Liver-Specific Disruption of the Murine Glucagon Receptor Produces α-Cell Hyperplasia

Evidence for a Circulating α-Cell Growth Factor

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Glucagon is a critical regulator of glucose homeostasis; however, mechanisms regulating glucagon action and α-cell function and number are incompletely understood. To elucidate the role of the hepatic glucagon receptor (Gcgr) in glucagon action, we generated mice with hepatocyte-specific deletion of the glucagon receptor. GcgrHep−/− mice exhibited reductions in fasting blood glucose and improvements in insulin sensitivity and glucose tolerance compared with wild-type controls, similar in magnitude to changes observed in Gcgr−/− mice. Despite preservation of islet Gcgr signaling, GcgrHep−/− mice developed hyperglucagonemia and α-cell hyperplasia. To investigate mechanisms by which sig- naling through the Gcgr regulates α-cell mass, wild-type islets were transplanted into Gcgr−/− or GcgrHep−/− mice. Wild-type islets beneath the renal capsule of Gcgr−/− or GcgrHep−/− mice exhibited an increased rate of α-cell proliferation and expansion of α-cell area, consistent with changes exhibited by endogenous α-cells in Gcgr−/− and GcgrHep−/− mice. These results suggest that a circulating factor generated after disruption of hepatic Gcgr signaling can increase α-cell proliferation independent of direct pancreatic input. Identification of novel factors regulating α-cell proliferation and mass may facilitate the generation and expansion of α-cells for transdifferentiation into β-cells and the treatment of diabetes. Diabetes 62:1196–1205, 2013

The islets of Langerhans comprise distinct populations of differentiated endocrine cells whose functions are critical for maintenance of metabolic homeostasis. Considerable progress has been made in understanding the control of β-cell growth, function, and survival (1). Moreover, advances in understanding the developmental and adaptive control of β-cell formation coupled with insights gleaned from studies of adult and embryonic pancreatic endocrine stem cells have yielded new information regarding the cellular origin and formation of differentiated adult β-cells.

In contrast, much less is known about mechanisms governing the development, growth, and survival of the glucagon-secreting α-cell (2). Glucagon secretion is stimulated by exercise or hypoglycemia; conversely, glucagon secretion is suppressed during conditions of fuel abundance. However, development of diabetes is often associated with failure to suppress glucagon secretion in the fed state (3,4); hence, therapeutic efforts to suppress α-cell function for the treatment of type 2 diabetes are ongoing (4). Moreover, α-cell mass appears dynamic in the context of diabetes, with expansion of α-cell mass noted in the diabetic primate (5) and human pancreas (6).

The observation that functionally differentiated α-cells can arise from α-cell precursors (7–9) has engendered additional interest in the control of α-cell growth. α-Cell hyperplasia is frequently observed in settings of partial or complete glucagon deficiency (10,11) or resistance to glucagon action (12). Mice with targeted disruption of Pesk2 exhibit impaired generation of bioactive glucagon, mild hypoglycemia, and marked α-cell proliferation, findings that are rapidly reversed by glucagon administration (13). Similarly, transgenic expression of Pax4 in pancreatic endocrine cells results in relative glucagon deficiency and compensatory α-cell proliferation; exogenous glucagon administration in this setting also reduces α-cell pro- liferation (7). Both transient genetic reduction of glucagon receptor (Gcgr) expression in normoglycemic or diabetic mice using antisense oligonucleotides or complete genetic germline disruption of GCGR signaling are associated with α-cell hyperplasia (10,14). Collectively, these findings raise the possibility that α-cell transdifferentiation toward a β-cell phenotype may represent an alternative strategy for replenishment of β-cell mass in vivo.

Despite evidence linking reduction in Gcgr signaling to α-cell hyperplasia, the precise tissues and signals important for stimulation of α-cell proliferation remain unknown. Because levels of GLP-1, a potent stimulator of islet cell prolif-eration, are extremely high in mice with partial or complete attenuation of Gcgr signaling (10,14), we analyzed α-cell mass in Gcgr−/−/Glp1r−/− mice (15). Although elimination of the Glp1r in Gcgr−/−/ mice reversed improvements in β-cell function, fasting glycemia, and inhibition of gastric emptying, Gcgr−/−/Glp1r−/− mice continued to exhibit marked islet and α-cell hyperplasia. Similarly, mice with liver-specific disruption of Gso exhibit α-cell hyperplasia despite genetic elimination of the Glp1r (16). Hence, although GLP-1 controls
α-cell function, the Glp1r is not required for development of α-cell hyperplasia in Gcgr−/− mice.

To elucidate mechanisms and tissues through which reduction in GCGR signaling promotes α-cell hyperplasia, we have assessed the importance of the liver and the tissue environment. Surprisingly, selective elimination of the hepatic Gcgr in GcgrHep−/− mice was sufficient to recapitulate the phenotype of α-cell hyperplasia in the endogenous pancreas, suggesting that reduction of GCGR signaling in liver originates one or more signals that promote α-cell proliferation. Remarkably, transplantation of GcgrHep+/− islets under the kidney capsule of Gcgr−/− or GcgrHep−/− mice resulted in stimulation of α-cell proliferation and hyperplasia in transplanted islets, implying the existence of one or more circulating factors capable of promoting α-cell hyperplasia independent of the normal pancreatic microenvironment.

RESEARCH DESIGN AND METHODS

Animals. Gcgr−/− mice on a C57BL/6 background were maintained as previously reported and generated by heterozygous-heterozygous breeding (10). Albumin-Cre (stock 005574) (17) and FLPe (stock 005703) transgenic mice were from Jackson Laboratory. GcgrFlox chimeric mice carrying one loxP site between exons 5 and 6 and two loxP sites in a neomycin cassette inserted between exons 12 and 13 of the Gcgr gene were generated in the C57BL/6 background, and the neomycin cassette was removed using the FLPe-FRT system. GcgrHep−/− mice were generated by breeding GcgrFlox mice and Albumin-Cre mice. All animals were maintained on a standard rodent chow under a normal 12 h light/12 h dark cycle. All wild-type Gcgr−/+ control groups were used as littermates; 1% cholic acid diet was obtained from Harlan. Experiments were conducted according to protocols and guidelines approved by the Mount Sinai Hospital Animal Care Committee or the Vanderbilt University Institutional Animal Care and Use Committee. The metabolic phenotype observed in GcgrHep−/− mice was similar in males and females.

Glucose challenge and measurement of plasma metabolites. Glucose tolerance tests were performed as described (18,19). For insulin and glucagon challenge, mice were fasted 24 h prior to glucose or insulin injection. Glucose challenge and measurement of plasma metabolites. Glucose tolerance tests were performed as described (18,19). For insulin and glucagon challenge, mice were fasted 24 h prior to glucose or insulin injection.

Insulin was measured using a mouse insulin ELISA kit (Alpco). Insulin and glucagon were measured using a mouse Milliplex assay (Millipore). SDF-1 levels were measured using a mouse Milliplex assay (all from Millipore) by the Vanderbilt Hormone and Analytical Core.

Statistical analysis. Statistical significance was assessed by one-way or two-way ANOVA using Bonferroni multiple comparison posttest and, when appropriate, by unpaired Student t test using GraphPad Prism 5 (GraphPad Software; San Diego, CA). A P < 0.05 was considered to be statistically significant.

RESULTS

Generation of mice with hepatocyte-specific deletion of the glucagon receptor. To generate GcgrHep−/− mice, GcgrFlox mice were mated with Alb-Cre mice (Supplementary Fig. 1A). Gcgr expression in the liver was abundant and comparable in wild-type and GcgrFlox mice and was virtually absent in GcgrHep−/− mice (Supplementary Fig. 1B). In contrast, Gcgr mRNA transcripts were expressed at normal levels in other tissues, including pancreas, kidney, and jejunum (Supplementary Fig. 1C–E).

Deletion of the Gcgr in hepatocytes leads to mild hypoglycemia and abrogates the hyperglycemic response to acute glucagon challenge. GcgrHep−/− mice exhibited normal body weight compared with littermate controls (Supplementary Fig. 2A) and reductions in fasting (Fig. 1A) and random glucose levels (Supplementary Fig. 2B), similar to Gcgr−/− mice (11,16). Plasma glucose levels increased briskly in response to exogenous glucagon in control mice; however, GcgrHep−/− mice failed to exhibit a glycemic response after acute glucagon challenge (Supplementary Fig. 2C), findings that also were reported for Gcgr−/− mice (10).

Both Gcgr−/− and GcgrHep−/− mice display improved glucose tolerance, decreased fasting insulin, and increased plasma glucagon levels. Intraportal injection (Fig. 1B) and oral (Fig. 1F) glucose tolerance were improved in GcgrHep−/− mice compared with littermate controls, findings that are similar to those described for Gcgr−/− mice (10). Plasma glucagon levels were markedly elevated in GcgrHep−/− mice (Fig. 1C, G) and similar to levels reported for Gcgr−/− mice (10,11). Furthermore, plasma insulin levels were reduced in the fasting state, but were not significantly different during glucose challenge in GcgrHep−/− mice compared with wild-type and GcgrFlox littermate controls (Fig. 1D, H), as reported in Gcgr−/− mice (10). Nevertheless, fasting glucose and insulin were lower, and insulin-to-glucose ratios were significantly increased in GcgrHep−/− versus control mice after glucose challenge (Fig. 1B, D–F, H, I). The similarity of the metabolic phenotypes in Gcgr−/− and GcgrHep−/− mice reveals a critical role for the hepatic Gcgr in the phenotype of Gcgr−/− mice.

GcgrHep−/− mice exhibit increased insulin sensitivity. Insulin challenge resulted in a greater initial decline in blood glucose in GcgrHep−/− mice (Fig. 2A) but no difference in overall glucose excursion (Fig. 2B), as previously
shown in Gcgr<sup>−/−</sup> mice (10). Furthermore, Gcgr<sup>−/−</sup> mice displayed increased insulin sensitivity compared with Gcgr<sup>+/+</sup> littermate controls (Fig. 2C, D). Insulin sensitivity was similarly increased in Gcgr<sup>Hep−/−</sup> mice (Fig. 2E, F).

The comparable phenotypes of Gcgr<sup>−/−</sup> versus Gcgr<sup>Hep−/−</sup> mice indicate that the impact of glucagon on glucose tolerance and insulin sensitivity is largely mediated by hepatic glucagon receptors.

**Disruption of GCGR signaling in the liver results in increased pancreas weight, α-cell proliferation, and α-cell hyperplasia.** Gcgr<sup>−/−</sup> mice exhibit markedly increased plasma glucagon levels, increased mass of the entire pancreas and islet, and α-cell hyperplasia (Fig. 3A, B) (10,11). Although total β-cell mass is increased in Gcgr<sup>Hep−/−</sup> and Gcgr<sup>−/−</sup> mice (Supplementary Fig. 3A, B), this increase is secondary to the enlargement of the pancreas itself, because β-cell mass per gram of pancreas is similar in 20-week-old Gcgr<sup>−/−</sup> and wild-type littermate controls (Fig. 3C). Additionally, glucagon and GLP-1 content in the pancreas of Gcgr<sup>−/−</sup> mice is markedly increased, whereas insulin and C-peptide content is unchanged when corrected for changes in pancreatic mass (Supplementary Fig. 2A). The increase in α-cell mass (Fig. 3D) is accompanied by a significant increase in α-cell proliferation (Fig. 3E–G). Despite preservation of Gcgr expression in the pancreas (Supplementary Fig. 1C), Gcgr<sup>Hep−/−</sup> mice exhibited markedly elevated plasma GLP-1 and glucagon levels (Supplementary Fig. 1C, G), increased pancreatic weight (Fig. 3B), and absolute β-cell mass (Supplementary Fig. 3B), but normal β-cell mass per gram of pancreas (Fig. 4A), comparable with the phenotype of Gcgr<sup>−/−</sup> mice (Fig. 3 and Supplementary Fig. 3A, B). Although GIP is produced in α-cells (23), plasma GIP levels were normal in Gcgr<sup>−/−</sup> and Gcgr<sup>Hep−/−</sup> mice (Supplementary Fig. 4C, D). Despite preservation of pancreatic Gcgr expression, Gcgr<sup>Hep−/−</sup> mice exhibited increased α-cell proliferation with islet and α-cell hyperplasia (Fig. 4A, D–G), indicating that this process is mediated by loss of hepatic GCGR signaling.

**Increased α-cell proliferation in islets transplanted into Gcgr<sup>−/−</sup> mice.** The findings of islet and α-cell hyperplasia arising in Gcgr<sup>Hep−/−</sup> mice implies that...
disruption of GCGR signaling in the liver initiates signals that are, in turn, communicated to the pancreas, perhaps through neural pathways or circulating factors, promoting \( \beta \)-cell proliferation. To determine if the proliferative signals are dependent on the pancreatic location, we transplanted 14-week-old \( Gcgr^{+/+} \) (wild-type) islets into 14-week-old \( Gcgr^{+/+} \) and \( Gcgr^{-/-} \) mice and allowed them to engraft for 8 weeks (Fig. 5A). We also transplanted \( Gcgr^{-/-} \) islets into the contralateral kidney of the same \( Gcgr^{+/+} \) and \( Gcgr^{-/-} \) mice. In this approach, the endogenous pancreatic islets in the transplant recipient serve as a control. Isolated islets from 14-week-old \( Gcgr^{-/-} \) mice before transplantation had higher glucagon and GLP-1 content, but lower insulin and C-peptide content when compared with \( Gcgr^{+/+} \) islets (Fig. 5B). Remarkably, transplantation of \( Gcgr^{+/+} \) islets under the kidney capsule of \( Gcgr^{-/-} \) mice resulted in a striking increase in glucagon and GLP-1 content of the grafts (Fig. 5C). A small increase in insulin also was observed in \( Gcgr^{+/+} \) grafts in \( Gcgr^{-/-} \) recipient mice. The area of glucagon-positive cells in the transplanted \( Gcgr^{+/+} \) islets also was increased in \( Gcgr^{-/-} \) recipients (Fig. 5E, F, I). Furthermore, \( Gcgr^{+/+} \) islets transplanted into \( Gcgr^{-/-} \) mice exhibited significantly increased \( \alpha \)-cell proliferation compared with \( Gcgr^{-/-} \) islets.

**FIG. 2.** Ablation of the \( Gcgr \) in hepatocytes increases insulin sensitivity. A: Intraperitoneal (IP) insulin tolerance test in 13-week-old male \( Gcgr^{Hep-/-} \) and littermate controls fasted for 5 h (\( n = 8-10 \) mice). B: Area under the curve (AUC) glucose from the IP insulin tolerance test shown in A. Hyperinsulinemic euglycemic clamp performed in conscious \( Gcgr^{-/-} \) (C, E) or \( Gcgr^{Hep-/-} \) (E, F) males and littermate controls fasted for 5 h (\( n = 5-7 \) mice). C and E: Glycemic excursion during stabilization and steady-state phase. D and F: Glucose infusion rates during steady state to maintain euglycemia. Data are mean ± SEM. *\( P < 0.05 \), **\( P < 0.01 \) vs. wild-type mice.
the grafts of Gcgr+/+ proliferation was extremely low after transplantation of Gcgr mice after 8 weeks. Gcgr+/+ recipients. Recipients recapitulated the phenotype observed in recipients, similar to the rate of proliferation 1 month after transplantation compared with those transplanted into GcgrHep mice (Fig. 7A–F). The magnitude of increase in glucagon area in Gcgr+/+ islets transplanted into GcgrHep−/− recipients after 4 weeks was less than after transplantation of Gcgr+/+ islets into Gcgr−/− mice after 8 weeks.

Analysis of potential mechanisms contributing to α-cell hyperplasia. Because β-cell hyperplasia arising as a result of liver insulin resistance has been linked to increased ERK1/2 phosphorylation through a liver–pancreas vagal signal, we assessed whether loss of hepatic glucagon signaling leads to enhanced ERK1/2 phosphorylation (25). Western blot analysis of hepatic extracts from wild-type, Gcgrlox−/−, and GcgrHep−/− mice revealed similar levels of ERK1/2 phosphorylation across genotypes (Supplementary Fig. 5).

Plasma levels of interleukin-6, associated with α-cell hyperplasia (26), were similar in control, Gcgr−/−, and GcgrHep−/− mice (Supplementary Fig. 6A, B). Plasma levels of SDF-1, known to induce expression of prohormone convertase-1, GLP-1, and islet cell proliferation (27), were similar in Gcgr−/−, GcgrHep−/−, and littermate controls (Supplementary Fig. 6C, D). Total bile acid levels were significantly increased in plasma from GcgrHep−/− and Gcgr−/− mice (Supplementary Fig. 6E, F), consistent with recent observations (28).

Because bile acids increase the secretion of proglucagon-derived peptides and promote cell proliferation, we fed wild-type mice a diet enriched with 1% cholic acid and assessed α-cell mass. Plasma bile acid levels were significantly increased after cholic acid feeding, in association with reduced levels of glucose and insulin (Supplementary Fig. 6G–J); however, pancreas weight (Supplementary Fig. 6J) and plasma glucagon levels (Supplementary Fig. 6K) were not increased. The α-cell mass was modestly increased, whereas β-cell mass was unchanged in cholic
acid–fed mice (Supplementary Fig. 6L, M). Therefore, it seems unlikely that increased bile acids produce the phenotypes of hyperglucagonemia and increased α-cell mass in Gcgr−/− mice. Taken together, the findings from GcgrHep−/− mice and transplantation experiments support the existence of one or more circulating factors that arise after elimination of liver GCGR signaling, which promote α-cell proliferation independent of the normal pancreatic location.

DISCUSSION

By comparing mice with whole-body and hepatocyte-specific inactivation of the Gcgr, we found that the predominant phenotypes arising in Gcgr−/− mice are recapitulated by selective loss of the Gcgr in hepatocytes. Both Gcgr−/− mice and GcgrHep−/− mice had similar reductions in fasting glucose and improvements in oral and intraperitoneal glucose tolerance and insulin sensitivity. Gcgr−/− mice and GcgrHep−/− mice also had development of similar increases in pancreatic and α-cell mass. Notably, normal islets transplanted into either Gcgr−/− or GcgrHep−/− mice displayed a marked increase in α-cell proliferation. This increased rate of α-cell proliferation at an extrapancreatic site establishes the liver as a critical organ for initiation of signals promoting the proliferation of islet α-cells after reduction or elimination of GCGR signaling.

Reduced or absent glucagon receptor signaling produced by several complementary approaches (inactivation or a reduction in glucagon and/or its receptor by genetic manipulation, immunoblockade, or antisense oligonucleotides) leads to α-cell expansion (10,14,29). Conversely, increased glucagon receptor signaling by administration of glucagon several times daily for up to 20 days produces marked atrophy of α-cells in the endocrine pancreas (30). Experimental hyperglucagonemia in rodents with transplantable glucagonomas also produces rapid diminution of islet and α-cell mass, apoptosis of α-cells, and depletion of proglucagon mRNA expression and immunoreactivity of glucagon in the pancreas (31,32). In the transplantation studies presented here, α-cell proliferation and glucagon content are reduced when Gcgr−/− islets are transplanted into Gcgr−/− recipients versus Gcgr+/+ recipients (Figs. 5 and 6). This is similar to the reversal of α-cell hyperplasia observed in Psk2−/− mice rescued with glucagon supplementation and GCGR antibody-treated mice on removal of treatment and restoration of glucagon signaling.

Our findings of robust α-cell hyperplasia in GcgrHep−/− mice are consistent with reports of islet and α-cell hyperplasia arising in Lgsko mice with liver-specific gene activation of the Gsα subunit (12,16). In contrast, disruption of Gsα in the entire pancreas (33) or selectively in β-cells (34) does not produce α-cell hyperplasia. Furthermore, direct attenuation of GCGR signaling in α-cells does not result in increased rates of cell proliferation (35). In agreement with findings from Lgsko mice, our data establish the liver as critical for generation of signals promoting α-cell hyperplasia pursuant to disruption of GCGR-dependent signal transduction pathways. An increase in α-cell proliferation has been reported in the pancreas of Gcgr−/− embryos, raising the possibility...
that the signal triggering expansion of α-cell mass is attributable to the developmental loss of GCGR signaling (11). However, even partial reduction of GCGR signaling in adult mice (14,29,36) induces α-cell hyperplasia, and adult islets rapidly exhibit increased α-cell proliferation after transplantation into Gcgr−/− recipients. Because albumin-Cre-mediated recombination in hepatocytes occurs largely postnatally (17,37), GcgrHep−/− mice would not likely undergo significant elimination of Gcgr expression during embryonic development. Hence, the available evidence strongly suggests that signals triggering α-cell expansion do not require disruption of GCGR-regulated signaling pathways during liver or islet development.

Possible pathways by which reduced or absent hepatic glucagon receptor signaling leads to α-cell hyperplasia include, among others, a stimulus transmitted from liver via intraislet nerves; however, 1-week transplantation data suggest that islet innervation is not necessary for the α-cell hyperplasia. 

**FIG. 5.** Gcgr+/+ islets transplanted into Gcgr−/− mice have development of α-cell hyperplasia. A: Experimental schematic showing islets from 14-week-old Gcgr+/+ or Gcgr−/− mice transplanted beneath the right and left kidney capsule of 14-week-old Gcgr+/+ or Gcgr−/− mice, respectively. The kidneys containing the islet graft were removed for analysis 8 weeks later. B: Hormone content of freshly isolated islets of 14-week-old Gcgr+/+ (open bars) and Gcgr−/− (closed bars) mice. Data are mean ± SEM. *P < 0.05, ***P < 0.001 vs. Gcgr+/+ islets. C: Hormone content of Gcgr+/+ grafts removed from 22-week-old mice Gcgr+/+ (open bars) and Gcgr−/− (light grey bars) recipient mice 8 weeks after transplant. Data are mean ± SEM. *P < 0.05, **P < 0.01 vs. Gcgr+/+ recipients. D: Hormone content of Gcgr−/− grafts removed from 22-week-old mice Gcgr+/+ (closed bars) and Gcgr−/− (dark grey bars) recipient mice 8 weeks after transplantation. Data are mean ± SEM. *P < 0.05, ***P < 0.001 vs. Gcgr−/− recipients. Representative islet graft sections from Gcgr+/+ (donor) to Gcgr+/+ (recipient; E) and Gcgr−/− (donor) to Gcgr+/+ (recipient; F) mice stained for insulin (green) and glucagon (red). E′ and F′: Insets of E and F (white box). Representative islet graft sections from Gcgr−/− (donor) to Gcgr+/+ (recipient; G) and Gcgr−/− (donor) to Gcgr−/− (recipient; H) mice stained for insulin (green) and glucagon (red). G′ and H′: Insets of G and H (white box). White scale bar represents 50 μm. Percentage of islet grafts that stained positive for glucagon for Gcgr+/+ donor (I) and Gcgr−/− donor islets (J) into Gcgr+/+ and Gcgr−/− mice (n = 3 mice, 3 sections/islet graft). Percent glucagon area is defined as the percentage of glucagon area/total insulin + glucagon area. Data are mean ± SEM. ***P < 0.001 vs. Gcgr+/+ recipient grafts.
proliferation. Slightly lower blood glucose could stimulate α-cell hyperplasia; however, previous studies show that mild hypoglycemia arising in some murine models of disrupted glucagon action is not required or sufficient to stimulate α-cell hyperplasia (14,29,31,38–40). Impaired glucagon signaling in β-cells could stimulate α-cell hyperplasia by intrasul signal; however, GcgrHep−/− mice retain intact GCGR function in β-cells yet have development of severe hyperglucagonemia. Excessive insulin signaling in α-cells in the absence of adequate glucagon signaling promotes α-cell hyperplasia; a recent report suggests that insulin can induce α-cell proliferation, and Kawamori et al. (41) have shown that interruption of insulin receptor signaling in α-cells alone can reduce α-cell mass. However, interruption of insulin receptor signaling in α-cells is not sufficient to arrest α-cell hyperplasia observed in mice with global interruption of GCGR signaling (41). A circulating factor released from the liver or released from other tissues after loss of liver GCGR signaling could be responsible for regulating α-cell mass. The current results strongly support the latter possibility, namely the existence of one or more circulating factors regulating α-cell mass. Because α-cell proliferation increased to a greater extent in islets transplanted into Gcgr−/− recipients for 8 weeks, relative to findings in Gcgr+−/− islets grafted in GcgrHep−/− mice for 4 weeks, interpretation of quantitative differences in the magnitude of changes observed in islets transplanted into Gcgr−/− versus GcgrHep−/− mice will require additional experimentation.

Imai et al. (25) demonstrated that increased activity of a hepatic extracellular regulated kinase triggered β-cell proliferation in murine models of insulin resistance or deficiency, and pancreatic afferent vagotomy abolished this stimulation of β-cell proliferation and increased ERK1/2 phosphorylation through a liver–pancreas vagal signal. We did not find a change in ERK1/2 phosphorylation or in plasma levels of interleukin-6, a circulating factor that promotes α-cell proliferation in experimental models of diabetes (26). Similarly, plasma levels of SDF-1, a chemokine known to promote islet GLP-1 production and islet cell proliferation, were normal. Although plasma levels of bile acids are markedly elevated in Gcgr−/− mice, α-cell

FIG. 6. Gcgr+−/− islets transplanted into Gcgr−/− recipients exhibit increased α-cell proliferation. Islet graft sections from Gcgr+−/− (donor) to Gcgr+−/− (recipient; A) and Gcgr+−/− (donor) to Gcgr−/− (recipient; B) mice stained for Ki67 (red), glucagon (green), and dapi (blue). A′ and B′: Insets of A and B (white box from A, B). White arrows indicate Ki67-positive α-cells. Islet graft sections from Gcgr−/− (donor) to Gcgr+−/− (recipient; C) and Gcgr−/− (donor) to Gcgr−/− (recipient; D) mice stained for Ki67 (red), glucagon (green), and dapi (blue). C′ and D′: Insets of C and D (white box from C, D). White arrows indicate Ki67-positive α-cells. White scale bar represents 50 μm. Proliferation rate of α-cells in grafts of islets from 14-week-old Gcgr+−/− (E) or Gcgr−/− (F) mice (donors) transplanted beneath the right and left kidney capsule, respectively, of 14-week-old Gcgr+−/− (recipient) or Gcgr−/− (recipient) mice. The kidneys containing the islet graft were removed for analysis 8 weeks later (n = 3/mice, 3 sections/islet graft). Data are mean ± SEM. **P < 0.01 vs. control grafts (donor and recipient of same genotype).

FIG. 7. Wild-type islets transplanted into GcgrHep−/− recipients for 4 weeks have increased α-cell area and proliferation. Representative islet graft sections from Gcgr+−/− (donor) to GcgrHep−/− mice transplanted into Gcgr+−/− mice (A:recipient) or GcgrHep−/− mice transplanted into Gcgr−/− mice (B:recipient). C: Percent of islet grafts that stained positive for glucagon for Gcgr+−/− donor islets into Gcgr+−/− (recipient, open bars) and GcgrHep−/− (recipient, closed bars) mice (n = 4 mice, 3 sections/islet graft). Percent glucagon area is defined as the percentage of glucagon area/total insulin + glucagon area. Data are mean ± SEM. *P < 0.05 vs. GcgrHep−/− recipient grafts. Islet graft sections from Gcgr+−/− (donor) to GcgrHep−/− (recipient; D) and Gcgr−/− (donor) to GcgrHep−/− (recipient; E) mice stained for Ki67 (red), glucagon (green), and dapi (blue). D′ and E′: Insets of D and E (white box). White arrows indicate the Ki67-positive α-cells. White scale bar represents 25 μm. F: Proliferation rate of α-cells in Gcgr−/− islet graft (donor) transplanted into GcgrHep−/− (recipient, open bars) or GcgrHep−/− (recipient, closed bars) mice (n = 4 mice, 3 sections/islet graft). Data are mean ± SEM. **P < 0.01 vs. GcgrHep−/− recipient grafts.
mass appears only modestly increased and glucagon levels are not increased in cholic acid–fed mice. Hence, the mechanisms responsible for induction of α-cell proliferation remain elusive and require further investigation.

Insulin-resistant states also have been shown to trigger β-cell proliferation in transplanted islets. Transplantation of murine islets under the kidney capsule of lean or obese normoglycemic insulin-resistant mice resulted in stimulation of β-cell proliferation and expansion of islet mass in transplanted islets (42). The findings in the current report are similar in that islet cell proliferation at an exapancreatic site appears to be induced by a circulating factor. Gu et al. (43) transplanted human islets underneath the kidney capsule of streptozotocin-treated mice, and then administered a single course of a monoclonal antibody directed against the glucagon receptor. Although the GCGGR antibody improved glucose tolerance and increased plasma levels of glucagon and GLP-1, the extent, if any, of α-cell proliferation in the islet transplant was not examined (43). Because of efforts to develop therapies targeting the GCGGR for the treatment of type 2 diabetes, it is important to establish whether α-cell proliferation and hyperplasia can occur in adult human islets in response to acute interruption of GCGGR signaling.

The α-cell hyperplasia arising pursuant to chronic reduction or elimination of GCGGR signaling also has been reported in humans. Zhou et al. (44) reported a 60-year-old woman presenting with a pancreatic mass, extremely elevated levels of glucagon, and persistent hyperglucagonemia; marked α-cell hyperplasia was detected after surgical resection of the pancreatic mass, and sequencing of genomic DNA revealed a homozygous missense mutation in the GCGGR gene, resulting in markedly reduced receptor affinity. Furthermore α-cell hyperplasia, adenomatosis, and hyperglucagonemia have been reported in patients without histological or clinical evidence of malignancy, some of whom may harbor loss-of-function mutations in the GCGGR (45).

Recent evidence for enhanced proliferation of α-cells in the pancreas from patients with newly diagnosed type 1 diabetes (46), coupled with reports demonstrating conversion or transdifferentiation of α-cells to β-cells (7–9), have rekindled therapeutic interest in understanding the signals and mechanisms regulating α-cell proliferation. Our data combining disruption of the GCGGR and islet transplantation establish a new model for analysis of the control of α-cell hyperplasia. Together with recent efforts directed at promoting generation of functional β-cells from α-cells (7,5), identification of this circulating factor may provide new opportunities for regeneration of functional β-cell mass for the treatment of diabetes.

ACKNOWLEDGMENTS

This work was supported by grant MOP93739 from the Canadian Institutes of Health Research to D.J.D., by a Canada Research Chair in Regulatory Peptides and the Banting and Best Diabetes Centre–Novo Nordisk Chair in Incretin Biology to D.J.D., the Juvenile Diabetes Research Foundation International, the VA Research Service, the National Institutes of Health (DK59637, DK69603), the Beta Cell Biology Consortium (DK72473, DK80572), the Vanderbilt Mouse Metabolic Phenotyping Center (DK59637), and the Vanderbilt Diabetes Research and Training Center (DK20593, Islet Procurement and Analysis Core, Hormone Assay and Analytical Services Core, and Cell Imaging Shared Resource).

No potential conflicts of interest relevant to this article were reported.

C.L., A.M.R., E.D.D., C.D., S.A., V.d.C., and I.M. designed and carried out experiments and wrote and reviewed the manuscript. P.M.V., M.J.C., A.C.P., and D.J.D. designed experiments, reviewed results, and wrote the manuscript. A.C.P. and D.J.D. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors give special thanks to Courtney Thompson and Greg Poffenberger (Vanderbilt University) for technical assistance.

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