Islets in Type 2 Diabetes: In Honor of Dr. Robert C. Turner

Susan Bonner-Weir[1] and Timothy D. O'Brien[2]

The UK Prospective Diabetes Study (UKPDS) orchestrated by Robert Turner showed that, even with treatment, in type 2 diabetes a progressive β-cell dysfunction in a large number of patients was seen with the eventual need for additional oral antihyperglycemic medication and even insulin treatment (1). Such a progression could be from a continued loss of β-cells, a progressive functional change, or from the combination of both. During the decades when type 2 diabetes was considered mainly a disease of insulin resistance, many older pathology studies that focused on the islets in diabetic pancreata were ignored. However, with the renewed appreciation of the role of the pancreatic β-cell in type 2 diabetes, new studies have focused on what happens to the β-cells in type 2 diabetes. This perspectives in diabetes article will present what we know and still need to know about the islets in type 2 diabetes.

Islet architecture in human pancreas. The islets of most mammalian species have a nonrandom pattern with a core of β-cells surrounded by a discontinuous mantle of non-β-cells one to three cells thick (2,3). However, islets of human and other primates have a more complex arrangement with many different islet profiles, including cloverleaf patterns. The profile differences have led to controversy about whether they actually have a mantle-core arrangement (3) or were random (4–6). In three dimensions, human islets can be considered as composites of several mantle-core subunits (7) or as lobulated with mantle-core lobules (3). In smaller islets, the rodent mantle core subunit arrangement is maintained, but in larger islets irregular fusion of such subunits are seen (Fig. 1). Most of the non-β-cells are found along penetrations of islet vasculature between subunits and the periphery (3,4), thus maintaining a mantle-core arrangement. Histologically, islets in the type 2 diabetic pancreas do not appear to differ from those of the nondiabetic pancreas, except for the presence of amyloid, as discussed below. Many years ago, pathologists reported "hydropic degeneration" seen as vacuolization in islets from diabetic persons. This vacuolization was due to extraction during histological processing (8) of large glycogen stores accumulated during poor metabolic control; it is less commonly reported now that it is understood. Fibrosis, particularly along the islet microvasculature, has also been previously reported but has since been found to occur equally in nondiabetic pancreata (9).

Cell composition of human islets. The β-cell composition in human islets has been reported in a number of studies presented as percentage on the basis of cell number or cell volume, with ranges of 52–75% in nondiabetic adults (10–16). The measure of proportion is further complicated because the probability of seeing a nucleus in a 1-μm optical section is higher in non-β-cells than in β-cells since the nuclear volumes are comparable but the cell volume of β-cells is more than twice that of non-β-cells. Unlike rodent islets, the islets within a single human pancreas are highly variable in composition (Fig. 1); there are occasional large islets seen with a majority of glucagon-positive cells (11,15), and islets from the pancreatic polypeptide (PP)-rich uncinate process are mainly PP cells, with β-cells being only $32.7 \pm 7.8\%$ as compared with $65.5 \pm 4.9\%$ in the rest of pancreas (12). (Similar values have been reported by Stefan et al. [10].) Because of this variability within a pancreas, it is imperative that a large number of islets are measured. In our studies (S.B.-W.) using ultrastructural analysis to determine cell type and cell boundaries, we found $72.8 \pm 1.7\%$ β-cells/islet in islets isolated from 41 pancreata. However, in studies using laser-scanning confocal microscopy on fewer islets, the β-cell number in islets was estimated to be 55% (2–5 islets/section, 5 pancreata) (5) or $53.9 \pm 2.5\%$ (32 islets isolated from 6 pancreata) (6). Thus, for comparison of cell composition in islets from type 2 diabetic and nondiabetic pancreata, it is important to use data from the same study. For example, by measuring cell volume, Butler et al. (16) found that islets from lean nondiabetic subjects have $52.0 \pm 4.1\%$ β-cells, but islets from lean diabetic subjects have only $38.0 \pm 3.9\%$; Yoon et al. (15) reported $59 \pm 10.3\%$ and $68.8 \pm 12.2\%$ for nondiabetic control subjects but $38.3 \pm 12.4\%$ for diabetic subjects; and Maclean and Ogilive (17) found 74.8% for nondiabetic subjects and 63% for diabetic subjects. Thus, in three studies, there is at least a tendency for the percentage of β-cells per islet to be decreased in type 2 diabetes.

Pancreatic weight. Pancreatic volume changes with age and obesity but is quite variable as measured by computed tomography (18). Most studies do not provide the pancreatic weights or volume, but even with similar (European) adult populations, major differences have been reported. Rahier et al. (14) reported mean pancreatic weight from 20 control patients as 85.4 g, from 4 type 1 diabetic patients as 40 g, and from 7 type 2 diabetic patients as 70.9 g; data from only the type 1 diabetic pancreata were reported to be statistically different. However, Kloppel et al. (13) divided their data between obese and nonobese and found no differences in the more selective measure of pancreatic
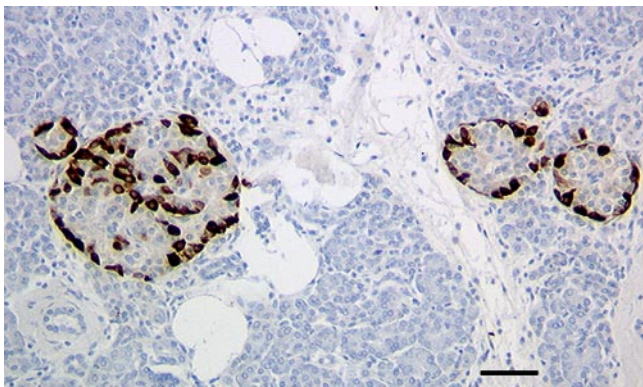
FIG. 1. Nonrandom distribution of glucagons-positive cells in human islets. In normal adult human pancreas, there is a nonrandom distribution of glucagon cells (brown) similar to that seen in rodents. In some small islets, glucagon cells form a mantle around a core of β-cells while larger islets seemed composed of irregular subunits of mantle-core organization. Immunoperoxidase staining for glucagon (brown); hematoxylin. Magnification bar = 50 um. (Please see http://dx.doi.org/10.2337/db07-1842 for a high-quality digital representation of this figure.)

parenchyma volume (equivalent of pancreatic volume without the fat cells) among nonobese control subjects, nonobese type 2 diabetic subjects, and obese type 2 diabetic subjects (40 ± 11, 30 ± 10, and 40 ± 13 ml, respectively), but a twofold increase in pancreatic parenchyma volume in obese nondiabetic subjects (80 ± 28 ml). Thus, in this study, pancreata from obese type 2 diabetic subjects are reduced in expected volume as compared with those from obese nondiabetic subjects. Using a different technique to make such estimates, a recent study measured pancreatic parenchymal volume by computed tomography: with 460 lean and 230 obese patients, the parenchyma volume was still significantly increased, but only by 10%, in the obese (48.7 ± 18.0 [SD] cm^3) compared with the lean subjects (43.2 ± 15.7 cm^3); here, type 2 diabetes pancreata ($n = 165$) were 7–8% decreased in parenchymal volume without stratification as to obesity (18). The trends of changes in pancreatic weight/volume are consistent in these three studies, even if the values differ. The finding that pancreatic volume/weight differs with obesity and with diabetes is important in evaluating recent studies that only measured relative β-cell volume (percent relative to pancreas) and not absolute β-cell mass/volume, which is the product of the relative β-cell volume and the pancreatic volume/weight.

Changes in β-cell mass in obesity. As early as 1933, it was suggested that many obese individuals had abnormally high islet volume density (number of islets per area) (19). However, it was not until 1985 that increased β-cell mass in nondiabetic obese people was clearly shown, albeit with a low number of pancreata (13). β-cell mass is used to indicate the total volume of β-cells within a pancreas without regard to number or size of the β-cells. In rodents it has been clear that there is a compensatory increase in β-cell mass in response to insulin resistance or obesity (20), so we assume a similar increase in humans is compensatory. In 2003 two studies with more human pancreata convincingly showed a compensatory increase in β-cells with increasing BMI. Using a morphometric approach on multiple sections of each of nine weighed pancreata, Yoon et al. (15) showed a linear relation between body weight and β-cell mass in a Korean population. Butler et al. (16) showed increased β-cell volume

density in 35 pancreata from nondiabetic obese people from Minnesota, about a 50% increase in the percentage of pancreatic volume comprised of β-cells (%, relative β-cell volume) ($2.6 \pm 0.4\%$ in obese nondiabetic individuals vs. $1.7 \pm 0.3\%$ in lean nondiabetic individuals). Because the evidence discussed above suggests that, in obesity without diabetes, pancreatic parenchymal volume also increases, the actual increase of β-cells may be enhanced to even a greater degree.

Changes in β-cell mass in type 2 diabetes. The controversial aspect of islet pathology in type 2 diabetes has been whether there is a decrease in β-cell mass or just a functional decrease. Older studies such as that by Maclean and Ogilvie (17) showed decreased β-cell mass in diabetes but did not distinguish between type 1 and type 2 diabetes, even though the age at onset roughly categorizes their cases. While several groups have maintained there is no significant decrease in β-cell mass in type 2 diabetes (11,14,21,22), most studies have shown a 40–60% decrease, particularly compared with pancreata from nondiabetic individuals of similar body weight or BMI. Using morphometric analysis on multiple sections throughout each pancreas, studies several decades ago estimated absolute β-cell mass (pancreatic weight × relative β-cell volume) in pancreata from diabetic subjects. One study reported 40% decreased β-cell mass (in 26 type 2 diabetic vs. 37 nondiabetic pancreata) (23); another reported 62.5% in nonobese and 50% decreased β-cell mass nonobese and obese type 2 diabetic pancreata compared with those from body weight–matched nondiabetic subjects (13). In their study of pancreata from 14 Japanese type 2 diabetic patients, Sakuraba et al. (12) reported only a 30% decrease in β-cell mass, although in the 7 of those subjects who were on insulin therapy, there was a 40% decrease. Surprisingly, a reduction of β-cell mass shown clearly by the three aforementioned studies was not accepted by the field until the publication of previously mentioned articles in 2003 (i.e., refs. 15,16), even though these latter studies only provided relative β-cell volume (percent pancreas) rather than actual β-cell mass.

The study that swayed the balance of thought was that of Butler et al. (16). That study had a large number of autopsied pancreata from patients with good clinical records and categorized by body weight index. Additionally, it had measurements of various determinants of β-cell mass that suggested possible mechanisms (see below). There was a 63% decrease in relative β-cell volume in 41 obese type 2 diabetic compared with 35 obese nondiabetic and a 41% decrease in lean type 2 diabetic subjects compared with 17 lean nondiabetic subjects. Importantly, this study included measurements of 15 obese subjects with impaired fasting glucose in whom the relative β-cell volume was decreased 40% compared with obese nondiabetic subjects. One criticism of this study has been that only one random section was evaluated in this study; however, several other studies (12,15,17) have shown that, with the exception of the PP-rich uncinate process, most sections across the pancreas have similar densities of islets and of β-cells. Even so, differences in pancreatic parenchyma volume in obese and lean, diabetic and nondiabetic subjects may amplify the differences in actual β-cell mass; the data to determine this are lacking due to the common procedures of autopsies today.

Mechanisms of changes in β-cell mass in type 2 diabetes. Changes in β-cell mass in type 2 diabetes is thus fairly certain, but such findings do not mean that there
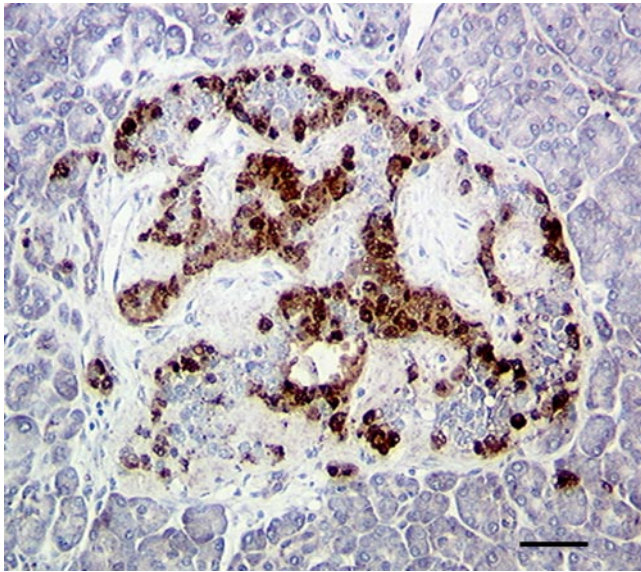
FIG. 2. Amyloid deposits along the islet capillaries. In islets with severe amyloid deposits such as this one, there is a loss of insulin-positive (brown) β-cells and a distorted islet structure. Immunoperoxidase staining for insulin (brown); hematoxylin. Magnification bar = 25 um. (Please see http://dx.doi.org/10.2337/db07-1842 for a high-quality digital representation of this figure.)

cannot be progressive functional changes in human β-cells, as seen in rodents with chronic hyperglycemia (24). Another issue is what causes the decreased β-cell mass or relative volume. The current concept is that there is a slow continuous turnover of β-cells within the pancreas with a careful balance, or even positive input, of cell renewal and cell loss. It cannot be ruled out that there is some impairment of compensatory growth mechanisms, such that a person who cannot compensate for increasing obesity develops type 2 diabetes. Even so, the favored scenario is that there is an imbalance in loss and renewal of β-cells that eventually results in the decreased β-cell mass. Cell renewal is by replication of preexisting β-cells and by differentiation from non-β-cell precursors. Mitotic figures or Ki67-positive cells are rarely reported in islets in adult human pancreas; mitotic figures in islets have been reported mainly in cases of liver disease (25), and Ki67-positive islet cells were fewer than 0.03 cells/islet (16). However, insulin-positive cells within ducts, considered neogenesis, were more frequent in obese versus lean pancreata but not different between diabetic and nondiabetic pancreata in either obese or lean individuals (16). Thus, the current favored view is that apoptosis or loss of cells is increased and a target for prevention. However, increased apoptosis has not consistently been shown. While Butler et al. (16) found a tendency of increased frequency of apoptosis/islet in obese type 2 and statistical significance only in lean diabetic subjects, Sakuraba et al. (12) found no evidence of apoptosis in either diabetic or nondiabetic individuals. However, in the latter study, there were signs of oxidative stress and decreased protective superoxide dismutase enzymes. The presence of amyloid deposits in type 2 diabetes (Fig. 2) has led to the suggestion that it is causal for diabetes since islets with amyloid deposits have decreased percentage of β-cells, and islet amyloid polypeptide (IAPP) fibrils have been shown to induce apoptosis (26). Yet, the finding that only 10% of those persons with impaired fasting glucose had any amyloid-positive islets but already a 40% deficit of relative

β-cell volume has suggested a lack of causality; such an interpretation ignores some of the complexity of IAPP and islet amyloidosis as discussed below.

Role of amyloidogenesis in β-cell loss and malfunctioning. More than 20 years ago the striking association between β-cell loss and the occurrence of islet amyloidosis in both humans and similar animal models led to the pursuit of the biochemical identity of this form of amyloid. At that time we hypothesized that "the presence of islet amyloid may represent an important clue to the basic derangements of islet cells that occur in patients with age-associated impairment of glucose tolerance and overt diabetes mellitus" (27). In pursuit of this clue to the pathogenesis of type 2 diabetes, two independent groups (including Robert Turner's) reported the amino acid sequence of human IAPP (amylin) in 1987 (28,29), and since then enormous strides have been made in the understanding of the pathogenesis of islet amyloidosis and its potential role in the loss of β-cells in type 2 diabetes. IAPP was shown to be copackaged and cosecreted with insulin as a normal product of the β-cell (28,30). IAPP, therefore, must be normally prevented from undergoing aggregation and polymerization into fibrils. It was thus clear that in order for IAPP to undergo amyloidogenesis (and to produce islet amyloid [IA]), pathologic alterations in synthesis, protein trafficking and chaperoning, secretion, or degradation, or combinations of these mechanisms must occur in association with type 2 diabetes. While much still needs to be elucidated concerning the molecular pathogenesis of IAPP amyloidogenesis, there is growing evidence supporting a role for this process in β-cell malfunction and loss in the development and progression of type 2 diabetes.

Cytotoxicity of IAPP. In order for IA or amyloidogenesis to play any role in the pathogenesis of type 2 diabetes, IAPP or aggregates must be shown to be directly cytotoxic to β-cells and/or to set off a molecular cascade of events that are cytotoxic. There is now abundant evidence of cytotoxic effects of amyloidogenic proteins in general, including IAPP (26,31–33). For example, it was found that incubation of human but not rodent IAPP was shown to be cytotoxic to β-cells under in vitro conditions (26). Because rodent IAPP is nonamyloidogenic, these findings also suggested a role for amyloidogenesis in this toxic effect. Likewise, COS-1 cells transfected with human IAPP showed rapid cell death associated with marked fibril formation within the endoplasmic reticulum (ER), and cell lines expressing human IAPP (hIAPP) could not be established in these cells (34). By comparison, cells transfected with rodent IAPP (rIAPP) were viable and readily established long-term stably transfected cell lines. Using this same system it was subsequently shown that the cell death in the hIAPP-transfected cells was due to apoptosis. Several other studies have further documented pro-apoptotic effects of hIAPP on β-cells and neurons. Interestingly, the effects of hIAPP on neurons closely resemble those of the Alzheimer's disease–associated Aβ (β-protein). Mechanisms involved in Aβ cytotoxicity include oxidative damage by reactive oxygen species, lipid peroxidation, reduced mitochondrial transmembrane potential, and destabilization of intracellular calcium homeostasis (35). Of special interest is the finding that membrane lipid peroxidation initiated by Aβ is also associated with impaired glucose transport into cultured rat hippocampal neurons (36). If similar alterations were induced in β-cells by IAPP fibrillogenesis, this may be yet another

mechanism by which IAPP fibrillogenesis impairs normal β-cell function.

While mature amyloid fibrils were initially thought to be the likely mediators of hIAPP toxicity, it has become increasingly apparent that it is actually the relatively small soluble oligomers of hIAPP that are most toxic to β-cells (26,33). Several studies have now shown that prefibrillar oligomers act by disrupting lipid bilayers and can lead to membrane fragmentation (37). Other studies have demonstrated that nonselective ion-permeable membrane pores form upon exposure to hIAPP oligomers (31). This mechanism may further lead to destabilization of the intracellular ionic milieu and lead to generation of reactive oxygen species and free radical formation. Furthermore, these activities of hIAPP have been linked to induction of apoptosis and, thus, potentially to β-cell death in type 2 diabetes (32,33).

Recently it has also been demonstrated that toxic oligomers (and not monomers or mature amyloid fibrils), formed by different amyloidogenic proteins including hIAPP, Aβ, synuclein, transthyretin, and prion protein, share a common epitope (38). Antibodies raised to this epitope using toxic oligomers of Aβ$_{1-40}$ also bind to toxic oligomers generated from the other amyloidogenic proteins and, in cell culture, block the cytotoxic effects of each of these diverse oligomers. These findings suggest a common molecular mechanism involved in the pathogenesis of several different disease conditions, all of which share the commonality of amyloid formation as part of the pathologic condition. In addition, antibodies obtained against this marker for the toxic epitope have allowed examination of the presence and location of these oligomers in hIAPP-transgenic mouse models of type 2 diabetes (39). In both models examined in this study, the toxic oligomer epitope was detected intracellularly in β-cells and was not found in extracellular locations. Toxic oligomers were also not found in nontransgenic mice of the background strain or in mice transgenic for rIAPP. In addition, vaccination of the hIAPP transgenic mice against the toxic epitope of Aβ$_{1-40}$ failed to prevent hIAPP-associated β-cell death or induction of diabetes in either of these mouse models. These findings together are consistent with an intracellular location for the earliest stages of IAPP amyloidogenesis, as suggested by previous studies (34). In addition, toxic IAPP oligomers may trigger the programmed cell death cascade by signaling at the β-cell surface (40,41). Evidence from RIN cells demonstrated that hIAPP but not rIAPP stimulated apoptosis involving a JNK1-mediated signaling cascade (40). More recently it was also shown in mouse islets and in two different insulinoma β-cell lines that exposure to solutions of hIAPP elicited increased expression and activation of Fas and Fas-associated death domain and led to β-cell apoptosis (41). Anti-Fas/FasL antibodies blocked the apoptotic effects of hIAPP, further implicating this mechanism in hIAPP induction of apoptosis. Interestingly, the Fas/FasL antagonist, Kp7-6, also blocked hIAPP-induced apoptosis and was also found to be an inhibitor of hIAPP fibrillogenesis, thus providing a further linkage between amyloidogenesis and the toxic and apoptotic effects of hIAPP. Although currently it appears that hIAPP toxic oligomers form intracellularly, it is possible that they may transit the secretory pathways or otherwise arrive in the extracellular compartment and initiate the apoptotic signaling cascades in that fashion; there is still much to be learned in this area.

The studies noted above provide potential mechanisms whereby IAPP amyloidogenesis may damage β-cells and ultimately lead to apoptosis and diminished β-cells in type 2 diabetes. However, factors that might trigger this cascade of IAPP fibrillogenesis are largely unknown. A role for increased expression and synthesis of IAPP in amyloidogenesis has long been suggested as a likely participating factor due to the tendency for parallel regulation of insulin and IAPP (42,43). Although insulin and IAPP tend to be regulated in parallel, such as their upregulation in insulin resistance, significant divergence from this parallel expression may occur under some physiologic or pathologic conditions that may play roles in amyloidogenesis (43). Marked hyperglycemia and corticosteroids have both been shown to result in disproportionate upregulation of IAPP versus insulin, thus altering the ratio of IAPP to insulin secreted by the β-cells. Further support for a role in increased IAPP expression in IA development is found in studies of human IAPP transgenic mice. IA formation occurs in these mice only under conditions of high IAPP expression due to the presence of high hIAPP gene copy number, due to upregulation of IAPP expression by exposure to hormones or diet that induce insulin resistance, or by breeding genetic obesity into hIAPP transgenic mice (44–46). However, the mechanisms coupling the increased hIAPP synthetic/secretory demand in β-cells with initiation of amyloidogenesis are currently unknown but may involve events that trigger ER stress. β-cells, like other "professional" secretory cells, are known to be particularly sensitive to perturbations of ER function that may lead to misfolded proteins (47). In the case of increased hIAPP synthesis, such an event could allow generation of toxic oligomers with subsequent triggering of the ER stress response that includes *1*) early and transient suppression of protein synthesis; *2*) activation of genes encoding components of the ER protein translocation, folding, secretion, and degradation machinery; and *3*) induction of programmed cell death. Precisely such responses, which included upregulation of C/EBP homologous protein (CHOP) expression followed by nuclear translocation and apoptosis, were demonstrated recently in INS-1 cells induced to express hIAPP (48). INS-1 cells similarly transfected with rIAPP or GFP showed no such responses. Likewise, β-cells in HIP rats (transgenic for hIAPP) showed similar upregulation of caspase-12 and CHOP in β-cells and increased apoptosis in comparison to wild-type rats. Finally, in human β-cells, perinuclear CHOP expression was found to be more frequent in obese type 2 diabetic patients than obese or lean nondiabetic individuals, and nuclear CHOP was significantly more common in obese diabetic patients than in either obese or lean nondiabetic patients (49). These findings strongly implicate misfolding of IAPP in the ER of β-cells in the pathogenesis of type 2 diabetes.

IAPP amyloidogenesis as cause of type 2 diabetes? As noted above, there is now general consensus that development of type 2 diabetes in humans is associated with a significant decrease of β-cells. A recent study found that obese patients with type 2 diabetes exhibited a 60% reduction in β-cell volume, while obese patients with impaired fasting glucose (IFG) exhibited a 40% reduction in β-cell volume as compared with nondiabetic obese control subjects (16). Interestingly, ~88% of the patients with type 2 diabetes and 10% of those with IFG had detectable IA deposits. Similarly, in spontaneous animal models of diabetes in macaques, domestic cats, and HIP

rats, development of IA is detectable in pre-diabetic animals and corresponds to β-cell functional decline and loss of β-cells. In an elegant longitudinal study of *Macaca nigra*, Howard evaluated β-cell function and, using serial pancreatic biopsies, evaluated islet morphology, including IA formation (49). He found that monkeys with mild IA also had mild decrements in glucose clearance and incremental insulin response and that development of diabetes was associated with relatively severe IA. Thus, there is a strong association between development of IA and the development of impaired β-cell function and β-cell loss. However, the amount of IA detectable in the islets does not always correlate well with the degree of β-cell loss, as seen in the human patients with IFG (16). This may be interpreted to mean that IAPP amyloidogenesis is not causative but merely follows as a secondary phenomenon. An alternative interpretation based on the current understanding of hIAPP amyloidogenesis would be that a correlation with detectable (mature) IA deposits per se is not necessary (or even expected) since the toxic components of this process are the early soluble oligomers that may or may not proceed to form mature amyloid fibrils. The soluble oligomers are not detectable by Congo red or other commonly used amyloid stains. Thus, detectable IA deposits may, in fact, be of relatively less pathologic importance and may indeed be a secondary phenomenon. It was recently shown that the fibrillar inclusions formed by mutant huntingtin in Huntington's disease neurons were protective rather than detrimental to the cells in which they occurred (50). Neurons that failed to form inclusions were more susceptible to cell death. A similar situation may occur in β-cells in which generation of toxic oligomers leads to cell death, whereas formation of mature amyloid fibrils may be a late indicator of the process, and their formation may even be protective.

The evidence cited above presents considerable evidence for a role of hIAPP amyloidogenesis in β-cell injury and induction of programmed cell death. But why doesn't the proliferative response of the endocrine pancreas replenish the damaged β-cells? If much of the regenerative capacity of the β-cells comes from the existing β-cells, the explanation may then be related to increased susceptibility of cells undergoing cell division to the effects of cytotoxic insults, such as hIAPP toxic oligomers. In support of this hypothesis, it has been found that RIN and HeLa cells, when exposed to hIAPP toxic oligomers, had increased apoptosis within 3 h of mitosis (51). Thus, the β-cell deficiency in type 2 diabetes may, at least partially, result from a failure to adaptively increase β-cell mass due to the increased vulnerability of replicating β-cells to apoptosis.

It can now be seen that the morphologic and molecular clues to diabetes that were sought by Dr. Turner and many others, and provided by IA and IAPP, have led us down many interesting pathways that now also appear to be leading us ever closer to an understanding of the molecular pathogenesis of type 2 diabetes.

REFERENCES

1. Turner RC: The U.K. Prospective Diabetes Study: a review. *Diabetes Care* 21 (Suppl. 3):C35–C38, 1998
2. Orci L, Unger RH: Functional subdivision of islets of Langerhans and possible role of D-cells. *Lancet* 2:1243–1244, 1975
3. Erlandsen SL, Hegre OD, Parsons JA, McEvoy RC, Elde RP: Pancreatic islet cell hormones distribution of cell types in the islet and evidence for the presence of somatostatin and gastrin within the D cell. *J Histo Cyto* 24:883–897, 1976
4. Grube D, Eckert I, Speck PT, Wagner HJ: Immunohistochemistry and microanatomy of the islets of Langerhans. *Biomed Res* 4 (Suppl.):25–36, 1983
5. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A: The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A* 103:2334–2339, 2006
6. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC: Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 53:1087–1097, 2005
7. Orci L: The microanatomy of the islets of Langerhans. *Metabolism* 25:1303–1313, 1976
8. Toreson WE: Glycogen infiltration (so-called hydropic degeneration) in the pancreas of human and experimental diabetes mellitus. *Am J Pathol* 27:327–347, 1951
9. Gepts W, In't Veld PA: Islet Morphologic Changes. *Diab Metab Rev* 3:859–872, 1987
10. Stefan Y, Grasso S, Perrelet A, Orci L: The pancreatic polypeptide-rich lobe of the human pancreas: definitive identification of its derivation from the ventral pancreatic primordium. *Diabetologia* 23:141–142, 1982
11. Rahier J, Goebbels RM, Henquin JC: Cellular composition of the human diabetic pancreas. *Diabetologia* 24:366–371, 1983
12. Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S: Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese type II diabetic patients. *Diabetologia* 45:85–96, 2002
13. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU: Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* 4:110–125, 1985
14. Rahier J, Wallon J, Loozen S, Lefevre A, Gepts W, Haot J: The pancreatic polypeptide cells in the human pancreas: the effects of age and diabetes. *J Clin Endocrinol Metab* 56:441–444, 1983
15. Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim DG, Lee IK, Bonner-Weir S: Selective β-cell loss and a-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endoc Metab* 88:2300–2308, 2003
16. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
17. Maclean N, Ogilvie RF: Quantitative estimation of the pancreatic islet tissue in diabetic subjects. *Diabetes* 4:367–376, 1955
18. Saisho Y, Butler AE, Meier JJ, Monchamp T, Allen-Auerbach M, Rizza RA, Butler PC: Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. *Clin Anat* 20:933–942, 2007
19. Ogilvie RF: The islands of Langerhans in 19 cases of obesity. *J Pathol Bacteriol* 37:473–481, 1933
20. Bonner-Weir S: Islet growth and development in the adult. *J Mol Endocrinol* 24:297–302, 2000
21. Guiot Y, Sempoux C, Moulin P, Rahier J: No decrease of the beta-cell mass in type 2 diabetic patients (Abstract). *Diabetes* 50 (Suppl. 1):S188, 2001
22. Clark A, Jones LC, de Koning E, Hansen BC, Matthews DR: Decreased insulin secretion in type 2 diabetes: a problem of cellular mass or function? (Abstract). *Diabetes* 50 (Suppl. 1):S169–S171, 2001
23. Saito K, Yaginuma N, Takahashi T: Differential volumetry of A, B, and D cells in the pancreatic islets of diabetic and non-diabetic subjects. *Tohoku J Exp Med* 129:273–283, 1979
24. Jonas JC, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC: Chronic hyperglycemia triggers loss of pancreatic β cell differentiation in an animal model of diabetes. *J Biol Chem* 274:14112–14121, 1999
25. LeCompte PM, Merriam JC: Mitotic figures and enlarged nuclei in the islands of Langerhans in man. *Diabetes* 2:35–39, 1962
26. Lorenzo A, Razzaboni B, Weir GC, Yankner BA: Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* 368:756–760, 1994
27. Johnson KH, O'Brien TD, Westermark P: Medical intelligence: islet amyloid, islet amyloid polypeptide and diabetes mellitus. *N Engl J Med* 321:513–518, 1989
28. Westermark P, Wernstedt C, Heldin C-H, Wilander E, Hayden DW, O'Brien TD, Johnson KH: Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a novel neuropeptide-like protein also present in normal islet cells. *Proc Natl Acad Sci U S A* 84:3881–3885, 1987
29. Cooper GJS, Willis AC, Clark A, Turner RC, Sim RB, Reid KBM: Purifica-

tion and characterization of a peptide from amyloid-rich pancreata of type 2 diabetic patients. *Proc Natl Acad Sci U S A* 84:8628–8632, 1987

30. Butler PC, Chou J, Carter WB, Wang YN, Bu BH, Chang D, Chang JK, Rizza RA: Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. *Diabetes* 39:752–756, 1990

31. Mirzabekov TA, Lin M-C, Kagan BL: Pore formation by the cytotoxic islet amyloid peptide amylin. *J Biol Chem* 271:1988–1992, 1996

32. Tucker HM, Rydel RE, Wright S, Estus S: Human amylin induces "apoptotic" pattern of gene expression concomitant with cortical neuronal apoptosis. *J Neurochem* 71:506–516, 1998

33. Janson J, Ashley RH, Harrison D, McIntyre S, Butler PC: The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. *Diabetes* 48:491–498, 1999

34. O'Brien TD, Butler PC, Kreutter DK, Kane LA, Eberhardt NL: Intracellular amyloid associated with cytotoxicity in COS-1 cells expressing human islet amyloid polypeptide. *Am J Pathol* 147:609–616, 1995

35. Mattson MP, Goodman Y: Different amyloidogenic peptides share a similar mechanism of neurotoxicity involving reactive oxygen species and calcium. *Brain Res* 676:219–224

36. Mark RJ, Pang Z, Geddes JW, Uchida K, Mattson MP: Amyloid beta-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation. *J Neurosci* 17:1046–1054, 1997

37. Brender JR, Dürr UHN, Heyl D, Budarapu MB, Ramamoorthy A: Membrane fragmentation by an amyloidogenic fragment of human islet amyloid polypeptide detected by solid-state NMR spectroscopy of membrane nanotubes. *Biochim Biophys Acta* 1768:2026–2029, 2007

38. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG: Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300:486–489, 2003

39. Lin C-Y, Gurlo T, Kayed R, Butler AE, Haataja L, Glabe CG, Butler PC: Toxic human islet amyloid polypeptide (h-IAPP) oligomers are intracellular, and vaccination to induce anti-toxic oligomers antibodies does not prevent h-IAPP-induced β-cell apoptosis in h-IAPP transgenic mice. *Diabetes* 56:1324–1332, 2007

40. Zhang S, Liu J, Dragunow M, Cooper GJS: Fibrillogenic amylin evokes islet β-cell apoptosis through linked activation of a caspase cascade and JNK1. *J Biol Chem* 278:52810–52819, 2003

41. Zhang S, Liu H, Yu H, Cooper GJ: Fas-associated death receptor signaling evoked by human amylin in islet β-cells. *Diabetes* 57:348–356, 2008

42. Fehmann HC, Weber V, Goke R, Goke B, Arnold R: Co-secretion of amylin and insulin from isolated rat pancreas. *FEBS Lett* 262:279–281, 1990

43. O'Brien TD, Westermark P, Johnson KH: Islet amyloid polypeptide and insulin secretion from isolated perfused pancreas of fed, fasted, glucose-treated, and dexamethasone-treated rats. *Diabetes* 40:1701–1706, 1991

44. Couce M, Kane L, O'Brien TD, Kreutter D, Soeller W, Butler PC: Induction of insulin resistance in mice transgenic for human islet amyloid polypeptide causes islet amyloidosis and beta cell dysfunction. *Diabetes* 45:1094–1101, 1996

45. Soeller WC, Janson J, Hart SE, Parker JC, Carty MD, Stevenson RW, Kreutter DK, Butler PC: Islet amyloid-associated diabetes in obese A(vy)/a mice expressing human islet amyloid polypeptide. *Diabetes* 47:743–750, 1998

46. Hull RL, Andrikopoulos S, Verchere CB, Vidal J, Wang F, Cop M, Prigeon RL, Kahn SE: Increased dietary fat promotes islet amyloid formation and β-cell secretory dysfunction in a transgenic mouse model of islet amyloid. *Diabetes* 52:372–379, 2003

47. Harding HP, Ron D: Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes* 51 (Suppl. 3):S455–S461, 2002

48. Huang C-J, Lin C-Y, Haataja L, Gurlo T, Butler AE, Rizza RA, Butler PC: High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress-mediated β-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. *Diabetes* 56:2016–2027, 2007

49. Howard CF, Jr: Longitudinal studies on the development of diabetes in individual *Macaca nigra*. *Diabetologia* 29:301–306, 1986

50. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S: Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431:805–810, 2004

51. Ritzel RA, Butler PC: Replication increases β-cell vulnerability to human islet amyloid polypeptide-induced apoptosis. *Diabetes* 52:1701–1708, 2003