Oxidative and nitrosative stress in β-cell apoptosis: their contribution to β-cell loss in type 1 diabetes mellitus

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ABSTRACT

The loss of β-cell mass consequential to the activation of pro-apoptotic signalling events is increasingly recognised as a causal and committed stage in the development of autoimmune, type 1, diabetes mellitus (DM). While the mechanisms responsible for targeted β-cell loss are multifaceted and difficult to define at a prediabetic stage, there is a need, from a therapeutic perspective, to understand the molecular mechanisms involved. Over recent years the use of animal and cell-line models of DM, together with investigations in isolated *ex vivo* human islets, have greatly increased our understanding of the processes involved in the pathogenesis of type 1 DM. From this work, several biochemical pathways have emerged that may have future potential for therapeutic intervention. This review looks at the current opinions on the role of apoptosis in β-cell loss at the molecular level, focusing on a central mechanism for oxidative and nitrosative stress, and suggests biochemical pathways that may have future potential for therapeutic intervention.

KEY WORDS: Apoptosis.
Diabetes mellitus.
Nitric Oxide.
Oxidative stress.

Introduction: apoptosis and β-cell loss

The process of programmed cell death, apoptosis, occurs via the activation of highly ordered and tightly regulated intracellular signalling pathways, which involve diverse triggers and effector molecules, including extracellular signals, intracellular ATP levels, phosphorylation cascades, procaspase cleavage and activation, and modification of the expression of pro- and anti-apoptotic genes. While undoubtedly this process is necessary and essential during normal development, tissue homeostasis and in the clearance of damaged cells, the inappropriate activation of death-inducing intracellular machinery can contribute significantly to pathological events associated with cell loss and impaired biological function. This is increasingly recognised as a direct contributor to β-cell loss associated with the pathogenesis of type 1 diabetes mellitus (DM).[1,2] Understanding the basic molecular mechanisms responsible for β-cell loss under these conditions continues to be a major aim of diabetes research, not least to identify new molecular targets for therapeutic intervention strategies in the treatment of type 1 DM but also following islet cell transplantation to improve long-term functionality of grafted islets.

Type 1 DM is classified as an autoimmune disease, where the inappropriate activation of the immune system against the β-cell is thought to result from an environmental trigger in genetically predisposed individuals, leading to a reduction in β-cell mass as a consequence of excessive β-cell apoptosis. Although the initiating events and mechanisms predisposing to the development of autoimmune disease states remain poorly understood, a crucial role for pro-inflammatory cytokines as soluble mediators of β-cell death in type 1 DM has been described.[3,4] This review will cover some of the current opinions relating to β-cell apoptosis in type 1 DM, with a particular emphasis on the role of pro-inflammatory cytokines as effector molecules and downstream chronic oxidative and nitrosative stress. It will consider evidence from clonal cell lines, primary rodent β-cells and islet cultures as well as some of the more limited data available in human islets. The differences in the susceptibility to, and the consequences of, cytokine exposure between various model systems used to study β-cell apoptosis will be considered.

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Pathogenesis of type 1 DM: cell-cell killing and pro-inflammatory cytokines as soluble effectors

Early observations in Wistar rat islets suggested that interleukin-1β (IL-1β), present in the supernatant of activated macrophage cultures, was cytotoxic to islet cultures,[3,5] an effect found to be specific to β-cells[6] and which appears to involve synergistic regulation by other inflammatory cytokines also produced from activated immune cells.[7–10] These include interferon-γ (IFNγ) and tumour necrosis factor-α (TNFα). These observations suggested that β-cell loss was initiated by soluble mediators secreted by activated mononuclear immune cells (macrophages and antigen presenting cells [APC]) and auto-active T cells. Nerup *et al*.[11] proposed the Copenhagen model to describe the processes involved in the coordination of

immune cells in type 1 DM, proposing that the stimulation of β-cell dysfunction by factors secreted by activated mononuclear immune cells leads to increased β-cell and APC expression of 'self antigens,' which become recognised by subsequently infiltrating T cells. These may also be involved directly in the initiation of apoptosis and can stimulate B cells to produce autoantibodies. This hypothesis is supported by the observation that the production of β-cell-targeted autoantibodies correlates with deteriorating glycaemic control.[12]

Direct T-cell-mediated effects on β-cell apoptosis by both $CD4^+$ and $CD8^+$ T cells are associated with diverse and multiple stimuli and effector molecules, as well as the potential for the former to stimulate autoantibody production. A clearer understanding of the primary contributor to β-cell loss is needed, whether that be the effects of pro-inflammatory cytokines as soluble mediators, secreted by both macrophages/APCs and auto-active T cells, or through direct cell-cell contact in recognition-linked mechanisms of death.[13] The processes involved in the regulation of immune cells in the pathogenesis of type 1 DM have recently been reviewed elsewhere[14] and remain poorly understood.

What has become clear is that the effects of pro-inflammatory cytokines on β-cell function depend on the perturbation of multiple gene regulatory networks. Oligonucleotide arrays have allowed the characterisation of the gene signalling networks stimulated by pro-inflammatory cytokines.[15,16] Around 66 of these genes were found to be dependent on NF-κB activation and, using a NF-κB 'super-repressor,' Heimberg *et al*.[17] observed significant protection from cytokine-induced toxicity. These genes include the up-regulation of the inducible form of nitric oxide synthase (iNOS), leading to nitric oxide (NO) production, and manganese superoxide dismutase (MnSOD), as well as proteins involved in the endoplasmic reticulum (ER) stress response, reduced β-cell function and (*in vivo*) increased immune cell infiltration. In Wistar rat islets, John *et al*.[18] suggested that several of the genes up-regulated in response to IL-1β treatment were NO-dependent, as 23 out of 105 transcripts found to be modified by cytokine exposure were inhibited by the inclusion of the arginine analogue, and NOS inhibitor, N_G-monomethyl-L-arginine (L-NMMA); thus, several cytokine-induced signalling networks may be NO-dependent. It has also been shown that, in RIN-r β-cells, cytokine-derived NO is a primary contributor to cell loss, and inhibition of iNOS activity protects cells from cytokine-induced apoptosis to a similar extent to that seen with adenoviral over-expression of a mutant IκB, preventing the activation of the transcription factor.[19] Proteomic analysis has suggested that the JAK/STAT signalling pathway mediates the response to IFNγ and, more recently, Pirot *et al*.[20] have suggested that IFNγ can sensitise cells to IL-1β-induced ER stress through a reduction in ER defence capability. Chen *et al*.[21] suggested that, in *ex vivo* rodent and human islets, IL-1β increases the expression of monocyte chemoattractant protein-1 (MCP-1), which can be enhanced by IFNγ. IL-1β also promotes an increase in intracellular adhesion molecule-1 (ICAM-1) expression, as well as increasing the surface presentation of self antigens.

Overall, it appears that β-cell apoptosis in response to cytokines depends on the severity of the perturbation of multiple key signalling networks, with a central role for the transcription factor NF-κB and the JAK/STAT pathways.[2] Although the predominant effects of their activation appear to be pro-apoptotic and lead to β-cell loss,[22] the up-regulation of anti-apoptotic genes following cytokine-induced activation of NF-κB has also been described in human islets and MIN6 cells.[23] A fuller understanding of the pro-apoptotic component may lead to the development of therapies aimed at shifting the delicate balance of pro- and anti-apoptotic signalling pathways to promote long-term β-cell survival in the face of cytokine toxicity.

Cytokines and ROS/RNS

While undoubtedly the pathways mediating cytokine toxicity are likely to be multifaceted, a central role for oxidative and nitrosative stress has been proposed.[24,25] Sources of ROS and reactive nitrogen species (RNS) in the vicinity of the pancreatic β-cell, as stimulated by cytokines secreted from mononuclear immune cells, are shown in Figure 1. The up-regulation of MnSOD, although itself an important antioxidant enzyme through the conversion of superoxide to hydrogen peroxide (H_2O_2), may contribute to β-cell ROS production as a result of increasing H_2O_2 levels, particularly in cell types with such low catalase and glutathione peroxidase expression[24] (both catalysing the removal of excess H_2O_2 into water and molecular oxygen). iNOS was first described as a contributor to β-cell dysfunction by Southern *et al*.,[26] who found that exposure of Sprague-Dawley rat islets to IL-1β inhibited insulin secretion, an effect that could be reversed using L-NMMA. In later studies, it was observed that the effects of an IL-1β/NO generating system were involved in the induction of β-cell apoptosis,[27,28] which appeared to require synergistic regulation by other inflammatory cytokines. Other studies

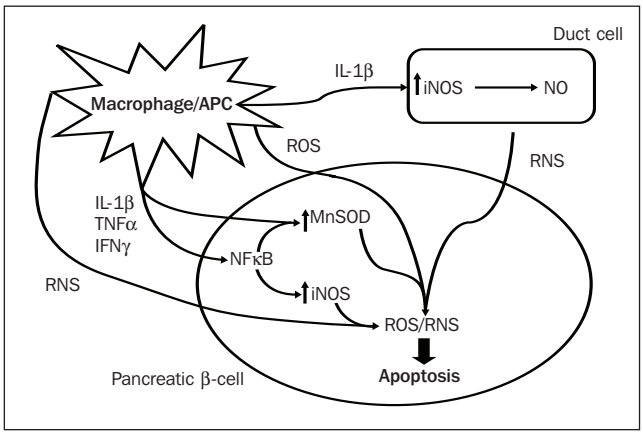

Fig. 1. Sources of ROS/RNS. Infiltrating macrophages secrete cytokines which lead to the up-regulation of iNOS and MnSOD via the activation of NFκB, producing an array of ROS/RNS in both β-cells and others (e.g., pancreatic duct cells). Despite its usual antioxidant role, the up-regulation of MnSOD may contribute to cytokine-toxicity through increased H_2O_2 production in the face of poor catalase and glutathione peroxidase expression. Other ROS/RNS produced by the macrophage and those secreted by the iNOS-active duct cells lead to exogenous sources of ROS/RNS in the vicinity of the β-cell. Downstream effects of excessive ROS/RNS build-up in the β-cell can lead to the initiation of apoptotic signal cascades.

also suggested a significant contribution of cytokine-induced oxidative stress,[24,29] which may also depend on the relative contributions of both ROS and RNS.[25] Ankarcrona *et al*.[27] observed that the effects of IL-1β alone were sufficient to up-regulate significant iNOS expression in rat β-cells, while Rabinovitch *et al*.[30] found that human islets were far less susceptible to cytokine-induced iNOS expression, where similar effects of NO using chemical NO donors have also been described.[31]

Transient effects of NO donors appeared to induce significant nitrosative stress-induced apoptosis in rodent islets, which was not found to be the case in human islets, suggesting that the physiological significance of an NO-generating system in human β-cells may be slight, perhaps reflecting the higher levels of key antioxidant defence enzymes expressed in human islets.[32] However, in later studies, the long-term effects of NO were observed to be equally deleterious to human islet function as to rodent islets, with extended culture of human islets following NO donor treatment inducing characteristic morphological changes associated with β-cell apoptosis.[33] Additionally, it has also been observed that cytokine-derived NO may sensitise human islets to T cells via the up-regulation of Fas.[34] Furthermore, Storling *et al*.[35] have suggested that the activation of JNK and inhibition of protein kinase B/AKT in response to NO contributes significantly to cytokine toxicity.

iNOS inhibitors and iNOS knockout models have suggested that NO production may not be the primary mechanism responsible for β-cell apoptosis, particularly in human islets,[30] and this suggests that iNOS does not therefore fulfil the 'one gene fits all' hypothesis.[36] However, the high chemical reactivity of the NO radical and the suggestion that significant NO production may occur *in vivo* in activated macrophages (via a feedback mechanism) and pancreatic duct cells,[37] coupled to some of the more recently observed NO-dependent effects of cytokines on β-cell viability, have combined to maintain interest in the role of NO and its derivative RNS, including peroxynitrite,[38] in β-cell death and apoptosis.

Using human islet preparations, Li and Mahato[39] have recently suggested (and were the first to do so) that iNOS-targeted siRNA can impact on islet functionality and apoptosis in response to pro-inflammatory cytokines, where a significant reduction in caspase-3 activity and apoptosis was observed in response to iNOS siRNA (although to a lesser extent than in clonal β-cell lines). It was suggested that earlier observations of a lack of any significant NO contribution may be due to low penetrance of siRNA constructs, in that only peripheral islet cells may have been affected by the silencing strategy, given the large size of human islet clusters (up to 3000 cells) when compared to the more convenient and frequently used clonal β-cell lines or dispersed islets.[39] It is of greater physiological relevance, particularly for transplantation, to use intact islets and thus retain intra-islet signalling cascades.

Islet transplantation represents a promising therapy for the treatment of type 1 DM; however, in rodent models, transplanted islets have been shown to undergo apoptosis in a matter of days following transplantation,[40] and, until recently, strategies aimed at improving the long-term survival and functionality of grafted islet preparations were generally ineffective. However, more recent studies have provided striking evidence for the benefit of anti-rejection therapy, virus antigen incorporation and over-expression of cytokine receptor antagonists[41] as suitable approaches to help control host defences, and micro-encapsulation has been shown to protect islet cells from immune attack.[42] However, non-specific intra-islet inflammation due to isolation and transplantation effects may contribute significantly to grafted islet failure. In support of these observations, Montolio *et al*.[43] have recently observed significant intra-islet expression of both IL-1β and iNOS in rodent islet cells syngeneically transplanted into the kidney capsule of male Lewis rats. Given these observations, and from a therapeutic perspective, a fuller understanding of the signalling pathways impacted on by ROS/RNS may be instrumental in the development of long-term solutions to islet transplantation.

β-cell susceptibility to ROS/RNS

Reactive oxygen species and RNS are able to interact with a range of subcellular proteins and signalling pathways as well as regulate gene expression, and the effects of oxidative and nitrosative stress on β-cell apoptosis are recurring themes in DM research; the susceptibility of β-cells to ROS and RNS has been proposed to be as a result of their low level of endogenous antioxidant defence capacity.[32] Indeed, Tiedge *et al*.[24] observed catalase (CAT) and glutathione peroxidase (GPx) enzyme expression to be as low as 5% of the levels seen in liver cells in Wistar rats. Interestingly, Sigfrid *et al*.[44] have observed that NO can directly inhibit any remaining CAT enzyme activity in RINm5F β-cells, an effect that was not associated with a reduction in CAT messenger

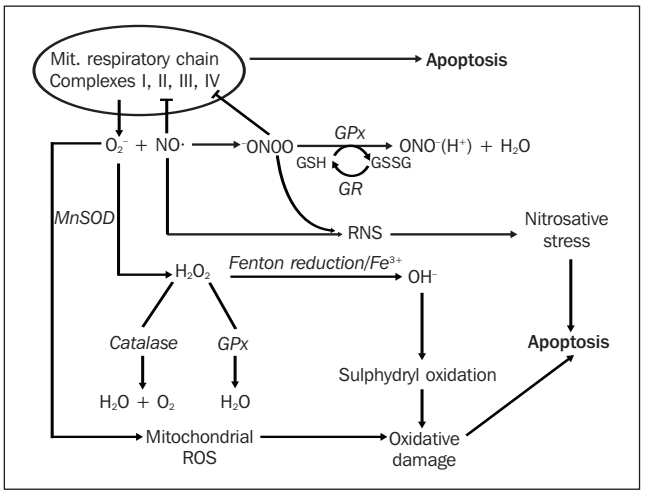

Fig. 2. Reactions and interactions of ROS/RNS. The reactions of NO, including the formation of peroxynitrite (–ONOO), can lead to the production of a range of RNS capable of inducing significant nitrosative damage. Prolonged nitrosative stress can have deleterious effects on mitochondrial respiration and can induce apoptosis. Significant contributions of ROS, O_2– and H_2O_2, as a result of reduced β-cell antioxidant enzyme (MnSOD, catalase and glutathione peroxidase [GPx]) expression can lead to severe oxidative damage. H_2O_2 can be converted to the highly damaging hydroxyl radical (OH–) by transition metal-catalysed Fenton reduction and can cause sulphydryl oxidation of target proteins, DNA damage and cell death. The key antioxidant enzymes catalase, MnSOD, GPx and GR are shown in italics.

RNA (mRNA) or protein content and which may potentially increase endogenous ROS production *in vitro*. The high chemical reactivity of ROS makes it difficult to define clearly their precise role and, although at low concentrations, ROS have been suggested to act as second messengers controlling gene transcription primarily through activation of the transcription factors NF-κB and AP-1.[45,46]

Increased ROS concentrations in cytokine-treated β-cells can lead to a multitude of different signalling components being interrupted, leading to apoptosis. Several studies have demonstrated a crucial role for increased oxidative stress in animal and human models of type 1 DM through over-expression of antioxidant enzymes.[47–49] In the non-obese diabetic (NOD) mouse model of spontaneous diabetes, increased markers of oxidative stress can be detected at a prediabetic stage when compared to oxidant- (and diabetes-) resistant NOD.Lc7 mice and wild-type litter mates;[50] these effects have also been shown to be reversed with a metalloporphyrin SOD mimetic.[51] The use of soluble SOD mimetics, acting as sustainable catalysts in the removal of excess toxic ROS, may offer greater scope than gene-based approaches to increase endogenous antioxidant enzyme expression or increased dietary consumption of radical scavengers such as vitamin C and E. Future research on the usefulness of oxidant scavenging mimetics in combination with strategies aimed at controlling increased nitrosative stress, such as β-cell-targeted inhibitors of iNOS expression/function, may help mute the intra-islet inflammation observed following transplantation.

Given that both iNOS and MnSOD are up-regulated in response to cytokine treatment, the latter enzyme catalysing the dismutation of superoxide into H_2O_2, it seems likely that increases in both ROS and RNS are involved in the pro-apoptotic actions of cytokines. Given their high chemical reactivity, it would also seem likely that neither subset of reactive species will act independently of the other; indeed, the reaction between NO and superoxide leads to the production of the highly damaging species peroxynitrite, which has been associated with DNA damage and β-cell necrosis. Human islets, for example, have been found to be highly sensitive to peroxynitrite toxicity.[38,52] The complexities of the reactions between distinct ROS/RNS and their effects on biological function are beyond the scope of this review; however, a simplified schematic of their reactions and interactions is shown in Figure 2.

In NOD mice the use of guanidinoethyldisulphide (GED), both a cell-permeable iNOS inhibitor and ROS scavenger, prevented the development of diabetes and conferred protection from cytokine-induced loss of β-cell mass.[25] In support of this role for both oxidative and nitrosative stress as contributors to β-cell death, a more recent study by Chen *et al*.[53] found that protection from oxidative stress by over-expressing CAT and MnSOD transgenes, alone, did not alter cytokine toxicity in mouse islets, which remained associated with increased NO. This is contradictory to previous studies suggesting that over-expression of antioxidant enzymes can confer some protection from cytokine-induced effects in RINm5F cells.[47] However, more conflicting data may suggest that MnSOD expression is required for triggering cytokine toxicity,[54] although this may still occur secondarily to NO-dependent effects.[55] These findings, collectively, may suggest a role for strategies aimed at the control of iNOS expression/function in combination with mitochondrial-targeted over-expression of antioxidant enzymes, for the protection of β-cells from cytokine-induced apoptosis.

Calcium signals in cytokine- and ROS/RNS-induced β-cell toxicity

While approaches aimed at controlling the expression of proteins involved in increased oxidative and nitrosative stress may improve the fate of the β-cell, understanding the molecular signalling events downstream of excessive ROS/RNS generation continues to be a major aim of research, with the hope of identifying suitable targets for small molecule intervention. In the context of both cytokine-induced apoptosis and cytokine-derived ROS/RNS, there is increasing evidence for a role for calcium channel activation and the disruption to both ER and mitochondrial function in mediating the susceptibility of β-cells to physiologically relevant apoptosis-inducing agents.[56–58]

Early observations of calcium-dependent apoptotic processes in response to NO donor treatment in MIN6 cells[59] have been proposed to be mediated by the release of calcium from intracellular stores.[56,57,60] These effects may lead to significant perturbation of intracellular calcium homeostasis, the activation of an ER stress response and increased mitochondrial calcium influx, culminating in the activation of multiple pro-apoptotic signalling pathways and cytochrome

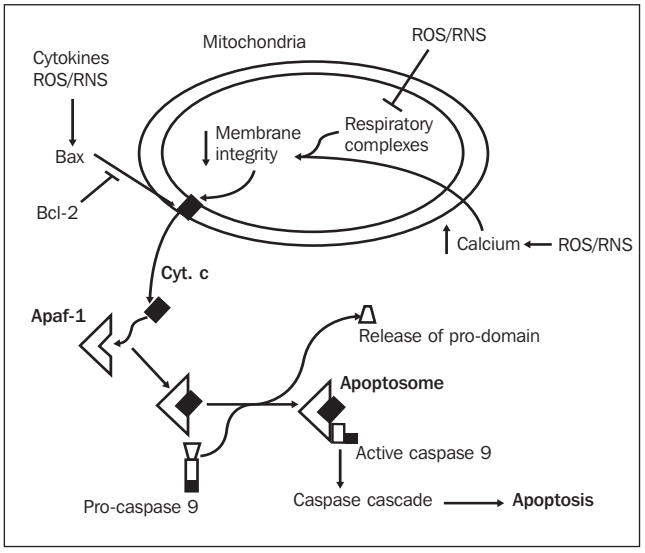

Fig. 3. Cytochrome c release and the induction of apoptosis. Both ROS and RNS are capable of inhibiting the function of components of the respiratory chain as well as increasing mitochondrial calcium concentrations. These processes can lead to disruption of the mitochondrial membrane potential (Ψ_M), activation of cyclophillin D and opening of the permeability transition pore (PTP), allowing the release of cytochrome c from the intermembrane space. Activation of the pro-apoptotic protein (Bax), a member of the Bcl-2 family, can also initiate cytochrome c release through oligomerisation and insertion into the outer mitochondrial membrane; the resulting pore is large enough to allow the release of cytochrome c. In the cytosol, cytochrome c binds to apoptosis protease activating factor-1 (apaf-1) and its binding partner procaspase 9. The formation of this complex leads to proteolytic cleavage of the pro-domain, activating caspase 9 and inducing a caspase signalling cascade leading to apoptosis.

c release from the intermembrane space of the mitochondria. It is widely argued that NO, H_2O_2 and their derivatives can directly modulate the activity of mitochondrial respiratory components,[61–63] which may impair mitochondrial membrane integrity and lead to cytochrome c release in β-cells.[58,64] For example, the over-expression of anti-apoptotic Bcl-2 has been shown to inhibit cytokine-induced mitochondrial dysfunction and apoptosis,[65] and, more recently, it has been suggested that cytokine-derived NO in RIN-r β-cells stimulates mitochondrial cytochrome c release and apoptosis, which can be inhibited by over-expression of the Bcl-2 family protein Bcl-$_{XL}$.[64] Effects of ROS/RNS on impaired mitochondrial function and cytochrome c release and the role of cytosolic cytochrome c in inducing caspase activation and apoptosis are shown in Figure 3.

The activation of ER calcium extrusion pathways in MIN6 and INS-1E cells, and also in primary rat β-cells, coupled to the down-regulation of the ER calcium ATPase SERCA in response to NO has been shown to lead to significant ER calcium release and activation of an ER stress response; an effect prevented by iNOS inhibition in cytokine-treated β-cells.[56,57] A comprehensive analysis of the effects of H_2O_2 on calcium homeostasis in MIN6 cells has suggested that intracellular calcium fluxes from both the extracellular space and intracellular calcium stores induce apoptosis and suggest an important role for calcium fluxes in ROS-induced β-cell death.[58] Chang *et al*.[66] concluded that cytokine-induced β-cell apoptosis was dependent on the activation of L-type calcium channels, having observed that the L-type calcium channel antagonist nifedipine inhibited cytokine-induced intracellular calcium increases and apoptosis after 48 hours' exposure. This effect may result from the inhibition of NF-κB activation by calcium antagonists;[67] downstream effects on apoptosis may, in this context, be a result of decreased iNOS expression and nitrosative stress (Watson and Loweth, unpublished findings). In animal models of hepatic injury, nifedipine, acting as an inhibitor of oxidative stress, has been shown to significantly reduce iNOS expression in resident liver macrophages (Kupffer cells) in response to IFNγ and LPS treatment, which was coupled to a reduction in hepatocyte damage[68] and in vascular disease.[69]

The intracellular calcium release channels of the ER, notably the ryanodine receptor (RyR) and IP3 receptor, have been shown to mediate the pro-apoptotic response to the SERCA inhibitor thapsigargin (TG) in primary mouse β-cells.[70] However, the activation of the RyR by second messengers, including metabolites of glucose, may also contribute significantly to glucose-stimulated insulin secretion.[71] Although intracellular calcium increases contribute significantly to physiological insulin secretion, pathophysiological effects in response to ROS/RNS may also contribute to cell death mechanisms. Understanding the impact of distinct ROS/RNS on calcium channel activation may ultimately help to develop therapies aimed at controlling and normalising their activation under pathophysiological conditions of excessive and prolonged opening.

β-cell apoptosis and the ER

Downstream effects of excessive and prolonged release of calcium from the ER can lead to the activation of ER stress signalling pathways, and this occurs when the cellular demand for ER function outweighs the ER's functional capacity. It has been suggested that β-cells are one of the most susceptible cell types to ER stress, given their highly developed ER necessary in meeting the demands of *in vivo* insulin secretion.[72] The ER stress signalling pathways and their role in various cell types and disease states, including diabetes, have been extensively reviewed.[72,73] Among their effects, it is known that activation of ER stress signalling pathways may contribute to cytokine toxicity in type 1 DM,[2] and the effects of cytokines and cytokine-derived ROS/RNS may, in this context, be as a result of ER calcium depletion.[56–58] Therefore, given their highly conserved and tightly regulated nature, the ER stress signalling pathways may serve as potential targets for therapeutic intervention in the control of the chronic oxidative and nitrosative stress implicated in the development of type 1 DM, potentially through the inhibition of ER calcium release pathways. Having said this, the role of ER calcium extrusion channels in TG-induced β-cell ER stress and apoptosis,[70] as discussed previously, may not necessarily indicate their suitability for small molecular intervention, given the suggested role of the luminal ER calcium pool in mediating insulin secretion upon second messenger stimulation.[71] In addition, small molecule inhibitors targeting stress response pathways, in particular the ER stress inhibitor salubrinal, appear poorly tolerated by β-cells and have been shown to perpetuate the pro-apoptotic effects of cytokines and free fatty acids.[74,75]

Other more recent observations may suggest that cytokine-induced ER stress in β-cells is secondary to other pro-apoptotic signalling events[76] and Chambers *et al*.[77] have suggested that cytokine-induced β-cell death only correlates transiently with induction of the ER stress response pathways. They also suggest that the role of NO in this context may be two-fold and, while the cytokine-induced ER stress response may well be NO-dependent, they suggest that this radical may also be directly involved in the coordination of anti-apoptotic ER chaperone expression.[77] This dual effect may be responsible for their proposed

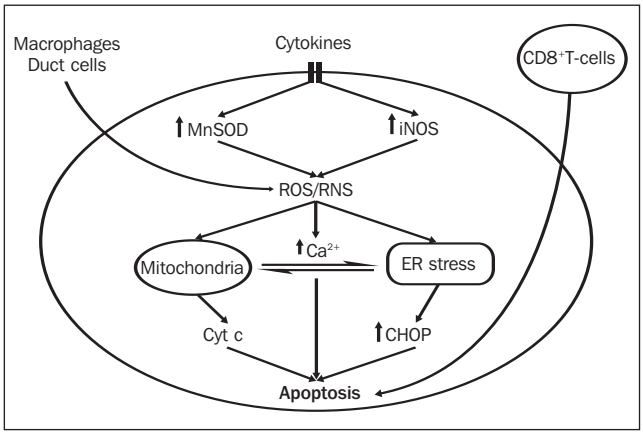

Fig. 4. ROS/RNS and β-cell apoptosis. The activation of β-cell apoptosis is stimulated by diverse and multiple triggers, including the effects of the T cell in recognition-linked mechanisms of cell death and through pro-inflammatory cytokines as soluble effectors. Coupled to the exogenous sources of ROS/RNS, produced by macrophages and pancreatic duct cells, cytokine combinations can induce significant accumulation of toxic intracellular ROS and RNS through the expression of MnSOD (leading to increased H_2O_2 production) and iNOS, respectively.

divergence of cytokine-induced β-cell death from the ER stress pathway after prolonged exposure. Nonetheless, it appears that the ER stress pathway regulates the susceptibility of β-cells to cytokine-induced nitrosative stress, as NO has been shown to be the primary contributor to cytokine-induced pro-apoptotic CHOP expression.[56,57] A member of the C/EBP homology protein family, CHOP expression is greatly increased following activation of an ER stress response. The role of CHOP in ER stress-induced apoptosis has been extensively reviewed[78] and it has been shown that disruption to the *CHOP* gene using knockout mice delays ER stress-induced β-cell apoptosis.[79]

Given that recent observations suggest that iNOS siRNA can improve human islet viability in response to cytokine treatment,[39] and that anti-inflammatory cytokines can attenuate pro-inflammatory cytokine effects as a result of decreased nitrosative stress,[55] interest in deciphering the contributions of ROS/RNS to cell death mechanisms, particularly with respect to the β-cell's ER, continues to be a major aim of diabetes research. An overview of some of the actions of cytokines, ROS and RNS and their interplay, as reviewed here, is shown in Figure 4.

Conclusions

Although the ROS/RNS-mediated component of cytokine-induced apoptosis may represent only one route to β-cell death, the complex interplay between distinct ROS and RNS may well be a significant contributor to β-cell loss.[24,25] Their combined effects may include both the synergistic downstream activation of pro-apoptotic ER stress signalling events[56,57] and disruption to mitochondrial function,[58,64] acting together to coordinate an apoptotic response. Studies such as those reviewed here are essential to aid our understanding of how cytokines and ROS/RNS impact on diverse signalling pathways and further research will be needed to allow a comprehensive evaluation of the therapeutic potential of strategies aimed at controlling nitrosative and oxidative stress in the protection of pancreatic β-cells in type 1 DM. One important outcome of understanding the inflammatory response(s) mediating β-cell destruction and, in particular, their susceptibility to oxidative/nitrosative stress may be improved islet transplantation strategies, as a result of perturbation of endogenous islet inflammation. □

References

1 Eizirik DL, Mandrup-Poulsen T. A choice of death – the signal transduction of immune-mediated beta-cell apoptosis. *Diabetologia* 2001; **44**: 2115–33.

2 Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes – many differences, few similarities. *Diabetes* 2005; **54**: (Suppl 2): S97–S107.

3 Mandrup-Poulsen T, Bendtzen K, Nerup J, Dinarello CA, Svenson M, Nielsen JH. Affinity-purified human interleukin-I is cytotoxic to isolated islets of Langerhans. *Diabetologia* 1986; **29**: 63–7.

4 Sandler S, Eizirik DL, Sternesjö J, Welsh N. The role of cytokines in the regulation of pancreatic beta cell function. *Biochem Soc Trans* 1994; **22**: 26–30.

5 Bendtzen K, Mandrup-Poulsen T, Dinarello CA *et al.* Human interleukin 1 is cytotoxic to insulin and glucagon-producing islets of Langerhans. *Eur J Clin Invest* 1986; **16**: A40.

6 Mandrup-Poulsen T, Egeberg J, Nerup J, Bendtzen K, Nielsen JH, Dinarello CA. Ultrastructural studies of time-course and cellular specificity of interleukin-1-mediated islet cytotoxicity. *Acta Pathol Microbiol Immunol Scand C* 1987; **95**: 55–63.

7 Cunningham JM, Green IC. Cytokines, nitric oxide and insulin-secreting cells. *Growth Regul* 1994; **4**: 173–80.

8 Cetkovic-Cvrlje M, Eizirik DL. TNF-alpha and IFN-gamma potentiate the deleterious effects of IL-1 beta on mouse pancreatic islets mainly via generation of nitric oxide. *Cytokine* 1994; **6**: 399–406

9 Mandrup-Poulsen T. The role of interleukin-1 in the pathogenesis of IDDM. *Diabetologia* 1996; **39**: 1005–29

10 Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. *Biochem Pharmacol* 1998; **55** (8): 1139–49.

11 Nerup J, Mandrup-Poulsen T, Helqvist S *et al*. On the pathogenesis of IDDM. *Diabetologia* 1994; **37**: S82–S89.

12 Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001; **358**: 221–9.

13 Mathis D, Vence L, Benoist C. Beta cell death during progression to diabetes. *Nature* 2001; **414**: 792–8.

14 Knip M, Siljander H. Autoimmune mechanisms in type 1 diabetes. *Autoimmunity* 2008; **7** (7): 550–7.

15 Cardozo AK, Heimberg H, HeremansY *et al*. A comprehensive analysis of cytokine-induced and nuclear factor-kappa B-dependent genes in primary rat pancreatic beta cells. *J Biol Chem* 2001; **276**: 48879–86.

16 Cardozo AK, Kruhoffer M, Leeman R, Orntoft T, Eizirik DL. Identification of novel cytokine-induced genes in pancreatic beta-cells by high-density oligonucleotide arrays. *Diabetes* 2001; **50**: 909–20.

17 Heimberg H, Heremans Y, Jobin C *et al*. Inhibition of cytokine-induced NF-kappa B activation by adenovirus-mediated expression of a NF-kappa B super-repressor prevents beta-cell apoptosis. *Diabetes* 2001; **50**: 2219–24.

18 John NE, Andersen HU, Fey SJ *et al*. Cytokine- or chemically derived nitric oxide alters the expression of proteins detected by two-dimensional gel electrophoresis in neonatal rat islets of Langerhans. *Diabetes* 2000; **49**: 1819–29.

19 McCabe C, Samali A, O'Brien T. Beta cell cytoprotective strategies: establishing the relative roles for iNOS and ROS. *Biochem Biophys Res Comm* 2006; **342**: 1240–8.

20 Pirot P, Eizirik DL, Cardozo AK. Interferon-gamma potentiates endoplasmic reticulum stress-induced death by reducing pancreatic beta cell defence mechanisms. *Diabetologia* 2006; **49**: 1229–36.

21 Chen MC, Proost P, Gysemans C, Mathieu C, Eizirik DL. Monocyte chemoattractant protein-1 is expressed in pancreatic islets from prediabetic NOD mice and in interleukin-1 beta-exposed human and rat islet cells. *Diabetologia* 2001; **44**: 325–32.

22 Ortis F, Pirot P, Naamane N *et al*. Induction of nuclear factor-kappaB and its downstream genes by TNF-alpha and IL-1beta has a pro-apoptotic role in pancreatic beta cells. *Diabetologia* 2008; **51** (7): 1213–25

23 Sarkar SA, Kutlu B, Velmurugan K *et al*. Cytokine-mediated induction of anti-apoptotic genes that are linked to nuclear factor kappa-B signalling in human islets and in a mouse beta cell line. *Diabetologia* 2009; **52**: 1092–101.

24 Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between

antioxidant enzyme gene expression and antioxidative defence status of insulin-producing cells. *Diabetes* 1997; **46**: 1733–42.
25 Suarez-Pinzon WL, Mabley JG, Strynadka K, Power RF, Szabo C, Rabinovitch A. An inhibitor of inducible nitric oxide synthase and scavenger of peroxynitrite prevents diabetes development in NOD mice. *J Autoimmun* 2001; **16**: 449–55.
26 Southern C, Schulster D, Green IC. Inhibition of insulin secretion by interleukin-1-beta and tumour necrosis factor-alpha via an L-arginine-dependent nitric oxide-generating mechanism. *FEBS Lett* 1990; **276**: 42–4.
27 Ankarcrona M, Dypbukt JM., Brune B, Nicotera P. Interleukin-1 beta-induced nitric oxide production activates apoptosis in pancreatic Rinm5F cells. *Exp Cell Res* 1994; **213**: 172–7.
28 Kaneto H, Fujii J, Seo HG *et al*. Apoptotic cell death triggered by nitric oxide in pancreatic beta cells. *Diabetes* 1995; **44**: 733–8.
29 Rabinovitch A, Suarez-Pinzon WL, Strynadka K, Lakey JRT, Rajotte RV. Human pancreatic islet beta-cell destruction by cytokines involves oxygen-free radicals and aldehyde production. *J Clin Endocrinol Metab* 1996; **81**: 3197–202.
30 Rabinovitch A, Suarez-Pinzon WL, Strynadka K *et al*. Human pancreatic islet beta-cell destruction by cytokines is independent of nitric oxide production. *J Clin Endocrinol Metab* 1994; **79** (4): 1058–62.
31 Eizirik DL, Sandler S, Welsh N *et al.* Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. *J Clin Invest* 1994; **93**: 1968–74.
32 Welsh N, Margulis B, Borg LAH *et al*. Differences in the expression of heat-shock proteins and antioxidant enzymes between human and rodent pancreatic islets – implications for the pathogenesis of insulin-dependent diabetes mellitus. *Mol Med* 1995; **1**: 806–20.
33 Eizirik DL, Delaney CA, Green MHL *et al*. Nitric oxide donors decrease the function and survival of human pancreatic islets. *Mol Cell Endocrinol* 1996; **118**: 71–83.
34 Stassi G, De Maria R, Trucco G *et al.* Nitric oxide primes pancreatic beta cells for Fas-mediated destruction in insulin-dependent diabetes mellitus. *J Exp Med* 1997; **186** (8): 1193–200.
35 Storling J, Binzer J, Anderson AK *et al*. Nitric oxide contributes to cytokine-induced apoptosis in pancreatic beta cells via potentiation of JNK activity and inhibition of Akt. *Diabetologia* 2005; **48**: 2039–50.
36 McDaniel ML, Corbett JA, Kwon G, Hill JR. A role for nitric oxide and other inflammatory mediators in cytokine-induced pancreatic beta-cell dysfunction and destruction. *Adv Exp Med Biol* 1997; **426**: 313–9.
37 Pipeleers D, Hoorens A, Marichal-Pipeleers M, van de Casteele M, Bouwens L, Ling Z. Role of pancreatic beta cells in the process of beta cell death. *Diabetes* 2001; **50** (Suppl 1): S52–7.
38 Delaney CA, Tyrberg B, Bouwens L, Vaghef H, Hellman B, Eizirik DL. Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death. *FEBS Lett* 1996; **394**: 300–6.
39 Li F, Mahato RI. iNOS gene silencing prevents inflammatory cytokine-induced beta-cell apoptosis. *Mol Pharm* 2008; **5**: 407–17.
40 Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 2002; **51**: 66–72.
41 Tellez N, Montolio M, Estilles E, Escoriza J, Soler J, Montanya E. Adenoviral overproduction of interleukin-1 receptor antagonist increases beta-cell replication and mass in syngeneically transplanted islets, and improves metabolic outcome. *Diabetologia* 2007; **50**: 602–11.
42 Qi M, Strand BL, Morch Y *et al*. Encapsulation of human islets in novel inhomogeneous alginate-Ca2+/Ba2+ microbeads: *in vitro* and *in vivo* function. *Artif Cells Blood Substit Immobil Biotechnol* 2008; **36**: 403–20.
43 Montolio M, Biarnes M, Tellez N, Escoriza J, Soler J, Montanya E. Interleukin-1 beta and inducible form of nitric oxide synthase expression in early syngeneic islet transplantation. *J Endocrinol* 2007; **192**: 169–77.
44 Sigfrid LA, Cunningham JM, Beeharry N *et al.* Cytokines and nitric oxide inhibit the enzyme activity of catalase but not its protein or mRNA expression in insulin-producing cells. *J Mol Endocrinol* 2003; **31**: 509–18.
45 Nose K, Shibanuma M, Kikuchi K. Transcriptional activation of early-response genes by hydrogen peroxide in a mouse osteoblastic cell line. *Eur J Biochem* 1991; **201**: 99–106
46 Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of NF-κB transcription factor and HIV-1. *EMBO J* 1991; **10**: 2247?58.
47 Lortz S, Tiedge M, Nachtwey T, Karlsen AE, Nerup J, Lenzen S. Protection of insulin-producing RINm5F cells against cytokine-mediated toxicity through overexpression of antioxidant enzymes. *Diabetes* 2000; **49**: 1123–30.
48 Tiedge M, Lortz J, Munday R, Lenzen S. Protection against the co-operative toxicity of nitric oxide and oxygen free radicals by overexpression of antioxidant enzymes in bioengineered insulin-producing RINm5F cells. *Diabetologia* 1999; **42**: 849–55.
49 Gurgul E, Lortz S, Tiedge M, Jorns A, Lenzen S. Mitochondrial catalase overexpression protects insulin-producing cells against toxicity of reactive oxygen species and proinflammatory cytokines. *Diabetes* 2004; **53**: 2271–80.
50 Haskins K, Kench J, Powers K *et al.* Role for oxidative stress in the regeneration of islet beta cells? *J Investig Med* 2004; **52** (1): 45–9.
51 Haskins K, Bradley B, Powers K *et al*. Oxidative stress in type 1 diabetes. *Ann N Y Acad Sci* 2003; **1005**: 43–54.
52 Delaney CA, Pavlovic D, Hoorens A, Pipeleers DG, Eizirik DL. Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells. *Endocrinology* 1997; **138**: 2610–4.
53 Chen HN, Li XY, Epstein PN. MnSOD and catalase transgenes demonstrate that protection of islets from oxidative stress does not alter cytokine toxicity. *Diabetes* 2005; **54**: 1437–46.
54 Lortz S, Gurgul-Convey E, Lenzen S, Tiedge M. Importance of mitochondrial superoxide dismutase expression in insulin-producing cells for the toxicity of reactive oxygen species and proinflammatory cytokines. *Diabetologia* 2005; **48**: 1541–8.
55 Souza KLA, Gurgul-Convey E, Elsner M, Lenzen S. Interaction between pro-inflammatory and anti-inflammatory cytokines in insulin-producing cells. *J Endocrinol* 2008; **197**: 139–50.
56 Oyadomari S, Takeda K, Takiguchi M *et al*. Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. *Proc Natl Acad Sci USA* 2001; **98**: 10845–50.
57 Cardozo AK, Ortis F, Storling J *et al*. Cytokines down-regulate the sarcoendoplasmic reticulum pump Ca2+ ATPase 2b and deplete endoplasmic reticulum Ca2+, leading to induction of endoplasmic reticulum stress in pancreatic beta-cells. *Diabetes* 2005; **54**: 452–61.
58 Choi SE, Min SH, Shin HC, Kim HE, Jung MW, Kang Y. Involvement of calcium-mediated apoptotic signals in H_2O_2-induced MIN6N8a cell death. *Eur J Pharmacol* 2006; **547**: 1–9.
59 Nakata M, Uto N, Maruyama I, Yada T. Nitric oxide induces apoptosis via Ca2+-dependent processes in the pancreatic beta-cell line MIN6. *Cell Struct Funct* 1999; **24**: 451–5.

60 Willmott NJ, Galione A, Smith PA. Nitric oxide induces intracellular Ca2+ mobilization and increases secretion of incorporated 5-hydroxytryptamine in rat pancreatic beta cells. *FEBS Lett* 1995; **371**: 99–104.
61 Lizasoain I, Moro MA, Knowles RG, Darley Usmar V, Moncada S. Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem J* 1996; **314**: 877–80.
62 Burwell LS, Nadtochiy SM, Tompkins AJ, Young S, Brookes PS. Direct evidence for S-nitrosation of mitochondrial complex I. *Biochem J* 2006; **394**: 627–34.
63 Long XL, Goldenthal MJ, Wu GM, Marin-Garcia J. Mitochondrial Ca2+ flux and respiratory enzyme activity decline are early events in cardiomyocyte response to H_2O_2. *J Mol Cell Cardiol* 2004; **37**: 63–70.
64 Holohan C, Szegezdi E, Ritter T, O'Brien T, Samali A. Cytokine-induced beta-cell apoptosis is NO-dependent, mitochondria-mediated and inhibited by BCL-X-L. *J Cell Mol Med* 2008; **12**: 591–606.
65 Rabinovitch A, Suarez-Pinzon W, Strynadka K *et al*. Transfection of human pancreatic islets with an anti-apoptotic gene (*bcl*-2) protects beta cells from cytokine-induced destruction. *Diabetes* 1999; **48** (6): 1223–9.
66 Chang I, Cho NJ, Kim S *et al*. Role of calcium in pancreatic islet cell death by IFN-gamma/TNF-alpha. *J Immunol* 2004; **172**: 7008–14.
67 Steffan NM, Bren GD, Frantz B, Tocci MJ, Oneill EA, Paya CV. Regulation of Ikb-alpha phosphorylation by PKC-dependent and Ca2+-dependent signal-transduction pathways. *J Immunol* 1995; **155**: 4685–91.
68 Mustafa SB, Olson MS. Effects of calcium channel antagonists on LPS-induced hepatic iNOS expression. *Am J Physiol* 1999; **277** (2 pt 1): G351–60.
69 Yamagishi S, Nakamura K, Matsui T. Role of oxidative stress in the development of vascular injury and its therapeutic intervention by nifedipine. *Curr Med Chem* 2008; **15**: 172–7.
70 Luciani DS, Gwiazda KS, Yang TLB *et al*. Roles of IP3R and RyR Ca2+ channels in endoplasmic reticulum stress and beta-cell death. *Diabetes* 2009; **58**: 422–32.
71 Johnson JD, Kuang SH, Misler S, Polonsky KS. Ryanodine receptors in human pancreatic beta cells: localization and effects on insulin secretion. *FASEB J* 2004; **18**: 878–84.
72 Araki E, Oyadomari S, Mori M. Endoplasmic reticulum stress and diabetes mellitus. *Intern Med* 2003; **42**: 7–14.
73 Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 2006; **7**: 880–5.
74 Cnop M, Ladriere L, Hekerman P *et al*. Selective inhibition of eukaryotic translation initiation factor 2 alpha dephosphorylation potentiates fatty acid-induced endoplasmic reticulum stress and causes pancreatic beta-cell dysfunction and apoptosis. *J Biol Chem* 2007; **282**: 3989–97.
75 Diakogiannaki E, Welters HJ, Morgan NG. Differential regulation of the endoplasmic reticulum stress response in pancreatic beta-cells exposed to long-chain saturated and monounsaturated fatty acids. *J Endocrinol* 2008; **197**: 553–63.
76 Akerfeldt MC, Howes J, Chan JY *et al*. Cytokine-induced beta-cell death is independent of endoplasmic reticulum stress signalling. *Diabetes* 2008; **57**: 3034–44.
77 Chambers KT, Unverferth JA, Weber SM, Wek RC, Urano F, Corbett JA. The role of nitric oxide and the unfolded protein response in cytokine-induced beta-cell death. *Diabetes* 2008; **57**: 124–32.
78 Oyadomari S, Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ* 2004; **11**: 381–9.
79 Oyadomari S, Koizumi A, Takeda K *et al*. Targeted disruption of the *Chop* gene delays endoplasmic reticulum stress-mediated diabetes. *J Clin Invest* 2002; **109**: 525–32.