

Expert Views in Diabetes

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Poulsen is former president of the Danish Society for Internal Medicine and of the Scandinavian Society for the Study of Diabetes. He is a member of the Juvenile Diabetes Research Foundation International Medical Science Review Board and former member of the Minkowski Prize Committee of the European Association for the Study of Diabetes (EASD). In addition, Professor Mandrup-Poulsen is chairman of the Data and Safety Monitoring Committee of the Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk (TRIGR) study. In 1994, Professor Mandrup-Poulsen received the Oskar Minkowski Award of the EASD and in 2001, he was honoured with the Knud Lundbæk Award of the Scandinavian Society for the Study of Diabetes. He has authored more than 250 scientific papers, mainly on the aetiology and pathogenesis of type 1 diabetes. Professor Mandrup-Poulsen's research group currently consists of 18 members, and focuses on cytokine-induced pro-apoptotic signalling in β -cells.

Inflammatory mediators and islet β -cell failure: a link between type 1 and type 2 diabetes?

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This commentary will consider the role of inflammatory mediators in type 1 and 2 diabetes. I am not going to discuss the role of inflammatory mediators in insulin resistance but will focus exclusively on the role of these mediators in the pancreatic β -cell.

β -cell failure in type 1 and type 2 diabetes

Type 1 diabetes is characterized by mononuclear infiltration in the islets of Langerhans. It is now proposed that rather than being an autoimmune disease, which implies that specific T-cells kill the β -cells, β -cell destruction is the end-stage result of an integrated inflammatory response in the islets. Therefore, type 1 diabetes can be considered an inflammatory disease of the pancreatic islets.

With regards to type 2 diabetes, there is loss of pancreatic β -cell mass compared with the obese, normoglycaemic individual. And so, what causes type 2 diabetes is inadequate insulin secretion to compensate for insulin resistance. Progressive β -cell loss is caused by apoptosis, and we now know that the β -cells are not only susceptible to stimuli from the immune system that will cause them to die, but that many metabolic factors including glucose, free fatty acids (FFAs), and amyloid polypeptide can cause β -cell death.

Type 1 diabetes: proposed cellular mechanisms of β -cell death

In order to understand the background to the signalling pathways and molecular mechanisms relevant to type 2 diabetes, we must first consider the evidence relevant to type 1

diabetes. In my area of investigation, there are two fields of scientists who are fighting each others' views: one school of thought believes that type 1 diabetes is essentially just a disease of a specific T-cell recognizing a β -cell epitope and then killing the β -cell – that is all there is to it. And, therefore, this school focuses all attention on trying to stop the cytotoxic (or CD8⁺) T-cell from killing the β -cell.¹ The other school of thought believes that immune activation of T-cells in the interplay with macrophages and antigen-presenting cells in the islet micro-environment, leads to inflammatory responses that ultimately cause β -cell destruction.¹ These two models are probably not mutually exclusive, but what is the

evidence related to these two schools of thought? Where is the agreement and what is the controversy?

The pathogenesis of type 1 diabetes: the agreement

We all agree that certain antigen-presenting cells take up β -cell antigen and present it to the immune system, and that the immune system and its specific recognition component, that is, the T-cell system, are both needed for disease development. We also agree that type 1 diabetes depends upon the processing of such antigens, and their presentation to the specifically reactive T-cells. Both the CD4⁺ T-helper cells and CD8⁺ T-cells are necessary, but CD4⁺ T-cells are sufficient, and can transfer disease in the absence of CD8⁺ T-cells. Native CD8⁺ T-cells are necessary for causing this response, but they are commonly not sufficient.¹⁻⁴

CD8⁺ T-cells: the controversy

The controversy is really related to the role of the cytolytic CD8⁺ T-cell, because its precise role is unclear. Is it a classical class 1-restricted cytolytic T-cell that kills the β -cell via contact-dependent effector mechanisms? This effector mechanism can be the insertion of the tubular complex perforin into the cell membrane, which allows a flux of the toxic molecule granzyme into the cytosol of the target cell, triggering the caspase pathway and causing apoptosis of the target cell. Or it could be another apoptotic death contact, the Fas/FasL system. A novel member of the Fas family, the TNF-related apoptosis-inducing ligand (TRAIL) is also incriminated, as is membrane TNF (mTNF).^{2,3} The problem is that none of these mechanisms seem to be essential for development of type 1 diabetes in animal models. Thus, the CD8⁺ T-cell may really be nothing but an immunomodulatory cell, the

action of which is not to directly kill the target cell, but to potentiate CD4 T-cell activity and local inflammation.

IL-1, TNF- α and IFN- γ : the 'filthy three'

So, there is little evidence to suggest that the cytotoxic T-cell is causing diabetes because it kills the β -cells directly: it is much more likely that it acts as an inflammatory cell that potentiates the inflammatory environment, leading to the secretion of the so-called 'filthy three', the pro-inflammatory cytokines par excellence – IL-1, TNF- α and IFN- γ . IL-1 and TNF- α are mainly secreted by monocytic cells, such as macrophages, whereas IFN- γ is a potent T-cell factor, bringing in the T-cell as an inflammatory effector cell. Among other events, IL-1, in synergy with TNF- α and IFN- γ , causes the liberation of nitric oxide (NO), a free radical which is toxic to β -cells and leads to necrosis and apoptosis in islet cells.

If IL-1 is blocked by administering the soluble receptor of IL-1 (which traps and thereby neutralizes IL-1), disease in an accelerated model of type 1 diabetes is prevented.⁵ This occurs in the absence of impaired T-cell function, suggesting that this effect is exerted at the level of the β -cell and not at the level of the immune system. If TNF-antagonism with IL-1 antagonism is combined, accelerated diabetes in the NOD mouse is prevented.⁶

How do inflammatory cytokines confer specificity of β -cell killing?

During the ontogeny of β -cell mass, an important transcription factor is needed; the so-called Pdx1 homeobox transcription factor. If immature α -/ β -cell-like phenotypes are transfected with Pdx1, driving them to become mature β -cells, severe sensitization to cytokine-mediated apoptotic

signalling ensues.⁷ We are now trying to identify the proteins driven by Pdx1 that cause this exquisite sensitization of β -cells to cytokines.

Another interesting point that could explain selective killing in the inflammatory infiltrate, is that molecules that downregulate the signalling of IL-1 in β -cells seem to be inadequate in β -cells, and thereby render the β -cell more sensitive to destruction by the cytokines. And we now have evidence to suggest that in the β -cells, cytokines lead to an aberrantly prolonged response via the JNK and NF κ B pathways.⁸ A very particular feature of the β -cell is that it uses 70% of its protein biosynthetic machinery to produce insulin, and therefore is very sensitive to stress in the endoplasmic reticulum (ER) protein-folding complex. As a part of glucose-induced insulin secretion, the β -cell is also one of the most active calcium-handling cells, and calcium is a strong apoptotic stimulus if it is aberrantly regulated in too high concentrations, in the wrong compartment, for too long a time. Another peculiar thing is that the β -cell is as efficient in producing NO as a macrophage, but the neighbouring α - and δ -cells cannot produce NO, probably because the NO-synthesizing enzyme, NO synthase, is completely dependent on NF κ B for its transcription. This may be deleterious to the cell because the β -cell, as we now know, is very sensitive to oxidative stress.

Mechanistic model for cytokine-induced β -cell apoptosis

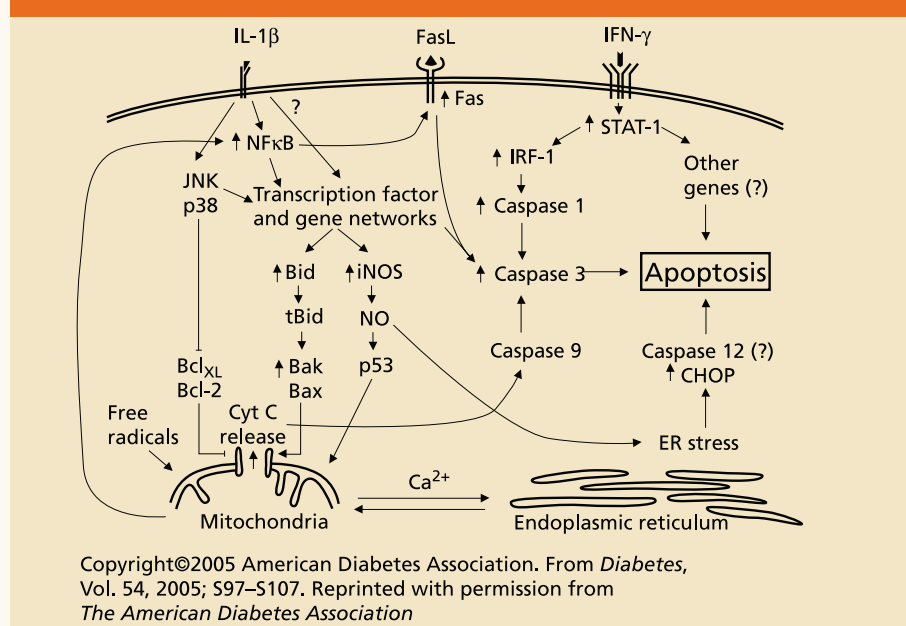
In terms of signalling, the 'filthy three' have diverse, but also convergent, signalling pathways in cells. The model in Figure 1 suggests that there are certain master switches in the transcription network: one of these is NF κ B and the other is

JNK.⁹ Activated JNK is known to translocate to the mitochondria where it can, amongst other things, activate a pro-apoptotic member of the Bcl-2 protein family (Figure 1). When this protein is activated, it translocates from the inner- to the outer-mitochondrial membrane, where it disturbs the balance between the pro- and the anti-apoptotic Bcl-2 family members. JNK inactivates an anti-apoptotic member of the Bcl-2 family, Bcl_{XL}, and thus the balance between the pro- and anti-apoptotic regulation of the mitochondrial pathways is perturbed. Ultimately, a number of apoptotic proteins that are sequestered in the space between the inner and the outer membrane enter the cytosol, and a known sequence of caspase activations leads to apoptosis.¹⁰

The other pathway is the ER stress pathway. When β -cells are exposed to cytokines, there is activation of NF κ B and generation of NO. This leads to a decrease in the influx of calcium that potentiates the unfolded protein response, leading to ER stress. During ER stress, the PERK system is activated and the attenuation of translation will lead to a lesser import of protein into the ER. Stopping protein import into the ER is a rational response, but unfortunately, if sustained, will lead to the activation of the transcription factor ATF-4, and when this enters the nucleus, a very bad molecule called CHOP is transcribed. This molecule, via its effect on other transcriptional promoters, leads to the elaboration of what is called downstream-of-CHOP (DOC) molecules, which then kills the cells, probably via mitochondrial effects, such as prevention of Bcl-2 and promotion of Bax activity.¹¹⁻¹⁴

All of this illustrates that we know (in quite a lot of detail) the molecular mechanisms of

Figure 1. Mechanistic model for cytokine-induced β -cell apoptosis⁹



cytokines causing β -cell apoptosis, and that there is probably a very important cross-talk between the mitochondrion and the ER system.

Type 2 diabetes: progressive β -cell apoptosis induced by metabolic factor

What is the role of inflammatory mediators in β -cell apoptosis in type 2 diabetes? This work was pioneered by Marc Donath's group who, in 2001, first made the interesting observation that the promoting effect of high glucose on human islet cell β -cell apoptosis is mediated via the Fas system.¹⁵ They showed that apoptosis in human islets seen with increasing glucose was associated with upregulation of the Fas death receptor. And it eventually led to the activation of an effector caspase; the dangerous, point-of-no-return caspase-3.

Looking at patient sections, this group showed that there is expression of Fas protein in the type 2 diabetic pancreases, and this is associated with Fas mRNA expression.¹⁵ To further prove that Fas was involved, they used an antagonistic Fas antibody: using high glucose, they could block the effect of glucose on apoptosis using this antagonistic antibody.

So, this set of data showed that glucose induces β -cell apoptosis in human islets via the Fas system.

In investigating what could then cause regulation of Fas, their attention turned to IL-1, because this is a potent inducer of Fas expression. The link they needed to show was that glucose somehow regulates IL-1. In a seminal paper, they showed that by incubating human islets with increasing concentrations of glucose, they secreted IL-1 protein in a dose- and time-dependent manner,¹⁶ in association with increased synthesis of the pro-IL-1 β molecule and mRNA for IL-1 in β -cells.

Now, to prove that IL-1 was the regulator of Fas β expression caused by glucose, they showed that high-glucose induction of Fas was downregulated by IL-1Ra, a natural competitive inhibitor of IL-1 binding to its receptor. In terms of mechanisms, they showed that by using an inhibitor of the NF κ B signalling pathway, which you would expect to be activated due to IL-1 signalling, glucose-induced apoptosis in human islets was reduced. Signalling was later shown to involve also the ERK signalling pathway and calcium channels.¹⁷

Later studies showed that in human diabetic islets, compared with control islets, the expression of IL-1Ra was reduced. Then, using small interfering RNA molecules to downregulate IL-1Ra, an induction of apoptosis was seen, and this was correlated with induction of the effector caspase-3.¹⁸ Finally, when investigating whether leptin could cause β -cell apoptosis in human islets and whether that involved the IL-1 system, they showed that the expression of IL-1Ra is severely downregulated, whereas IL-1 secretion is induced by leptin, and IL-1Ra blocks leptin-induced apoptosis.¹⁸ When feeding *Psammomys obesus* a high-fat diet, type 2 diabetes is promoted. Donath's group showed that this was associated with islet IL-1 expression.¹⁶

Model for inflammatory β -cell dysfunction and apoptosis in type-2 diabetes

So, how can all these data be fitted together into a comprehensive model? It has been suggested that the type 2 diabetic state leading to increased FFA levels causes the synthesis of NO and reactive oxygen species (ROS) that can induce β -cell dysfunction and apoptosis. Glucose can add to this, this is well established, but the new part of this model is the

IL-1 pathway that adds a further stimulus to the production of free oxygen radicals. Glucose-induced IL-1-mediated signalling through the ERK/NF κ B/calcium pathways leads to dysfunction and apoptosis of β -cells. IL-1 can further activate the Fas/FasL system, sentencing the cells to apoptosis, and leptin, which is now known to induce IL-1 in the β -cells, can also downregulate IL-1Ra. Finally, it will further aggravate Fas/FasL activation, so that FLIP – an inhibitor of Fas – is reduced, adding to this vicious circle (Figure 2).

Contradictory evidence

Other studies have questioned this model. In human islets, there was no significant upregulation of either Fas, IL-1, or IL-1Ra mRNA expression, although there was a trend in that direction.¹⁹ The difference between type 2 and control subjects in terms of islet mRNA expression for IL-1 has not been confirmed. In the islets of an immune-mediated type 1 diabetes animal model, the Ztm-iddm rat, there was plenty of IL-1 and iNOS protein, and a few cells staining for caspase, but this was not possible to demonstrate in the type 2 *Psammomys obesus* model.⁹

Conclusions

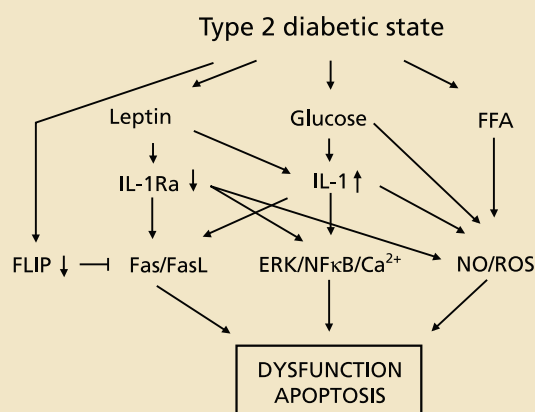
β -cell failure and destruction in type 1 and type 2 diabetes may

be caused by convergence of immunologic and metabolic stimuli on a common mechanism, involving inflammatory mediators sharing intracellular signalling pathways, but confirmatory evidence is in demand; in particular, intervention studies in patients. If correct, this hypothesis may, however, have important consequences for strategies aimed at intervening in the natural course of β -cell replacement therapy for these two disorders.

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Figure 2. Model for inflammatory β -cell dysfunction and apoptosis in type 2 diabetes



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