Glucocorticoids: novel agents to stimulate beta-cell neogenesis?

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All forms of diabetes lead to impaired glucose homeostasis, and together they exert a significant financial burden on health services worldwide (1). In each case, the exact aetiology of the disease may differ, but, in a similar outcome, insufficient endogenous insulin is produced to manage the body's metabolic requirements. In type 2 diabetes (T2D), increased provision of insulin is required to counteract insulin resistance (IR) in the primary sites of glucose storage and utilisation, i.e. the liver and muscle. In this phenotype of disease insulin has a greatly diminished efficacy at these target sites; this, in turn, leads to impaired glucose uptake and necessitates increased levels of insulin to manage blood glucose levels. Alternatively, a targeted autoimmune attack causes the selective depletion of beta-cells in type 1 diabetes (T1D), ultimately leading to a lifelong requirement of exogenous insulin provision. As stated, irrespective of the disease phenotype, more insulin than can be endogenously generated is required to maintain normoglycemia, thus mechanisms to promote increased insulin release from beta-cells are attractive therapeutic targets, and where much research effort is focussed. One method by which this could be achieved is to identify strategies which, at best increase, or at the least, maintain pancreatic beta-cell mass in patients. There are several potential routes to achieving this goal with the most obvious aim to prevent beta-cell destruction. However, research aimed at promoting beta-cell mass "recovery" by increasing proliferation of existing beta-cells within the pancreas, inducing differentiation of other endocrine cells into beta-cells, as well as attempts to stimulate beta-cell neogenesis are gaining momentum. Sadly, these seemingly sensible goals are difficult to realise, particularly in the context of the human condition.

Studying beta-cells in humans is a challenging proposition, and studying living beta-cells *in situ* is, as yet, not a possibility. Therefore, much of what we know in humans is derived from the study of post-mortem tissue, where discrete data points from individuals who have died are combined to extrapolate the birth, life and death of a beta-cell in vivo.

Histological studies of the human pancreas have revealed that proliferation of the existing mature human beta-cells is vanishingly low (approximately 0.2% of beta-cells/24h). Therefore, to achieve the aim of inducing proliferation of residual beta-cells in disease, much effort has been invested in identifying factors which can stimulate beta-cell growth; and although several mitogenic compounds have been identified in mouse studies (2), only a small subset of these compounds can modestly enhance proliferation of human beta-cells. An example of this is harmine (and other 'harmalogs'), which stimulates proliferation through inhibition of the kinase, DYRK1A (resulting in a ~1.5-2% proliferation rate in human cells) (3). Encouragingly, a recent study has revealed that the combination of harmine with inhibitors of TGFβ signalling has a much more potent effect on human and rodent beta-cell proliferation, and these compounds synergise together to stimulate as many as

18% of cells to replicate (4). However, these effects were not restricted to beta-cells; alpha-cells and ductal cell were also stimulated to proliferate. It is thus important to identify strategies to better focus the mitogenic signal delivered by such compounds to ensure that these treatments do not cause unwanted tumorigenic side-effects.

Neogenesis, the process of forming new beta-cells from progenitor populations within the pancreas, is also an attractive alternative; particularly for those people who have suffered an aggressive autoimmune-mediated destruction of beta-cells, or for those who have had long-standing disease, and have few, if any, residual beta-cells remaining from which to proliferate (5). This process is understood to be integral to the regulation of beta-cell mass during pancreatic development, but it has also been reported to be potentially important during periods of metabolic stress, such as pregnancy or obesity. The evidence supporting these observations has largely come from the study of rodent models. In such models, beta-cell neogenesis has been reported to be experimentally induced by partial pancreatectomy, pancreatic ductal ligation or β-cell ablation (6-8). However, other studies have been unable to replicate these findings (9, 10). Thus the potential utility of these processes remains unclear. In humans, it has been similarly challenging to gather conclusive evidence for beta-cell neogenesis. Lineage tracing experiments in humans are impossible to carry out. As such, the identity of the progenitor cells within the human pancreas from which new adult beta-cells could be derived remains a matter of debate; moreover, it remains uncertain whether beta-cell neogenesis occurs at all in the adult human pancreas. However, if, under certain conditions, the neogenesis of beta-cells is a *bone fide* response, identifying novel agents to stimulate this process is a tantalising prospect.

Glucocorticoids (GC) are a group of steroid hormones, whose anti-inflammatory properties are used in the treatment of inflammatory diseases such as asthma, multiple sclerosis or rheumatoid arthritis. Importantly a common sequela of chronic administration of these drugs is the development of insulin resistance, increased serum insulin and, ultimately, diabetes. Therefore understanding the mechanisms induced by GCs in IR and increased insulin levels is important.

It is asserted that chronic treatment with GC can lead to an elevation in insulin release upon glucose challenge in humans (11). A proportion of this response will be to compensate for the already well-characterised insulin-resistant state that long-term GC treatment can stimulate. However, a variety of beta-cell studies have reported there is a functional change in insulin secretion when cells are treated with GC *in vitro*. One such study reported that *in vitro* treatment of beta-cells or islets can inhibit insulin secretion (12). Conversely, a recent report by Fine *et al.* did not find an overall change in insulin release when cells were treated with GC. These investigators revealed that despite GC

reducing glucose stimulated calcium influxes, a concomitant elevation in intracellular cAMP appeared to maintain the insulin secretory response (13).

One explanation then for increased circulating insulin in GC treated patients, if there is no change or even a decrease, in beta-cell secretion, is that there are elevated numbers of beta-cells. Interestingly, it has been reported that GC treatment can also increase beta-cell mass. In one study, dexamethasone treatment of rats increased absolute beta-cell mass, with a ~4-fold rise in the number of proliferating beta-cells compared to untreated control animals (14). However, beta-cell neogenesis has hitherto not been examined in this context.

A recent article by Courty *et al*., published in *Diabetes*, has revealed that the chronic treatment of mice with glucocorticoids (4-8 weeks) stimulated an increase in islet size, beta-cell mass and beta-cell to alpha cell ratio (15). Detailed analysis revealed increased numbers of proliferating beta-cells as determined by Ki-67 and BrdU staining. However, an increased number of insulin-positive cells were also found away from the islets sites, within ducts of treated animals, and more islets were found to be in close association with these ducts. Additionally, significantly more islets/mm^2 (islet density) were observed in the mice that had undergone chronic GC treatment. The authors, therefore, suggested that taken together these data provided evidence of both increased proliferation and neogenesis of beta-cells, in mice, following GC treatment.

The pancreatic duct is regularly touted as the most likely source of newly formed beta-cells. Concurrent with this hypothesis, as stated above, these investigators observed elevated numbers of beta-cells within ducts, and increased numbers of islets near to these ducts. Indeed, analysis of pancreata from individuals who have undergone partial pancreatectomy reveals little evidence of beta-cell replication after the procedure, whilst increased numbers of insulin-positive cells can be found within the ductules (16). However, this response is not found in all cohorts and remains controversial (17). Furthermore, certain lineage tracing experiments outlined in the literature, also suggest ductal progenitors may not contribute to the neogenesis of beta-cells (18). Similar approaches have also discounted acinar and islet cells as the source of new beta-cells (16). Thus, there is no solid consensus as to the origin of these cells.

In concordance with these latter reports, Courty *et al.* were also unable to determine the source of the new beta-cells. Lineage tracing experiments revealed that they did not derive from Sox9+ progenitor cells from the pancreatic duct; nor did they derive from an endocrine precursor lineage, expressing Ngn3+ (15). These results are surprising since these lineages would represent the two most likely sources of beta-cells generated by neogenesis. However, important endocrine progenitor cells markers (e.g. Nkx2.2), and transcription factors (such as Nkx6.1 (19)) important to beta-cell fate

were found to be elevated in islets isolated from GC treated mice. Conversely, ARX (necessary for the development of alpha-cells (20)) was found to be reduced - this was in keeping with the investigator's finding that the beta to alpha cell ratio was affected in GC treated animals. When taken together, these findings could support the hypothesis that GC administration, *in vivo,* is associated with neogenesis of beta-cells in mice. However, following on from studies into ARX and Nkx6.1 (21), it might also suggest that alpha to beta-cell transdifferentiation, at least in part, may be responsible for the shift in the ratio of alpha to beta cells in these GC treated animals. Thus, the source of these nascent beta-cells remains unclear.

Importantly, Bonner-Weir (22) asserts that increased neogenesis of beta-cells associated with pregnancy, in mice, is reversed by apoptosis following the birth of the pups, however whilst beta-cell mass was found to reduce upon cessation of GC treatment in the work of Couty *et al.* (15), the islet density did not concomitantly return to pretreated levels. This is of importance since it implies that with appropriate stimulation neo-islets may persist beyond the cessation of GC treatment, although it is unclear how stable these islets are. However, it opens up the possibility that intermittent treatment with GC may promote the maintenance of neo-islets without generating long term IR.

Finally and importantly, the investigators also showed that pancreatic neogenesis might not be mediated by the direct effect of GC activity. The treatment of murine pancreatic buds with serum from glucocorticoid-treated mice was sufficient to stimulate beta-cell neogenesis (15). These data suggest that one or more circulating soluble factor(s) secreted in response to GC treatment (or insulin-resistance) is responsible for these effects, although the identity of this molecule was not established. It is unclear whether beta-cell neogenesis during pancreas development or the stimulation of new beta-cell formation by other experimental means (e.g. partial pancreatectomy) is mediated by a similar mechanism; and if so whether the same secreted factor (or group of factors) is responsible. However, if the soluble agents responsible can be identified, it opens up a possibility to potentially dissociate the beneficial stimulation of beta-cell neogenesis from the damaging effects of GC induced insulin resistance.

Despite the clear significance of these findings, it is important to keep in mind that these data have been generated from a whole animal mouse model, and as such have not been validated in human tissue or cells. As previously noted, there are a plethora of compounds or treatments reported to manipulate beta-cell mass in rodents, but whose efficacy does not translate to human beta-cells. For example, GLP-1 can effectively induce beta-cell replication in rodent models but does not induce proliferation of human beta-cells (2). Therefore, the concepts introduced by Courty *et al*. (15), whilst potentially important, will be difficult to study in humans. The challenges involved in identifying the

potential soluble factor responsible for beta-cell generation notwithstanding, if such neogenesis occurs in humans, even isolated human islets are likely not a sufficient model to use, since the progenitors of the newly formed beta-cells may be of extra-islet origin. Thus alternative strategies will need to be considered when extending this study to the human condition.

One initial step could be to examine available human pancreas samples. Unfortunately, this is a challenging proposition because biopsying or resecting pancreata from patients who have been treated long-term with GCs is not an option (the reasons for this are multifold and beyond the remit of this commentary but are introduced here (23, 24)). However, examining post mortem (donor) pancreas, recovered from patients who died with extensive clinical history (such as those available in the JDRF nPOD collection http://www.jdrfnpod.org//for-partners/npod-partners/), might provide a good starting point. Assessment of beta-cell proliferation (via Ki-67 staining) and potential neogenesis (as measured by mean islet density analysis and progenitor markers) in relevant GC treated patients, when compared to aged-matched, non-diabetic/non-GC treated adults, may illuminate if these phenomena also occur in humans.

There is much to be gained by discovering methodologies that protect or augment beta cells mass, particularly for people with diabetes. In this context, the study by Courty *et al.* (15) promises much: increased proliferation of beta-cells, increased beta-cell neogenesis, and indeed, the study even reports regeneration after beta-cell depletion, all linked to a mechanism associated with chronic GC treatment. These novel findings are important, informative and provide potential new avenues of study. However, critically, the caveat stands that these studies are performed in rodents, and therefore their relevance to the pathways regulating beta-cell mass in the human pancreas is unclear, especially when considering the number of agents identified in rodent studies that have failed to induce similar increases in beta-cell mass in humans. Thus, it is critical that these findings can be validated in human cells, since until that is achieved, GC will be another agent in a long list which, whilst effectively increasing beta-cell mass in rodents, do not do this in equivalent human cells.

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Footnote

Conflicts of Interest: The authors declare no conflicts of interest.

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