β-Cell Neogenesis in Type 2 Diabetes

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Type 2 diabetes is associated with inappropriate insulin secretion at diagnosis; whether this is due to a decrease in β-cell mass is unclear. Morphological studies of postmortem specimens have demonstrated a relatively small change or no change in the population of β-cells, with the most dramatic change being a 30% reduction (1–3). Some of the reduction in mass of β-cells arises from the destructive effects of islet amyloid fibrils (4). However, it remains to be shown if type 2 diabetes is associated with quantitative or qualitative (functional) changes in islet β-cells.

The human fetal endocrine pancreas develops from 10 weeks of gestation, with cells differentiating from pancreatic ductal stem cells (5,6). These cells are the precursors for both endocrine and exocrine cells; differentiating signals are associated with specific transcription factors (7,8). Differentiation of endocrine cells from the ductal epithelium continues throughout fetal life and is present in the neonate and infant, as shown by immunohistochemical labeling (6,9), but the number of ductal endocrine cells is reduced in the normal adult human pancreas (10). Ductal proliferation is associated with pancreatic disease, such as carcinoma and pancreatitis, when increased numbers of ductal endocrine cells are observed (11). In rodents and other animal models, the process of endocrine cell ontogeny is similar, but evidence is accumulating that differentiation of β-cells from ducts is increased in diabetes induced by partial pancreatectomy, and these new cells appear to ameliorate hyperglycemia (12,13). This suggests that the islet cell population is less fixed than previously supposed and that changes in cellular neogenesis could be associated with diabetes. To determine if there is regenerative capacity in human pancreatic duct cells in diabetes, postmortem specimens were examined by quantitative immunohistochemistry.

RESEARCH DESIGN AND METHODS

Human pancreatic tissue was obtained from nine subjects diagnosed with type 2 diabetes and nine similarly aged nondiabetic subjects (40–79 years). Permission from the local ethics committee and consent from the relatives was obtained. Postmortem examination was performed within 21 h of death (mean 8 h), and specimens were taken from the midbody region of the pancreas in all of the cases. Tissue was fixed in a 10% buffered formaldehyde and embedded in paraffin wax. Sections (5 μm thick) were cut from each block and immunolabeled using immunocytochemical techniques. Antigens were retrieved by incubating dewaxed slides at 120°C for 2 min in a pressure cooker in 0.01 mol/l citrate buffer. Exocrine ductal cells were identified with a mouse monoclonal antibody to cytokeratin19 (CK19) (Dako, Ely, U.K.). β-Cells were identified with guinea pig anti-insulin (ICN, Thame, U.K.). Antibody binding was visualized with biotinylated strepdavidin-HRP complex (CK19; Dako) or alkaline phosphatase (insulin; Dako). Exocrine fibrosis as a marker of chronic pancreatitis was identified with Van Gieson's stain and islet amyloid deposits with Congo red. Morphometric analyses were made on stained sections at a magnification of 200×, and 10 fields of the section were randomly selected. Quantitative analyses of stained areas and numbers of ductal endocrine cells were made with the semiautomatic IBAS Ks400 system (Kontron, Messergereate, Germany). The following parameters were assessed: CK19-positive ductal cell area, total area of insulin-positive cells, and the area of ductal insulin-positive cells. Morphometric data was expressed as a ratio to the pancreatic area examined. Statistical comparisons were made with the nonparametric Mann-Whitney U test for nonpaired data; $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Insulin-positive cells were found in CK19-positive ductular epithelium in both diabetic and nondiabetic subjects. The area density of ductal insulin cells was significantly greater in diabetic subjects ($P = 0.02$) compared with the nondiabetic tissue, but the area of insulin-positive cells was similar in the two groups (mean 3.4% of diabetic tissue, 3.1% of nondiabetic tissue) (Table 1). This increase was unrelated to the degree of fibrosis, which was significantly higher in the diabetic subjects (4.7% of diabetic tissue, 2.3% of nondiabetic tissue; $P < 0.01$) or to the degree of islet amyloidosis (Table 1). Insulin-positive cells situated in CK19-labeled ducts indicated that endocrine cells were newly differentiating from ductal stem cells, as has been described in the human fetal pancreas (6). These cells are found less frequently in the adult human pancreas (10), suggesting that there is little neogenesis of β-cells in adult humans. Ductal cells have also been shown to be mitotically active in fetal and adult tissue, indicating the location of stem cells for islet development, but mature islets have a very low mitotic index and are therefore unlikely to play a role in the production of new insulin-positive cells (6,14). The current data suggest that there is a greater stimulus for differentiation of new β-cells in type 2 diabetes compared with that in nondiabetic subjects, but the nature of the stimulus is unclear.

Chronic subclinical pancreatitis is a feature of type 2 diabetes (15) and could result in progressive erosion of the exocrine tissue and pancreatic fibrosis. Ductal hyperplasia and neogenesis of insulin-positive cells within and adjacent to ductular epithelium is seen in animal models of pancreatitis, such as duct-ligated rats (14). These endocrine cells, as in fetal tissue, extend into the exocrine parenchyma and have the appearance of neogenic islets. No such islet cell clusters were seen in type 2 diabetic tissue. The association of increased fibrosis and β-cell neogenesis suggests that

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CK19, cytokeratin19.

TABLE 1
Cell area density in diabetic and nondiabetic study subjects

	Fibrosis (%)	CK19 area density	Ductal β-cell area density ($\times 10^{-3}$)	β-Cell area density (ratio, μm^2)	Islet amyloid (% islet area)
Diabetic subjects					
1	5.9	0.04	0.03	0.06	8.7
2	6.2	0.08	0.13	0.04	0.3
3	3.7	0.02	0.17	0.03	53
4	2.7	0.08	0.21	0.05	13.8
5	3.6	0.07	0.08	0.04	0
6	2.7	0.03	0.08	0.02	18
7	4.1	0.05	0.13	0.02	12.3
8	9.9	0.03	0.03	0.03	4.3
9	3.7	0.04	0.08	0.02	10
Mean	4.7	0.05	0.1	0.034	13.4
Nondiabetic subjects					
1	3.3	0.06	0	0.03	0
2	3.3	0.08	0.02	0.02	0
3	2.4	0.07	0	0.05	0
4	2.3	0.09	0.02	0.03	0
5	2.3	0.10	0	0.05	0
6	0.8	0.07	0	0.03	0
7	1.5	0.06	0.02	0.02	0
8	ND	0.13	0	0.03	0
9	1.2	0.15	0	0.05	0
Mean	2.3	0.09	0.006	0.031	0

ND, no data; the patient was not included in the analysis.

chronic exocrine disease may be the stimulus for ductal differentiation of endocrine cells in type 2 diabetes.

In conclusion, these new data confirm that the total density of insulin-immunoreactive cells is not different in type 2 diabetes, suggesting that the secretory defect results from dysfunction rather than quantitative cellular changes. β-Cell neogenesis could be related to low-grade pancreatitis in diabetes in humans.

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