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Development of the Endocrine Pancreas

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Insulin availability is a key factor in embryonic and fetal growth, and infants born with nesidioblastosis or the Beckwith-Wiedemann syndrome, conditions associated with hyper-secretion of insulin, exhibit enhanced fetal somatic growth. Conversely, transient neonatal diabetes or pancreatic agenesis results in growth retardation. The temporal and structural development of the endocrine pancreas is a determining factor for both developmental hypo- or hyper-insulinemia, and developmental abnormalities, such as inappropriate expression of the transcription factor Pdx-1, lead to maturity onset diabetes in the young (MODY). However, the development of the endocrine pancreas is controlled by epigenetic as well as genetic variables, and an adverse fetal or neonatal environment can lead to inappropriate β-cell mass, and constitute a causative factor for subsequent type 2 diabetes. Understanding the mechanisms and trophic control of endocrine pancreatic development has become a priority strategy for the generation of islets of Langerhans from precursor cells *in vitro* with a view to islet transplantation, and for drug development for the targeted expansion or regeneration of β-cells *in situ* for the reversal of diabetes. This article will review current knowledge of the developmental control of the endocrine pancreas, and some resulting current strategies for manipulation of β-cell mass.

Pancreas Development in the Embryo and Fetus

The development of the pancreas has been studied in detail in the mouse embryo [1], allowing for subsequent analysis of targeted or general gene deletions or over-expression. Dorsal and ventral pancreatic buds appear at day E9.5 from mid-gut endoderm, and fuse by E16–17. A local block in signaling to the endoderm by Notch specifies the position of bud development from the mid-gut. Pancreatic mesenchyme accumulates around the dorsal gut epithelium and induces pancreatic bud formation and branching [2]. Each bud forms highly branched structures and the acini and ducts are distinguishable at E14.5. Endocrine cells appear early in bud development and represent 10% of the pancreas by E15.5. The appearance of endocrine cells is induced by the actions of morphogenetic factors derived from the lateral plate mesoderm, such as activin and bone morphogenic proteins, on progenitor cells expressing the characteristic transcription factor, Pdx-1 [1]. Endocrine cells develop from the pancreatic duct epithelial cells and exist initially as individual cells or small cell clusters close to the pancreatic ducts. Mature islets, with outer α-cells and pancreatic peptide/adremomedullin cells, and an inner mass of ß and *D* cells, form a few days before birth in rodent species and increase 2-fold over 2 days just prior to term, due to both β-cell replication and recruitment and maturation of undifferentiated β-cell precursors. A similar development is seen by early third trimester in the human. Putative endocrine precursors undergo a lineage progression and replication with α cells originally thought to constitute a default pathway [3]. The coordinated expression of specific transcription and growth factors are needed to generate the endocrine cell types during development.

Neogenesis of islets is rapid in the fetus and continues through neonatal life in the rat, but ceases shortly after weaning. This derives not only from ß-cell replication but also from the recruitment and maturation of undifferentiated ß-cell precursors [4], although their precise identity has yet to be determined. In contrast, the rate of mitosis in adult pancreatic ß-cells is normally low, approximately 3% replication rate of ß-cells per day [5]. The change from a very plastic endocrine compartment to one with only a limited capacity for renewal occurs over the first 3 months of postnatal life. Substantial remodeling of the pancreas occurs in the neonatal rodent with a transient wave of apoptosis occurring in the β-cells between 1–2 weeks of age [6,7]. The incidence of apoptotic cells within islets

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increases 3-fold at 14 days after birth, compared to either 4 or 21 days. However, ß-cell mass is not altered appreciably at the time of neonatal apoptosis as a new population of ß-cells compensates for the loss. Increased numbers of insulin-positive cells are seen near to the ductal epithelia after 12 days, suggesting the generation of new islets to maintain ß-cell mass. Some evidence suggests that a similar wave of ß-cell apoptosis occurs in the human fetus during third trimester [8]. This partial replacement of ß-cells neonatally in the rodent may mark a transition between cells with a slow glucose responsiveness for insulin release, to cells with acute glucose-stimulated insulin release that are better adapted to metabolic control in later adult life.

The development of endocrine cells within the pancreatic ductal epithelium is controlled by a specific expression sequence of transcription factors [9] (Fig. 1). Pdx1, initiates endocrine lineage commitment for cells within the pancreatic ducts, becomes restricted to differentiating ß, D and PP cells and is lost from presumptive β cells, and finally becomes restricted to mature ß-cells where it controls insulin and Glut-2 gene expression [10]. Pdx-1 $-/-$ mice form pancreatic ducts but little further endocrine differentiation or morphogenesis occurs [11]. The pre-endocrine cell type derived from ducts has a transcription factor expression signature of Pdx-1, neurogenin 3 (Ngn3), Isl-1, Nkx2.2, Beta 2 and Pax6. Subsequent differentiation of ß-cells requires the additional expression Nkx6.1 and Pax4 with a reduction in Ngn3 and Beta 2. Deletion of Pax-4 by homologous recombination in mice caused a complete loss of pancreatic ß- and D-cells, but an increased number of α cells [12]. Conversely, knock-out of the Pax-6 gene decreased the presence of all endocrine cell types in pancreas, but the presence of α cells was totally abolished [13]. Mice lacking Pax-4 and Pax-6 fail

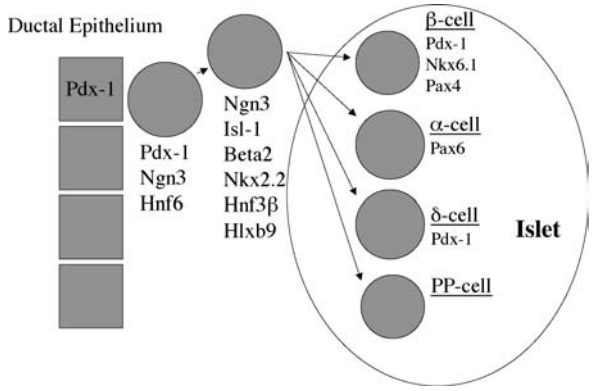

Fig. 1. *Ontogeny of expression of transcription factors in the developing endocrine pancreas from the generation of presumptive endocrine cells at the ductal epithelium to their expansion into mature islets.*

to develop any mature endocrine cells in the pancreas. Presumptive endocrine cells also express hepatocyte nuclear factor 6 (Hnf6), Hnf3β and Hlxb9, the latter controlling migration away from the ducts. Hnf3β is a transcriptional regulator of Pdx1, and is itself regulated by Hnf6. Hnf6 also controls expression of neurogenin 3 (Ngn3) which continues to be expressed throughout islet formation until the point of final commitment of the endocrine lineages.

The presence of organ-specific stem cells or precursors of the endocrine pancreatic lineages within the pancreatic ducts has been proposed, but no cell type of specific phenotype has yet been isolated. Cells immunopositive for nestin, CD34 and c-kit are located adjacent to the pancreatic ducts and within the islets [14]. Nestin is an intermediate filament protein that is abundantly expressed in neuro-epithelial stem cells in embryogenesis, but is absent from nearly all mature CNS cells. Immature pancreatic endocrine cells share characteristics with developing neuronal cells, since transcription factors such as Ngn3 are implicated in the development, phenotype determination and maintenance of function in both. C-*kit* is a transmembrane protein whose ligand is stem cell factor (SCF), which is of critical importance for early epithelial stem cell differentiation in haematopoiesis or gametogenesis. Recent studies suggest that SCF and c-*kit* are involved in the growth and development of hepatic and islet progenitor cells [15]. Isolated pancreatic ductal epithelial cells, or islets, are capable of a transdifferentiation *in vitro* to form proliferative, monolayers that express nestin. Such cells can subsequently re-differentiate into pancreatic endocrine, exocrine and hepatic phenotypes [16]. While nestin-expressing cells *in situ* were therefore originally thought to be endocrine cell precursors, they were subsequently shown through lineage tracing to be mesenchymal, and more closely associated with endothelial cell formation and vascular remodeling [17,18]. However, they appear intimately connected to endocrine cell development.

Herrera used cell-targeted diphtheria toxin expression to specifically delete glucagon, insulin and pancreatic polypeptide (PP) expressing cells, and concluded that glucagon-positive cells were not precursors for β-cells [19,20]. Studies with Cre-tagged cells showed that insulin and glucagon expressing cells derive from mutually exclusive cell populations, but that both can derive from Pdx-1 positive cells. Both insulin and glucagon precursors can express the PP gene, but somatostatin and insulin share more immediate precursors than do either with glucagon. An alternate approach has been to separate islet progenitor cells on the basis of marker gene expression. Genetic tagging of Ngn-3-expressing cells with yellow fluorescent protein allowed for their separation from the pancreas by

cell sorting, and the demonstration that these cells could develop into a β-cell phenotype *in vitro* [21]. This is in agreement with other reports that Ngn-3-expressing cells are β-cell precursors, and that differentiation is mediated by a down-stream series of Ngn-3-dependent genes [22]. While the ontogeny of transcription factor expression in the developing pancreas is well documented, the true hierarchical relationship of mature endocrine cells to precursor lineages is therefore still uncertain.

Paracrine Control of Islet Formation

Peptide growth factors contribute to endocrine cell formation and islet expansion in the pancreas, and originate from multiple tissue compartments including the ductal epithelium, the surrounding mesenchyme, and the vascular endothelium (Fig. 2). Fibroblast growth factors (FGF) are involved in early pancreatic induction from gut endoderm, and the high affinity FGF receptor, FGFR1, has been localized to ductal epithelial cells and to small developing islets [23]. FGFR4 is highly expressed in fetal rat islet development *in vitro* [24]. Major ligands for FGFR4 include FGF-1 and FGF-7. During development of the endocrine pancreas, FGF-7 is expressed within the mesenchyme adjacent to the pancreatic ducts [25]. Systemic injection of FGF-7 into adult rats for up to 2 weeks caused a rapid increase in DNA synthesis within the ductal epithelium followed by pancreatic duct hyperplasia [26]. When FGF-7 was specifically expressed within the embryonic liver of transgenic mice, pancreatic duct hyperplasia was seen, with increased numbers of ductal cells containing immunoreactive insulin [27]. FGF-10 has been also been shown to be expressed within pancreatic mesenchyme in the mouse embryo and its absence causes a failure of ductal

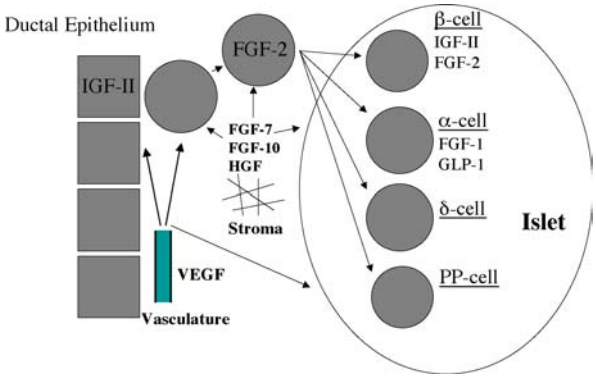

Fig. 2. *Reported cellular origins of and target tissues of peptide growth factors during the development of the endocrine pancreas. IGF - insulin-like growth factor, FGF—fibroblast growth factor, HGF—hepatocyte growth factor, and GLP-1—glucagon-like polypeptide-1.*

branching and an absence of Pdx1-expressing endocrine progenitor cells [25,28].

Vascular endothelial growth factor (VEGF) is a potent mitogen for endothelial cells both *in vitro* and *in vivo*, and also increases vascular permeability. Two of its high affinity, tyrosine kinase-type receptors are Flt-1 and Flk-1. Flk-1 mRNA is expressed within RINm2F islet cells, as well as in fetal rat islets where VEGF is able to increase insulin content [29]. In intact fetal rat pancreas immunoreactive Flk-1 was localized to pancreatic ductal cells and vascular endothelium, suggesting that ductal cells may also be a target for VEGF action. Using porcine fetal and adult rat ducts, VEGF was found to enhance cell replication and insulin content, implying that β-cell neogenesis was also occurring [30]. Lammert [31] demonstrated by targeted over-expression that VEGF acts as a morphogen to induce endocrine cell commitment within the pancreatic ducts, leading to islet cell hyperplasia. While VEGF therefore induces an angiogenic response in support of an increasing islet cell mass, it also acts directly as a mitogen and morphogen for ductal epithelium. Hepatocyte growth factor (HGF) is expressed within tissue mesenchyme in the embryo and fetus, and during postnatal tissue regeneration. Its receptor, the tyrosine kinase met, is expressed within adjacent epithelial tissues which are target sites for mitogenic and morphogenic actions of HGF. In the human fetus met is found on cells within the pancreatic ducts, while in adults *met* mRNA and peptide are localized to islet ß-cells. Conversely, HGF mRNA is abundant in pancreatic stroma, and is able to regulate the proliferation and differentiation of adjacent epithelial cells [32]. A transgenic over-expression of HGF to ß-cells was shown to increase insulin release and islet cell replication once islets were transplanted to donor animals [33].

The IGFs potentiate ß-cell growth, maturation, and function, and are expressed by ß-cells in early life. They appear to act via the type-1 IGF receptor and its activation of IRS-2 within the ß-cells. Complete disruption of IRS-2 in mice carrying a heterozygous mutation for the IGF-IR (*Irs-2−/−, Igf1r+/−* mice) resulted in a severe absence of β-cells in 4 week-old animals [34]. This phenotype was more pronounced than the 50–60% reduction in ß-cells observed in islets of *Irs2−/−* mice [34]. The analysis of *Igf1r+/− and Igf1r+/−, Irs2+/−* mice revealed also a reduction of 30–50% in the islet area of insulin-positive cells which was less severe than that in *Igf1r+/−, Irs2−/−* animals. These observations suggest that the IGF-IR and IRS-2 signaling pathway is critical for β-cell development. Interestingly, mice carrying a null mutation of IRS-1 and heterozygous mutation for IRS-2 (*Irs1−/−, Irs2+/−*) displayed insulin resistance associated with normal islet morphology but a 2-fold increase of the β-cell area at 4 weeks or 4 months of age [34].

These data suggest that IRS-1 is not necessary for the maintenance of the β-cell mass but that IRS-2 is crucial for a compensatory effect of the insulin resistance, causing islet hyperplasia. IRS-2 signaling is likely to be linked to Pdx-1 expression since Pdx-1 was able to restore β-cell mass and function in Irs-2 $-/-$ animals [35].

IGF-II mRNA is greatest in the fetal pancreas, being expressed within islet cells and focal clusters of ductal epithelial cells, but declines during the neonatal period in rats and mice. Conversely, IGF-I mRNA is low but detectable in fetal life, rises to adult levels within three weeks of birth, but is expressed mainly in the exocrine pancreas. Using transgenic mice we showed that over-expression of IGF-II caused a 4–5 fold increase in the mean islet size at birth, affecting all endocrine cell types, but that the total number of mature islets was not altered [36]. This implies that, *in vivo*, IGF-II functions as a growth factor for existing islet cells, but does not promote islet neogenesis. Between postnatal days 7 and 21 of normal development a transient wave of apoptosis destroys a proportion of ß-cells in both rat and mouse [6,7], to be replaced both from ductal neogenesis, and from proliferation of remaining islet cells. This normal islet remodeling is thought to change the overall phenotype of ß-cell mass from a fetal pattern of poor glucose responsiveness, to a postnatal phenotype of rapid glucose responsiveness. This developmental islet cell apoptosis coincides temporally with a diminished pancreatic expression of islet IGF-II [86]. IGFs can prevent apoptosis in many cell types, and we showed that endogenous IGF-II within isolated neonatal rat islets could protect them from cytokine-induced apoptosis [7]. This protection was lost by weaning when islets no longer expressed IGF-II, but could be restored with exogenous IGF-II. Functional proof that changes in IGF-II availability provoke developmental ß-cell apoptosis was obtained from transgenic mice over-expressing IGF-II in skin, leading to increased circulating levels which did not fall postnatally [37]. Here, the neonatal wave of ß-cell apoptosis was suppressed. In studies by others using transgenic mice expressing IGF-II within ß-cells the disruption in developmental apoptosis within the islets resulted in glucose intolerance in older animals [38]. A targeted over-expression of IGF-I to the β-cell promoted β-cell regeneration and was protective against diabetes [39]. It might be therefore be expected that pancreas-specific inactivation of IGF-I would limit endocrine cell proliferation and survival, and lead to a reduced islet mass. Conversely, such mice demonstrated enlarged islets and a resistance to streptozotocin (STZ) -induced β-cell loss and dietary induced diabetes [40]. Since deletion of the insulin genes also leads to islet overgrowth during development [41], it is possible that both insulin and IGF-I function as a negative feedback loop within the islets to regulate β-cell mass, and act as counter-regulatory factors to IGF-II and other trophic peptides such as glucagon-like polypeptide-1.

Glucagon-like polypeptide-1 (GLP-1) is expressed by the intestinal L cells in adult life under the control of nutritional glucose and lipids. It is generated by post-translational processing of the proglucagon gene by the proconvertase enzymes, PC1/3 and PC2. GLP-1 stimulates glucose-dependent insulin biosynthesis and release, suppresses glucagon release from the α-cells, reduces gastric motility, and promotes satiety. Glucagon can also increase β-cell mass through modulating the expression of Pdx-1, the glucose-dependent activity of the Pdx-1 N-terminal trans-activation domain, and the cytoplasmic to nucleus translocation of Pdx-1 allowing for Pdx-1-dependent gene transcription [42,43]. Pdx-1, however, does not cause a reduction in GLP-1 release and proglucagon gene expression [44]. It is possible that GLP-1 might also direct the trans-differentiation of other pancreatic cell lineages towards a β-cell phenotype, as occurs in AR42J rat acinar cells, PANC-1 human pancreatic ductal cells, or human islet-derived progenitor cells [45,46]. Coupled with a reported ability to promote β-cell proliferation while inhibiting apoptosis [47], GLP-1 is a multi-acting trophic agent for the expansion of β-cell mass. The extent to which it functions as such in development is surprisingly ambiguous. The fetal and neonatal pancreatic α-cells in the mouse or rat synthesize both glucagon and GLP-1 [48], the latter decreasing with age. Inhibition of the normally rapid degradation of GLP-1 in the circulation by dipeptidyl peptidase IV (DPP IV) inhibitors results in an enhanced regenerative response in the neonate following β-cell depletion by partial pancreatectomy. Conversely, the GLP-1 receptor null mouse has only mild hyperglycemia and moderate glucose intolerance [49] with a normal β-cell mass although islet architecture is abnormal with α-cells scattered throughout the islets. However, regeneration of β-cell mass in young mice following 70% pancreatectomy was impaired in GLP-1 receptor null animals [50].

Extracellular matrix is a dynamic molecular complex serving as a cellular scaffold and regulator of differentiation and survival. At the pancreatic endocrine-exocrine interface, basement membrane constitutes a peri-insular capsule that contains primarily laminin, a protein that has been shown to play a role in tissue regeneration after injury and in directing morphogenesis during embryonic development. The ability of ECM to control the fate of cells is ultimately dependent on a family of transmembrane integrin receptor molecules. Integrins bind to ECM ligands, form clusters and associate with intracellular cytoskeletal and signaling proteins in large multi-protein aggregates termed focal adhesions. Several growth factor receptors are physically associated with integrins and their

activation is required for growth factor signaling. Integrins also transmit signals directly through recruitment of non-tyrosine kinases from the focal adhesion kinase (FAK) and Src families; there is subsequent activation of downstream effectors of the PI3-K or MAP-K pathways, both of which are critical for regulation of cyclin-dependent kinases as well as cell cycle progression.

Integrin receptors and the ECM have been shown to be important determinants of islet cell biology, influencing survival, proliferation, and differentiation. Disruption of the islet-matrix relationship induces some islet apoptosis, and leads to a loss of stability, resulting in transdifferentiation of an islet to a ductal phenotype [51]. Integrin-ECM interactions may also be important in maintaining β-cell function, as observed when human islets embedded in type I collagen gels have a greater insulin secretory response [52]. Integrin $\alpha 3\beta$ 1 regulates the migration of $CK19^{+}$/PDX-1 putative pancreatic progenitors of human fetal pancreatic epithelial cells on netrin-1 [53]. The $\alpha 6\beta$ 1 is believed to enhance and regulate the insulin secretory response of rat islets; and β 1 integrin may be involved in early motile processes that are required for the formation of new islets by supporting migration of human fetal β cells [54]. This supports a role for $\alpha\beta$ 1 integrins in the process of neogenesis of new β cells from pancreatic progenitors, and in maturation and function of islet cells. Recent studies have demonstrated that β 1-integrin (and its associated $\alpha 3$, $\alpha 5$ and $\alpha 6$ subunits) is expressed at specific stages during islet cell development both in the human fetal pancreas and in the pancreas of the fetal and postnatal rat [55]. Functional blocking of β 1-integrin results in inhibition of islet cell adhesion, disruption of islet architecture, down-regulation of insulin gene expression, and a significant increase in the number of apoptotic islet cells [56], highlighting its important role in islet cell architecture, development, integrity and function.

The Plastic Potential of the Endocrine Pancreas

Regeneration of pancreatic β-cell mass following either toxin or autoimmune-mediated destruction is possible in the young rodent, but the extent of the recovery decreases with age and is incomplete in adult life. Similarly, there is histological evidence of islet cell neogenesis and a regenerative response in children and adolescents with type 1 diabetes [57]. Neogenesis was found in association with centroacinar and ductular cells leading to the formation of large, ß-cell-rich islets. A 90% pancreatectomy in the young rat induces regeneration of both exocrine and endocrine tissue. New ß-cells are derived by both neogenesis, and cell replication within the remaining islets. Enhanced IGF-I mRNA expression was reported in duct cells, capillary endothelium and new endocrine cells [58], while VEGF mRNA was rapidly up-regulated. Partial pancreatectomy in the juvenile rat leads to a reduction in the expression of Pdx1, GLUT 2 and insulin mRNAs, most likely due to the associated hyperglycemia. Islet cell neogenesis can be induced in the adult hamster by obstruction of the pancreatic duct. Ductal cell proliferation occurs within 14 days leading to a cell outgrowth after 21 days forming new, small islets containing either glucagon, insulin, or both. A second wave of proliferation occurs in islet cells after 8 weeks leading to a total two-fold increase in islet cell mass [59]. IGF-II mRNA is increased 10 days after pancreatic duct obstruction and coincident with the commencement of ductal cell proliferation. IGF-II released from ducts was capable of suppressing β-cell apoptosis during co-culture with islets [60]. These findings support the notion that an increase in ß-cell number can occur in early postnatal life, and that this involves changes in local growth factor and transcription factor expression,

We utilized a model in which the young rat is rendered diabetic with a moderate dose of STZ 4 days after birth. This results in destruction of approximately 60% of the ß-cells within 72 h and causes a transient hyperglycemia that is returned to the normal physiological range within 40 days post-STZ (61, Hill DJ, submitted). These changes are superimposed upon the normal islet remodeling seen in rodents between postnatal days 7 and 21. The ß-cell destruction caused by STZ is rapidly reversed with ß-cell mass having returned to some 70% of controls within 40 days. This is due both to a re-population from within islets, and from neogenesis at the pancreatic ducts. Increased mitotic activity is apparent in the pancreatic ductal epithelium as early as 48 h after STZ treatment, accompanied by an increased presence of FGF-7 within the adjacent mesenchyme. By 4 days post-STZ small clusters of cells immuno-positive for Pdx1 and Ngn3 have budded from the ducts. Cells immunopositive for nestin and/or c-*kit* are observed in controls throughout the study period adjacent to the ducts, while nestin-positive cells are also dispersed throughout islets. Their abundance increases in the ductal compartment within 4 days of STZ treatment, and this is maximal by 8 days. Within 8 days of STZ numerous, small insulin-positive cell clusters had appeared adjacent to the pancreatic ducts. Within pre-existing islets there was increased cell proliferation in both insulin- and glucagon-positive cells at 8 days, and α cell hyperplasia. This was associated with an increased pancreatic content and circulating levels of glucagon, but a decreased expression of Pax6, a transcriptional marker of mature α-cells. Pancreatic levels of GLP-1 were increased 8 days post-STZ compared to controls and the GLP-1/glucagon ratio changed in

favor of GLP-1. Atypical insulin-positive cells were found in the islet core after 8 days that co-localized glucagon, GLP-1 and the GLP-1 receptor. The model shows that the pancreas of the young rat can rapidly regenerate a 60% loss of β-cells, and that this is associated with a hyperplasia of α cells with an altered phenotype of increased GLP-1 synthesis. While this suggests the establishment of a regenerative paracrine milieu, the target cells of GLP-1 action are unclear. These could include a rare multipotential stem cell population, or the ability of differentiated endocrine cells to de-differentiate into a precursor cell phenotype, then capable of expansion and re-differentiation along a β-cell lineage. Cell lineage marking of β-cells in mice followed by partial pancreatectomy showed that new β-cells in the remaining organ derived almost exclusively from existing β-cells in adult life [62]. However, there is also clear evidence of a scarce sub-population of multi-potent islet precursor cells resident in both ducts and islets of the adult mouse pancreas [63].

This model of STZ depletion of β-cells suggests that islet plasticity in early life involves at least two elements. Firstly, islet cell neogenesis is induced at the pancreatic duct, possibly initiated by growth factors such as FGF-7, and secondly that a β-cell regeneration can occur from within mature islets. While the regenerative response is sub-effective in adulthood, and unable to reverse diabetes, a total elimination of β-cells with STZ within the islets of adult mice, followed by insulin replacement, caused two new cell populations to appear, some cells co-expressing somatostatin, insulin and Pdx-1, and others expressing only GLUT2 [64,65].

What then might become rate-limiting with age to islet plasticity? We reported a robust regeneration of β-cell mass in adult mice following transplantation of marrow-derived hematopoietic stem cells into animals rendered deficient in β-cells with STZ [66]. Marrow-derived cells preferentially colonized the damaged pancreas and almost 10% demonstrated markers of endothelial precursor cells (EPC) such as PECAM1. New β-cells were predominantly derived from endogenous cell populations rather than trans-differentiation of the transplanted marrow stem cells. A subsequent study demonstrated that a similar CD34 + ve/c-kit + ve marrow cell fraction was capable of integration into vascular endothelium in the pancreas of the diabetic animal [67]. Islet regeneration was also seen in non-obese diabetic (NOD) mice following administration of splenocytes together with complete Freund's adjuvant [68]. While the latter was partially due to a trans-differentiation of a splenocyte fraction into both pancreatic endocrine and duct cells, the ability of irradiated splenocytes to cause islet regeneration showed that this also involved an induction of regeneration by endogenous mechanisms. The loss of regenerative capacity in the untreated pancreas with age following β-cell depletion could therefore be related to the presence of endogenous EPCs. We found that a population of endogenous EPC-like cells exists in the neonatal pancreas of mouse and rat [69], and in the human fetus, and in rodents decreases with age along with islet regenerative capacity. These cells, which are immunopositive for CD34, nestin and c-*kit* are located adjacent to the pancreatic ducts and within the islets. The nature of the inductive signal from EPCs that initiates islet regeneration is unclear. However, one candidate is VEGF since a targeted over-expression of VEGF to the developing pancreas in a transgenic mouse model resulted in islet hyperplasia [31]. We have attempted to manipulate EPC abundance in late fetal and neonatal life using atorvastatin, since statins at low dose are known to mobilize EPCs in other tissues, increase VEGF expression, and prevent cell apoptosis [70]. Atorvastatin treatment resulted in a significant increase in islet mass in the neonatal rat affecting all endocrine cell types, while following STZ depletion of β-cells, there was an accelerated regenerative response (Fig. 3). Collectively, these findings suggest that substantial plasticity exists in the endocrine pancreas, even in adult life, but that regenerative capacity is lost because of a paucity of one or more inductive signals. One source of such inductive signals appears to be EPCs.

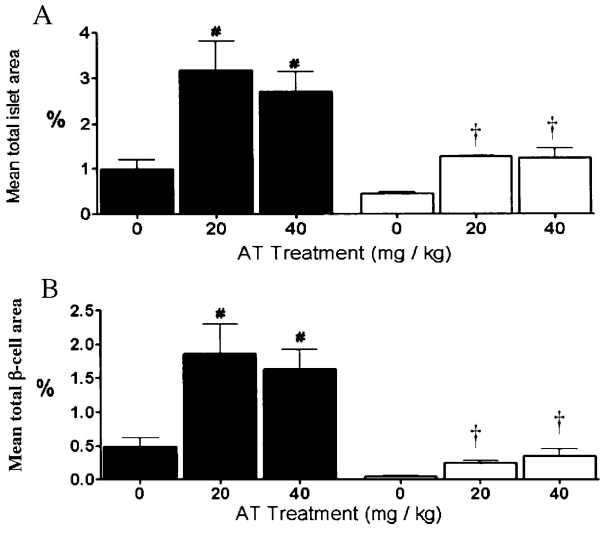

Fig. 3. *Mean (±sem) percentage of pancreas occupied by entire islets (A), or islet β-cells (B) in neonatal rats at postnatal day 14 following administration of atorvastatin (20 or 40 mg/kg) to the pregnant dams from gestational day 14. Animals were given either saline (closed bars) or streptozotocin (50 mg/kg) (open bars) at postnatal day 4. Figures represent 3 litters of rats comprising 12–18 animals at each time point.* $^{\#}p < 0.01$ *vs. saline alone,* $^{\dagger}p < 0.05$ *vs. streptozotocin alone.*

Environmental Programming of the Endocrine Pancreas

In the human a period of islet remodeling occurs in third trimester, and while considerable capacity for recovery of islet cell mass may occur following discrete fetal insults, this may impact on islet functionality and predispose to disease in adult life. Intrauterine growth retardation (IUGR) in rat or human is associated with a reduced pancreatic β-cell number at birth and is a major risk factor for type 2 diabetes and hypertension in later life [71]. While the mechanisms responsible for fetal environmental programming of islet phenotype, insulin availability, and risk of later diabetes in humans cannot be directly assessed, there is a strong body of evidence from animal models.

If maternal calorie intake was reduced by 50% in the rat from day 15 of gestation until term then β-cell mass and islet number were reduced in the newborn [72]. If normal diet was restored at birth, the β-cell mass returned to that of controls by weaning. However, continuation of energy restriction during neonatal life lead to irreversible changes in β-cell mass. A reduced dietary protein provided to rats throughout gestation caused a profound reduction in pancreatic weight at birth with reduced ß-cell mass and islet size [73]. The offspring of the low-protein-fed (LP) animals have been shown to exhibit abnormal circulating amino acid profiles, with a particular deficiency in taurine. If nutritional restriction is lifted at birth the islet morphology will partly recover, but if extended until weaning or beyond the changes are irreversible and lead to glucose intolerance in later life. Islets isolated in late fetal life following LP administration during pregnancy showed a lower basal insulin release and a blunted insulin release in response to glucose, arginine or leucine [74]. These models show a strong effect of nutritional sufficiency on fetal islet development, but that the neonatal period is also a time of islet plasticity that will have lifelong consequences for glucose homeostasis. The mechanisms by which LP diet alter islet development involve changes to local peptide growth factor presence and action. LP diet causes a decreased rate of ß-cell replication, an increase in apoptosis, and a lower pancreatic expression of IGF-II [73]. Analysis of ß-cell cycle kinetics *in situ* by detection of cell cycle-specific proteins suggested that cycle length was increased by LP, with an extended G1 phase. This may reflect a cell cycle block in which cells are not progressing to DNA synthesis due to the deficiency of a trophic progression factor, such as IGF-II, or result from a permanent re-programming of cell cycle kinetics which may be imprinted on a precursor cell population prior to β-cell differentiation. The pancreatic population of EPCs, as indicated by staining for immunoreactive nestin, CD34 and c-kit was decreased subsequent to LP diet in parallel with β-cell mass [69], as was the capillary density within islets and the presence of VEGF and its receptors [75]. In the Goto Kahizaki (GK) rat model of type 2 diabetes fetal IGF-II production is defective [76], and these animals have impaired regeneration of β-cells following partial pancreatectomy [77]. Induction of IUGR in the rat fetus using uterine vessel ligation yields offspring that are prone to type 2 diabetes in later life [78]. Some of the effects of dietary insult on fetal pancreatic development may be mediated by exposure to excess maternal glucocorticoids, due to a decrease in the placental expression of 11β-hydroxysteroid dehydrogenase type 2 that metabolizes glucocorticoids to inactive derivatives [79]. Cortisol has been shown to down-regulate the expression of Pdx-1 in the embryonic pancreas.

Strategies for the reprogramming of a developmentally-induced risk of diabetes have centred either on trophic hormone modulation, or on selective food supplementation at an epidemiological level, and the identification of key micronutrients at an experimental level. Rat pups with IUGR as a result of uterine vessel ligation in the mother were treated with the GLP-1 agonist, exendin-4. This increased the rate of β-cell proliferation and the expression of Pdx-1, and animals did not develop glucose intolerance as adults [80]. A similar rescue has been achieved with dietary micronutrients. Taurine is normally an abundant sulphur-containing amino acid in most tissues, including the pancreas, and is synthesized from methionine and cysteine. Taurine levels were shown to be reduced in maternal blood, and seriously depleted in fetal blood of rats given LP diet during pregnancy. Taurine supplementation alone was also able to reverse the deficits seen in pancreatic EPC presence, vascularity, VEGF presence, fetal and neonatal β-cell proliferation, and glucose and amino acid-dependent insulin release from islets isolated in late gestation [74,75,81] in offspring of LP-fed animals. The proportion of islet cells demonstrating immunoreactive IGF-II in early life was decreased following exposure to LP diet, but this was restored by taurine, which appeared to delay the age-related loss of IGF-II in neonatal islets. There is considerable evidence from other tissues for an anti-apoptotic action of taurine through mechanisms including an inhibition of the Fas pathway. LP diet caused an increase in the presence of immunoreactive Fas and Fas ligand within islets, being greatest at the time of neonatal developmental apoptosis at day 14. Both Fas and Fas ligand presence were reduced by taurine supplementation. In addition, taurine addition to culture medium or to maternal diet prevented IL-1 and TNFα induced apoptosis in isolated islets [82] and restored insulin secretion. Thus, it appears that selective

nutritional components can have a profound effect on endocrine pancreatic development, and have the potential to offset environmentally-induced damage in early life, and possibly the risk of transmission of type 2 diabetes.

Conclusions and Further Developments

Few areas of research have more momentum at present than strategies to generate functional β-cells and islets from precursor and stem cells. There has sometimes been a belief that a single trans-differentiation event can achieve this goal if a dominant trophic growth factor can be identified. Experience has shown that while it is remarkably easy to generate a proportion of cells with insulin gene expression, the glucose responsiveness, insulin storage capacity, and expandability of these few cells is usually poor. A thorough understanding of the ontological sequence by which functional β-cells emerge in the embryo and fetus is essential to deliver new therapies to reverse diabetes. This can be readily seen from the extensive literature on the biology of GLP-1 that has lead to the current evaluation of long-acting analogues, and DPP IV inhibitors to prevent the degradation of endogenous GLP-1, for the control and reversal of type 2 diabetes. Key levels of control in β-cell development are still uncertain. Consensus does not yet exist as to the dominant transcriptional control of the β-cell lineage, the homeostatic mechanisms that control the relative synthesis of GLP-1 versus glucagon within the α-cell are unknown, and the nature of the inductive signals for islet regeneration delivered from marrow derived stem cells, such as endothelial precursor cells, are poorly understood. However, the next advances in the reversal of diabetes will emerge from the study of pancreatic developmental biology.

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