A Switch From Prohormone Convertase (PC)-2 to PC1/3 Expression in Transplanted α-Cells Is Accompanied by Differential Processing of Proglucagon and Improved Glucose Homeostasis in Mice

Rhonda D. Wideman,[1] Scott D. Covey,[1] Gene C. Webb,[2] Daniel J. Drucker,[3] and Timothy J. Kieffer[1,4]

OBJECTIVE—Glucagon, which raises blood glucose levels by stimulating hepatic glucose production, is produced in α-cells via cleavage of proglucagon by prohormone convertase (PC)-2. In the enteroendocrine L-cell, proglucagon is differentially processed by the alternate enzyme PC1/3 to yield glucagon-like peptide (GLP)-1, GLP-2, and oxyntomodulin, which have blood glucose–lowering effects. We hypothesized that alteration of PC expression in α-cells might convert the α-cell from a hyperglycemia-promoting cell to one that would improve glucose homeostasis.

RESEARCH DESIGN AND METHODS—We compared the effect of transplanting encapsulated PC2-expressing αTC-1 cells with PC1/3-expressing αTCΔPC2 cells in normal mice and low-dose streptozotocin (STZ)-treated mice.

RESULTS—Transplantation of PC2-expressing α-cells increased plasma glucagon levels and caused mild fasting hyperglycemia, impaired glucose tolerance, and α-cell hypoplasia. In contrast, PC1/3-expressing α-cells increased plasma GLP-1/GLP-2 levels, improved glucose tolerance, and promoted β-cell proliferation. In GLP-1R$^{-/-}$ mice, the ability of PC1/3-expressing α-cells to improve glucose tolerance was attenuated. Transplantation of PC1/3-expressing α-cells prevented STZ-induced hyperglycemia by preserving β-cell area and islet morphology, possibly via stimulating β-cell replication. However, PC2-expressing α-cells neither prevented STZ-induced hyperglycemia nor increased β-cell proliferation. Transplantation of αTCΔPC2, but not αTC-1 cells, also increased intestinal epithelial proliferation.

CONCLUSIONS—Expression of PC1/3 rather than PC2 in α-cells induces GLP-1 and GLP-2 production and converts the α-cell from a hyperglycemia-promoting cell to one that lowers blood glucose levels and promotes islet survival. This suggests that alteration of proglucagon processing in the α-cell may be therapeutically useful in the context of diabetes. ***Diabetes* 56: 2744–2752, 2007**

From the [1]Department of Cellular and Physiological Sciences, Laboratory of Molecular and Cellular Medicine, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada; the [2]Department of Medicine, University of Chicago, Chicago, Illinois; the [3]Department of Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada; and the [4]Department of Surgery, Laboratory of Molecular and Cellular Medicine, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada.

Address correspondence and reprint requests to Dr. Timothy J. Kieffer, 2350 Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3. E-mail: tim.kieffer@ubc.ca.

Received for publication 23 April 2007 and accepted in revised form 6 August 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 17 August 2007. DOI: 10.2337/db07-0563.

AUC, area under the curve; GLP, glucagon-like peptide; IPGTT, intraperitoneal glucose tolerance test; PC, prohormone convertase; PCNA, proliferating cell nuclear antigen; PGDP, proglucagon-derived peptide; STZ, streptozotocin.

The proglucagon precursor gives rise to numerous peptides belonging to the glucagon superfamily of hormones (1). Despite significant peptide sequence homology, members of this superfamily exert diverse and sometimes opposing regulatory functions. Proglucagon is expressed in pancreatic α-cells, intestinal L-cells, and specific neurons in the central nervous system. Tissue-specific posttranslational processing by prohormone convertase (PC)-1/3 or PC2 results in a different profile of proglucagon-derived peptides (PGDPs) in these tissues. Thus, as a result of cleavage by PC2, the major bioactive PGDP arising in pancreatic α-cells is glucagon (2,3), which serves as a counterregulatory hormone opposing insulin action by increasing hepatic glucose output. While the activity of glucagon is critical for preventing hypoglycemia under normal conditions, hyperglucagonemia accompanies diabetes and may contribute to its pathology (4). Indeed, suppression of glucagon action using immunoneutralization, antisense oligonucleotides, or glucagon receptor antagonists improves glucose handling in both rodents (5,6) and humans (7,8).

In contrast to the α-cell, in the enteroendocrine L-cell and the brain, glucagon-like peptide (GLP)-1, GLP-2, and oxyntomodulin are released from proglucagon via the alternate processing enzyme PC1/3 (9,10). GLP-1 elicits effects that tend to lower blood glucose, including stimulation of insulin synthesis and secretion, inhibition of gastric emptying and food intake, and promotion of β-cell survival and proliferation (1,11). This complement of antidiabetic effects has led to much interest in developing long-acting GLP-1 mimetics as a treatment for diabetes. GLP-2 and oxyntomodulin also tend to lower blood glucose levels by promoting satiety and inhibiting gastric emptying (12,13). Thus, depending on whether it is processed by PC2 or PC1/3, proglucagon gives rise to products having either glucose-raising or glucose-lowering effects.

We hypothesized that expression of PC1/3 rather than PC2 in α-cells might shift the PGDP profile to become more like that of the L-cell and thereby convert the α-cell from a hyperglycemia-promoting cell to one that would lower blood glucose levels. We therefore sought to directly compare the in vivo effects of PC2- versus PC1/3-derived PGDPs on glucose homeostasis, β-cell survival, and morphology of the pancreas and jejunum in normal mice and in a mouse model of type 1 diabetes.

RESEARCH DESIGN AND METHODS

Tissue culture media, antibiotics, and fetal bovine serum were obtained from Invitrogen Canada (Burlington, Ontario, Canada). Active GLP-1 enzyme-linked

immunosorbent assay, total GLP-1 radioimmunoassay, and glucagon radioimmunoassay kits were from Linco Research (St. Charles, MO). Glucagon and GLP-2 enzyme immunoassay kits were from Alpco Diagnostics (Salem, NH). Western blotting reagents were from GE Healthcare (Buckinghamshire, U.K.). αTC-1 (clone 9) cells were from the American Type Culture Collection. Imaging and quantification was performed using an Axiovert 200 microscope (Carl Zeiss, Toronto, Ontario, Canada) connected to a digital camera (Retiga 2000R; QImaging, Burnaby, British Columbia, Canada) controlled with Openlab 5.0 software (Improvision, Lexington, MA).

Cell culture and analysis. αTCΔPC2 cells are an α-cell line derived from mice lacking active PC2, precluding glucagon production, and have been described previously (14). αTC-1 and αTCΔPC2 cells were cultured in high-glucose Dulbecco's modified Eagle medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37°C/5% CO_2, with media refreshment every 2–4 days. Experiments were performed using passages 10–14 or 20–29 for αTC-1 and αTCΔPC2 cells, respectively. Cells were seeded at equal density in six-well plates, and media were collected after a 24-h static incubation and assayed for glucagon or active GLP-1 content. Equal amounts of protein from cell lysates were electrophoresed on a 10% polyacrylamide gel and transferred to a 0.2-μm polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). After blocking (5% milk powder in TBS + 0.1% Tween-20), the membrane was incubated with anti-PC1/3 antiserum (1:1,000) and developed using horseradish peroxidase–conjugated secondary antibody (1:5,000) and chemiluminescence reagents.

Animal experiments. Male mice, age 8–10 weeks, were used for all experiments. CD-1 mice were from the University of British Columbia Animal Care Facility, and C57BL/6 mice were from Jackson Laboratories (Bar Harbor, ME). All experiments were approved by the University of British Columbia Animal Care Committee. Mice were maintained on a standard 12-h light/dark cycle and received a standard diet. Blood glucose and body weight were monitored two to three times weekly after a 4-h morning fast. Blood glucose monitoring and survival blood sampling was carried out on restrained unanaesthetized mice via the saphenous vein.

GLP-1R$^{-/-}$ mice (15) and wild-type littermates were maintained on a C57BL/6 background and genotyped using PCR primers that amplify 437 bp of the GLP-1R in transmembrane domains 2–4, the region disrupted in mice bearing a knockout allele (16) (forward 5′TACACAATGGGGAGCCCCTA3′; reverse 5′AAGTCATGGGATGTGTCTGGA3′) and primers that amplify 280 bp of the Neo^R insert (forward 5′CTTGGGTGGAGAGGCTATTC3′; reverse 5′AGGTGAGATGACAGGAGATC3′). PCR was carried out as follows using standard reaction mix with 1.5 mmol/l $MgCl_2$: 2 min at 94°C; 30 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min; and 72°C for 10 min. Wild-type mice had a single 437-bp product, GLP-1R$^{-/-}$ mice had a single 280-bp product, and GLP-1R$^{+/-}$ mice had both products.

Encapsulation and transplantation of cells. Cells were grown to ~80% confluence. After trypsinization, cells were washed in PBS without $CaCl_2$, and a cell count was performed using a hematocytometer. Cells were resuspended in a mixture of 1.5% sodium alginate (IE-1010; Inotech Biosystems International, Rockville, MD) and morpholinepropanesulfonic acid, transferred to a sterile encapsulator (Inotech Biosystems International), and encapsulated according to the manufacturer's instructions. Capsules (~700 μm) were washed in PBS without $CaCl_2$ and loaded to sterile syringes attached to 18-G catheters. Recipient mice were anesthetized using isoflurane, and an incision was made midway between the ventral midline and the animal's side, ~1.5 cm anterior of the hind leg. Capsules were injected to the intraperitoneal cavity, and the musculature and skin were closed with a running suture and a wound clip, respectively. For all experiments, 1.4–2.2 ml of capsules (~4.0×10^7 cells/mouse) were transplanted in <3 ml total volume. Transplants were completed within 1.5 h of cell encapsulation. Sham-operated mice received an equal volume of sterile saline under identical conditions.

In vivo glucose tolerance testing. Glucose tolerance tests were performed by oral gavage (oral glucose tolerance test) or intraperitoneal (intraperitoneal glucose tolerance test [IPGTT]) delivery of glucose (2 g/kg) to recipient mice after an overnight fast. Blood glucose was monitored for 2 h after glucose delivery. Where blood glucose levels exceeded the limit of detection for the blood glucose meter, samples were diluted 1:2 in saline.

Streptozotocin treatment of C57BL/6 mice. Streptozotocin (STZ) (Sigma, St. Louis, MO) was prepared in citrate buffer (pH 4.5) and delivered by intraperitoneal injection (50 mg/kg) for 5 consecutive days beginning 8 days posttransplant in C57BL/6 mice.

Plasma and tissue analysis of transplant recipients. At the indicated time after transplantation, mice were anesthetized with isoflurane, and a cardiac puncture was performed to collect plasma before cervical dislocation. Pancreas and jejunum (8–10 cm distal to the pylorus) were removed, rinsed in PBS, fixed in 4% paraformaldehyde, and paraffin sectioned at 3 μm. Plasma analytes were measured using total GLP-1 radioimmunoassay, GLP-2 enzyme immunoassay, and/or glucagon enzyme immunoassay kits.

Histology and morphometric analysis. After antigen retrieval in citrate buffer, pancreas sections were stained using guinea pig anti-insulin (1:900; Linco Research, St. Charles, MO), rabbit anti-glucagon (1:1,800; Sigma), mouse anti–proliferating cell nuclear antigen (PCNA) (1:200; BD Biosciences, San Jose, CA), and secondary antisera conjugated to Alexafluor 488 or 594 (1:800). Total insulin-/glucagon-positive area, as well as the number and average size of insulin-/glucagon-positive clusters, were determined in one section per mouse and normalized for sectional pancreatic area. The proportions of α- and β-cells per islet were determined by counting nuclei of all glucagon- and insulin-positive cells of five or more randomly selected islets for each mouse. α-Cells within two cells of the islet edge were considered "peripheral " and otherwise were considered "central." Some sections were costained for insulin and PCNA, and the number of insulin-positive/PCNA-positive cells was quantified in seven or more randomly selected islets per animal.

Jejunal crypt cell proliferation was assessed using a method described elsewhere (17). Sections were stained for PCNA, and for six or more upright intact crypts per mouse, the first 20 cells extending upward from the crypt base (position 1) were scored as PCNA$^-$ or PCNA$^+$. Crypt plus villus height was determined by measuring from the crypt base to the tip of the villus in 4–10 upright intact villi in hematoxylin-stained sections for each mouse.

Statistical analysis. Data are presented as means ± SE and were analyzed using the Student's *t* test or ANOVA with Bonferroni's post-test as appropriate ($P < 0.05$, $P < 0.01$, $P < 0.001$).

RESULTS

Characterization of α-cell lines expressing PC2 or PC1/3. To study the impact of expression of PC2 versus PC1/3 in the α-cell, we used two different α-cell lines. Using immunocytochemistry and immunoblotting, we confirmed that αTC-1 cells express PC2 (data not shown) but little or no PC1/3; αTCΔPC2 cells, in contrast, express PC1/3 but not bioactive PC2 (Fig. 1*A* and *B*). Consistent with minimal PC1/3 expression, αTC-1 cells secreted very little GLP-1 compared with PC1/3-expressing αTCΔPC2 cells (Fig. 1*C*) (6.6 ± 1.7 vs. 276.9 ± 96.0 ng/ml; $n \geq 2$). However, αTC-1 cells secreted 10-fold more glucagon than αTCΔPC2 cells (Fig. 1*C*) (113.0 ± 9.6 vs. 10.0 ± 1.9 ng/ml; $n \geq 2$). Thus, these cells allowed us to compare the in vivo effects of transplanting α-cells expressing either PC1/3 or PC2.

Transplantation of PC2- or PC1/3-expressing α-cells to normal and GLP-1R$^{-/-}$ mice. We sought to compare the impact of transplantation of PC2-expressing (αTC-1) or PC1/3-expressing (αTCΔPC2) α-cells in normal mice. αTC-1 recipients had slightly increased fasting blood glucose levels during the 14 days after transplant (Fig. 2*A*) ($P = 0.06$ at 8 days; $P = 0.054$ at 12 days; $P < 0.05$ at 14 days; $n \geq 7$), whereas blood glucose of αTCΔPC2 recipients was slightly lower than that of sham-operated mice 2 days posttransplant (8.3 ± 0.2 vs. 9.0 ± 0.2 mmol/l, respectively; $P < 0.05$; $n = 8$) but not different thereafter. Body weights were not different among the three groups (data not shown).

Glucose handling was assessed using an IPGTT 7 days posttransplant (Fig. 2*B*). While sham-operated mice and αTC-1 recipients behaved similarly, αTCΔPC2 recipients had a dramatic improvement in glucose tolerance, with both lower peak glucose levels (10.0 ± 0.2 vs. 16.7 ± 1.9 mmol/l for sham at 15 min; $n \geq 7$) and a 41% decrease in area under the curve (AUC) (Fig. 2*C*). To assess the contribution of GLP-1R signaling to the improved glucose tolerance observed after αTCΔPC2 transplant, we repeated transplant studies in GLP-1R$^{-/-}$ mice and wild-type littermates. GLP-1R$^{+/+}$ mice receiving αTCΔPC2 cells had a 33% decrease in peak glucose levels and a 25% decrease in AUC in an IPGTT performed 7 days posttransplant (Fig. 2*D* and *F*). In contrast, GLP-1R$^{-/-}$ mice receiving αTCΔPC2 cells had a mild decrease in peak glucose and no change in AUC compared with controls (Fig. 2*E* and *F*).

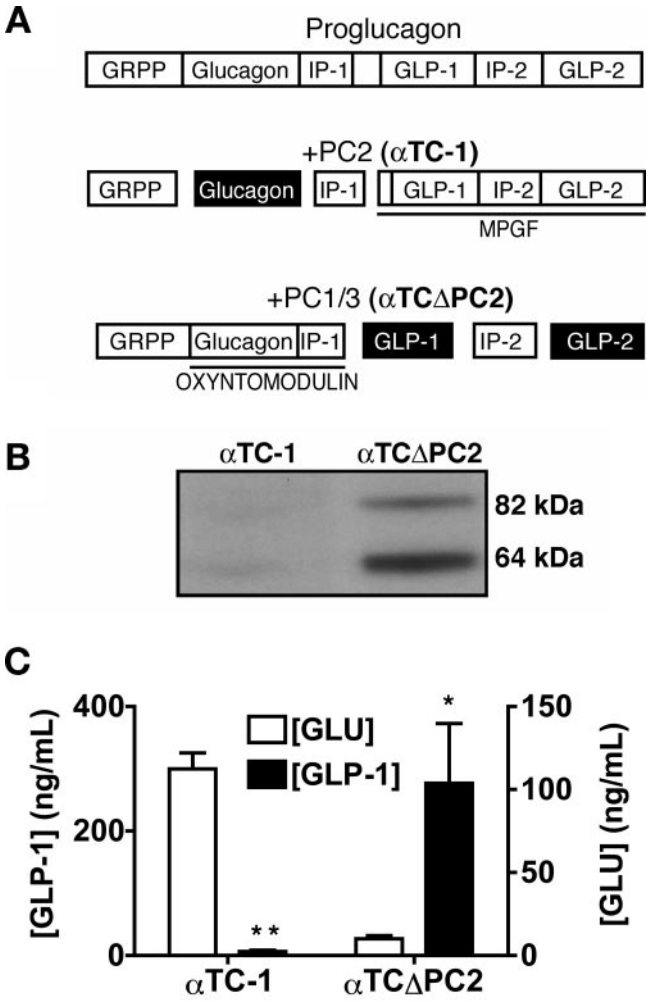

FIG. 1. αTC-1 cells express PC2 and produce glucagon, whereas αTCΔPC2 cells express PC1/3 and produce GLPs and oxyntomodulin. *A*: Proglucagon processing in α-cells expressing either PC2 or PC1/3. In PC2-expressing αTC-1 cells, proglucagon is processed to yield glucagon as the major bioactive product. PC1/3-expressing αTCΔPC2 cells process proglucagon differently, resulting in GLP-1 production and action of GLP-1, GLP-2, and oxyntomodulin. *B*: Western blot for PC1/3 in αTC-1 and αTCΔPC2 cell lysates. Blot is representative of four or more experiments. *C*: GLP-1 and glucagon (GLU) secretion from αTC-1 and αTCΔPC2 cells ($n \geq 2$). *$P < 0.05$, **$P < 0.01$. GRPP, glicentin-related polypeptide; IP, intervening peptide; MPGF, major proglucagon fragment.

Sixteen days posttransplant, CD-1 mice were killed and pancreas tissue collected for histological examination. Sham-operated and αTC-1 and αTCΔPC2 recipients had similar total and average insulin-positive area and number of insulin-positive areas (Fig. 3*A*–*E*). However, αTC-1 recipients had relatively fewer α-cells per islet (Fig. 3*F*) and a trend toward decreased total glucagon-positive area ($P = 0.11$) owing to decreased average size of glucagon-positive areas (Fig. 3*G*–*I*).

Administration of multiple low-dose STZ to mice after αTCΔPC2 or αTC-1 cell transplant. Because transplantation of PC1/3-expressing αTCΔPC2 cells or PC2-expressing αTC-1 cells resulted in opposite effects on glucose homeostasis in nondiabetic mice, we sought to examine the impact of αTCΔPC2 or αTC-1 cells in a mouse model of type 1 diabetes. C57BL/6 mice received sham surgery or encapsulated αTCΔPC2 or αTC-1 cells on day 0, and a low-dose regimen of the β-cell toxin STZ was administered from 8–12 days posttransplant. αTCΔPC2 recipients exhibited lower fasting blood glucose levels than sham-operated mice even before STZ was administered, and whereas control mice developed mild diabetes within ~10 days of the last dose of STZ, αTCΔPC2 recipients did not progress to diabetes (Fig. 4*A*). Body weights of control mice and αTCΔPC2 recipients did not differ (Fig. 4*B*). An IPGTT was performed 7 days post-transplant (before STZ administration), and consistent with our observations in CD-1 mice, αTCΔPC2 recipients exhibited a dramatic improvement in glucose tolerance, with lower blood glucose levels at all time points and a 55.6% decrease in AUC (Fig. 4*C*). Plasma GLP-1 was measured in blood samples collected just before glucose administration, and whereas GLP-1 was below the level of detection in control mice (<8.9 pg/ml), αTCΔPC2 recipients had plasma GLP-1 levels of 297.7 ± 36.7 pg/ml ($n \geq 6$). In an oral glucose tolerance test performed 20 days posttransplant (just before STZ-induced diabetes onset), αTCΔPC2 recipients again displayed improved glucose disposal, with lower peak blood glucose values and a 24.9% decrease in AUC (Fig. 4*D*).

In contrast to αTCΔPC2 recipients, αTC-1–transplanted mice had consistently higher blood glucose levels than sham-operated controls following transplant (Fig. 5*A*). An IPGTT was performed 7 days posttransplant, and unlike αTCΔPC2 recipients, αTC-1 recipients exhibited impaired glucose tolerance relative to control mice, with higher peak blood glucose levels and a 53.0% increase in AUC (Fig. 5*C*). Plasma glucagon was measured in blood samples collected just before glucose administration, and αTC-1 recipients had threefold greater glucagon levels than controls (799.6 ± 60.2 pg/ml vs. 246.6 ± 41.2 pg/ml; $n = 8$; $P < 0.001$). In response to STZ treatment, αTC-1 recipients developed hyperglycemia similar to control mice (Fig. 5*A*). Finally, an oral glucose tolerance test was performed 20 days posttransplant, and in contrast to αTCΔPC2 recipients, αTC-1 recipients did not exhibit improved glucose handling (Fig. 5*D*).

Plasma and histological analysis of STZ-treated αTCΔPC2 or αTC-1 recipient mice. Mice were killed 30 days posttransplant, and plasma and pancreatic tissue were collected for further analysis. αTCΔPC2-treated mice had threefold higher plasma GLP-1 and 1.8-fold greater plasma GLP-2 levels than sham-operated controls at this time (177.7 ± 56.5 vs. 59.9 ± 12.0 pg/ml for GLP-1, $n \geq 5$; 1.3 ± 0.2 vs. 0.7 ± 0.02 ng/ml for GLP-2; $n \geq 8$). αTC-1 recipients had 8.3-fold higher plasma glucagon levels than controls (494.6 ± 164.7 vs. 59.77 ± 11.37 pg/ml; $n = 8$).

Histological examination of the pancreas revealed that sham-operated STZ-treated mice had relatively more α-cells and fewer β-cells per islet than did αTCΔPC2-transplanted STZ-treated mice (Fig. 6*A* and *B*). Sham-operated mice exhibited disrupted islet architecture, with more centrally located α-cells, whereas mice receiving αTCΔPC2 cells were protected against this STZ-induced abnormality (28.0 ± 3.2 vs. 15.8 ± 1.6%, respectively; $P < 0.01$; $n \geq 7$). αTCΔPC2 recipients tended to have more insulin-positive pancreatic area ($P = 0.062$) and, on average, larger islets, although the number of islets was unchanged (Fig. 6*C*–*E*). αTCΔPC2 recipients also displayed an increased number of replicating (i.e., $PCNA^+$) β-cells as a percentage of total β-cells (Fig. 6*F*–*G*).

STZ-treated αTC-1–transplanted mice also displayed relatively fewer α-cells and more β-cells per islet (Fig. 7*A* and *B*). Sham-operated mice had more centrally located α-cells

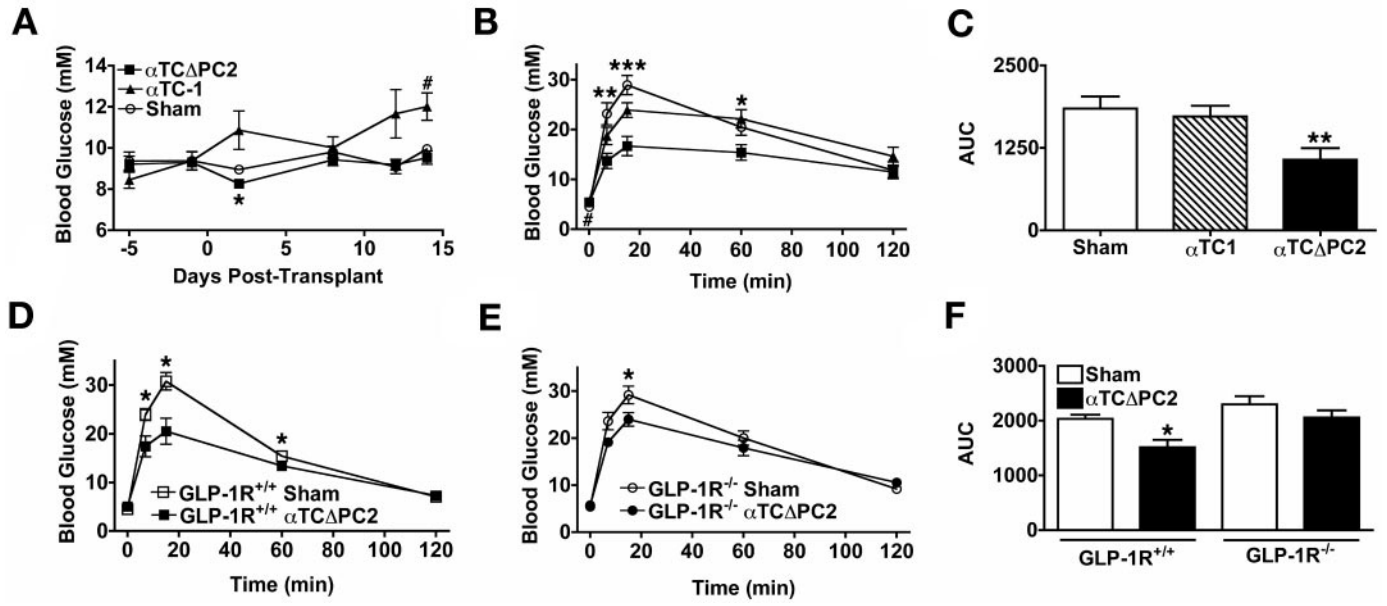

FIG. 2. Transplantation with encapsulated αTCΔPC2 cells improves glucose tolerance in normal mice. *A–C*: CD-1 mice were transplanted with 4.0 × 10^7 αTCΔPC2 or αTC-1 cells ($n \geq 6$). *A*: Four-hour morning fasted blood glucose. *B*: IPGTT (2 g/kg) performed 6 days posttransplant. *C*: AUC for IPGTT. #$P < 0.05$ (αTC-1 vs. sham), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (αTCΔPC2 vs. sham). *D–F*: IPGTT (2 g/kg) performed in GLP-1R$^{+/+}$ (*D*) or GLP-1R$^{-/-}$ (*E*) mice 7 days posttransplantation with 4.0 × 10^7 αTCΔPC2 cells (n = 5–10). *F*: AUC for IPGTTs. *$P < 0.05$ (αTCΔPC2 vs. sham).

than did αTC-1 recipients (28.8 ± 1.8 vs. 16.7 ± 2.4% of α-cells, respectively; $P < 0.01$; $n \geq 7$). However, whereas αTC-1 recipients exhibited no change in insulin-positive area, islet size, or islet number relative to sham-operated controls (Fig. 7*C–E*), they tended to have decreased total glucagon-positive pancreatic area (Fig. 7*F*; $P = 0.068$). The average glucagon-positive area per islet was decreased in αTC-1 recipients, although the number of glucagon-positive areas was unchanged (Fig. 7*G* and *H*). β-Cell replication as assessed by insulin/PCNA costaining was not altered in αTC-1 recipients relative to controls (data not shown).

We also examined jejunal sections for evidence of possible effects that any αTCΔPC2– or αTC-1–derived products might have had on this tissue. Compared with sham-operated controls, αTCΔPC2 recipients displayed increased crypt plus villus height and increased proliferation (i.e., PCNA$^+$ cells) in the region 16–20 cells up from the crypt base (Fig. 8*A* and *B*). In contrast, αTC-1 recipients displayed no change in crypt plus villus height or crypt cell proliferation compared with their respective controls (Fig. 8*C* and *D*).

DISCUSSION

In this study, we compared the effect of transplanting α-cells in which proglucagon was processed as normal via PC2 (αTC-1) or as in the L-cell via PC1/3 (αTCΔPC2) (Fig. 1). As has been reported previously (18,19), we observed that αTC-1 cells express PC2 but not PC1/3 and secrete glucagon but not GLP-1. In contrast, αTCΔPC2 cells, which lack bioactive PC2 (14), expressed abundant levels of PC1/3 and thus produced high levels of GLP-1 but very little glucagon. The upregulation of PC1/3 in the absence of PC2 seems to be specific to this cell line and not a more general feature of loss of function of PC2 in α-cells, since there is no apparent induction of PC1/3 expression in the α-cells of PC2$^{-/-}$ mice (2,20). Robust PC1/3 expression in αTCΔPC2 cells may reflect developmental arrest induced by transformation at a stage when PC2 and PC1/3 are normally coexpressed in the developing α-cell (14,20,21). High glucose culture conditions may have further promoted PC1/3 expression in αTCΔPC2 cells, since hyperglycemia has been shown to increase PC1/3 expression in rodent α-cells (22).

Alginate encapsulation provides a means of protecting transplanted cells from host immune attack and has been used to achieve long-term maintenance of transplanted islets in models of diabetes (23,24). We used cell encapsulation to allow us to examine the long-term delivery of PC2- versus PC1/3-derived PGDPs in mice in the absence of immunosuppression. Capsules remained interspersed throughout the intraperitoneal cavity of transplant recipients, although beyond ~3–4 weeks posttransplant, capsules occasionally hardened and clumped together. Because at the time of death mice receiving αTC-1 or αTCΔPC2 cells still had elevated plasma levels of glucagon and GLP-1, respectively, transplanted cells likely remained viable even 30 days posttransplantation.

CD-1 mice that received transplants of glucagon-producing αTC-1 cells displayed increased fasting blood glucose levels, whereas mice that received GLP-1–producing αTCΔPC2 cells exhibited greatly improved glucose tolerance (Fig. 2). Thus, by changing the processing enzyme present in α-cells from PC2 to PC1/3, transplanted cells were converted from hyperglycemia promoting to causing a robust improvement in glucose homeostasis, even in normal mice that were not glucose intolerant. Transplantation of αTCΔPC2 cells to GLP-1R$^{-/-}$ mice revealed that the improvement in glucose tolerance observed in normal mice was primarily due to GLP-1R signaling. Given that GLP-1R signaling has been implicated in β-cell survival (1,11,25), this finding led us to compare the effect of αTC-1 and αTCΔPC2 transplantation in a mouse model of diabetes.

In the low-dose STZ model, sham-operated mice developed hyperglycemia 1–2 weeks after STZ administration,

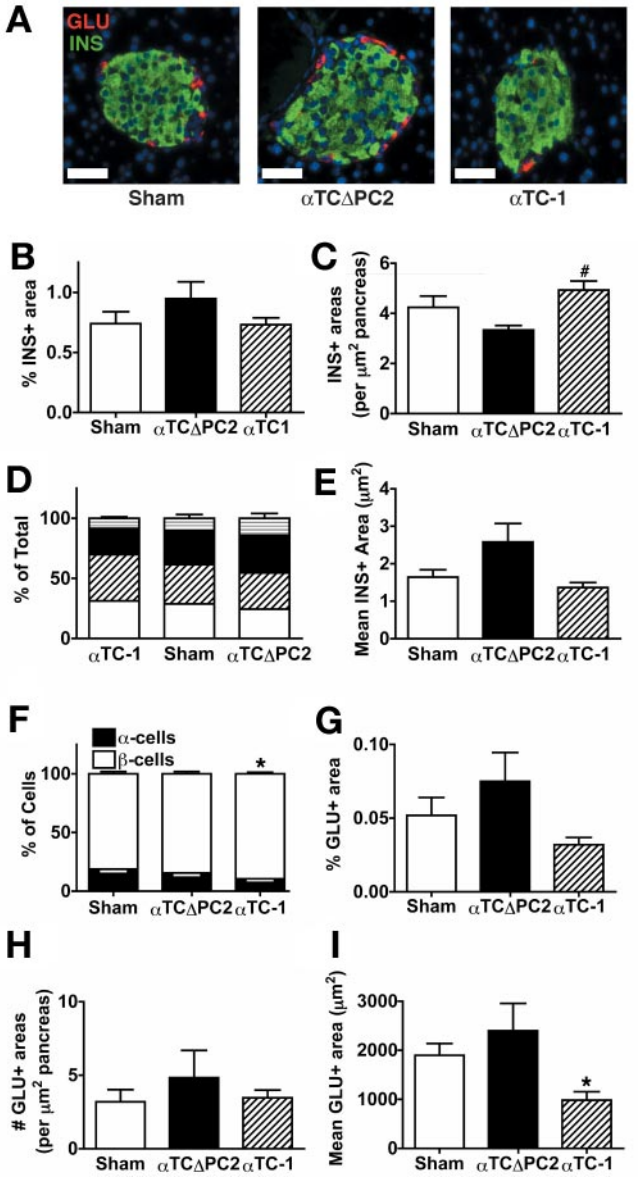

FIG. 3. Morphometric analysis of pancreata from mice transplanted with encapsulated αTCΔPC2 or αTC-1 cells. CD-1 mice transplanted with αTCΔPC2 or αTC-1 cells were killed 16 days posttransplant ($n \geq 4$). *A*: Insulin (green) and glucagon (red) in representative islets from transplant recipients. Scale bar = 100 μm. *B*: Total insulin-positive area per section, expressed as percentage of sectional pancreas area. *C*: Number of insulin-positive clusters per section, normalized for sectional pancreas area. *D*: Islet number, stratified by islet size, expressed as percentage of total islets per section. □, >2,500 μm²; ▨, 2,501–10,000 μm²; ■, 10,001–50,000 μm²; ▤, >50,001 μm². *E*: Average size of an individual insulin-positive area (μm²). *F*: Proportion of α- and β-cells, expressed as percentage of total α- and β-cells per islet. *G*: Total glucagon-positive area per section, expressed as percentage of sectional pancreas area. *H*: Number of glucagon-positive clusters per section, normalized for sectional pancreas area. *I*: Average size of an individual glucagon-positive area. *$P < 0.05$ (sham vs. αTC-1). #$P < 0.05$ (αTCΔPC2 vs. αTC-1). (Please see http://dx.doi.org/10.2337/db07-0563 for a high-quality digital representation of this figure.)

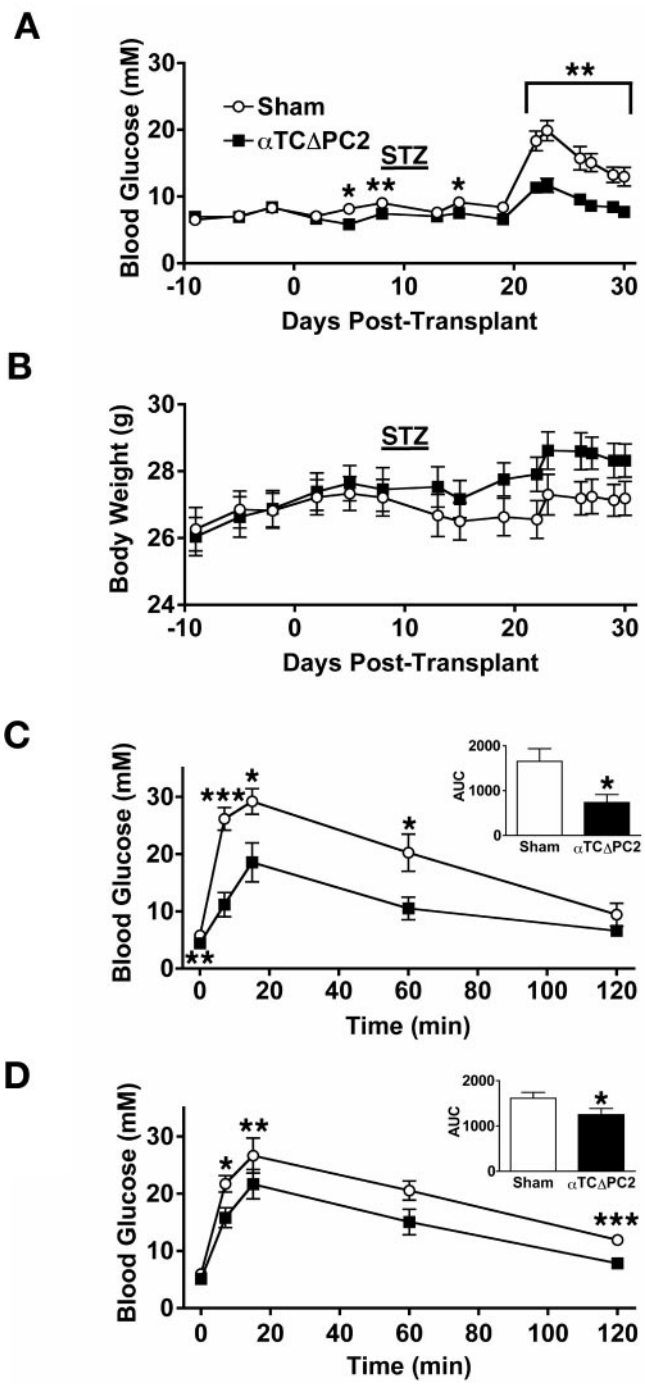

FIG. 4. Transplantation of encapsulated αTCΔPC2 cells prevents hyperglycemia onset in multiple low-dose STZ-treated mice. C57BL/6 mice received sham surgery or cell transplant on day 0 and daily STZ (50 mg/kg) from days 8 to 12 inclusive ($n \geq 8$). Four-hour morning fasted blood glucose (*A*) and body weight (*B*) of recipients is shown. *C*: IPGTT (2 g/kg) performed 7 days posttransplant. Inset: AUC. *D*: Oral glucose tolerance test (2 g/kg) performed 20 days posttransplant. Inset: AUC. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with sham.

and whereas αTCΔPC2 recipients were protected against diabetes development and had improved glucose tolerance (Fig. 4*A*), αTC-1 recipients developed hyperglycemia along with their respective controls and had impaired glucose tolerance (Fig. 5). Histological examination of the pancreas revealed that αTCΔPC2 recipients had greater insulin-positive area than controls (Fig. 6), whereas αTC-1 recipients showed no change compared with controls (Fig. 7). Interestingly, sham-operated mice displayed the disrupted islet architecture typical of the multiple low-dose STZ model (26), whereas αTCΔPC2 recipients did not (Fig. 6). The relative increase in the number of β-cells per islet, and the increases in islet area and β-cell replication observed in αTCΔPC2-treated mice, suggest that a PC1/3-derived PGDP may have stimulated β-cell replication. GLP-1 is the most likely candidate given its known trophic

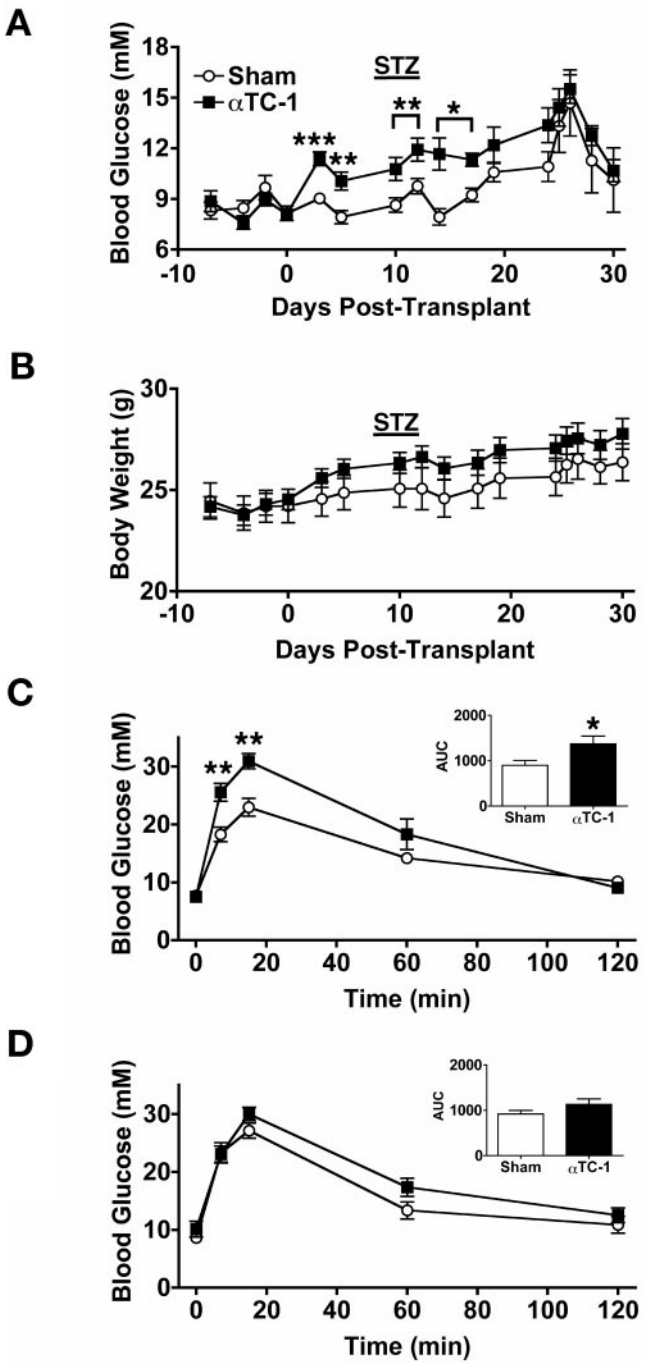

FIG. 5. Transplantation of encapsulated αTC-1 cells does not prevent hyperglycemia in multiple low-dose STZ-treated mice. C57BL/6 mice received sham surgery or cell transplant on day 0 and daily STZ (50 mg/kg) from days 8 to 12 inclusive ($n \geq 8$). Four-hour morning fasted blood glucose (*A*) and body weight (*B*) of recipients is shown. *C*: IPGTT (2 g/kg) performed 7 days posttransplant. Inset: AUC. *D*: Oral glucose tolerance test (2 g/kg) performed 20 days posttransplant. Inset: AUC. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with sham.

effects on β-cell mass (1,11,27). It is possible that GLP-1 produced by the transplanted cells might also have protected islets against STZ-induced apoptosis in the αTCΔPC2 recipients, consistent with GLP-1's known pro-survival effect on β-cells (1,11,25). Further supporting the concept that these effects are mediated by a PC1/3-derived PGDP is the observation that transplanted PC2-expressing αTC-1 cells afforded no protection against STZ-induced hyperglycemia and did not induce greater insulin-positive area or β-cell proliferation (Fig. 7).

Consistent with our observations in CD-1 mice transplanted with αTC-1 cells, αTC-1–transplanted C57BL/6 mice displayed increased fasting blood glucose levels even before STZ treatment (Fig. 5). αTC-1 transplant in this model also caused impaired glucose tolerance. It is unclear how transplanted αTC-1 cells impaired glucose tolerance in C57BL/6 but not CD-1 mice, but it is possible that different genetic backgrounds have different capacities to compensate for hyperglucagonemia. In both models, chronic stimulation of hepatic glucose output by the excessive glucagon coming from the transplanted αTC-1 cells may have led to the observed perturbations in blood glucose levels. Consistent with the notion that ectopic glucagon decreased the demand for pancreatic glucagon, αTC-1 recipients exhibited α-cell hypoplasia in the endogenous pancreas (Figs. 3 and 7). This observation supports previous studies in which α-cell atrophy resulted secondary to hyperglucagonemia induced by transplantation of a glucagonoma (28,29) or by glucagon minipump (30). Notably, α-cell hypoplasia was absent in CD-1 mice receiving αTCΔPC2 transplants, providing further evidence that this was a compensatory adaptation specific to the PC2 product glucagon.

Disorganization of islet architecture and a relative increase in number of α-cells have been widely reported in models of β-cell damage or dysfunction (26,31–34). It remains unclear whether these architectural changes reflect true α-cell hyperplasia or are an artifact of decreased islet size and the inward collapse of islets as β-cells are destroyed. It is also unknown whether this putative change in α-cell mass is secondary to hyperglycemia or is directly due to the loss of an intra-islet β-cell–derived signal. However, in the current study, αTC-1 recipients were protected against STZ-induced disorganization of islet architecture and α-cell hyperplasia, even in the face of hyperglycemia. Our studies therefore suggest that hyperglycemia itself is not sufficient to induce islet disorganization and α-cell hyperplasia.

In addition to GLP-1, it is likely that αTCΔPC2 cells also produce oxyntomodulin and GLP-2, since these are also products of PC1/3 cleavage and are cosecreted along with GLP-1 from the enteroendocrine L-cell (1,11,35). The observation that αTCΔPC2 transplant improved glucose tolerance in wild-type, but not GLP-1R$^{-/-}$, mice suggests that GLP-1 coming from the transplanted cells is the primary PC1/3-derived proglucagon product that mediates this effect. However, since GLP-1R$^{-/-}$ mice receiving αTCΔPC2 cells had lower blood glucose than controls 15 min after glucose delivery, some other product of αTCΔPC2 cells may also have beneficial effects on glucose handling. Oxyntomodulin has been reported to stimulate insulin secretion (36,37) and thus is a potential candidate contributing to glucose-lowering effects, although such actions might also be mediated in part through the GLP-1 receptor (38).

STZ-treated αTCΔPC2 recipients had increased plasma GLP-2 levels relative to controls and hyperplasia of the intestinal epithelium (Fig. 8*A* and *B*). Notably, these effects were absent in mice that received αTC-1 cells, which lack the capacity for GLP-2 production (Fig. 8*C* and *D*). Our observations in αTCΔPC2 recipient mice are consistent with the known role of GLP-2 as a proliferative factor for intestinal enterocytes (11,39,40) and agree with a recent study in which injection of a GLP-2 analog resulted in

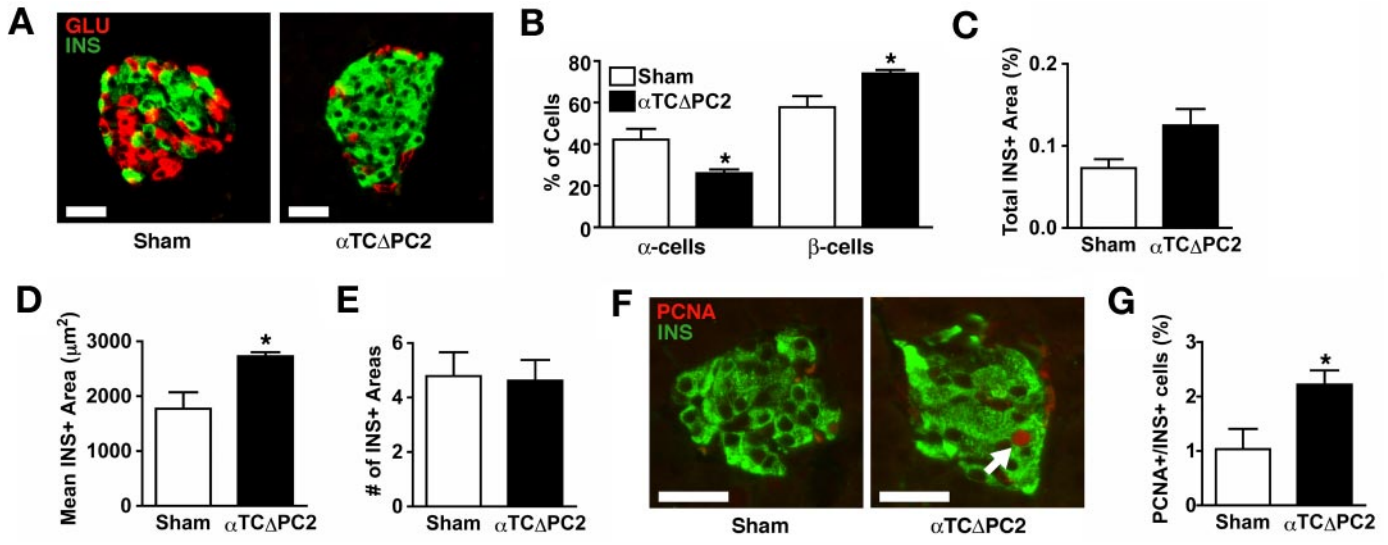

FIG. 6. Morphometric analysis of pancreata from STZ-treated mice receiving encapsulated αTCΔPC2 cells. Mice were killed 30 days posttransplant ($n \geq 3$). *A*: Insulin (green) and glucagon (red) in representative islets from recipients. Scale bar = 100 μm. *B*: Proportion of α- and β-cells in islets, expressed as percentage of total combined α- and β-cells per islet. *C*: Total insulin-positive area, expressed as percentage of sectional pancreas area. *D*: Average area of an individual insulin-positive region. *E*: Number of insulin-positive areas per animal, normalized for sectional pancreas area. *F*: Insulin (green) and PCNA (red) staining in representative islets from recipients. Scale bar = 50 μm. Arrow identifies a PCNA$^+$ β-cell. *G*: Number of PCNA$^+$ β-cells, expressed as a percentage of total insulin-positive cells. *$P < 0.05$ vs. sham. (Please see http://dx.doi.org/10.2337/db07-0563 for a high-quality digital representation of this figure.)

greater proliferation in the "rapidly proliferating transit zone" defined by cells 11–20 of the jejunal crypts (17). Since a role for GLP-2 in promotion of β-cell function or survival has not been described (41) and since the GLP-2 receptor has not been localized to the β-cell (42), we consider it unlikely that GLP-2 acted directly on the β-cell to contribute significantly to the phenotype of αTCΔPC2 recipient mice.

In summary, these studies demonstrate that simply switching the PC profile in proglucagon-expressing cells can render them either glucose raising (PC2) or glucose lowering (PC1/3). Transplantation of GLP-1–producing αTCΔPC2 cells prevented STZ-induced hyperglycemia, which supports the growing body of evidence suggesting that long-term activation of the GLP-1 receptor in mice promotes β-cell survival and proliferation (25,43,44). We have recently shown that delivery of PC1/3 to islets induces GLP-1 production, improves islet function and survival, and enhances islet transplantation outcomes in STZ-treated mice (45). The current work provides additional evidence that modulation of proglucagon processing in the α-cell promotes the release of a beneficial profile of PGDPs and could be useful for diabetes therapy. Moreover, αTCΔPC2 cells provide a model system for studying

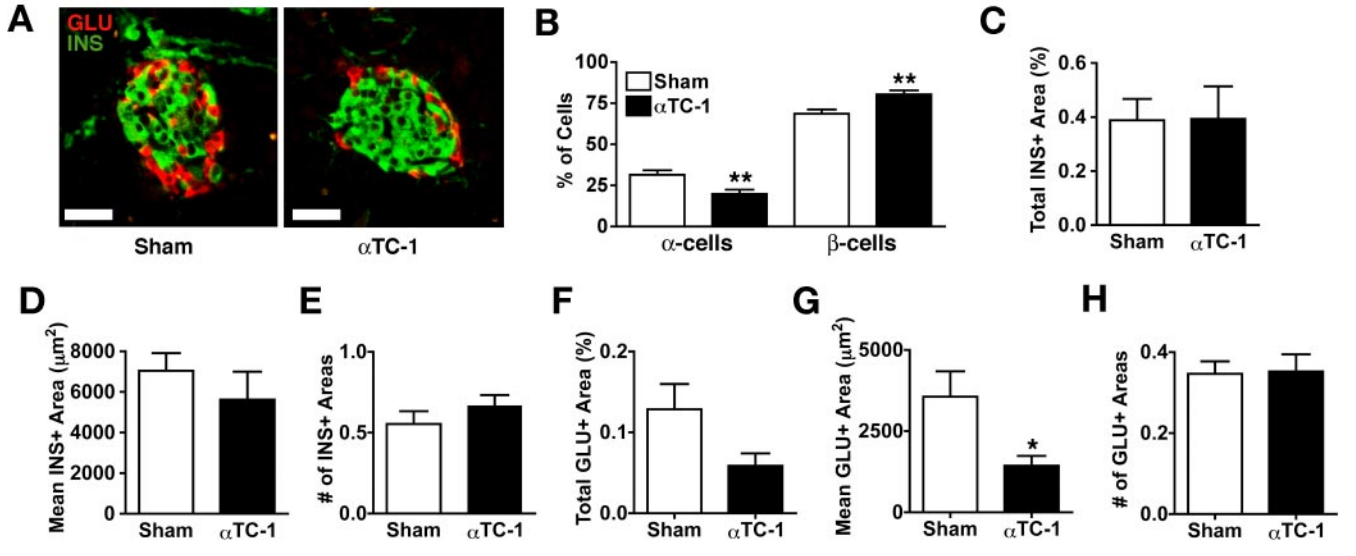

FIG. 7. Morphometric analysis of pancreata from STZ-treated mice receiving encapsulated αTC-1 cells. Mice were killed 30 days posttransplant ($n \geq 6$). *A*: Insulin (green) and glucagon (red) in representative islets from recipients. Scale bar = 100 μm. *B*: Proportion of α- and β-cells in islets, expressed as percentage of total combined α- and β-cells per islet. *C*: Total insulin-positive area per animal, expressed as percentage of sectional pancreas area. *D*: Average area of an individual insulin-positive cluster. *E*: Number of insulin-positive areas, normalized for sectional pancreas area. *F*: Total glucagon-positive area per section, expressed as percentage of sectional pancreas area. *G*: Average size of an individual glucagon-positive area. *H*: Number of glucagon-positive areas, normalized for sectional pancreas area. *$P < 0.05$, **$P < 0.01$ vs. sham. (Please see http://dx.doi.org/10.2337/db07-0563 for a high-quality digital representation of this figure.)

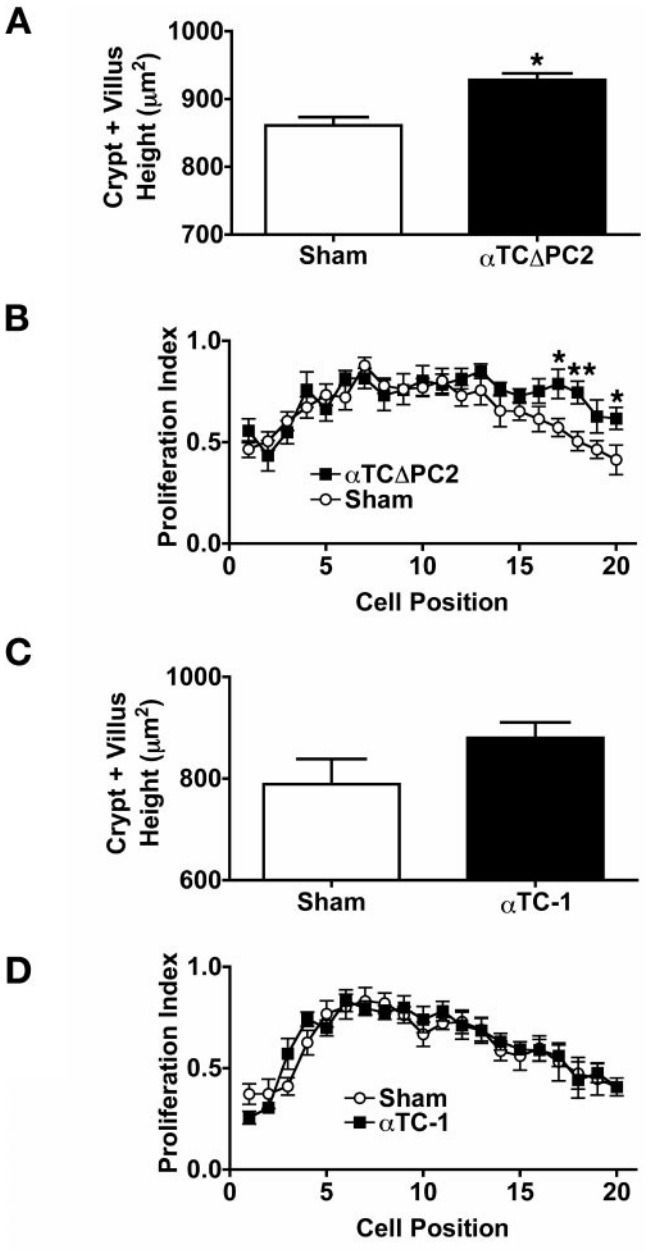

FIG. 8. Jejunal proliferation in STZ-treated mice receiving encapsulated αTCΔPC2 or αTC-1 cells. Jejunal proliferation was assessed in sections prepared from αTCΔPC2 (*A* and *B*) or αTC-1 (*C* and *D*) recipients 30 days posttransplantation. Crypt plus villus height was measured from the base of the crypt to the tip of the villus in duplicate for at least four upright intact villi per animal using a hematoxylin-stained slide (*A* and *C*). Distribution of $PCNA^+$ cells in jejunal crypts immunostained for PCNA (*B* and *D*). The cell at the midpoint of the crypt base was defined as position 1, and the first 20 cells extending upward from this position were scored as $PCNA^-$ (0) or $PCNA^+$ (1). *$P < 0.05$, **$P < 0.01$ compared with respective sham.

alternate proglucagon processing and examining the impact of continuous delivery of PC1/3-derived proglucagon products.

ACKNOWLEDGMENTS

This research was funded by grants from the Canadian Diabetes Association, the Canadian Institutes for Health Research, and the Juvenile Diabetes Research Foundation (JDRF). T.J.K. received a Career Development Award from JDRF and scholarship support from the Michael Smith Foundation for Health Research (MSFHR). R.D.W. received scholarship support from MSFHR and the Natural Sciences and Engineering Research Council.

We thank Dr. D. Steiner (University of Chicago) for helpful discussions and Dr. L. Devi (Mount Sinai School of Medicine) for providing PC1/3 antisera. We acknowledge the excellent technical support provided by C. Donald for these studies.

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