Programmed cell death associated with normal tissue remodelling does not induce inflammation. However, if the dead cells are not removed promptly by phagocytosis they can disintegrate and release cellular contents in a manner similar to pathological tissue damage which can trigger inflammation. In fact, accumulation of dead cells has been noticed in NOD mice and similarly, disintegrating, so called secondary necrotic cells were sufficient to induce inflammation, macrophage infiltration and pre-diabetic insulinitis in NOD mice (H.S. Kim et al., 2007).

The second wave of  $\beta$ -cell death is driven by the autoimmune reaction. This is an ongoing process gradually killing the  $\beta$ -cells and culminating in the disease phenotype. The mechanism of  $\beta$ -cell death induced by the autoreactive leukocytes has been extensively examined with consensus that the major form of  $\beta$ -cell death is apoptosis, however, under certain conditions and especially in rodent experimental models of T1DM, necrotic  $\beta$ -cell death can also contribute to  $\beta$ -cell loss.

**Apoptosis** is a physiological form of cell death involved in the elimination of cells that have served their function, are no longer needed or are damaged. It is an active, highly ordered and rapid process characterised by the detachment of the dying cell from its neighbours, cell shrinkage, condensation of chromatin, fragmentation of the nucleus and finally fragmentation of the cell into membrane bound particles, called apoptotic bodies which are engulfed by neighbouring cells or professional phagocytic cells (Samali et al., 1996). By this means, cells are eliminated without leakage of otherwise inflammatory cellular material.

The morphological changes typical of apoptosis are orchestrated by the caspase family of proteases (Samali et al., 1999). Caspases are activated by two distinct mechanisms. The extrinsic pathway is triggered by an extracellular pro-apoptotic stimulus, usually a cytokine that belongs to the death ligand subfamily of the TNF superfamily. Upon engagement of the death ligand with its cognate death receptor on the cell surface of the target cell, the receptors trimerise and induce the formation of a protein complex, called the death-inducing signalling complex (DISC). The DISC is an activation platform for caspases-8 and/or -10 (Peter & Krammer, 2003). Once these initiator caspases are activated they activate downstream effector caspases, which leads to a burst of caspase activity and subsequent proteolysis that dismantles the cells.

The second, so called intrinsic pathway is initiated at the level of mitochondria. Upon intracellular stress these organelles release cytochrome *c* that associates with the adaptor



protein APAF-1 to build a multimeric cytoplasmic protein complex termed the apoptosome, which functions to activate another initiator caspase, caspase-9 (Riedl & Salvesen, 2007). Mitochondrial release of cytochrome *c*, and thus activation of the intrinsic apoptosis pathway, is controlled by members of the Bcl-2 family of proteins (see section 2.3). Once cytochrome *c* is released and caspase-9 is activated, the same caspase cascade is triggered as during the extrinsic apoptotic pathway that leads to the final demise of the cell.

Interestingly, TNF $\alpha$  was shown to induce expression of an endogenous caspase inhibitor in  $\beta$ -cells that prevents apoptosis, the X-linked inhibitor of apoptosis protein (XIAP). This NF- $\kappa$ B-mediated induction of XIAP is inhibited by IFN $\gamma$  signalling, providing a mechanism for synergistic cytotoxicity of TNF $\alpha$  and IFN $\gamma$  in  $\beta$ -cells (H.S. Kim et al., 2005).

Apoptosis is distinguished from **necrosis**, a pathological, mostly uncontrolled mode of cell death. During necrosis cells swell, their membranes disintegrate and their content is released, inducing inflammation. Recently, an active mode of necrosis, termed necroptosis, has been described that can be induced upon activation of TNFR1 when caspase-8 activation is blocked (Vandenabeele et al., 2010). A possible role of necroptosis in initiation of diabetes seems worthy of further investigation in light of the known involvement of TNFR1 signalling in diabetes and of a recent study that provided evidence of necrotic  $\beta$ -cell death playing a role in initiating autoimmune-type diabetes (Steer et al., 2006).

## 2.2 The role of nitric oxide in cytokine-mediated $\beta$ -cell loss

It is thought that cytokine-induced  $\beta$ -cell stress and death is partly caused by intracellular production of ROS and NO. NO is a gaseous hydrophobic signalling molecule that readily diffuses through membranes and plays an essential role in various neurological, immunological and cardiovascular processes. The biosynthesis of NO is catalysed by nitric oxide synthases (NOS). In  $\beta$ -cells IL-1 $\beta$  signals up-regulation of iNOS and subsequent generation of NO. The main physiological effect of NO is mediated via the direct activation of guanylyl cyclase by NO leading to production of cyclic GMP (cGMP) and activation of cGMP-dependent signal transduction pathways. However, if present for a prolonged period or in high quantities, NO can nitrosylate specific cysteine residues of various proteins (S-nitrosylation) forming nitrosothiols and thereby affect the protein's activity, stability and localisation (Hess et al., 2005). In most cases this leads to rapid degradation of the nitrosylated proteins but a small subgroup of proteins have been shown to gain stability after nitrosylation (Paige et al., 2008). NO can have anti-apoptotic and cytoprotective effects in some cell types (McCabe et al., 2006), but can become toxic if present at high levels due to formation of ROS and protein nitrosylation which, amongst other things, also causes mitochondrial damage.

It has been shown that NO can induce both necrotic and apoptotic cell death (Bonfoco et al., 1995). With respect to  $\beta$ -cell destruction, it has been shown that endogenous levels of NO are sufficient to induce  $\beta$ -cell injury in rodent models of T1DM (Thomas et al., 2002) and increased levels of NO caused by cytokine-mediated iNOS induction cause cell death by both necrosis (Hoorens et al., 2001, Welsh et al., 1994) and apoptosis (Holohan et al., 2008). The relative involvement of NO in the destruction of  $\beta$ -cells in human and rodent islets is not fully elucidated. Several studies have shown that a combination of IL-1 $\beta$  with IFN $\gamma$  or TNF $\alpha$  induces cell death in rodent pancreatic islet cells, predominantly by induction of apoptosis but also partly by necrosis (D. Liu et al., 2000, Saldeen, 2000). In rodent  $\beta$ -cells the

cytokine-induced induction of necrosis seems to be dependent on iNOS-induced production of NO as the level of necrotic cell death was greatly reduced in purified  $\beta$ -cells from iNOS-deficient mice (D. Liu et al., 2000). Another study found that inhibition of iNOS in rat islets reduced both necrosis and apoptosis induction (Saldeen, 2000). In any case, the cytokine-induced production of NO seems to play a major role in mediating  $\beta$ -cell death in rodent experimental models of T1DM. Additionally, we recently demonstrated that a combination of IL-1 $\beta$  and IFN $\gamma$  induces the intrinsic apoptosis pathway in a synergistic manner in a rat insulinoma cell line (RIN-r) and showed that iNOS-mediated production of NO was both required and sufficient for apoptosis induction (Holohan et al., 2008). This is in agreement with previous findings that showed that apoptosis induced by a combination of IL-1 $\beta$  and IFN $\gamma$  is NO-dependent in a rat insulinoma cell line (Storling et al., 2005).

Human islets have been shown to be less sensitive to NO-induced damage compared to rodent cells. As such, inhibition of iNOS could not protect human islets from cytokine-induced cell death suggesting a NO-independent cytotoxicity. (Delaney et al., 1997, Eizirik & Mandrup-Poulsen, 2001, Hoorens et al., 2001). The resistance of human islets towards NO compared to rodent islets is speculated to be due to higher levels of heat shock protein 70 (Hsp70) in human  $\beta$ -cells (Burkart et al., 2000) which protects cells from the oxidative stress inflicted by NO (Welsh et al., 1994).

### 2.3 Role of the Bcl-2 family proteins

Cytokines can modulate the expression and/or activity of several members of the Bcl-2 family (Gowda et al., 2008, A. Stephanou et al., 2000, P. Wang et al., 2009, L. Zhang et al., 2008). The various interactions between the pro- and anti-apoptotic members of this family of proteins lie at the heart of the intrinsic pathway of apoptosis (Youle & Strasser, 2008). Bcl-2 family members are characterised by up to four conserved regions termed Bcl-2 homology (BH) domains. The pro-apoptotic multidomain family members Bax and Bak contain three BH domains and can be activated to form oligomeric structures in the outer mitochondrial membrane that trigger cytochrome *c* release, which then initiates the intrinsic pathway of caspase activation. Activation proceeds through interaction with BH3-only family members (harbouring only the third BH domain) that are induced or activated by cellular stress signals. Activation of Bax or Bak is counteracted by anti-apoptotic multidomain Bcl-2 family members (such as Bcl-2, Bcl-xL, or Mcl-1), which bind and sequester the BH3-only proteins.

Viral transduction of Bcl-2, the prototype member of the family, was shown to protect human islet cells from cytokine-induced apoptosis, giving a first indication that regulation of Bcl-2 family proteins by cytokines might contribute to  $\beta$ -cell apoptosis (Rabinovitch et al., 1999). Likewise, adenoviral transduction of Bcl-X<sub>L</sub> prevented cytokine-mediated apoptosis of RIN-r cells (Holohan et al., 2008).

Several recent studies have addressed the involvement of Bcl-2 family proteins in cytokine-induced  $\beta$ -cell death in more detail. Treatment of human or rat islets with inflammatory cytokines resulted in activation of the intrinsic pathway of apoptosis and involved activation of the pro-apoptotic BH3-only protein Bad by dephosphorylation (Grunnet et al., 2009). Dephosphorylation of Bad was also found in a second study analysing cytokine-treated rat islets, and in addition up-regulation of pro-apoptotic BH3-only proteins Bim and Bid was also detected (Mehmeti et al., 2011). In a different study it was shown that in primary rat  $\beta$ -cells cytokines as well as ER stress lead to increased expression of the pro-apoptotic BH3-only protein DP5/Hrk in a JNK-dependent manner (Gurzov et al., 2009). Up-

regulation of DP5 in  $\beta$ -cells is mediated by the transcription factor STAT-1 which is regulated by IFN $\gamma$  (Moore et al., 2011). In addition, inflammatory cytokines led to up-regulation of the pro-apoptotic BH3-only protein PUMA in primary rat  $\beta$ -cells as well as in human islets through a pathway involving NF- $\kappa$ B signalling, iNOS activation and ER stress (Gurzov et al., 2010). Furthermore, down-regulation of the anti-apoptotic multidomain Bcl-2 family member Mcl-1 turned out to be critically involved in the cytokine-induced apoptosis of the rat insulinoma cell line INS-1E (Allagnat et al., 2011). In summary, exposure to cytokines leads to alterations in expression of several Bcl-2 family members in  $\beta$ -cells in a manner that favours activation of the intrinsic pathway of apoptosis.

### 3. $\beta$ -cell stress in type 1 diabetes

#### 3.1 Endoplasmic reticulum stress

It has been suggested that endoplasmic reticulum (ER) stress is involved in  $\beta$ -cell destruction. Pancreatic  $\beta$ -cells are specialised cells that rapidly synthesise and secrete insulin in response to fluctuations in blood glucose levels (Pirot et al., 2007). This imparts a heavy burden on the ER and, consequently,  $\beta$ -cells are particularly susceptible to cellular conditions that impair the ER's ability to correctly fold nascent proteins. Under such conditions, the resultant accumulation of unfolded or damaged proteins within the ER lumen triggers the unfolded protein response (UPR), an adaptive signalling pathway that increases the folding capacity of the ER and restores homeostasis (Szegezdi et al., 2006). Although the initial UPR is a protective response, prolonged ER stress can lead to the initiation of apoptosis. Thus while under physiological conditions the UPR acts as a pro-survival mechanism in  $\beta$ -cells, chronic ER stress can lead to redirection of the UPR towards pro-apoptotic signalling.

Three ER-localized transmembrane proteins sense the accumulation of unfolded proteins in the ER lumen and initiate the UPR, PKR-like ER kinase (PERK), inositol-requiring enzyme 1  $\alpha$  (IRE1 $\alpha$ ) and activating transcription factor 6 (ATF6). These proteins transduce information from the ER to the nucleus by activating transcription factors that control genes involved in restoring ER function (Szegezdi et al., 2006). The PERK arm of the UPR has been the main focus of studies with regard to  $\beta$ -cell stress in diabetes, therefore this chapter will focus on PERK signalling in more detail. Upon accumulation of unfolded proteins, PERK is activated and induces a translational block by phosphorylating eukaryotic initiation factor 2  $\alpha$  (eIF2 $\alpha$ ). Phosphorylation of eIF2 $\alpha$  by PERK leads to inhibition of cap-dependent protein synthesis. This reduces the protein load of the ER while allowing cap-independent translation to persist and leads to preferential translation of the transcription factor ATF4. One target gene induced by ATF4 (in conjunction with ATF6) is C/EBP homologous protein CHOP, a transcription factor that is known to promote apoptosis (Zinszner et al., 1998).

##### 3.1.1 The role of PERK in $\beta$ -cell function

The PERK signalling branch of the UPR appears to be essential for the regulation of  $\beta$ -cell function. Stimulation of insulin production in mouse pancreatic islets leads to dephosphorylation of eIF2 $\alpha$  (P. Zhang et al., 2002) reversing the translational block caused by PERK signalling and allowing for increased biosynthesis of insulin. Studies with knockout mice showed that PERK is essential for  $\beta$ -cell function and survival (Harding et al., 2001, P. Zhang et al., 2002). Pancreatic  $\beta$ -cells of PERK $^{-/-}$  mice degenerated within the first four weeks after birth, and a diabetic phenotype could be observed (Harding et al.,

2001, P. Zhang et al., 2002).  $\beta$ -cell loss was associated with damaged rough ER and high levels of apoptosis in the pancreas (P. Zhang et al., 2002). However, a subsequent study discovered that the onset of diabetes in PERK<sup>-/-</sup> mice is due to developmental defects during  $\beta$ -cell proliferation and differentiation leading to a reduction in  $\beta$ -cell mass (W. Zhang et al., 2006). At the molecular level, down-regulation of PERK in rat  $\beta$ -cells was shown to induce deregulation of ER chaperones Grp78 and ERp72 and disruption of ER function leading to reduced insulin production and reduced cell proliferation (Feng et al., 2009).

### 3.1.2 Involvement of the UPR in cytokine-induced $\beta$ -cell death

There is some evidence to suggest that cytokines induce  $\beta$ -cell apoptosis by stimulating pro-apoptotic signalling of the UPR.  $\text{Ca}^{2+}$  levels in the ER are about four times higher than in the cytosol as high  $\text{Ca}^{2+}$  levels are required for ER function in aiding protein folding and posttranslational processing. Disruption of  $\text{Ca}^{2+}$  homeostasis causes severe ER stress resulting in accumulation of unfolded proteins in the ER and activation of the UPR. It was shown that cytokine-exposure leads to elevated basal cytosolic  $\text{Ca}^{2+}$  levels selectively in mouse pancreatic  $\beta$ -cells compared to glucagon-secreting  $\alpha$ -cells and this was associated with cytokine-induced apoptosis (L. Wang et al., 1999). In line with these results, it was shown that increased production of NO in rodent  $\beta$ -cells leads to depletion of ER  $\text{Ca}^{2+}$  levels (Oyadomari et al., 2001). Furthermore, overexpression of the ER-located  $\text{Ca}^{2+}$ -binding protein, calreticulin increased levels of  $\text{Ca}^{2+}$  in the ER and made cells more resistant to NO-induced apoptosis (Oyadomari et al., 2001). This suggests that NO-induced apoptosis in rodent  $\beta$ -cells is at least partly caused by ER stress induced by NO-mediated  $\text{Ca}^{2+}$  depletion. Some evidence suggests that NO may regulate  $\text{Ca}^{2+}$  levels in  $\beta$ -cells through down-regulation of the sarcoendoplasmic reticulum  $\text{Ca}^{2+}$  ATPase 2b (SERCA2b) (Cardozo et al., 2005). SERCA pumps  $\text{Ca}^{2+}$  from the cytoplasm into the ER thus maintaining ER  $\text{Ca}^{2+}$  levels. Rodent and human islet cells have been reported to express the isoforms SERCA2b and SERCA3 (Varadi et al., 1996). Treatment of rodent pancreatic  $\beta$ -cells with a combination of IL-1 $\beta$  and IFN $\gamma$  induced transcriptional down-regulation of SERCA2b and this was partially prevented by inhibition of iNOS. Furthermore, after inhibition of SERCA, the effect of cytokine exposure on ER  $\text{Ca}^{2+}$  was abolished (Cardozo et al., 2005). This suggests that NO-induced depletion of ER  $\text{Ca}^{2+}$  is at least in part mediated by SERCA down-regulation. The SERCA isoform SERCA2a has been shown to be specifically inactivated by peroxynitrite (ONOO<sup>-</sup>)-mediated nitration of a tyrosine residue within the channel-like domain *in vitro* (Viner et al., 1999). Peroxynitrite is produced in cells by a reaction between NO and the free radical superoxide (Pacher et al., 2007). SERCA2a differs from SERCA2b only in regions of the C-terminus (Dode et al., 1998) and it could be hypothesised that cytokine-induced NO production inhibits SERCA2b  $\text{Ca}^{2+}$ -ATPase activity by peroxynitrite-mediated nitration in the same way. Another possible mechanism by which NO might mediate reduction of ER  $\text{Ca}^{2+}$  levels is via activation of the ryanodine receptor-2. Ryanodine receptor-2 is a calcium channel located in the ER membrane that releases  $\text{Ca}^{2+}$  from the ER into the cytosol and has been reported to be expressed in mouse pancreatic  $\beta$ -cells (Islam et al., 1998). NO-induced poly-S-nitrosylation enhances the activity of this calcium channel (Xu et al., 1998) but whether this mechanism is relevant to cytokine-exposed  $\beta$ -cells remains to be determined. Treatment of rodent  $\beta$ -cells with a combination of IL-1 $\beta$  and IFN $\gamma$  induces the expression of CHOP in an NO-dependent manner (Fig. 4). This is in line with a number of other reports (Cardozo et al., 2001b, Cardozo et al., 2005). Inhibition of iNOS by N<sup>G</sup>-(1-iminoethyl)-L-ornithine (L-NIO) or N<sup>G</sup>-methyl-L-arginine (LMA) blocked cytokine-induced NO

production and expression of CHOP. In addition to CHOP, the UPR marker proteins Grp78 and phosphorylated eIF2 $\alpha$  were also found to be up-regulated after cytokine treatment, without affecting expression of spliced X-box binding protein 1 (sXBP-1) which is induced downstream of IRE1 $\alpha$  (Fig. 4). Overexpression of iNOS alone was sufficient for CHOP expression (Fig. 4) and treatment with the NO-donor molecule S-nitroso-N-acetyl-D,L-penicillamine (SNAP) induced expression of Grp78 and CHOP (Oyadomari et al., 2001). This suggests that NO is sufficient to activate the UPR in rodent pancreatic  $\beta$ -cells. Pancreatic islet cells from CHOP $^{-/-}$  mice were shown to be resistant to cytokine- and NO-mediated apoptosis compared to cells from CHOP $^{+/+}$  and CHOP $^{+/-}$  mice (Oyadomari et al., 2001). Together these results suggest that the apoptotic effects of cytokine-induced NO are mediated by activation of CHOP.

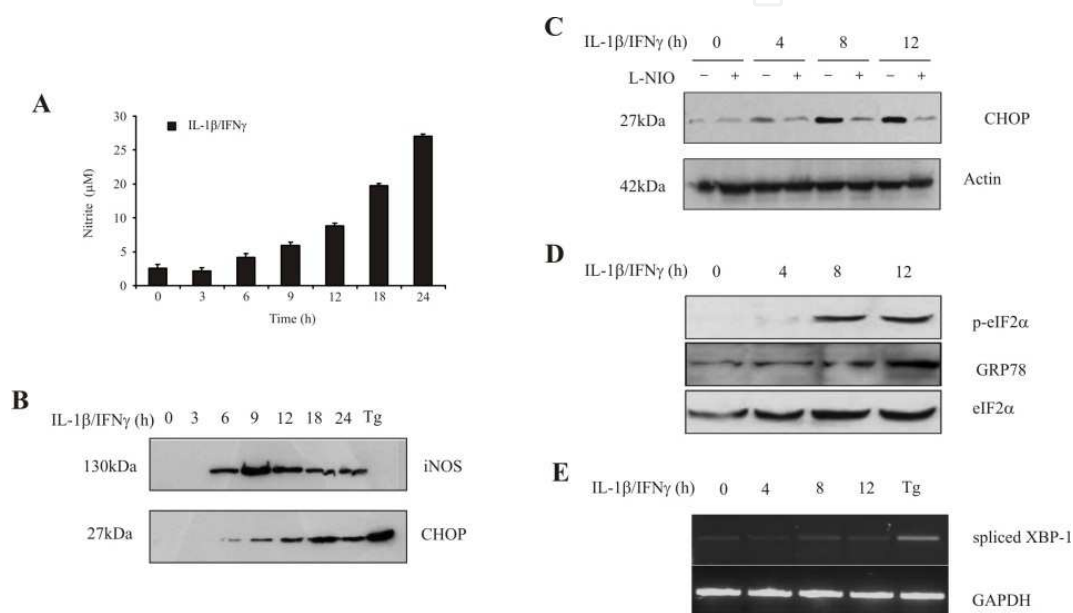


Fig. 4. IL-1 $\beta$ /IFN $\gamma$  induced the expression of the pro-apoptotic transcription factor CHOP and other UPR markers. (A) A time-course cytokine treatment (IL-1 $\beta$  and IFN $\gamma$ , 60 U/ml of each) was carried out and samples assayed for NO production. (B) The same samples were then analysed by Western blotting for iNOS and CHOP expression. This data demonstrates an increase in NO production, iNOS expression and CHOP induction occurring at 6 h post cytokine treatment, although CHOP is not strongly expressed until 9 h. (C) Samples were assayed for CHOP expression following cytokine treatment in the presence and absence of the iNOS inhibitor L-NIO. Cytokine-induced CHOP expression was decreased in the presence of L-NIO indicating that this is an NO-dependent process. (D) Alterations in the expression of ER stress-associated proteins after cytokine treatment were analysed by Western blotting. The expression of UPR markers Grp78 and phosphorylated eIF2 $\alpha$  (p-eIF2 $\alpha$ ) were up in response to cytokine treatment. (E) Production of spliced XBP-1 mRNA after cytokine treatment was determined by RT-PCR. Thapsigargin (Tg) treatment was used as a positive control. Cytokine treatment did not show an effect on the level of spliced XBP-1 mRNA. The images presented are representative of three independent experiments.

Conversely, another study suggested that although cytokine signalling induces ER stress as demonstrated by activation of PERK and JNK, induction of CHOP is not required for  $\beta$ -cell death in rodents (Åkerfeldt et al., 2008). In support of these findings, a recent study suggests

that CHOP is not required for  $\beta$ -cell death and the development of diabetes in NOD mice (Satoh et al., 2011). However, CHOP<sup>-/-</sup> NOD mice showed delayed production of insulin autoimmune antibodies (Satoh et al., 2011) suggesting a role for CHOP in the early onset of the autoimmune reaction leading to  $\beta$ -cell destruction. Therefore, cytokine-induced  $\beta$ -cell death may be partly mediated by induction of CHOP.

While a functional UPR (at least the PERK branch) appears to be essential for  $\beta$ -cell function and development, its downstream target CHOP has been associated with cytokine-induced  $\beta$ -cell destruction suggesting that PERK signalling regulates  $\beta$ -cell function and survival under physiological conditions but may switch towards pro-death signalling under conditions of cytokine-induced  $\beta$ -cell stress.

### 3.2 Oxidative stress

Cytokines induce multiple stress pathways in  $\beta$ -cells. Oxidative stress is induced by increased production of ROS and an imbalanced, low level of antioxidant enzymes (Sies, 1997). IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$  induce the production of ROS and NO by inducing iNOS (Rabinovitch & Suarez-Pinzon, 1998). Free oxygen and nitrogen radicals generated can react to form peroxynitrite, which is a very strong oxidant. Oxygen free radicals, nitrogen free radicals as well as the radical peroxynitrite can react with and damage a range of cellular proteins and in this way block metabolic functions and induce  $\beta$ -cell death (Azevedo-Martins et al., 2003).  $\beta$ -cells have been shown to be especially susceptible to such oxidative stress because they have particularly low levels of antioxidant enzymes (Lenzen et al., 1996, Tiedge et al., 1998).

## 4. Therapeutic strategies

The number of people affected by T1DM is approximately 20 million worldwide and is rapidly rising (Chabot, 2002). While exogenous administration of insulin is an effective treatment for acute hyperglycaemia in T1DM, it does not prevent secondary complications (White et al., 2008) and can in some cases lead to hypoglycaemia (Kort et al., 2011). Alternative therapeutic strategies include pancreas transplantation and islet transplantation. While whole pancreas transplantation is an invasive surgical method associated with major complications, islet transplantation is less invasive and associated with significantly lower morbidity and mortality. Successful islet transplantation would result in insulin independence, protection from hypoglycaemia, improvement of microvascular complications, improved patient survival and enhanced quality of life (Kort et al., 2011). The method is currently in clinical trials and has been used to treat around 1,000 individuals worldwide (Kort et al., 2011). Islet transplantation has many limitations, including limited availability of suitable islet graft donors, high cost and high rate of partial or total graft failure. Islet graft failure can be caused by allorejection, toxicity of immunosuppressive drugs that are required to reduce immune rejection, glucotoxicity, and recurrence of autoimmunity (Kort et al., 2011).

An approach to reduce  $\beta$ -cell death in islet grafts is the transfer of therapeutically useful genes into islet cells prior to transplantation (McCabe et al., 2006). The development of gene therapy techniques that can protect  $\beta$ -cells from autoimmune destruction may not only improve outcomes after islet transplantation but may also lead to preventive therapies for patients at high risk of developing T1DM (McCabe et al., 2006).

Various candidate transgenes are being examined for their potential in protecting  $\beta$ -cells under various stresses including cytokine-exposure and oxidative stress. The rational choice of therapeutic genes is helped by understanding the mechanism of  $\beta$ -cell destruction which has been the subject of this chapter. Potential targets will be reviewed in this section. Target genes studied to date encode regulators of the cytokine signal transduction pathways, molecules that inhibit  $\beta$ -cell apoptosis, antioxidant enzymes, immunoregulatory proteins and pro-survival cytokines (McCabe et al., 2006).

#### 4.1 Anti-apoptotic gene transfer

Apoptosis plays a major role in  $\beta$ -cell death in T1DM (see section 2.). The transfer of anti-apoptotic genes as a strategy to counteract islet destruction has been explored. Candidate genes include those expressing cytoprotective and anti-apoptotic heat shock proteins (Hsps) and anti-apoptotic Bcl-2 family proteins. Hsp70 is one of the major heat shock proteins in mammals and is thought to be responsible for the relative resistance of human  $\beta$ -cells to cytokine-induced stress and death (Burkart et al., 2000). Hsp70 can protect cells under conditions of stress by directly inhibiting the transduction of the apoptotic signal, by decreasing the amount of oxidative stress and also by reducing ER stress via its chaperone activity. It has been shown that pre-conditioning by heat shock could protect pancreatic islet cells from insults by NO, ROS and the cytotoxic drug streptozocin and this increased resistance correlated with induced expression of Hsp70 (Bellmann et al., 1995). Another Hsp that is potentially capable of protecting  $\beta$ -cells is heme oxygenase (HO-1), also known as Hsp32. HO-1 exerts its cytoprotective effects mainly by reduction of oxidative stress (McCabe et al., 2006) and overexpression of HO-1 could protect cytokine-exposed islet cells from apoptosis (Pileggi et al., 2001, Ye & Laychock, 1998). Bcl-2 family proteins, such as the anti-apoptotic Bcl-2, are major regulators of the apoptotic signalling cascade. It has been suggested that an impaired induction of anti-apoptotic Bcl-2 plays a role in cytokine-induced dysfunction and cell death of human islet cells relative to porcine islets (Piro et al., 2001). Moreover, overexpression of Bcl-2 was shown to protect  $\beta$ -cells from cytokine-induced apoptosis (Y. Liu et al., 1996) and increase the longevity of islet grafts after transplantation (Contreras et al., 2001). Several mechanisms by which Bcl-2 might exert  $\beta$ -cell protection have been suggested (McCabe et al., 2006). These include inhibition of cytochrome c release from mitochondria, inhibition of ER stress-induced apoptosis and blocking of  $\text{Ca}^{2+}$  release from the ER. It was shown that Bcl-2 overexpression can reduce ER stress-induced apoptosis in islet cells (Contreras et al., 2003). Both of these mechanisms have been associated with cytokine-induced  $\beta$ -cell death. Another candidate transgene may be the gene encoding the cellular FADD-like IL-1 $\beta$ -converting enzyme (FLICE)-like inhibitory protein (cFlip) as its overexpression has been shown to inhibit the activation of caspase-8 in  $\beta$ -cells exposed to TNF $\alpha$  (Cottet et al., 2002).

#### 4.2 Anti-cytokine gene transfer

Inhibition of NF- $\kappa$ B, a main effector of cytokine-signalling, was shown to reduce cytokine-induced apoptosis in rodent  $\beta$ -cells *in vitro* (Baker et al., 2001, Heimberg et al., 2001) and *in vivo* (Eldor et al., 2006) and Fas-induced apoptosis in human islet cells (Giannoukakis et al., 2000). It should be noted that active NF- $\kappa$ B has been shown to be an essential factor in mediating glucose-stimulated insulin secretion (Norlin et al., 2005) and while NF- $\kappa$ B inhibition may protect  $\beta$ -cells from apoptosis it may also interfere with insulin secretion.

JNK is another candidate target for anti-cytokine gene therapy. Inhibition of JNK has been shown to protect pig islet cells from apoptosis and loss of JNK function after isolation and also after transplantation suppresses IL-1 $\beta$  induced apoptosis in insulin-secreting rodent cell lines (Nikulina et al., 2003, Noguchi et al., 2005). Other potential transgenes interfering with cytokine signalling include feedback inhibitors, e.g. SOCS (Yasukawa et al., 2000). It is thought that a compromised ability to up-regulate SOCS in response to cytokine exposure makes  $\beta$ -cells particularly susceptible to cytokine-induced damage (Karlsen et al., 2001, Yasukawa et al., 2000). The overexpression of SOCS-3 in response to IL-1 $\beta$  was shown to be slower in  $\beta$ -cells compared to other cell lines (Karlsen et al., 2001). It was also demonstrated that SOCS-3 overexpression can protect rodent  $\beta$ -cells from cytokine-induced death (Karlsen et al., 2001).

#### 4.3 Anti-oxidant gene transfer

The protective effects of several antioxidant enzymes including catalase, glutathione peroxidase and the superoxide dismutases (SODs) MnSOD and CuZnSOD have been investigated. While results have not been entirely consistent, many studies have demonstrated that activation or overexpression of these enzymes can protect  $\beta$ -cells against oxidative stress or cytokine-induced destruction at least to some extent (Benhamou et al., 1998, Bertera et al., 2003, Hohmeier et al., 1998, Lortz & Tiedge, 2003, Lortz et al., 2000). These studies have shown that antioxidant gene transfer is a promising strategy in prolonging islet graft longevity. However, it has also been observed that transfer of antioxidant genes alone could not protect  $\beta$ -cells long term against toxicity caused by cytokine exposure and oxidative stress.

### 5. Conclusion

In recent years basic biomedical research has delivered a wealth of knowledge about the pathways by which inflammatory cytokines sensitise  $\beta$ -cells to cell death during the course of T1DM pathogenesis. Although the picture is still incomplete, we have learned about the major stresses to which  $\beta$ -cells are exposed. Some of the molecular players mediating these stresses have been identified. In particular, pro-inflammatory cytokines IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$  have been implicated as main mediators of  $\beta$ -cell stress and death during T1DM. It emerges that these cytokines synergistically activate transcriptional programs that lead to NO signalling, oxidative stress, ER stress, as well as modulation of Bcl-2 family protein expression. How these pathways precisely intersect has not yet been fully clarified. Studies elucidating these mechanisms may provide the knowledge to improve therapy. Islet transplantation, a therapeutic approach that would overcome the need of continuous insulin administration, is still in its infancy. Modern gene transfer techniques offer a huge potential for improvement to islet transplantation as it can help overcoming the cellular and autoimmune-mediated stress transplanted islets are exposed to. The experiments mentioned at the end of this chapter are encouraging that the accumulating knowledge of the molecules and pathways mediating  $\beta$ -cell stress will help to develop gene therapeutic approaches alleviating these stresses, thus improving survival of transplanted islets.

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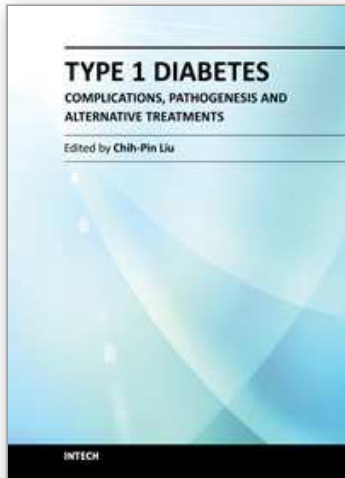
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This book is intended as an overview of recent progress in type 1 diabetes research worldwide, with a focus on different research areas relevant to this disease. These include: diabetes mellitus and complications, psychological aspects of diabetes, perspectives of diabetes pathogenesis, identification and monitoring of diabetes mellitus, and alternative treatments for diabetes. In preparing this book, leading investigators from several countries in these five different categories were invited to contribute a chapter to this book. We have striven for a coherent presentation of concepts based on experiments and observation from the authors own research and from existing published reports. Therefore, the materials presented in this book are expected to be up to date in each research area. While there is no doubt that this book may have omitted some important findings in diabetes field, we hope the information included in this book will be useful for both basic science and clinical investigators. We also hope that diabetes patients and their family will benefit from reading the chapters in this book.

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