Glycoprotein 130 receptor signaling mediates α-cell dysfunction in a rodent model of type 2 diabetes

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Abstract
Dysregulated glucagon secretion accompanies islet inflammation in type 2 diabetes. We recently discovered that interleukin (IL)-6 stimulates glucagon secretion from human and rodent islets. IL-6 family cytokines require the glycoprotein 130 (gp130) receptor to signal. In this study, we elucidated the effects of α-cell gp130 receptor signaling on glycemic control in type 2 diabetes. IL-6 family cytokines were elevated in islets in rodent models of this disease. gp130 receptor activation increased STAT3 phosphorylation in primary α-cells and stimulated glucagon secretion. Pancreatic α-cell gp130 knockout (αgp130KO) mice showed no differences in glycemic control, α-cell function, or α-cell mass. However, when subjected to streptozotocin plus high-fat diet to induce islet inflammation and pathophysiology modeling type 2 diabetes, αgp130KO mice had reduced fasting glycemia, improved glucose tolerance, reduced fasting insulin, and improved α-cell function. Hyperinsulinemic-euglycemic clamps revealed no differences in insulin sensitivity. We conclude that in a setting of islet inflammation and pathophysiology modeling [...]
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Islet inflammation (1,2) and pancreatic α-cell dysfunction (3–6) contribute to hyperglycemia in patients with type 2 diabetes. Pancreatic islets from patients with type 2 diabetes are infiltrated with macrophages (7,8), express elevated proinflammatory cytokines (9,10), and express features of fibrosis (11), consistent with reports from animals and primates with this disease (7,12–18). The detrimental effects of inflammation on islet β-cell function were recently confirmed, when the interleukin (IL)-1 receptor antagonist reduced hyperglycemia and improved β-cell insulin secretion in patients with type 2 diabetes (1).

Pancreatic α-cell dysfunction is detectable in the early stages of type 2 diabetes, where the inverse relationship between pulsatile insulin and glucagon secretion is lost (19). This dysregulation is associated with relative hyperglucagonemia, which contributes to the development of fasting hyperglycemia associated with frank type 2 diabetes (6). Indeed, inhibition of glucagon action by various means reduces hyperglycemia in rodent models of this disease (5,6).
We recently identified IL-6 as a key cytokine elevated during islet inflammation in rodents with type 2 diabetes (7,20), with effects on α-cell glucagon secretion, GLP-1 secretion, and survival (21,22). IL-6 is part of a family of IL-6–type cytokines that all share the same common signal transducer for signaling, the glycoprotein 130 (gp130) (23). In support of a role for IL-6 cytokines in human disease, a recent study of global gene expression in human islets identified a group of coexpressed genes associated with type 2 diabetes. Within this group of genes, expression of two IL-6 family cytokines, IL-6 and IL-11, were both increased in islets from patients with type 2 diabetes: IL-6 was increased 2.75-fold, while IL-11 mRNA was increased 1.61-fold (24).

Despite these advances, there remain significant gaps in our knowledge regarding how the gp130 receptor regulates α-cell function. In our previous reports, we were unable to distinguish systemic IL-6 effects from tissue-specific IL-6 cytokine-mediated effects on the α-cell. In addition, we did not evaluate how IL-6 family cytokines regulate glycemia via actions on glucagon or GLP-1 secretion in a setting of islet inflammation and decreased β-cell mass modeling type 2 diabetes (21,22). Thus we generated α-cell gp130 receptor knockout (αgp130KO) mice and investigated the role of the α-cell in a nongenetic rodent model of type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Animals**

Male rodents were used for all experiments. Goto-Kakizaki (GK) rats (Taconic), Wistar rats (Taconic), BKS.Cg-m<sup>+/–</sup>Lepr<sup>db</sup>/BomTac (db/db) mice (Taconic), C57BL/6J mice (CDM), and Gt(ROSA)26Sor<sup>cre</sup>/Rosa26Sortm4(ACTB-tdTomato, EGFP)Luo<sup>fl/fl</sup> (mT/mG) mice (The Jackson Laboratory) were from commercial sources. Gp130<sup>fl/fl</sup> and Ggc-Cre mice were provided by W. Müller (25) and P. Herrera (26), respectively. Ggc-Cre mice were bred with mT/mG mice to produce Ggc-Cre<sup>fl/m</sup> mice. All rodents were housed at the Child and Family Research Institute (CFRI) animal facility, maintained on a 12-h light/dark cycle, and fed a normal chow diet (13 kcal% fat). All procedures were approved by the University of British Columbia Committee on Animal Care or the Principles of Laboratory Care (Denmark).

**Streptozotocin, High-Fat Diet, and Streptozotocin/High-Fat Diet Mouse Models**

Streptozotocin (STZ; Sigma-Aldrich) was prepared in acetate buffer and administered (25 mg/kg) via intraperitoneal injections for 5 consecutive days at 7–9 weeks of age. Control mice were administered an intraperitoneal injection of acetate buffer. Some mice were put on high-fat diet (HFD; 58 kcal% fat with sucrose; Research Diets) 3 weeks following intraperitoneal injection of acetate buffer or STZ.

**Islet Isolation and Immunocytochemistry**

Mouse and rat islets were isolated and cultured as described (7,27,28). For pSTAT3 experiments, islets were dispersed and plated on 804G extracellular matrix–coated 8-chamber slides (Nunc) (29). Islet cells were serum starved followed by treatment with IL-6, hyper-IL (HIL)-6 (30), leukemia inhibitory factor (LIF), or IL-27 (100 ng/mL was used for all cytokine treatments unless otherwise stated). Cells were paraformaldehyde fixed, permeabilized with methanol, blocked with 5% goat serum (Invitrogen), and incubated with rabbit anti-pSTAT3 (1:100; Cell Signaling Technology) and mouse anti-glucagon (1:1,000; Sigma-Aldrich). Slides were incubated with Alexa 488 donkey anti-rabbit (1:250; Jackson ImmunoResearch) and Alexa 594 donkey anti-mouse (1:450; Jackson ImmunoResearch) and mounted using Vectashield with DAPI (Vector Laboratories). Imaging was acquired with a BX61 microscope and Leica SP5 II confocal imaging system and quantified using Image-Pro Analyzer. An average of 1,130 ± 261 glucagon-positive α-cells were counted per experimental condition.

Pancreatic α-cell apoptosis was assessed by TUNEL staining as described (21) and imaged using a BX61 microscope. An average of 3,164 ± 722 glucagon-positive α-cells were counted per experimental condition.

**Flow Cytometry**

Isolated islets were dispersed and incubated with Fc block (1:100; eBioscience). Islet cells were stained with CD45-eFluor 450 (1:250; clone 30-F11; eBioscience), CD11b-PE (1:1,200; clone M1/70; eBioscience), Ly-6c allophycocyanin (1:1,200; clone HK1.4; eBioscience), and the viability dye eFluor 506 (1:1,000; eBioscience). Unstained, single stains, and fluorescence minus one controls were used for setting gates and compensation with the help of the CFRI FACS core facility using a BD LSR II instrument (BD Biosciences). Cells were gated on live cells and CD45<sup>+</sup> cells followed by CD11b<sup>+</sup>Ly6c<sup>+</sup> cells. Islets were pooled from two mice per treatment per time point.

**Secretion Assays**

Islets were plated on 804G extracellular matrix–coated plates for secretion assays. Aprotinin (250 kallikrein inhibitor units/mL; Sigma-Aldrich) and dipeptidyl peptidase-4 inhibitor (50 μmol/L; Millipore) were added to each well, and islets were treated with IL-6 or HIL-6. Conditioned media were collected, and 70% acid ethanol was added to islets for total hormone content. Glucagon and GLP-1 were measured by radioimmunoassay (Millipore). Glucose-stimulated insulin secretion from islets was performed as previously published (7). For total pancreatic insulin, glucagon, and GLP-1 content, hormones were extracted using 70% acid ethanol and determined using Luminex technology (Millipore). Total pancreatic protein concentration was determined using the Pierce BCA Protein Assay kit (Thermo Scientific). Ex vivo secretion of IL-6 and LIF was determined by Luminex, and soluble IL-6 receptor (sIL-6R) secretion was assayed by ELISA (31).

**Physiological Measurements**

For blood sampling, aprotinin (250 kallikrein inhibitor units/mL plasma; Sigma-Aldrich) and dipeptidyl peptidase-4 inhibitor (50 μmol/L; Millipore) were added to the
collection tubes. Mice were fasted overnight and injected intraperitoneally with 1.5 g/kg body weight glucose for glucose tolerance tests (intraperitoneal glucose tolerance test [IPGTT]). For insulin tolerance tests (ITTs), mice were fasted 2 h and injected intraperitoneally with 1 unit/kg insulin (Novolin GE, Novo Nordisk). Plasma insulin and glucagon were measured using ELISA (ALPCO and Merodia, respectively), total GLP-1 was measured using Meso Scale Discovery technology, and plasma IL-6 was measured using Luminex technology (Millipore).

Hyperinsulinemic-Euglycemic Clamps
Hyperinsulinemic-euglycemic clamps were performed as previously described (32–34). Mice were fasted overnight and anesthetized with acepromazine, midazolam, and fentanyl, with an initial dose by intraperitoneal injection and top-up doses by subcutaneous injection. Once fully immobilized from anesthesia, the tail vein was cannulated, and a 1-h basal infusion of $^{3}$H-D-glucose (1.2 μCi/h) was started to determine steady-state tracer. Duplicate blood samples were taken from the saphenous vein at the end of the basal period to measure fasting blood glucose levels, for determination of endogenous glucose production, and for insulin measurements. Hyperinsulinemia was induced with a constant infusion of insulin (3.5 mU/kg/min). To maintain euglycemia (∼5.0 mmol/L), a variable infusion of D-glucose was started simultaneously. Once euglycemia had been achieved, steady state was maintained for 60 min, after which triplicate blood samples were taken for further analysis. Briefly, insulin concentrations were measured by ELISA, and plasma samples were counted using a 1450 MicroBeta TriLux LSC and Luminescence Counter (PerkinElmer) after extraction by trichloroacetic acid precipitation. Brieﬂy, the rate of $^{3}$H-D-glucose appearance was determined as the ratio of the specific activity of glucose to the rate of $^{3}$H-D-glucose appearance. Subsequently, endogenous glucose production (μmol kg$^{-1}$ min$^{-1}$) could be calculated as the difference between whole-body glucose uptake and exogenous glucose infusion.

Immunohistochemistry
Antigen retrieval was performed on sectioned tissues using 10 mmol/L citrate buffer (Fisher Scientiﬁc) followed by blocking with 1% goat serum and donkey/goat anti-mouse IgG (1:30; eBioscience). Sections were incubated with guinea pig anti-insulin (1:1,000; Millipore) and mouse anti-glucagon (1:2,000) followed by incubation with Alexa 488 donkey anti-mouse (1:250; Jackson ImmunoResearch) and Alexa 594 donkey anti-mouse (1:450). Sections were mounted using Vectashield. Images were acquired using a BX61 microscope.

Pancreatic Gcg-Cre X mT/mG and mT/mG cryosections were paraformaldehyde fixed and equilibrated through a sucrose gradient. Tissue was frozen in optimum cutting temperature compound (Tissue-Tek). Sections were permeabilized with 0.1% Triton X-100 for 30 min and then blocked in 5% goat serum and 1% BSA (Fisher Scientiﬁc). Sections were incubated in 1% BSA and 0.1% Triton X-100 with mouse anti-glucagon (1:2,000) followed by incubation with Alexa 647 goat anti-mouse (1:250; Abcam) and mounted with Vectashield. Images were taken on a Leica SP5 II confocal imaging system.

For pancreatic α- and β-cell mass analysis, all islets in three sections (spaced 200 μm apart) were analyzed using Image-Pro Analyzer. Insulin and glucagon-positive area was quantiﬁed using the area/count function. Endocrine cell mass was calculated as previously described (21).

Gene Expression Analysis
Total RNA was extracted from islets using the MN Nucleospin RNA II kit (Macherey-Nagel), RNA was reverse transcribed and quantitative PCR was performed using PrimeTime primers/probes (IDT) in a ViiA 7 real-time PCR system (ABI) as previously described (35). Differential expression was determined by the 2$^{-}$ΔΔCT method (36) with RploP and 18S as reference genes.

Statistical Analysis
Data are expressed as means ± SEM/SD with the number of individual experiments presented in the ﬁgure legends. All data were analyzed using the nonlinear regression analysis program Prism (GraphPad), and signiﬁcance was tested using Student t test or ANOVA with post hoc tests for multiple comparison analysis. Signiﬁcance was set at $P < 0.05$.

RESULTS
IL-6 Family Cytokines Are Elevated in Pancreatic Islets From Rodent Models of Type 2 Diabetes
We analyzed islet IL-6 family cytokine expression in two rodent models, the GK rat and db/db mouse. Both the GK rat and the db/db mouse displayed elevated blood glucose and declining insulin levels at 12 and 15 weeks of age, respectively, suggestive of β-cell failure (Fig. 1A, B, D, and E). Isolated islets from 8–12-week-old GK rats had signiﬁcantly increased levels of Il6, Lif, Clcf1, and Osm mRNA relative to Wistar controls (Fig. 1C); similar effects were observed in islets from 15-week-old db/db mice (compared with Fig. 1C and F). Interestingly, both IL-6 family cytokines and glycemia were more elevated in db/db mice relative to GK rats. Thus elevated IL-6 family cytokine expression accompanies hyperglycemia and declining insulin levels in rodent models of type 2 diabetes, and the magnitude of islet IL-6 cytokine expression may contribute to the prevailing hyperglycemia in these models.

gp130 Receptor Activation Stimulates STAT3 Phosphorylation and Glucagon Secretion From α-Cells
To determine if gp130 receptor signaling is activated in α-cells following IL-6 treatment, dispersed islets were stimulated with IL-6 or HIL-6 (IL-6 fused to the sIL-6R [37]; HIL-6 can stimulate all gp130 receptors in the absence of an IL-6R) and immunostained for pSTAT3. IL-6 induced phosphorylation of STAT3 in α-cells within 30 min to a similar degree as HIL-6 (Fig. 2A). Next we assessed the effect of different IL-6 doses and time of exposure on glucagon secretion in islets. IL-6 increased glucagon secretion in a concentration- and time-dependent
manner, with no significant differences in glucagon content (Fig. 2B and C). Consistent with our pSTAT3 data, IL-6 stimulated glucagon secretion to a similar degree as HIL-6 (Fig. 2D and E), suggesting that α-cell gp130 receptor signaling is maximally activated by IL-6 and supporting our previous observation that pancreatic α-cells express the IL-6 transmembrane receptor (21). To ensure that IL-6 did not induce α-cell death, causing glucagon release into the incubation medium, islets were treated with IL-6 and apoptosis visualized by TUNEL staining. IL-6 protected from cell death induced by a mixture of cytokines (IL-1β, TNFα, and IFNγ) (Fig. 2F), consistent with our previous observations under nutrient stress (21). Collectively, these data indicate that gp130 signaling within α-cells leads to phosphorylation of STAT3 and stimulation of glucagon secretion.

**Generation and Validation of αgp130KO Mice**

To assess Cre recombinase activity within α-cells, Gcg-Cre mice were crossed with mT/mG reporter mice. Pancreatic sections revealed expression of EGFP in a proportion of α-cells stained with glucagon, demonstrating active Cre recombinase in 44.1 ± 1.5% of α-cells (Fig. 3A and B), similar to previous reports using Gcg-Cre mice (38).

Next we assessed pSTAT3 levels in response to gp130 receptor activation in fl/fl controls and αgp130KO islets. Stimulation of pSTAT3 with IL-6 and LIF was significantly decreased within α-cells of αgp130KO islets (Fig. 3C). IL-27 failed to induce pSTAT3 within control islets, suggesting lack of IL-27 receptors on α-cells. Finally, we assessed glucagon secretion in response to gp130 receptor activation in αgp130KO islets. IL-6 stimulated glucagon secretion was reduced by ~50% in αgp130KO islets (Fig. 3D). Thus αgp130KO islets have Cre-mediated recombination occurring in a proportion of α-cells and reduced gp130 receptor signaling, coinciding with a 50% reduction in gp130 receptor–mediated glucagon secretion.

To rule out any α-cell or intestinal L-cell developmental abnormalities in αgp130KO mice, we assessed several
physiological parameters in αgp130KO mice. αgp130KO mice displayed no difference in body weight, glucose tolerance, glucose-stimulated insulin secretion, or insulin sensitivity at 16–18 weeks of age (Fig. 3F–I). Fasting GLP-1 levels were unchanged, and pancreatic α-cell function was normal, as assessed by glucose-mediated glucagon suppression during a GTT and hypoglycemia-mediated glucagon secretion during an ITT (Fig. 3J and K). Data represent mean ± SEM from n = 3 mice performed in three independent experiments (A), n = 3–6 mice performed in two independent experiments (B and C), n = 6 mice performed in three independent experiments (D and E), and n = 4 mice in two independent experiments (F). ★P < 0.05; ★★P < 0.01; ★★★P < 0.001 vs. untreated control; #P < 0.05 vs. cytokine mix as tested by ANOVA with Dunnett (A–E) or Newman-Kuels posttest (F). Ctrl, control; cyt, cytokine; CM, cytokine mix.

Pancreatic α-Cell Dysfunction and Islet Inflammation in the STZ/HFD Mouse Model of Type 2 Diabetes
To investigate the role of the α-cell gp130 receptor in a mouse model of human type 2 diabetes, we generated a non-genetic model displaying features of type 2 diabetes. Mice were administered STZ, HFD, or STZ in combination with HFD as depicted in Fig. 4A. STZ alone caused glucose intolerance, tended to reduce insulin secretion in response to glucose in vivo, had minimal effects on glucose-mediated glucagon suppression and hypoglycemia-induced glucagon secretion in vivo, and tended to reduce β-cell mass and increase α-cell mass (Fig. 4B–L). HFD
alone increased body weight, caused glucose intolerance, increased insulin secretion in response to glucose in vivo, impaired glucose-mediated glucagon suppression, had no effect on hypoglycemia-induced glucagon secretion in vivo, and had minimal effects on β- and α-cell mass (Fig. 4B–L).

STZ in combination with HFD caused increased body weight, fed hyperglycemia, glucose intolerance, and marked α-cell dysfunction (Fig. 4B–H). In addition, β-cell mass was reduced by ~50%, in parallel with impaired glucose-stimulated insulin secretion from islets ex vivo and reduced
Figure 4—Pancreatic α-cell dysfunction and islet inflammation in STZ-, HFD-, and STZ/HFD-treated mice. Schematic of chow, STZ (25 mg/kg), HFD, and STZ/HFD treatment groups (A) with weekly fed blood glucose monitoring (B). IPGTT (C) (1.5 g/kg glucose), ITT (D) (1 unit/kg insulin), and fed insulin (E) after 9 weeks. Pancreatic β- and α-cell mass (J and K) in treated mice with representative images (L). Glucose-stimulated insulin secretion (M) and insulin content (N) of islets ex vivo after 9 weeks. Pancreatic islet infiltrating monocytes (CD11b+Ly6c+ cells) in chow and STZ-treated mice (O). Expression levels of IL-6 family cytokines post-STZ injection and during HFD relative to chow mice (P). IL-6, sIL-6R, and LIF protein secretion from 100 islets/well isolated 3 weeks post-STZ (Q). Islet-derived IL-6 (R) from 100 islets/well and
islet insulin content (Fig. 4J–N). α-Cell mass was also significantly increased in STZ/HFD mice (Fig. 4K). Thus treatment with STZ plus HFD resulted in a mouse with impaired α- and β-cell function and hyperglycemia.

To characterize islet inflammation post-STZ, islet infiltrating monocytes (CD11b+Ly6c- cells) were analyzed by flow cytometry. The number of islet monocytes tended to increase at weeks 0.5 and 1 post-STZ and were significantly increased at week 2 post-STZ (Fig. 4O). To determine if islet IL-6 family cytokine expression was increased, we analyzed islet gene expression at multiple times post-STZ and during subsequent HFD feeding (Fig. 4P). IL-27 mRNA was increased ∼1 week post-STZ, Osm mRNA was increased 2 weeks post-STZ, and IL-6 and Lif mRNA were increased 3 weeks post-STZ. Other IL-6 family cytokines were unchanged (Fig. 4P and not shown). Consistent with these mRNA data, IL-6 and LIF protein secretion were increased in islets isolated 3 weeks post-STZ, with no change in sIL-6R secretion (Fig. 4Q). Islet IL-6 secretion was not significantly increased at week 9 in our treatment groups (Fig. 4R), indicating that IL-6 cytokines are only transiently increased following STZ and not further increased by HFD. However, plasma IL-6 levels were significantly increased in our STZ/HFD group at the end of our study (Fig. 4S). Taken together, these data show that STZ/HFD-treated mice have many hallmark characteristics of type 2 diabetes, including pancreatic α-cell dysfunction and increased expression of pancreatic islet IL-6 family cytokines.

**Partial Knockout of the α-Cell gp130 Receptor Protects From Hyperglycemia Following STZ/HFD**

To determine the consequence of α-cell gp130 signaling during islet inflammation and in a pathophysiological setting modeling type 2 diabetes, we subjected gp130KO mice to STZ alone or STZ followed by HFD feeding. gp130KO mice displayed no difference in body weight following STZ or STZ/HFD (Fig. 5A). When subjected to STZ alone, gp130KO mice showed mildly improved glucose tolerance, with no differences in insulin sensitivity, insulin secretion, or α-cell function in vivo (Fig. 5C–I). However, following STZ/HFD, gp130KO mice had decreased fasting glycemia, improved glucose tolerance, and decreased fasting insulin and showed improvements in glucose modulated glucagon secretion in vivo (Fig. 5B–I). This was despite decreased fasting GLP-1 levels (Fig. 5E). ITTs indicated no difference in insulin sensitivity in gp130KO mice (Fig. 5F). No differences in pancreatic glucagon, insulin, or GLP-1 content (Fig. 5J–L) were observed between genotypes, along with no differences in β- and α-cell mass (Fig. 5M–O). STZ/HFD-treated Ggc-Cre mice did not show any glucose intolerance compared with wild-type mice excluding any phenotypic effect of Cre recombinase expression in α-cells (Supplementary Fig. 1). Further, gp130KO mice on HFD alone showed no phenotypic differences in glucose tolerance or α- and β-cell function (Supplementary Fig. 2).

Finally, to determine if changes in insulin sensitivity contributed to the protective phenotype in STZ/HFD-treated mice, we performed hyperinsulinemic-euglycemic clamps. There were no differences in glucose infusion rate, whole-body insulin-stimulated glucose uptake, or hepatic insulin sensitivity between genotypes (Fig. 6). Interestingly, hepatic glucose production under fasting conditions tended to be reduced in gp130KO mice (Fig. 6D), consistent with reduced fasting glycemia in these mice. Taken together, these data show that gp130 receptor signaling contributes to α-cell dysfunction in a nongenetic model of type 2 diabetes, and partial inhibition of gp130 receptor signaling improves glucose tolerance and protects from hyperglycemia through improved α-cell function.

**DISCUSSION**

Normally, glucagon secretion is increased during fasting and suppressed as blood glucose rises following a meal in a counterregulatory fashion to insulin. However, during type 2 diabetes, glucagon secretion is inappropriately elevated, contributing to hyperglycemia (5,6). Pioneering studies by Müller et al. (39) and elegant clamp studies by Shah et al. (3) have demonstrated that lack of glucagon suppression contributes to postprandial hyperglycemia in people with type 2 diabetes. The main mechanism underlying this hyperglucagonemia is thought to be β-cell dysfunction and a decrease in β-cell-derived secretory products that are known to have inhibitory effects on α-cell secretion, including insulin, γ-aminobutyric acid, and zinc ions (40). All of these factors inhibit glucagon secretion, and their secretion is likely decreased in frank type 2 diabetes. Our data demonstrate that islet inflammation also contributes to α-cell dysfunction, with a central role for gp130 receptor signaling in this process and with implications for type 2 diabetes.

gp130KO mice were protected from glucose intolerance following STZ alone, but not following HFD alone. This was consistent with STZ treatment causing a transient increase in IL-6 and LIF secretion from islets, while
HFD alone did not increase islet IL-6 family cytokine expression. One caveat in our study is that isolating islets induces IL-6 mRNA expression and secretion (41). It is therefore likely that islet IL-6 is elevated for a more prolonged period of time than depicted in Fig. 4P. Interestingly, islet IL-6 cytokine mRNA levels correlated very well with macrophage infiltration following STZ. We recently found that depletion of islet macrophages reduced islet amyloid polypeptide–induced islet IL-6 mRNA expression (42). Whether islet macrophages are the cellular source of IL-6 family cytokines causing a-cell dysfunction should be addressed in future studies.

Figure 5—αgp130KO mice display improved glucose homeostasis and a-cell function following STZ/HFD. Body weight (A) and fasting glycemia (B) of fl/fl and αgp130KO mice at 16–18 weeks of age following STZ or STZ/HFD. IPGTT (C) (1.5 g/kg) with quantification of area under curve above basal (D), fasting GLP-1 (E), and ITT (F) (1 unit/kg) with corresponding plasma insulin (G) and glucagon levels (H–I). Insulin (J), glucagon (K), and GLP-1 (L) pancreatic content of fl/fl and αgp130KO mice following STZ/HFD. Pancreatic β- and a-cell mass (M–N), and representative islet images (O) of STZ/HFD fl/fl and αgp130KO mice. Representative image indicates glucagon (red), insulin (green), and nuclei (blue; DAPI). Scale bars represent 50 μm. Data represent mean ± SEM from n = 4–6 mice per genotype treated with STZ (A–I), n = 22–23 mice per genotype treated with STZ/HFD (A–D), n = 4 mice per genotype (E), n = 2–8 mice per genotype treated with STZ/HFD (F), n = 11–14 mice per genotype treated with STZ/HFD (G–I), n = 4–8 mice per genotype (J–N) from 1–4 independent cohorts of mice. Black border white bars/triangles represent STZ/HFD fl/fl, and red border red bars/squares represent STZ/HFD αgp130KO. ★P < 0.05; ★★P < 0.01 as tested by Student t test. In C, ★P < 0.05 and #P < 0.05 as tested by Student t test compared with fl/fl controls. AUC, area under the curve; KO, αgp130KO; ns, not significant.
Interestingly, the combination of STZ plus HFD did not prolong or increase islet IL-6 family cytokine expression, but it did result in further impairments in α-cell function compared with STZ or HFD treatments alone. One possible explanation for this synergism is that HFD mediates α-cell dysfunction by causing α-cell insulin resistance and that STZ exacerbates these effects by decreasing insulin and inducing inflammation. This might explain the synergistic effect of STZ plus HFD on glucose-mediated glucagon suppression in vivo during an IPGTT. With respect to ITT responses, hypoglycemia-induced glucagon secretion is strongly driven by the parasympathetic nervous system (43,44). Thus STZ acts synergistically with HFD to impair this response. Regardless of the mechanism of action, these hypotheses are consistent with effects of gp130 receptor signaling on α-cell function and glycemic control being more pronounced in the STZ/HFD model, compared with STZ or HFD treatments alone. Thus gp130 receptor signaling acts as a modifier of α-cell function in a synergistic manner with HFD-induced effects on α-cell function.

Improved α-cell function in αgp130KO mice treated with STZ/HFD resulted in reduced fasting hyperglycemia, likely due to reduced hepatic glucose output. Reduced glycemia was likely responsible for the decreased fasting insulin observed in αgp130KO mice. We ruled out any contribution of increased insulin sensitivity in αgp130KO mice treated with STZ/HFD by performing hyperinsulinemic-euglycemic clamps. However, reduced fasting glycemia may have improved β-cell function in αgp130KO mice, likely by reducing the effects of glucotoxicity on the β-cell. This sequence of events may also help explain how a transient increase in islet IL-6 cytokines can have long-lasting effects on glycemic control.

Two independent groups recently investigated the effect of gp130 receptor cytokines on the α-cell. McGuinness and colleagues found that IL-6−/− mice had a blunted glucagon response to endotoxin that was restored by replacement of IL-6 (45). They went on to show that IL-6 amplifies adrenergic-dependent glucagon secretion from mouse islets (46). In a separate study, Fernández-Millán et al. showed that α-cell mass expansion and hyperglucagonemia during suckling in rats is partly IL-6−/− dependent, contributing to hyperglycemia postweaning (47). These studies, our previous work (21), and data presented here support the notion that IL-6 cytokines are modulators of α-cell glucagon secretion. Taking these findings into consideration, we propose that gp130 receptor actions are context-dependent and that IL-6 cytokines modulate α-cell glucagon secretion to allow for increased glucose output during normal physiology (e.g., during acute infection or postexercise), while also driving excessive α-cell glucagon secretion and dysfunction during type 2 diabetes.

Exposure of human islets to IL-6 for 4 days or enriched human pancreatic α-cells to IL-6 enhances GLP-1 secretion in addition to glucagon secretion, perhaps via upregulation of pro(hormone)-convertase 1/3 (22). Mouse islets exposed to IL-6 for 2 days did not have elevated GLP-1 secretion. Dedifferentiation of α-cells to a pre-α-cell state has been shown to induce GLP-1 secretion (48). In addition, prolonged culture of human islets in
vitro results in increased numbers of glucagon-positive cells due to conversion of β-cells to α-cells (49). We surmise that signals causing α-cell dedifferentiation or conversion of β-cells to α-cells act together with IL-6 cytokines to stimulate GLP-1 secretion from α-cells. In the future, the mechanisms underlying IL-6-stimulated α-cell GLP-1 secretion will need to be determined and their role tested in vivo.

Despite decreased systemic GLP-1 levels in ogp130KO mice (likely due to receptor deletion in L cells), activation of α-cell gp130 receptor signaling in a setting of reduced β-cell mass and HFD-induced insulin resistance had detrimental effects on normal α-cell function and glycemic control. This may suggest that effects of gp130 receptor cytokines on α-cell function are more important for glycemic control than effects on L-cell GLP-1 secretion in type 2 diabetes. While it remains to be determined what the mechanisms are that facilitate IL-6 cytokine actions on glucagon secretion, insight into how IL-6/gp130 influences α-cell secretory processes and/or α-cell differentiation may allow us to identify druggable targets that modify the actions of the α-cell and could thereby be used for the treatment of type 2 diabetes.

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