Prior streptozotocin treatment does not inhibit pancreas regeneration after 90% pancreatectomy in rats

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1Elliott P. Joslin Research Laboratories, Joslin Diabetes Center, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215; and 2Diabetes Research Laboratory, School of Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Finegood, Diane T., Gordon C. Weir, and Susan Bonner-Weir. Prior streptozotocin treatment does not inhibit pancreas regeneration after 90% pancreatectomy in rats. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E822–E827, 1999.—The effects of residual β-cell mass and glycemia on regeneration of endocrine pancreas after 90% pancreatectomy were investigated. Streptozotocin or buffer alone was injected into 4-wk-old male Lewis rats (day 0). On day 7, varying numbers of syngeneic islets were transplanted under the kidney capsule to achieve varying degrees of glucose normalization. On day 14, a 90% pancreatectomy or sham pancreatectomy was performed. On day 19, rats were killed and the pancreas was fixed for quantitative morphometric determination of β-cell mass. Focal areas of regenerating pancreas were observed in all animals that underwent partial pancreatectomy. The percentage of remnant pancreas classified as foci was unaffected by streptozotocin treatment or by plasma glucose. Moderate to severe hyperglycemia did not promote regeneration of the pancreatic β-cell mass; rather the total endocrine cell mass was inversely related to the plasma glucose level ($r = -0.5$, $P < 0.01$). These data suggest that the precursor population for both endocrine and exocrine tissue is not susceptible to damage by streptozotocin and that local effects of residual β-cell mass are not important for regeneration after a 90% pancreatectomy.

islets of Langerhans; precursor cells

SUBSTANTIAL REGENERATION of both exocrine and endocrine tissue occurs after a 90% partial pancreatectomy in the young adult rat (4, 7). Regeneration occurs through two pathways: 1) replication of preexisting differentiated exocrine and endocrine cells and 2) proliferation and differentiation of ductal epithelial cells to form new pancreatic lobules (2, 4, 7). The latter is seen as focal areas of regeneration that consist of duct-like “tubular complexes,” or small ductules surrounded by loose connective tissue. Cells that immunostain for glucagon appear early in the development of these new lobules. As development progresses, cells that immunostain for insulin appear as new islets, as small aggregates of cells, and as single cells in ductules. In the final stages of regeneration, exocrine cells differentiate and form acini, the lumens of the terminal ductules decrease, and the normal morphology of a pancreatic lobe is assumed.

The origin of the tubular complexes has been a matter of controversy. Some investigators suggest they arise from dedifferentiation of acinar tissue (9, 10, 28), but there is also strong evidence from our own laboratory to suggest that the ductular profiles can be the result of a regenerative process originating from the ductal tree (2). We demonstrated that the regeneration begins with increased proliferation of the common pancreatic duct at 24–36 h, followed by increased proliferation of the main ducts at 36–48 h and increased proliferation of small ducts at 48 h postpancreatectomy. Areas of proliferating small ductules called focal areas of regeneration have a high proliferative rate through 72 h. These data also provide strong evidence to suggest that both the endocrine and exocrine tissues of the focal areas arise from pluripotent precursor cells. In support of the pluripotent precursor hypothesis are demonstrations that β-cell specific genes, such as GLUT-2 (18) and the homeodomain transcription factor Pdx-1 (16), are expressed in ductal epithelial cells of the developing embryo. Whether the precursor cells of the normal adult animal have sufficient β-cell-specific characteristics to be susceptible to the β-cell toxin streptozotocin (STZ) has not been investigated.

Various hormones and growth factors have been shown to affect the proliferation of the ductal epithelium and differentiation into endocrine and exocrine cell types, including transforming growth factor (TGF-α) (1, 27), gastrin (26, 27), TGF-β (5, 21), epidermal growth factor (1, 24), hepatocyte growth factor-scatter factor (17), and insulin-like growth factor I (22). It is not known, however, whether metabolic factors such as glucose play a role. Glucose is a well-known stimulus for proliferation of preexisting β-cells (3), but its role in endocrine and/or exocrine cell neogenesis is unclear. Insulin is also a well-known growth factor, and the mass of β-cells is thought to have a trophic effect on the exocrine pancreas via a local islet acinar portal circulation (19). Thus both glucose and insulin could influence the magnitude of the regenerative response of endocrine and/or exocrine tissue after partial pancreatectomy. The purpose of this study was twofold: to determine if a massive depletion of endogenous β-cells influences the regenerative response to a 90% pancreatectomy and to determine the susceptibility of ductal precursor cells to STZ.

MATERIALS AND METHODS

Experimental animals. Male Lewis rats (Harlan Sprague Dawley, Indianapolis, IN) were used as both recipients and islet donors. Rats were housed in a temperature-controlled room with a 12:12-h light-dark cycle. All animals had continuous access to standard rat chow and water throughout the...
study. All procedures were approved by the institutional animal care committee.

After a few days of acclimation, rats (4 wk of age, −100 g body wt) were randomly assigned to one of four experimental groups: 1) STZ treatment, islet transplant, and partial pancreatectomy (n = 19); 2) sham STZ, islet transplant, and partial pancreatectomy (n = 8); 3) STZ, islet transplant, and sham partial pancreatectomy (n = 5); and 4) STZ and partial pancreatectomy (n = 6). Young rats were used to facilitate the partial pancreatectomy procedure. Body weight and plasma glucose levels were recorded on days 0, 3, 7, 11, 14, 17, and 19, where day 0 is the day of the initial STZ administration.

STZ (Sigma, St. Louis, MO) was freshly dissolved in sodium citrate buffer (pH 4.5) at a concentration of 35 mg/ml. STZ was administered intraperitoneally as one (on day 0) or two (days 0 and 3) doses of 70–90 mg/kg. Citrate buffer alone was injected in controls (group 2). Animals not achieving hyperglycemia (plasma glucose >14 mM) after the first dose of STZ were administered a second dose on day 3. Plasma glucose was checked again on day 7, and only animals with a plasma glucose >14 mM were retained in the study. Plasma glucose determinations were made on tail vein blood samples with a Beckman Glucose Analyzer II.

Islets were isolated from donor rats (weighing 180–230 g) with previously described methods (14). Freshly isolated islets were transplanted under the kidney capsule of recipient rats on day 7 after the initial administration of STZ (14). To achieve different degrees of glucose normalization, variable numbers of 150-µm islet equivalents were used (group 1: 857 ± 87, range 127–1,526; group 2: 931 ± 98, range 704–1,526; group 3: 984 ± 113, range 704–2,171 islet equivalents; P = 0.72 for the difference among groups).

On day 14 after diabetes induction (7 days after the islet transplantation), rats in groups 1, 2, and 4 underwent a 90% partial pancreatectomy (4). Rats in group 3 underwent a sham partial pancreatectomy.

Immunocytochemistry. On day 19 (5 days after the partial pancreatectomy), the rats were killed with an overdose of amobarbital sodium and the pancreas or pancreatic remnant was excised, lightly blotted, weighed, and placed in Bouin's fixative. Paraffin sections (4–6 µm) of these tissues were lightly blotted, weighed, and placed in Bouin's fixative. Paraffin sections (4–6 µm) of these tissues were dehydrated, embedded in paraffin, and 4-µm sections were cut and mounted on glass slides. Sections were stained with a cocktail of antibodies (anti-glucagon, anti-somatostatin, and anti-insulin) and counterstained with hematoxylin.

RESULTS

Body weight. Body weights for each group at baseline, after STZ treatment, islet transplantation, and 90% partial pancreatectomy are given in Table 1. Body weight increased after STZ or buffer injection, after islet transplantation, and after partial pancreatectomy or sham operation in all four groups (P < 0.05), except for group 4 where there was a small but significant weight loss after the partial pancreatectomy (P = 0.04). Although there were small differences in body weight among groups at the beginning of the study and as a result of the STZ treatment (P < 0.05), recovery after the transplant procedure resulted in no differences among groups before the pancreatectomy.

Plasma glucose. Before STZ administration there was no difference in fed-state plasma glucose among groups of animals (Table 1). At 1 wk after STZ treatment, plasma glucose was significantly elevated in STZ-treated rats (22.6 ± 0.9 mM, P = 0.0001). Unexpectedly, there was also a small but significant rise in the plasma glucose level in rats receiving only citrate buffer (group 2, P = 0.003). Transplantation of islets under the kidney capsule resulted in a significant decrease in plasma glucose levels in diabetic rats (groups 1 and 3 combined: from 23.9 ± 0.8 to 15.0 ± 1.2 mM, P = 0.0001, range of decrement: 2.1–19.2 mM). Transplantion of islets into non-STZ-treated rats had no effect on their plasma glucose level (group 2, P = 0.10). There was also no change in the glucose level in rats not receiving a transplant (group 4, P = 0.14).

The plasma glucose remained stable after partial pancreatectomy in groups (1, 2, and 3) that received a transplant before the surgical resection of the pancreas or sham partial pancreatectomy. In group 4 (rats without a transplant), plasma glucose levels increased as a result of the pancreatectomy (P = 0.001). Because the group receiving islets after STZ injection (group 1)...

Table 1. Fed-state body weight and plasma glucose at baseline and after STZ treatment, islet transplantation, and 90% partial pancreatectomy

<table>
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<th>Group</th>
<th>Baseline</th>
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<td>Body weight, g</td>
<td>Plasma glucose, mM</td>
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<td>Group 1</td>
<td>104 ± 2*</td>
<td>150 ± 3*</td>
<td>189 ± 3</td>
<td>197 ± 4***</td>
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<td>Group 2</td>
<td>98 ± 5†</td>
<td>159 ± 4*</td>
<td>199 ± 5</td>
<td>205 ± 5*</td>
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<tr>
<td>Group 3</td>
<td>99 ± 5*</td>
<td>142 ± 4*</td>
<td>184 ± 5</td>
<td>211 ± 3†</td>
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<td>Group 4</td>
<td>89 ± 2t</td>
<td>128 ± 3t</td>
<td>184 ± 6</td>
<td>178 ± 8t</td>
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<tr>
<td>Group 1</td>
<td>8.1 ± 0.1</td>
<td>24.3 ± 0.9*</td>
<td>16.4 ± 1.3*</td>
<td>14.2 ± 1.6*</td>
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<tr>
<td>Group 2</td>
<td>7.7 ± 0.1</td>
<td>8.2 ± 0.2t</td>
<td>7.7 ± 0.1*</td>
<td>7.5 ± 0.1†</td>
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<tr>
<td>Group 3</td>
<td>8.2 ± 0.3</td>
<td>22.4 ± 1.7†</td>
<td>9.8 ± 0.7†</td>
<td>8.2 ± 0.2†</td>
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<tr>
<td>Group 4</td>
<td>7.6 ± 0.2</td>
<td>17.2 ± 2.4†</td>
<td>21.1 ± 2.7†</td>
<td>23.8 ± 0.7*</td>
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Data are means ± SE. Group 1: streptozotocin (STZ), islet transplant (Tx), and partial pancreatectomy (Px), n = 19; group 2: Tx and Px, n = 8; group 3: STZ and Tx, n = 5; group 4: STZ and Px, n = 6. Differences between groups at each time were determined by Tukey’s post hoc test; groups with different means (P < 0.05) have a different symbol. For example, at baseline, body wt for groups 1 and 4 is different, but group 1 is not different from group 2 or 3, and group 4 is not different from groups 2 or 3. Nos. in parentheses, control population for particular procedure.
received a large range of islet equivalents, there was a wide range in plasma glucose concentration after partial pancreatectomy. Fed-state glucose levels in these animals at the time they were killed ranged from 6.8 to 26.1 mM and were inversely correlated with the number of islet equivalents transplanted ($r = -0.49, P = 0.03$).

Exocrine and endocrine cell mass. There were no differences in the weight of the remnant pancreas among groups at 5 days after partial pancreatectomy (Fig. 1). The average weight of the remnants was 21% of the weight of the pancreas in the group that received the sham pancreatectomy. As expected, there were no areas characteristic of pancreatic regeneration in group 3 (STZ and islet transplant, but no partial pancreatectomy). In all other groups, focal regeneration was observed regardless of whether the animals were pretreated with STZ or not (Fig. 1). Focal areas of regeneration comprised 0.2–27.9% of the pancreatic mass; this percentage did not differ among groups that underwent partial pancreatectomy (Fig. 1).

Endocrine tissue, both $\beta$-cells and non-$\beta$-cells, identified by positive staining for glucagon, somatostatin, and pancreatic polypeptide was found in the focal areas of regenerating tissue in 13 of the 25 animals that received STZ (52% total; group 1: 10 of 19; group 4: 3 of 6) and in four of eight animals that were not pretreated with STZ (50%). As expected, the total mass of non-$\beta$-cell endocrine tissue was significantly greater in the animals not subjected to a partial pancreatectomy (Fig. 1). There was also no relationship between the mass of the focal area and the degree of hyperglycemia in animals receiving both STZ and a partial pancreatectomy (data not shown). Similarly, the mass of endocrine tissue in the focal regions was not related to the plasma glucose level ($P = 0.63$, data not shown).

Role of STZ treatment and hyperglycemia in regeneration. Although focal areas of pancreatic regeneration were found in all of the animals that underwent a partial pancreatectomy, there were no differences among groups 1, 2, and 4 in the weight of the remnant pancreas, the mass of the focal area, or the mass of the non-$\beta$-cell endocrine tissue. This lack of difference was present despite a significant difference in the $\beta$-cell mass of the remnant in those animals that received STZ compared with the non-STZ-treated group (group 2: 0.31 ± 0.09 vs. group 1: 0.11 ± 0.02 or group 4: 0.06 ± 0.03%, $P < 0.05$).

Fig. 2. Top: $\beta$-cell mass of pancreas in groups 1-4. Bottom: non-$\beta$-cell endocrine mass in groups 1-4. $\beta$-cell and non-$\beta$-cell mass was determined by immunocytochemical staining with a cocktail of antibodies to glucagon, somatostatin, and pancreatic polypeptide. Data are means ± SE. Bars with different letters are significantly different by post hoc Tukey’s Studentized range test at 0.05 level.

Fig. 1. Top: weight of pancreas (endocrine tissue, exocrine tissue, and focal areas of regeneration) in groups 1-4. Bottom: focal areas of regeneration as a percentage of remnant pancreas weight in groups 1-4. Group 1, n = 19; group 2, n = 8; group 3, n = 5; group 4, n = 6. Data are means ± SE. Bars with different letters are significantly different by post hoc Tukey’s Studentized range test at 0.05 level. STZ, streptozotocin; Tx, islet transplant; Px, partial pancreatectomy.
focal regions between animals with and without endocrine tissue in the focal areas (Table 2). Although the prevailing plasma glucose level was different in groups 1, 2, and 4 (Table 1), there was no difference in the plasma glucose level between rats with positive non-β-hormone staining in focal areas of regeneration compared with rats without non-β-hormone staining in foci when group was considered a factor (2-way ANOVA; P = 0.77). The difference in the mass of the focal regions between animals with and without endocrine tissue in the focal areas remained significant when group was considered a factor (2-way ANOVA; P = 0.0002).

The mass of endocrine tissue in the entire remnant was inversely proportional to the post-partial pancreatectomy plasma glucose level (Fig. 3, r = −0.50, P = 0.01). Although groups 1 and 4 had identical pancreatic procedures, group 4 had much higher plasma glucose levels and significantly less endocrine cell mass in the remnant pancreas than normoglycemic group 1 animals that were treated with STZ (plasma glucose >26 mM: 0.29 ± 0.10 vs. plasma glucose <9.6 mM: 0.96 ± 0.23 mg, P < 0.05). Because of the large variation in plasma glucose levels among the rats in group 1, there was insufficient power to detect a difference in either β-cell or non-β-endocrine cell mass between groups 1 and 4 (Fig. 2).

**DISCUSSION**

The effects of a 90% partial pancreatectomy on pancreatic regeneration have been well described (2, 4, 7). Increased proliferation is initially seen in the common pancreatic duct, then subsequently in smaller ducts, followed by increased proliferation in the ducts in focal areas of the regenerating tissue. This suggests that the ductal tree expands by proliferation from the common pancreatic duct outward, making new lobes of the ductal tree, and that the tubular complexes, which are characteristic of the focal areas of regeneration, arise from ductal precursors. Also in support of the ductular origin of the regenerating tissue is the demonstration by Bouwens et al. (6) that cytokeratins, which are specifically expressed in ductal cells but not acinar cells of the normal rat pancreas, are also expressed in cells in the focal areas of regeneration after partial pancreatectomy. The possibility that the ductal precursors are pluripotent is suggested by the observation of cells with various endocrine and hepatocyte markers in ducts and regenerating islets of interferon-γ transgenic mice (12) and the presence of both exocrine and endocrine cells in the focal areas of regeneration after pancreatectomy (2). Embryologically, ductal progenitor cells are known to express various proteins that are relatively specific to the β-cell, such as GLUT-2 (18), Pdx-1 (16), and glutamic acid decarboxylase (12).

In this study, we demonstrate that treatment with the β-cell toxin STZ sufficient to induce diabetes does not inhibit pancreatic regeneration after a 90% partial pancreatectomy. Both endocrine and exocrine cell types were found in focal areas of regeneration, and there were no qualitative or quantitative differences in regeneration between animals pretreated with STZ and those that were not given STZ. These data suggest that the precursor cell population is not susceptible to damage by STZ. This lack of susceptibility may be due to the fact that the cells do not express sufficient β-cell characteristics, e.g., the expression of GLUT-2 is too low, or they lack other essential characteristics that make the mature β-cell particularly susceptible to toxins such as STZ or alloxan (13). Our results are consistent with the recent reports by Movassat et al. (15) and by Wang and colleagues (25). Wang et al. found that treatment of newborn rats with 100 µg/g of STZ dramatically reduced the number of β-cells in islets but had little effect on the number of β-cells found in aggregates of less than six endocrine cells. Presumably, these small aggregates are neogenic and are derived from differentiation of a precursor cell population. The lack of an effect of STZ on these aggregates suggests that this may be a less differentiated, immature β-cell population or that they arose after the STZ was administered.

Our second hypothesis was that metabolic factors would affect the regenerative response after pancreatec-
Glucose is a well-known stimulus for proliferation of preexisting β-cells, such as during a continuous infusion of 50% glucose in vivo (3) or exposure of isolated islets or β-cells in vitro (8, 23). Hyperglycemia may also play a role in stimulating β-cell replication after neonatal STZ administration (15). After a partial pancreatectomy, marked β-cell regeneration occurs in the presence of moderate hyperglycemia, but the relative influence of this hyperglycemia on new β-cells arising from ductal precursors vs. those arising from preexisting β-cells is uncertain. In the present experiments, however, there was no correlation between the fed plasma glucose level and the mass of the focal areas of regeneration, which suggests that glucose has no effect on ductal proliferation. Additionally, the plasma glucose levels in animals with endocrine cells in foci and those without endocrine cells in foci were not different (Table 2). These results suggest that glucose itself is not a stimulus for either exocrine or endocrine differentiation after pancreatic resection.

Insulin is a well-known trophic agent that acts on many cell types, including pancreatic exocrine cells. Atrophy of the exocrine pancreas is a characteristic of established type I diabetes mellitus, where few β-cells remain (19). Although the atrophy is thought to be due to the disappearance of insulin, Rahier et al. (20) found no correlation between the weight of the pancreas and the age at onset or the duration of diabetes. We had hypothesized that pancreatic regeneration after a 90% partial pancreatectomy would be impaired by reduction of the preexisting β-cell mass. In contrast to our expectation, a reduction of the β-cell mass >70% by pretreatment with STZ had no effect on regeneration of either endocrine or exocrine tissue in the remnant pancreas. These data suggest that locally exerted trophic effects of insulin are not important during regeneration after pancreatic resection. However, because insulin was supplied systemically by the islets transplanted under the kidney capsule, these data cannot exclude a trophic effect of circulating insulin.

In contrast to our observations that suggest STZ did not affect the precursor population is the report by Gu et al. (11). They injected 500 mg/kg (in 2 doses) of STZ into transgenic mice expressing interferon-γ in their pancreatic β-cells. In this model of ongoing islet cell regeneration, the high dose of STZ inhibited replication of pancreatic duct cells, suggesting that the ducts are at least partially susceptible to the deleterious effects of STZ. Whether the difference between their observations and ours is due to the difference in animal models, the three- to fourfold higher dose of STZ used by Gu et al., or the prevailing glucose level is unclear. Despite the effect of STZ on duct cell replication, Gu et al. concluded that duct cell proliferation is not enhanced by hyperglycemia and that islet regeneration is not dependent on the existing β-cell mass. These observations in the interferon-γ transgenic mouse model are consistent with our observations of pancreatic regeneration after a 90% pancreatectomy.

The finding that focal areas with endocrine cells in the foci are threefold larger than those without endocrine cells in the foci is probably a reflection of the dynamics of focal area development. Presumably, not all of the focal areas were initiated at exactly the same time, so taking samples at a single point in time will result in focal areas of different “ages.” Both the size and the appearance of endocrine tissue probably indicate that the focal area is older or more “mature” (see Fig. 3 in Ref. 2).

In summary, our results suggest that the precursor population that contributes to regeneration of both endocrine and exocrine tissue after a 90% pancreatectomy is not susceptible to the β-cell toxin STZ. Locally exerted trophic effects of insulin secreted by the pancreatic remnant are also quantitatively unimportant to pancreatic regeneration. The effects of hyperglycemia, however, may be complex. There is no evidence that hyperglycemia enhances the formation of new endocrine cells from ductal precursor cells. The possibility that moderate hyperglycemia has a trophic influence on preexisting β-cells in the form of hypertrophy or replication has not been excluded, but severe hyperglycemia, or something associated with it, appears to inhibit the development of the endocrine cell mass.

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