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Transplanting fragments of diabetic pancreas into activated omentum gives rise to new insulin producing cells

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Abstract

To determine if pancreatic progenitor cells can be induced to form insulin producing cells in vivo, we auto-transplanted fragments of streptozotocin-induced diabetic pancreas into omentum pre-injected with a foreign material. As shown previously, omentum pre-activated in this manner becomes rich in growth factors and progenitor cells. After auto-transplanting diabetic pancreas in the activated omentum, new insulin secreting cells appeared in the omentum in niches surrounding the foreign particles—a site previously shown to harbor progenitor cells. Extracts of these omenta contained measurable insulin. Four of eight diabetic animals treated in this manner became normoglycemic. This shows that new insulin producing cells can be regenerated from diabetic pancreas by auto-transplanting pancreatic fragments into the activated omentum, an environment rich in growth factors and progenitor cells.

Keywords: β-Cells; Diabetes; Pancreas; Omentum; Progenitors; Growth factors; Streptozotocin; Insulin; Pdx-1; Auto-transplantation

In the treatment of type I diabetes, whole organ pancreas transplantation offers the best hope of cure but is limited by a shortage of human donors [1]. Transplanting viable islets in the portal vein as an alternative has been plagued by implant failure due to poor vascularization and immunosuppressive drug toxicity [2]. A new and exciting approach would be to regenerate β-cells in vivo from their progenitors present in the pancreatic ductal tissue, as shown in culture conditions [3–5]. Such regeneration of β-cells from existing diabetic pancreas has already been achieved by immunologically blocking the continuous autoimmune destruction of islets [6–9]. These experiments confirm the feasibility of regenerating β-cells in vivo, but this immunological approach may not be immediately applicable in humans.

Here, we attempt to regenerate β-cells in vivo from streptozotocin (STZ) diabetic pancreas by auto-transplanting pancreatic fragments into the omentum. The omentum has well-recognized healing properties after it is deliberately brought in close proximity with injured tissues (omental transposition) [10–13]. The omentum, after contact with injured tissues, brings about repair by supplying the injured tissue with angiogenic, growth and chemotactic factors [14–16]. Such factors, in our preliminary studies, could be further augmented when the omentum was activated by placing inert foreign materials in it [17]. We hypothesized that in this activated state the omentum could present a stimulating environment for allowing resting adult β-cell progenitors to differentiate and grow to maturity.

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Methods

Animal experiments. All experiments were conducted as per the guidelines and approval of the Institutional Animal Care Committee.

Auto-transplantation of STZ diabetic pancreas in activated omentum. One week prior to auto-transplantation normal Sprague–Dawley rats (male; 150–200 g; Harlan Laboratories, Indianapolis IN) were injected with 5 mL of polydextran particle slurry (PD) (Biogel P-60, 120 μM; Biorad Laboratories, Richmond, CA) (1:1 in phosphate-buffered saline (PBS)) intraperitoneally to pre-activate the omentum [17]. After one week, rats were tested for normal blood glucose level by a hand-held glucometer (day 1). At day 2, diabetes was induced by a single intravenous injection of streptozotocin (65 mg/kg in saline containing 10 mM citrate pH 5.0). At day 3, the rats were tested for blood glucose levels and were considered diabetic if their blood glucose levels were >350 mg/dL. On this day the diabetic rats were laparotomized under anesthesia and subjected to 80% pancreatectomy (described below). The removed pancreas was gently dispersed to a granular suspension by a manual sintered-glass homogenizer, mixed with 2 mL of sterile polydextran particles (for further activating omentum) and introduced in the abdominal cavity of the same animal ($n = 8$; transplanted group I) under sterile conditions. Appropriate control rat groups (groups II–IV) were included [18]. On the same day (day 3) all animal groups (except normal control) were implanted with a subcutaneous mini-osmotic pump to deliver insulin at the rate of 1.25 IU/day for 28 days (Alzet, Durect Corporation, Cupertino CA). Animals were monitored weekly for blood glucose levels until day 31 when the mini-osmotic pumps were removed. Following withdrawal of insulin the animals were further monitored for blood glucose levels for two weeks before termination. At termination, omenta and pancreas were harvested, weighed, and processed for histology and measurement of insulin content.

Additional control groups of rats were set up for comparing insulin levels achieved in the omenta containing transplanted pancreas fragments (of experimental rats) with those in activated omentum alone (without pancreas), or normal rat pancreas or diabetic rat pancreas [19].

Partial pancreatectomy. Laparotomy was performed under total anesthesia and approx. 80% of pancreas was removed surgically by excising most of the pancreas except the head (duodenal side). A narrow strip of the lower margin of the length of the pancreas that embeds the critical artery supplying the spleen was also spared to protect the spleen.

Processing of omental and pancreas tissue for determination of insulin. For determination of insulin in the omentum and pancreas whole tissues were harvested at the time of termination of animals, washed in ice-cold PBS and wet-weighed. The tissue was homogenized in 5 volumes of ice-cold PBS using polytron homogenizer. For further extraction 0.1% Triton X-100 was added to the homogenate and frozen until assay. At the time of assay the homogenates were thawed and centrifuged at 13,000 rpm for 30 min to remove unsolubilized tissue. The clear supernatants were used for insulin assay using ELISA kits designed for rat insulin (Crystal Chem Inc., Downer's Grove IL). The assay has a sensitivity of 0.1–10 ng/mL.

Immune-staining for insulin and PDX-1 in pancreas and omental tissue. Tissues were fixed in Histochoice® (Amresco, Solon OH), embedded in paraffin and 5 μM sections collected on glass slides. Following deparaffinization the sections were incubated with the primary anti-insulin antibody (Sigma Chemical Co., St. Louis, MO) or anti-PDX-1 antibody (Chemicon International, Inc., Temecula, CA) followed by the appropriate fluorescene labeled secondary antibody. Sections of omentum were counterstained with ethidium bromide (10 μg/mL) to highlight cell nuclei. Finally, the sections were washed and wet-mounted in 1:1 glycerol:PBS mixture, viewed under epifluorescence and digitally photographed (Nikon, New York, NY). Positive control slides included sections of normal pancreas for visualization of insulin positive islets. The positive staining for insulin in the omental tissue was confirmed by blocking the insulin staining by co-incubating purified insulin in the primary antibody step of the immune staining.

Statistical analysis. Quantitative data are presented as means ± standard error of mean and statistical comparisons were made using the Student's t test (one-tail), considering $P < 0.05$ as significant. Image analysis was used for determining 'number of insulin positive cells/unit area' in the omental tissue [20].

Results

Previously we have shown that one week after injection of polydextran particles in the peritoneal cavity of rats the native omentum, which consists mostly of adipose tissue, expanded its non-adipose part (milky spots mass) by 20-fold. VEGF and other growth factors increased 2- to 4-fold, blood vessel density 3-fold, and blood content 2-fold. Stromal cell derived factor-1α (SDF-1α), chemokine receptor-4 (CXCR-4), and Wilm's tumor-1 (WT-1) positive cells associated with tissue regeneration increased in the activated omentum, mostly at locations immediately surrounding the polydextran particles [17].

Following a single iv injection of STZ rats become diabetic within 24 h with blood glucose levels of 420 ± 30 mg/dL ($n = 20$) compared to normal levels of 102 ± 10 mg/dL ($n = 20$). If untreated the rats remain hyperglycemic indefinitely with progressive loss of weight (in younger growing rats as used in this study weight loss is measured as absence of weight gain) becoming significant only after 3–4 weeks of diabetes. When treated with insulin (delivered via mini-osmotic pumps) the weight gain is restored and blood glucose normalizes to 110 ± 14 mg/dL through the life of the pump.

To test our hypothesis fragments of STZ diabetic rat pancreas were auto-transplanted in the pre-activated omentum. Following this the rats were maintained on insulin infusion for four weeks to control hyperglycemia and allow normal differentiation of progenitor β-cells. Fig. 1

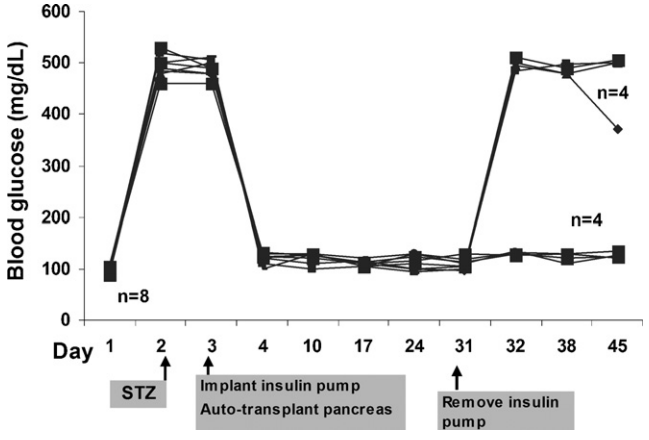

Fig. 1. Blood glucose levels in the eight auto-transplanted diabetic rats during the course of the experiment. Rats were induced diabetes with STZ, and soon after, their own pancreas was transplanted into the activated omentum. To maintain normal glucose level during the regeneration (4 weeks) these rats were continuously infused with insulin (via osmotic pump). Following the removal of insulin pumps blood glucose continued to be normal in 4/8 rats (successfully treated). The remaining 4/8 diabetic rats reverted to the hyperglycemic state (treatment unsuccessful). Control diabetic groups (see text; groups II–IV) remained hyperglycemic (not shown).

presents blood glucose levels in the eight auto-transplanted diabetic rats during the course of the experiment. Before auto-transplantation all the eight rats were severely hyperglycemic. After transplantation of diabetic pancreas and during insulin infusion for four weeks (via osmotic pump) the blood glucose levels remained normal. Following the removal of insulin pumps after four weeks of normoglycemia, blood glucose continued to be normal in 4/8 rats until the termination of the experiment (successfully treated). The remaining 4/8 diabetic rats reverted to the hyperglycemic state. The diabetic control rats (groups II–IV) remained normal until the insulin pumps were present but became hyperglycemic after removal of the insulin pumps (data not shown).

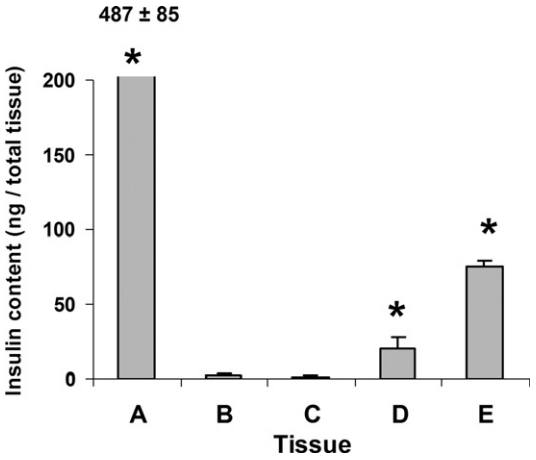

Fig. 2. Insulin content in the omentum of auto-transplanted rats. Data is presented as mean with limit bars showing standard error of mean. Number of tissues in each case is four. Asterisk (*) denotes statistically significant difference at $P < 0.05$ from all other groups. Normal rat pancreas contained approx. 500 ng of insulin (A) while STZ diabetic rats had negligible amounts (B). In the four rats in which hyperglycemia were reversed the omental tissue contained significant amounts of insulin (E). Although this constituted only 15% of the content of normal rat pancreas it was 50 times higher than the insulin content of diabetic pancreas. Omentum of the four rats that remained hyperglycemic after treatment showed lower amounts of insulin (D) than the successfully treated rats, but significantly higher than the insulin content of either diabetic pancreas or activated omentum alone from normal (or diabetic) rats (without pancreas transplant) (C).

In the four successfully treated rats (in which hyperglycemia was reversed), the insulin content of the omentum was ~75 ng/total tissue, which is 75 times higher than the insulin content of STZ diabetic pancreas (~1.0 ng/total tissue) (Fig. 2). However, this amount of insulin constituted only 15% of the content in a normal rat pancreas (~500 ng/total tissue). Omentum of the four rats that remained hyperglycemic showed lower amounts of insulin (~20 ng/total tissue) than the successfully treated rats, but significantly higher than the insulin content of either diabetic pancreas or activated omentum alone from normal (or diabetic control) rats (without pancreas auto-transplant) (~1.0 ng/total tissue).

Omenta from the eight auto-transplanted diabetic rats shown in Fig. 1 and from control rats were immunostained for insulin. Fig. 3C shows a representative photomicrograph of the omental tissue from the four rats which were successfully treated by auto-transplantation. It shows niches of insulin secreting cells in the cluster of cells surrounding the polydextran particles (12.3 ± 1.8 insulin positive cells/10,000 sq pixels; $n = 10$). Rats that reverted to hyperglycemic state after auto-transplantation showed few-

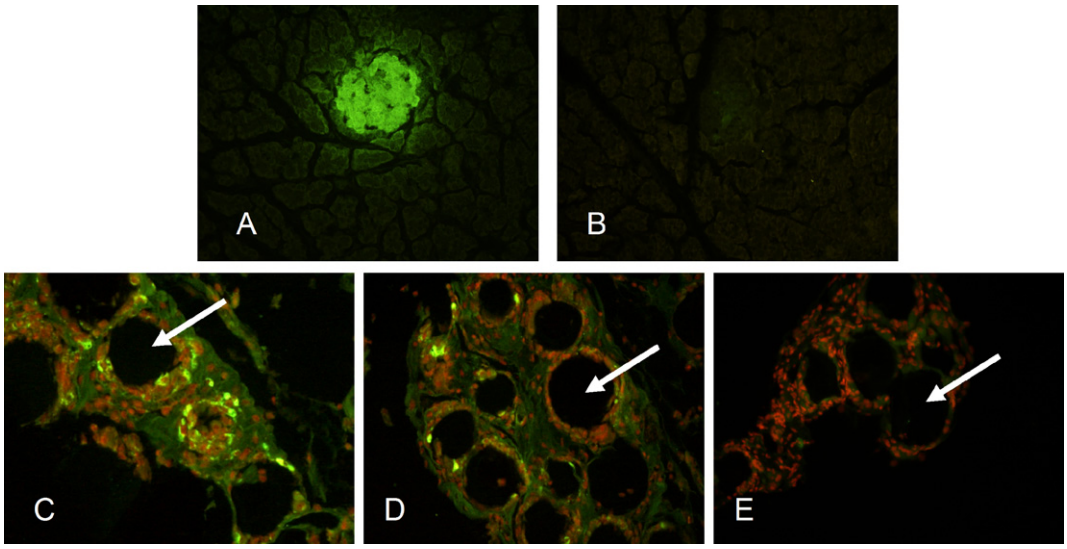

Fig. 3. Detection of insulin positive cells in the omenta of auto-transplanted rats by immunocytochemical staining. Note that a normal rat pancreatic islet is highly positive for insulin (A) and a STZ diabetic pancreatic islet is negative for insulin (B). Omenta from the four auto-transplanted diabetic rats which were successfully treated showed niches of insulin positive cells in the cluster of cells surrounding the polydextran particles (arrows) (C). Omenta from the four rats that remained hyperglycemic after auto-transplantation of pancreas showed fewer insulin producing cells in the omentum than the successfully treated rats (D). Omenta from diabetic control rats (groups II–VI) were negative for insulin cells as were activated omenta alone from normal control rats (E). Tissues C–E were counterstained with ethidium bromide to highlight cell nuclei.

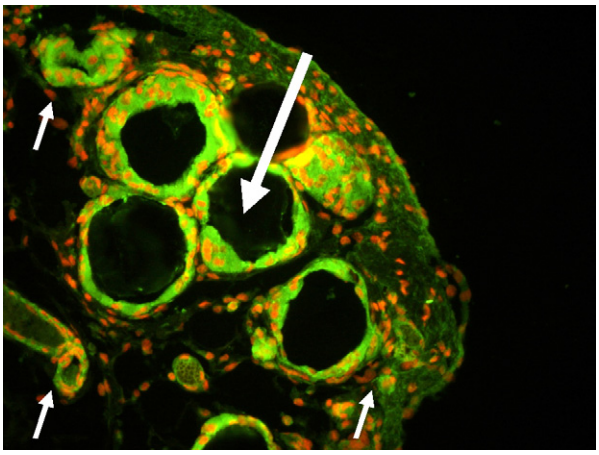

Fig. 4. Activated omentum immuno-stained for PDX-1 (pancreatic duodenal homeobox-1), a developmental marker, expressed in cells that can potentially differentiate to β-cells. Both, in the activated omentum alone (shown) as well as in the activated omentum containing auto-transplanted pancreas (not shown) the cells immediately surrounding the polydextran particles (large arrow) and blood vessels (small arrows) were highly positive for PDX-1. Tissue was counterstained with ethidium bromide to highlight cell nuclei.

er insulin producing cells in the omentum (Fig. 3D) (2.4 ± 0.4 insulin positive cells/10,000 sq pixels; $n = 10$; difference is statistically significant at $P < 0.05$). Insulin positive cells were undetectable in omenta from control rats (groups II–VII) (Fig. 3E). Also shown for comparison are a normal rat pancreatic islet highly positive for insulin and a STZ diabetic pancreatic islet negative for insulin. It should be noted that insulin by immune-staining is typically undetectable in STZ diabetic pancreas.

The omental tissues were stained for PDX-1 (pancreatic duodenal homeobox-1), a developmental marker, expressed in cells that can potentially differentiate to β-cells [21,22]. Both, in the activated omentum alone as well as in the activated omentum containing auto-transplanted pancreas the cells immediately surrounding the polydextran particles (and blood vessels) were highly positive for PDX-1 (Fig. 4).

Discussion

In this study, we have shown that pre-activated omentum, which becomes rich in growth factors, chemotactic factors, and progenitor cells, offers a potential in vivo site for producing new insulin secreting cells from pancreatic progenitors. We used the omentum because it has remarkable healing properties. More recently the omentum has also shown promise as a suitable site for sustaining the function of transplanted islets and for growing organs from embryonic anlagen [23,24].

Taking advantage of the growth factor-rich environment created in the omentum by foreign body stimulation, we auto-transplanted in it fragments of diabetic pancreas to regenerate new β-cells. By immunocytochemical staining we found that new insulin secreting cells had appeared in the omentum. Then we homogenized the omentum containing pancreatic fragments and found in the extracts measurable amounts of insulin, consistent with the number of insulin positive cells seen by staining. We also found that diabetic animals containing higher amounts of insulin in the omentum became normoglycemic (successfully treated) whereas those with lesser amounts remained hyperglycemic. When pancreatic fragments were transplanted into native (unactivated) omentum, measurable insulin in the omentum was negligible and insulin positive cells were not seen. Moreover these animals remained hyperglycemic, showing that the activated omentum was responsible for regenerating insulin producing cells.

The amounts of extractable insulin in the omentum of successfully treated rats were about 15% of those measured in the pancreas of normal rats. Such a finding was in accord with our unpublished observations and those of others [25] that 80–90% pancreatectomy (remaining pancreas = 10–20%) in normal rats does not result in significant hyperglycemia. This is because when the β-cell mass is reduced (as in the successfully treated rats) the sensitivity of the remaining β-cells to ambient glucose increases, and this helps maintain normoglycemia by increasing insulin release [25,26]. Clearly, this putative increased sensitivity to glucose was insufficient to restore normoglycemia in the rats that had remained hyperglycemic despite having some degree of β-cell regeneration.

It has been controversial whether the adult pancreas contains progenitor β-cells, and if so what is their origin [27]. On one hand it is possible that the activated omentum facilitated the multiplication of the few surviving β-cells in the diabetic pancreas [25]. Conceivably, on the other hand, the progenitor cells present in the activated omentum could have differentiated into new β-cells facilitated by high growth factors and cues from the fragments of the pancreas tissue lying in the vicinity. This hypothesis is consistent with our observations that the cells enveloping the polydextran particles are likely to be adult progenitor cells—because they express stem cells markers such as SDF-1a, CXCR-4, and PDX-1. It would suggest that our approach of using the activated omentum to regenerate β-cells from progenitors is reasonable and practical.

Acknowledgments

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[18] Controls consisted of (1) PD-activated diabetic rats from which the pancreas were removed but not transplanted and so discarded (non-transplanted group II), (2) non PD- activated diabetic rats in which pancreas were auto-transplanted without polydextran particles (non-activated, transplanted group III), (3) PD-activated diabetic rats in which pancreas were not removed, and therefore not auto-transplanted (STZ diabetic rats with activated omentum, group IV) and (4) normal non-diabetic rats without PD-activation of omentum and without pancreatectomy and auto-transplantation, but sham laparotomized (normal sham control group V) ($n = 4$ in each of group II–V).
[19] Additional controls included (1) normal rats (for harvesting normal pancreas) ($n = 4$, same as group V), (2) normal rats in which omentum was activated by injection of PD (for harvesting normal activated omentum; group VI, $n = 4$), and (3) 6 week STZ diabetic rats without PD-activation of omentum (for harvesting diabetic pancreas; group VII, $n = 4$).
[20] Image analysis was performed on ten random pictures of immunocytochemically stained tissue using the ImageJ software (JAVA imaging software inspired by the National Institute of Health and available free at http://rsb.info.nih.gov).
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