The pancreatic β-cell: birth, life and death

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Abstract
Defective insulin secretion is a hallmark of all forms of diabetes. Whereas Type 1 diabetes has long been known to result from the immune-mediated destruction of β-cells, Type 2 diabetes appears to involve both loss of β-cell mass and glucose sensitivity in the face of extrapancreatic insulin resistance. We summarize here the proceedings of a Biochemical Society Focused Meeting, held at the St Thomas campus of King’s College London in December 2007, which highlighted recent research advances targeting the β-cell.

Introduction
It has become increasingly clear in the last few years that pancreatic β-cell failure or destruction lies at the heart not only of Type 1, but also of Type 2 diabetes. Although these two diseases have strikingly distinct aetiology (Type 1 is an early-onset autoimmune disease involving β-cell destruction [1], whereas Type 2 diabetes is seen later in life and usually involves both insulin resistance and β-cell failure and/or loss [2]) the ability to protect, rejuvenate or reintroduce β-cells into patients with either form has recently become a focus of vigorous research. Importantly, and as discussed by Philippe Froguel (Imperial College) at the meeting, whole-genome association studies for Type 2 diabetes [4–7] have implicated a new set of genes which, unexpectedly, all appear to be involved in β-cell function and/or development. The recent demonstration of the feasibility of generating pluripotent stem cells from the skin cells of non-human primates [8], and thus a genuine chance ultimately to obtain new β-cells, into patients with either form has now become a focus of vigorous research. Importantly, and as discussed by Philippe Froguel (Imperial College) at the meeting, whole-genome association studies for Type 2 diabetes [4–7] have implicated a new set of genes which, unexpectedly, all appear to be involved in β-cell function and/or development. The recent demonstration of the feasibility of generating pluripotent stem cells from the skin cells of non-human primates [8], and thus a genuine chance ultimately to obtain new β-cells, into patients with either form has recently become a focus of vigorous research. Importantly, and as discussed by Philippe Froguel (Imperial College) at the meeting, whole-genome association studies for Type 2 diabetes [4–7] have implicated a new set of genes which, unexpectedly, all appear to be involved in β-cell function and/or development. The recent demonstration of the feasibility of generating pluripotent stem cells from the skin cells of non-human primates [8], and thus a genuine chance ultimately to obtain new β-cells, into patients with either form has recently become a focus of vigorous research. Importantly, and as discussed by Philippe Froguel (Imperial College) at the meeting, whole-genome association studies for Type 2 diabetes [4–7] have implicated a new set of genes which, unexpectedly, all appear to be involved in β-cell function and/or development. The recent demonstration of the feasibility of generating pluripotent stem cells from the skin cells of non-human primates [8], and thus a genuine chance ultimately to obtain new β-cells, into patients with either form has recently become a focus of vigorous research. Importantly, and as discussed by Philippe Froguel (Imperial College) at the meeting, whole-genome association studies for Type 2 diabetes [4–7] have implicated a new set of genes which, unexpectedly, all appear to be involved in β-cell function and/or development. The recent demonstration of the feasibility of generating pluripotent stem cells from the skin cells of non-human primates [8], and thus a genuine chance ultimately to obtain new β-cells, into patients with either form has recently become a focus of vigorous research.

Meeting highlights
In the first session, ‘Birth’, Marcus Stoffel (Zurich) presented early, but very recent, findings on the role of ‘microRNAs’ (miRNAs) in the control of β-cell function. This group of 19–22 nt single-stranded RNAs have been shown in recent years to be critical regulators of many processes in several cell types, and several of these, including miRNA-375 [9], miRNA-124 [10] and miRNA-9 [11] are implicated in β-cell development and/or function. Having published previously that one of these, miRNA-375, was highly abundant in β-cells, and a potent inhibitor of glucose-stimulated insulin secretion, data were shown describing the effects of deleting this miRNA selectively in β-cells: mice become hyperglycaemic, with decreased mass of both β- and α-cells. Moreover, models of insulin resistance and compensatory β-cell growth (fa/fa and db/db) mice showed decreased levels of miRNA-375, whereas mice with a double knockout of this gene and leptin receptors died at 12 weeks: this study identified miRNA-375...
as an important regulator of compensatory changes. Clearly, the role of miRNAs in this tissues is complex, but the use of ‘antagomiRs’ [12], shown by Professor Stoffel in proof-of-principle experiments to elicit a remarkable knockdown of target miRNAs in vivo, provides hope that they may represent an exciting new target for therapies aimed at regulating β-cell mass. Henrik Semb (Lund) [13] and Raphaël Scharfmann (Paris) [14] then discussed the development of the β-cell, and reminded us that perhaps the greatest blockade to generating an ‘infinite’ supply of ‘artificial’ β-cells, obtaining a ‘definitive endoderm’, has now been achieved and new strategies (the ‘Novocell’ strategy) exist to generate insulin-expressing cells from ES (embryonic stem) cells. Both emphasized the fact that these cells did not secrete insulin in a regulated manner in response to glucose, and that much remains to be done both to define the molecular machinery through which ‘real’ β-cells pull off this trick [15], and to coax ES-derived cells into doing so. Roles for EGF (epidermal growth factor) receptor signalling were stressed by Timo Otonkoski (Helsinki) [16], who emphasized that important differences may exist between rodent models and human islets in terms of developmental trajectory.

The provenance of ‘new’ β-cells in adults has been an area of particular controversy in recent years. Although it used generally to be thought that most or all arose from neogenesis, a landmark paper by Dor, Melton and colleagues [17] questioned this through the use of ‘lineage tracing’ whereby the expression of a particular gene (e.g. insulin) can be flagged by deletion of a stop codon in a ‘floxed’ gene in area of particular controversy in recent years. Although it used generally to be thought that most or all arose from neogenesis, a landmark paper by Dor, Melton and colleagues [17] questioned this through the use of ‘lineage tracing’ whereby the expression of a particular gene (e.g. insulin) can be flagged by deletion of a stop codon in a ‘floxed’ gene in

β-cells, which may or may not represent mature α-cells, are involved. Such findings are clearly of importance.

Autocrine signalling in the β-cell has also been another area of controversy after a slew of papers, starting with a description of the effects of deleting insulin receptors from β-cell in mice [20] or in vitro [21,22], suggested the hormone might ‘act back’ on the β-cell in a positive manner. Shanta Persaud (King’s College) [23] showed new data on human cells illustrating the importance of insulin binding in preventing apoptosis; whether this is through insulin or IGF-1 (insulin-like growth factor 1) receptors remains unresolved.

In the ‘Life’ session, advances in imaging single insulin vesicles, and even single SNARE (soluble N-ethylmaleimidesensitive factor-associated protein receptor) molecules, were discussed by Imperial College’s Sebastian Barg [24], while Frans Schuit (King’s College London) [25] provided a description of one of two exciting emerging concepts at the meeting: that of ‘disallowed’ genes in the β-cell; quite why some of these (such as the lactate/monocarboxylate transporter, MCT-1, and lactate dehydrogenase) are eliminated very selectively in β-cells [26–28] (but are abundant in almost all other cell types) is uncertain. Nevertheless, it is evident that, in developing ‘artificial’ β-cells, it will be necessary to achieve not only the overexpression of classical β-cell genes (insulin being the cardinal example), but also efficient shut-down of members of this latter family.

The second important new concept emerged in Yuval Dor’s (Jerusalem) presentation: that of ‘metabolic licence’ for β-cell survival and proliferation. Using an inducible Cre/LoxP technology to inactivate an upstream kinase for the ‘master metabolic regulator’ AMPK (AMP-activated protein kinase) [29,30], in only a fraction of β-cells, Dor’s laboratory found that LKB1- (also called STK11 or Peutz–Jeghers kinase) deleted mice displayed enhanced glucose tolerance and that only β-cells lacking LKB1 (and therefore with suppressed AMPK activity) were able to proliferate. These results imply that cells with active AMPK, and thus under metabolic stress, were ‘unlicensed’ to divide. Although the interpretation of these experiments requires several caveats (for example, the relationship between LKB1 and AMPK is far from monogamous since the former phosphorylates many other downstream targets [31], and AMPK is a target for at least two other upstream kinases [32]), the results tie in nicely with previous findings that activated AMPK suppresses glucose-stimulated insulin secretion and gene expression [33,34] and leads to β-cell death [35,36]. Given that important antihyperglycaemic agents, including the biguanides (e.g. metformin) and thiazolidinediones, act on extra-pancreatic tissues to activate AMPK, new agents which do not activate AMPK in the β-cell may provide interesting therapeutic advantages.

Both Type 1 and Type 2 diabetes have significant genetic components. As emphasized by Philippe Froguel (Imperial College) and Anna Gloyn (Oxford) [37], human genetics, and particularly whole-genome scans, have demonstrated that strong associations exist with genes where polymorphisms affect disease (see above). Although the associated polymorphisms are frequently observed in non-coding regions of genes (and may even report associations with non-ascribed genes), Mererwyn Loder (Imperial College) [38] showed data that silencing the expression of the TCF7L2 gene, most strongly associated with Type 2 diabetes, in β-cells, affects both the development of embryonic β-cells and glucose-stimulated insulin secretion from mature cells; whether levels of TCF7L2, a transcription factor acting downstream of the Wnt signalling pathway, are increased or decreased in individuals with the at-risk allele requires further clarification. Polymorphisms in a second gene, encoding the insulin vesicle-localized zinc transporter, ZnT8 (SLC30A8), seem likely to disrupt Zn2+ transport into vesicles, perhaps affecting both co-crystallization of Zn2+ and insulin, and cytosolic Zn2+ concentrations (G.A. Rutter and T. Nicolson, unpublished work).

β-Cell destruction in Type 1 diabetes

In a session discussing the death of β-cells in Type 1 diabetes, Linda Wicker (Cambridge) [39] described common mechanisms in the genetic control of autoimmune diabetes in humans and the NOD (non-obese diabetic) model of spontaneous diabetes. She reminded us that the MHC
class II genes are the primary genetic determinants in this multigenic immune-mediated disease and that non-MHC candidate genes identified recently have relatively weak effects. These include the INS genes that regulate insulin expression, CTLA4, involved in T-cell regulation, PTPN22, also involved in immune regulation and also associated with rheumatoid arthritis, and IL2RA (CD25). New candidate genes have now been identified which include IFI1H1, PTPN2, ERBB3 and KIAA0350 [40]. It should be noted that all of the new candidate genes preferentially have effects on immune cells, whereas the genes that are important for Type 2 diabetes are β-cell-specific genes and there is no overlap between the genes involved in Type 1 and Type 2 diabetes.

Susan Wong (Bristol) [41] gave an overview of the mechanisms involved in the β-cell damage and destruction in Type 1 diabetes by a major cellular player, the cytotoxic CD8 T-cell. She discussed the fact that there is evidence, in NOD mice, that CD8 T-cells can damage the islets by multiple mechanisms. These include release of cytotoxic granules containing perforin that allows granzymes to enter the target islets. These islets are involved in the β-cell damage and destruction in Type 1 diabetes. Cytotoxic granule release is also involved in immune regulation and also associated with inflammatory cytokines that include IFN (interferon) γ and TNFα (tumour necrosis factor α), directed towards the β-cell by specific recognition of their target β-cell antigens. It is likely that all of these mechanisms play some role, and the dominance of one or other pathway may vary for CD8 T-cells with different antigen specificity as well as different phases of the disease process. A multi-pronged attack may be required to control CD8 T-cells, including the generation and increase of regulatory T-cells that can modulate their effects.

Decio Eizirik (Brussels) [42] continued the discussion of β-cell damage, specifically focusing on the role of cytokines in β-cell destruction. The pathway activated through IL (interleukin) -1β is particularly important, leading to NF-κB (nuclear factor κ B) activation and ultimately to ER (endoplasmic reticulum) stress, as a precursor to apoptotic cell death. He drew particular attention to CHOP (C/EBP (CCAAT/enhancer-binding protein)-homologous protein), a transcription factor of the C/EBP family that increases expression in response to ER stress [43]. One possible therapeutic mechanism would be to block CHOP expression by siRNA (short interfering RNA). It was emphasized, however, that it is unlikely that a single treatment will be effective and that multiple hits would be required to protect the β-cells from damage.

Chantal Mathieu (Leuven) [44] elaborated further on cytokine-induced damage in Type 1 diabetes by discussing the downstream proteins in the IFNγ pathway. She discussed the importance of STAT-1 (signal transducer and activator of transcription 1) signalling, the first transcription factor downstream of the IFNγ receptor. In STAT-1-knockout mice, the chemokines normally induced by IL-1 and IFNγ in β-cells, such as MCP (monocyte chemoattractant protein) and IP10 (IFNγ-inducible protein 10), are inhibited. The STAT-1-knockout mice are protected from low-dose-STZ (streptozotocin)-induced diabetes, a model sometimes used to represent autoimmune-type diabetes. Islet grafts using STAT-1-knockout islets are also protected against primary non-function, but not against recurrent autoimmune diabetes [45]. Regulation at the level of STAT-1 is important, as knocking out IRF-1 (IFN regulatory factor-1), which is regulated by STAT-1, but is also subject to other regulation independently of STAT-1, will protect islets in vitro. However, islet damage is accelerated in vivo. As with the previous speaker, she emphasized the importance of targeting multiple pathways to protect islets.

Danielle Melloul (Jerusalem) [46] described her approach to targeting the pro-inflammatory NF-κB, a transcription factor central to apoptosis of β-cells. The model used a tetracycline-regulated system targeting NF-κB, and she had generated a transgenic mouse designated Tolβ [tetracycline-on ΔIκB (inhibitor of NF-κB) in β-cells]. This model uses rTFA (reverse tetracycline transactivator) under the RIP (rat insulin promoter) such that ΔIκB was only expressed in doxycycline-treated islets. The islets were protected from cytokine-induced apoptosis in vitro and from diabetes induced by multiple low-dose-STZ in vivo. Her results using this inducible system contrasted with previously published work where constitutive expression of a repressor of NF-κB in the islets accelerated diabetes [47]. Dr Melloul suggested that the ability to temporally regulate NF-κB in the islets may allow specific time windows where regulation of apoptosis is beneficial to be exploited.

**β-Cell destruction in Type 2 diabetes**

Continuing on the theme of cytokine-induced β-cell damage and destruction, Marc Donath (Zurich) [48] highlighted the fact that in all models of diabetes, both Type 1 and Type 2, islet inflammation is a prominent feature and that there is a clear increase in inflammatory cells in Type 2 diabetes, as well as in Type 1 diabetes. The metabolic stress induces chemokines and cytokines and the inflammatory stress is important for stimulation of islet regeneration. In their studies in Type 2 diabetes, patients treated with IL-1 receptor antagonists had shown a significant reduction in HbA1c and had a decrease in CRP (C-reactive protein) and IL-6, indicating that IL-1 is an important mediator of impaired glycaemia in Type 2 diabetes [49]. Thus this type of anti-inflammatory treatment may be important for the future. It should be noted that IL-1 receptor antagonist treatment is currently also undergoing trials in Type 1 diabetes.

Sigurd Lenzen (Hannover) [50] discussed the vulnerability of the β-cell to reactive oxygen species-mediated and nitric oxide-mediated stress, both of which are important mediators of β-cell damage induced by cytokines. Although these pathways differ in molecular mechanisms, they damage β-cell mitochondrial function and cause ER stress, and both pathways should be considered in examining the downstream mediators of cytokine damage.

Miriam Cnop (Brussels) [51], in discussing fatty acid and glucolipotoxicity, picked up on the earlier theme of ER stress pathways (Eizirik [42]). Her studies had shown that there is early loss of β-cell function in first degree relatives in Type 2 diabetes and highlighted the importance of β-cell deficiency
and increased β-cell apoptosis in patients with Type 2 diabetes. Non-esterified (‘free’) fatty acids, palmitate being particularly potent, activate an ER stress response in β-cells, inducing apoptosis. The palmitate induces phosphorylation of PERK [PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase] and eIF2α (eukaryotic initiation factor 2α), protein synthesis inhibition (a major function of the ER) and induction of CHOP. In testing the role of PERK–eIF2α in contributing to β-cell death, the selective inhibitor of eIF2α dephosphorylation, salubrinal, was used, but, surprisingly, the salubrinal-induced eIF2α phosphorylation had a pro-apoptotic effect in β-cells and specifically potentiated the deleterious effects of non-esterified fatty acids. Links with the effects of glucotoxicity will be important.

As discussed by Daniel Drucker (Toronto) [52], the delivery of stabilized forms of content derived mechanisms such as GLP-1 (glucagon-like peptide 1), or inhibition of GLP-1 degradation [‘DPP IV (dipeptidyl peptidase IV) inhibition’] appear to promise new treatment for Type 2 diabetes. GLP-1 potentiation of glucose-induced insulin secretion has been proposed to stimulate β-cell proliferation. It has therefore been of interest to see whether GLP-1 may simulate β-cell growth in models of Type 1 diabetes mellitus; despite encouraging reports of improvements in NOD mice, work in Dr Drucker’s laboratory suggested the improvement may be small, and may, at least to some extent, be due to effects on regulatory T-cells.

Finally, in a session on β-cell ‘revival’, Stephanie Amiel (King’s College) [53] described the successes and limitations of human islet transplantation in the U.K. and elsewhere. Work in our laboratories is supported by grants from the Medical Research Council (U.K.), the Wellcome Trust, Juvenile Diabetes Research Foundation, Diabetes UK, National Institutes of Health, the European Union and Diabetes Vaccine Development Center (Australia).

Conclusions
Vigorous and exciting research on the β-cell, as discussed at this meeting, continues to provide hope for new therapies for all forms of diabetes and we hope that future meetings on this theme will see further progress towards a cure.

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