β-Cell-specific glucocorticoid reactivation attenuates inflammatory β-cell destruction

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Progression and severity of type 1 diabetes is dependent upon inflammatory induction of nitric oxide production and consequent pancreatic β-cell damage. Glucocorticoids (GCs) are highly effective anti-inflammatory agents but have been precluded in type 1 diabetes and in islet transplantation protocols because they exacerbated insulin resistance and suppressed β-cell insulin secretion at the high-doses employed clinically. In contrast, physiological-range elevation of GC action within β-cells ameliorated lipotoxic β-cell failure in transgenic mice overexpressing the intracellular enzyme 11β-hydroxysteroid dehydrogenase type 1 (MIP-HSD1H19+/− mice). Here, we tested the hypothesis that elevated β-cell 11beta-HSD1 protects against the β-cell destruction elicited by streptozotocin (STZ), a toxin that dose-dependently mimics aspects of inflammatory and autoimmune β-cell destruction. MIP-HSD1H19+/− mice exhibited an episodic protection from the severe hyperglycemia caused by a single high dose of STZ associated with higher and sustained β-cell survival, maintained β-cell replicative potential, higher plasma and islet insulin levels, reduced inflammatory macrophage infiltration and increased anti-inflammatory T regulatory cell content. MIP-HSD1H19+/− mice also completely resisted mild hyperglycemia and insulitis induced by multiple low-dose STZ administration. In vitro, MIP-HSD1H19+ islets exhibited attenuated STZ-induced nitric oxide production, an effect reversed with a specific 11beta-HSD1 inhibitor. GC regeneration selectively within β-cells protects against inflammatory β-cell destruction, suggesting therapeutic targeting of 11beta-HSD1 may ameliorate processes that exacerbate type 1 diabetes and that hinder islet transplantation.

Keywords: glucocorticoids, 11beta-hydroxysteroid dehydrogenase type 1, type 1 diabetes, inflammation, beta-cells, anti-inflammatory agents, insulin secretion, streptozotocin

INTRODUCTION

Type 1 diabetes is a chronic disease characterized by inflammatory β-cell destruction secondary to an initial autoimmune targeting of the islets (1). Inflammatory macrophages are key to the development and maintenance of islet damage (2). Pro-inflammatory cytokines derived from macrophages and damaged β-cells further suppress β-cell function in part through induction of nitric oxide production (3, 4). As type 1 diabetes progresses, pro-inflammatory cytokines inhibit β-cell regeneration, stimulate peripheral insulin resistance and maintain insulitis (1).

Glucocorticoids (GCs) are used clinically due to their potent anti-inflammatory and immunosuppressive effects (5) but were excluded as a treatment for type 1 diabetes and in transplant protocols (6) because they promoted peripheral insulin resistance and suppressed β-cell function at the high-doses employed (7–9). However, the prevailing dogma that GC action on β-cells is purely deleterious has been increasingly challenged (10–14). Exposure of normal mouse islets to GCs can improve aspects of secretory function through suppression of inflammatory signaling (10). Moreover, pre-treatment of islets with GCs (11) or localized exposure of transplanted islets to GCs contained within their surrounding implant matrix (12) has shown improved efficacy and graft survival. Crucially, transgenic mice with modest β-cell-specific elevation of the intracellular GC regenerating enzyme 11β-hydroxysteroid dehydrogenase (HSD11b1; 11beta-HSD1; MIP-HSD1 mice) exhibited protection from lipotoxic β-cell failure in vivo as a result of increased islet number, arising from a post-developmental effect, and function, due to enhanced secretory capacity and cell survival signaling (14).

The beneficial effects of β-cell-specific 11beta-HSD1 elevation (14) were manifest in a chronic high-fat feeding obesity model. Although obesity is associated with a low-grade inflammation of the islets (15), the protective mechanisms found in MIP-HSD1 islets were not obviously anti-inflammatory (14). Therefore, the impact of intra-β-cell GC regeneration on the processes of cellular damage occurring in profoundly inflammatory contexts relevant to type 1 diabetes remains unknown. To address this we tested the hypothesis that elevated β-cell 11beta-HSD1 protects against the profound β-cell destruction or inflammatory insulitis driven by distinct doses of the β-cell toxin streptozotocin (STZ).
MATERIALS AND METHODS

ANIMALS
All experiments conformed to local ethical guidelines of the University of Edinburgh and the UK Home Office Animals (Scientific Procedures) Act (1986). Male MIP-HSD1\(^{+/+}\) and C57BLKS/J (KsJ) littermate control mice (1:4) were housed in standard conditions on a 12 h light/dark cycle and fed standard rodent chow (Special Diet Services, Edinburgh, UK). Age-matched 10–12-week-old male mice were used for all the experiments.

STREPTOZOTOCIN TREATMENTS
Mice were intraperitoneally injected with a single bolus of STZ (180 mg/kg body weight) or for five consecutive days with 40 mg/kg/body weight STZ dissolved in 10 mmol/l sodium citrate (pH 4.5) or vehicle. Blood glucose was measured (OneTouch Ultra, Johnson and Johnson, Bucks, UK) from a tail venipuncture. Mice were sacrificed at 3 and 10 days (single dose) or 15 days (multiple dose) after injection. Insulin was measured by ELISA (Crystal Chem, Downers Grove, IL, USA).

IMMUNOHISTOCHEMISTRY
Pancreata were fixed in 4% paraformaldehyde, paraffin embedded, sectioned (4 \(\mu\)m), and immunostained with guinea pig anti-insulin (1:300) (AbCam, Cambridge, UK), rabbit anti-Mac-2 (1:150) (Cedarline, ON, Canada), rabbit anti-FOXp3 (1:150) (eBioscience, Hatfield, UK), rabbit anti-NEUROG3 (1:1000), and rabbit anti-SOX9 (1:8000) (Millipore Corporation, Bedford, MA, USA). For chromogen labeling with diaminobenzidine (DAB) (DakoCytomation, Carpinteria, CA, USA), biotinylated anti-guinea pig and anti-rabbit (AbCam) secondary antibodies were used. Image and quantification of positive cells in islet areas were carried out using KS300 software (3.0 CarlZeiss Vision, GmBH) or computerized image analysis (MCID Basic 7.0 software) for analysis of the whole sections. For immunofluorescence, sections were incubated with rabbit anti-ki67 (1:3000, Dakocytomation) then goat anti-rabbit peroxidase (Abcam) followed by Tyramide green 488 (Perkin Elmer, Cambridge, UK) then incubated with rabbit anti-PDX1 (1:1000, Millipore). After antigen retrieval, sections were incubated with goat anti-rabbit Alexa Fluor 546 (1:200, Molecular probes, Paisley, UK) and DAPI (1:1000, Sigma Aldrich, Dorset, UK) and visualized using a Leica fluorescence microscope. Quantification for PDX1 and KI67 was performed using Image J software (http://www.ncbi.nlm.nih.gov).

ISLET ISOLATION AND PREPARATION
Pancreata were digested with collagenase XI (Sigma Aldrich) and islets were hand-picked under a stereomicroscope in Hank’s Balanced Salt Solution, 10% FBS, 6.1 mmol/l d-glucose, 2 mmol/l 11-dehydrocorticosterone with or without 10 mmol/l STZ diluted in sodium citrate 10 mmol/l and with or without L-NAMe (Sigma) 5 mmol/l for 72 h on 8 \(\mu\)m inserts (Millipore). Pictures of the islets were taken using a Zeiss microscope and media were collected for measurement of nitric oxide.

NITRIC OXIDE (NO) PRODUCTION
Total NO in the media was assayed as nitrite, the stable breakdown product of NO, using a Sievers chemiluminescence analyzer (Analytix, Sunderland, UK). Islets were homogenized in lysis buffer as described in (14) and protein content evaluated by Biorad assay (BioRad Laboratories, Hercules, CA, USA).

STATISTICS
Data are expressed as mean±SEM and were analyzed using one-way ANOVA (Newman–Keuls post hoc test).

RESULTS

MIP-HSD1\(^{+/+}\) MICE RESIST HIGH-DOSE STZ-INDUCED HYPERGLYCEMIA
We began by administering a high-dose of STZ (180 mg/kg body weight) known to completely ablate β-cell function (16). High-dose STZ caused marked and comparable hyperglycemia by 2 days in MIP-HSD1\(^{+/+}\) and control non-transgenic littermates (KsJ), indicating comparable ablation of β-cell function. However, after day 3, during the inflammatory-response phase of islet destruction, MIP-HSD1\(^{+/+}\) mice began to exhibit episodic phases of significantly less severe hyperglycemia than KsJ mice (Figures 1A,B) suggestive of partial recovery of function in existing β-cells and/or spontaneous regeneration of new β-cells. Plasma insulin levels were significantly higher in MIP-HSD1\(^{+/+}\) than in KsJ mice at day 3 and 10 (Figure 1C), consistent with their residual islet insulin staining and further supporting an islet-specific β-cell recovery (Figure 1D). Notably, circulating corticosterone levels were markedly elevated by high STZ, but to a similar degree in KsJ and MIP-HSD1\(^{+/+}\) mice (nmol/l: KsJ vehicle: 131 ± 28, KsJ high STZ: 535 ± 155, MIP-HSD1\(^{+/+}\) vehicle: 138 ± 45 MIP-HSD1\(^{+/+}\) high STZ: 683 ± 125, no significant effect of genotype), supporting a role for local β-cell GC regeneration as the underlying driver of genotype-specific effects.

MIP-HSD1\(^{+/+}\) MICE MAINTAIN HIGHER β-CELL MASS AND REPLICATIVE CAPACITY AFTER STZ
The remarkable resilience of MIP-HSD1\(^{+/+}\) β-cells against high-dose STZ could be due to higher β-cell survival or increased spontaneous β-cell regeneration. Of note, there was low and comparable β-cell proliferation (~2% of total islet cell number double-positive for the proliferation marker Ki67 and the β-cell marker PDX1) in vehicle-treated KsJ and MIP-HSD1\(^{+/+}\) mice (Figure 2A, left lane and quantification Figure 2B). Islet PDX1-positive β-cell number was severely reduced by day 3 and continued to fall by day 10 to undetectable levels in KsJ mice treated with STZ. Replicating Ki67 single-positive cells in islets of STZ-treated KsJ mice are likely infiltrating immune cells. Ki67/PDX1 double-positive cells were undetectable in KsJ islets indicating a complete loss of β-cell replicative capacity (Figure 2A, upper row, middle, and right lane and quantification, Figure 2B). Despite a marked reduction in PDX1-positive cells, a substantial β-cell number remained in STZ-treated MIP-HSD1\(^{+/+}\) islets, although the comparable hyperglycemia between genotypes from day 2 to 3 indicates these β-cells underwent a period of initial secretory dysfunction (Figure 1). MIP-HSD1\(^{+/+}\) mice also maintained their Ki67/PDX1 double-positive cell number in their remaining islets (Figure 2A bottom row and quantification Figure 2B) suggesting that their β-cell replicative capacity is maintained after STZ. This could not be accounted for by β-cell neogenesis from potential progenitor cell types, as assessed with SOX9...
and NEUROG3 (17, 18) immunostaining that did not differ by genotype 3 or 10 days after STZ (Figures S1 and S2 in Supplementary Material). Apoptosis was not detected in islets of either genotype by a number of methods suggesting that this process does not account for the differences between the genotypes under these experimental conditions (Figure S3 in Supplementary Material).

**MIP-HSD1**<sup>+/+</sup> ISLETS HAVE REDUCED MACROPHAGE AND INCREASED T REGULATORY CELL INFILTRATION AFTER STZ

Inflammatory macrophage infiltration is an early event in autoimmune (2) and STZ-induced islet damage (19). MIP-HSD1<sup>+/+</sup> islets had fewer (~40%) infiltrated macrophages, as assayed by the macrophage marker Mac-2, compared to KsJ controls at 3 and 10 days after the single high-dose STZ injection (Figure 3A and quantification, Figure 3B).

**T** regulatory cells (Treg; FOXP3<sup>+</sup> cells) mediate inflammatory resolution and retard the progression of diabetes (20). Treg cell numbers increased modestly (twofold) in both normal KsJ and MIP-HSD1<sup>+/+</sup> islets/peri-islet area 3 days post-STZ. Treg cell numbers continued to increase significantly only in MIP-HSD1<sup>+/+</sup> islets by day 10 post-STZ (Figure 3C, and quantification Figure 3D).

**MIP-HSD1**<sup>+/+</sup> MICE RESIST MULTIPLE LOW-DOSE STZ-INDUCED HYPERGLYCEMIA

To test responses to a more subtle diabetic insult that recapitulates some of the inflammatory and autoimmune aspects of type 1 diabetes (21, 22), a low-dose of STZ (40 mg/kg/BW) was administered for five consecutive days. KsJ mice showed significant hyperglycemia from day 4 that reached a modestly diabetic plateau by day 12 (Figure 4A), had reduced islet insulin staining (Figure 4B) and increased islet macrophage numbers, whereas MIP-HSD1<sup>+/+</sup> mice maintained normal glycemia and pancreatic morphology and displayed abrogated macrophage infiltration (Figure 4C, and quantification, Figure 4D).

**β-CELL 11β-HSD1 ACTIVITY CURTAILS ISLET STZ-INDUCED NITRIC OXIDE PRODUCTION**

Inflammatory mediators induce production of nitric oxide (NO) that causes β-cell destruction and acts as an important chemoattractant for macrophages (1, 3, 4). STZ can induce inflammatory pathways and islet damage in part by generating NO (23). Incubation of normal KsJ islets with STZ stimulated NO production (Figure 5A, white bars) and caused islet disintegration (Figure 5C, middle lane, top row). The STZ-induced rise in NO and islet damage was attenuated by the iNOS inhibitor aminoguanidine (Figure 5D).
FIGURE 2 | MIP-HSD1\textsuperscript{tg/+} mice exhibit enhanced \( \beta \)-cell survival and maintained replicative capacity after STZ treatment. (A) Representative immunofluorescence images of paraffin-fixed pancreata co-stained with rabbit anti-Ki67 (green), rabbit anti-PDX1 (red), and DAPI (blue) from KsJ littermate (upper row), MIP-HSD1\textsuperscript{tg/+} (lower row) mice after vehicle treatment (Veh, left lane), 3 (middle lane) and 10 days post-STZ treatment (right lane). Arrows indicate double-positive stained cells, magnification \( \times 400 \). (B) PDX1 and Ki67 positive cells were counted within the islets as defined morphologically by the islet capsule boundary using Image J software. Proliferating \( \beta \)-cells were measured as a ratio of PDX1, Ki67 double-positive cells over total PDX1-positive cells. Ki67 and PDX1 double-positive staining was undetectable (nd) in KsJ mice 3 and 10 days post-STZ treatment. Values represented mean \( \pm \) SEM, differences were analyzed by one-way ANOVA, Newman–Keuls test (\( n = 6–12 \)).

FIGURE 3 | MIP-HSD1\textsuperscript{tg/+} mice have reduced islet macrophage infiltration and increased T regulatory cell numbers after STZ administration. (A) Immunohistochemical staining for Mac-2 with (B) quantification and (C) FOXP3 immunohistochemical staining with (D) quantification. (A,C) Pancreata from KsJ littermate (upper rows), MIP-HSD1\textsuperscript{tg/+} (lower rows) mice after vehicle (left lane) or 3 (middle lane) and 10 days (right lane) post-STZ treatment were paraffin-fixed and sectioned. (C,D) Mac-2 and FOXP3 positive cells per islet area were quantified using Zeiss (KS300 3.0) software. Values represent mean \( \pm \) SEM. Differences were analyzed by one-way ANOVA and Newman-Keuls test, * \( P < 0.05 \) and *** \( P < 0.001 \) for STZ vs. Veh; * \( P < 0.05 \) and ** \( P < 0.01 \) for MIP-HSD1\textsuperscript{tg/+} vs. KsJ control; § \( P < 0.05 \) and §§§ \( P < 0.001 \) STZ D10 vs. STZ D3 (\( n = 6–10 \)). Bars = 50 \( \mu \)m, magnification \( \times 400 \).

inhibitor, L-NAME (Figures 5A,C, right lane). NO production was suppressed after STZ treatment of MIP-HSD1\textsuperscript{tg/+} islets (Figure 5B, black bars) and islet disintegration was attenuated (Figure 5C, middle lane, bottom row). The specific 11beta-HSD1 inhibitor UE2316 (14) reversed suppression of NO found in MIP-HSD1\textsuperscript{tg/+} islets in the presence of the 11beta-HSD1 substrate 11-dehydrocorticosterone, confirming that \( \beta \)-cell 11beta-HSD1 activity inhibits NO production.
Modest elevation of 11beta-HSD1 activity in beta-cells (14) has conferred improved beta-cell survival and a sustained capacity for spontaneous beta-cell regeneration in the context of severe inflammatory beta-cell destruction. Although the protection against hyperglycemia is modest across the short-time course of the present studies, the continued survival and replenishment of functional beta-cells after high-dose STZ points to a remarkable and unexpectedly effective protective role for local GC regeneration. Hyperglycemia induced by multiple low-dose STZ, a regimen that invokes some beta-cell destruction, although the protection against hyperglycemia is modest across the short-time course of the present studies, may counteract triggering of more aggressive inflammatory responses after beta-cell insult.

Under normal physiological conditions, beta-cell mass is maintained through slow rates of renewal and turnover (26). Hyperglycemia can prompt islet mass compensation predominantly through hypertrophy of existing beta-cells (27), beta-cell proliferation (26), and neogenesis from progenitors, at least in pancreatectomy models (18). However, we found no evidence for altered ductal (SOX9) or islet endocrine (NEUROG3) progenitor cells as the basis of maintained beta-cell mass in STZ-treated MIP-HSD1^tg/+ mice, in agreement with recent findings that beta-cell neogenesis does not come from ductal cell progenitors in the adult pancreas (17). Thus, altered GC regeneration selectively impacts upon beta-cell replication in the STZ-injured adult pancreas. Moreover, MIP-HSD1^tg/+ islets exhibit a hyper-functionality associated with increased Cdkn1a (P21) expression suggesting accelerated functional maturation of MIP-HSD1^beta-cells (14), consistent with the role of GCs in terminal differentiation. Newly generated MIP-HSD1 beta-cells likely achieve functionality more rapidly than normal beta-cells. In support of this notion, overexpression of the pro-differentiation factor P21 in beta-cells promotes resistance to high-dose STZ by increasing progenitor differentiation (28).


discussion

FIGURE 4 | MIP-HSD1^tg/+ mice completely resist hyperglycemia induced by multiple low-dose injection of STZ. Twelve-week-old mice were injected i.p. either with sodium citrate vehicle or STZ (40 mg/kg/body weight) for five consecutive days. (A) Blood glucose levels measured immediately before injections (day 0) and day 4, 8, 12, and 15 after vehicle injection in littermate KsJ mice (○, n = 8) or MIP-HSD1^tg/+ mice (●, n = 7) and after STZ injection in littermate KsJ mice (●, n = 8) or MIP-HSD1^tg/+ mice (○, n = 12). Blood glucose level in MIP-HSD1^tg/+ STZ-treated mice was significantly elevated only KsJ STZ-treated mice. Values represented mean ± SEM. Differences were analyzed by one-way ANOVA, Newman–Keuls test, ***P < 0.001 for KsJ STZ vs. KsJ Veh; **P < 0.01 and **P < 0.05 for MIP-HSD1^tg/+ STZ vs. KsJ STZ.

(B) Preservation of islet structure in MIP-HSD1^tg/+ shown by insulin staining of paraffin-fixed pancreata from KsJ mice (upper row) and MIP-HSD1^tg/+ mice (bottom row) 10 days after the end of vehicle (Veh, left lane) or STZ (right lane) treatment. Scale = 400 µm magnification ×50. (C) Reduced macrophage infiltration in MIP-HSD1^tg/+ shown by Mac-2 staining of paraffin-fixed pancreata from KsJ mice (upper row) and MIP-HSD1^tg/+ mice (bottom row) 10 days after the end of vehicle (Veh, left lane) or STZ (right lane) treatment. Bars = 50 µm, magnification ×400. (D) Quantitation of Mac-2 positive cells per islet area using Zeiss (KS300, 3.0i) software. Values represent mean ± SEM, differences were analyzed by one-way ANOVA, Newman–Keuls test, ***P < 0.001 for STZ vs. Veh; **P < 0.01 for MIP-HSD1^tg/+ STZ vs. KsJ STZ (n = 5–10).

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Increased T regulatory cell numbers also suggests that a greater generation long after the STZ has been metabolized. GCs suppression of Th1 cells and, intriguingly, may indicate enhanced suppression of self-antigens needed to determine the contribution of altered β-cell GCs to this process. Macrophage infiltration in diabetogenic islets is an early event (1-4). Depletion (19) or inactivation (2) of macrophages prevents the progression of type 1 diabetes, highlighting the importance of the inflammatory mechanism per se in islet destruction. Reduction of macrophage infiltration in MIP-HSD1<sup>tg/</sup>* mice is consistent with a reduced inflammatory insult that will work, at least in part, through longer-term curtailment of cytokine-mediated NO generation long after the STZ has been metabolized. GCs suppress a number of distinct pro-inflammatory signaling pathways that will also contribute to overall improvement in islet function (5). The higher Treg cell influx into MIP-HSD1<sup>tg/</sup>* mice (black bars) compared to islets from KsJ (white bars) with vehicle ( ), STZ ( ), or with STZ and L-NAME incubated with vehicle ( ), STZ ( ), or STZ and L-NAME ( ), with L-NAME ( ), or with STZ and L-NAME ( ) shows attenuation of nitric oxide production in isolated islets from MIP-HSD1<sup>tg/</sup>* mice treated with STZ with or without 11beta-HSD1 inhibitor UE2316 ( ) for 72 h. Values represent mean ± SEM, differences analyzed by one-way ANOVA, Newman-Keuls test. ***P < 0.001 for STZ vs. Veh; **P < 0.01 for MIP-HSD1<sup>tg/</sup>* STZ vs. KsJ STZ, **P < 0.01 STZ + L-NAME vs. STZ (n = 6). (B) NO production in primary cultures of islets from MIP-HSD1<sup>tg/</sup>* mice with or without 11beta-HSD1 inhibitor UE2316 ( ) for 72 h. Values represent mean ± SEM, differences analyzed by ANOVA, Newman-Keuls test. ***P < 0.001 for STZ vs. Veh; **P < 0.01 for STZ + UE2316 vs. STZ (n = 6). (C) Representative light microscopy images of islets from KsJ (upper row) and MIP-HSD1<sup>tg/</sup>* mice (lower row) incubated with vehicle ( ), STZ ( ), or with STZ + L-NAME ( ). Magnification ×400.

REFERENCES
1. Eizirik DL, Colli ML, Orts F. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. Nat Rev Endocrinol (2009) 5:219–26. doi:10.1038/nrendo.2008.21
2. Hutchings P, Rosen H, O’Reilly L, Simpson E, Gordon S, Cooke A. Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. Nature (1990) 348:639–42. doi:10.1038/348639a0
3. Corbett JA, Mikhail A, Shimiizu J, Frederick K, Musko TP, McDaniel ML, et al. Nitric oxide production in islets from nonobese diabetic mice: aminoacylase-sensitive and -resistant stages in the immunological diabetic process. Proc Natl Acad Sci U S A (1993) 90:8992–5. doi:10.1073/pnas.90.19.8992
4. Flodström M, Tyrberg B, Eizirik DL, Sandler S. Reduced sensitivity of inducible nitric oxide synthase-deficient mice to multiple low-dose streptozocin-induced diabetes. Diabetes (1999) 48:706–13. doi:10.2337/diabetes.48.4.706
5. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Ann N Y Acad Sci (2012) 1261:55–63. doi:10.1111/j.1749-6632.2012.06633.x
6. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med (2000) 342:260–8. doi:10.1056/NEJM200002243420201
7. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. J Clin Invest (1997) 99:414–23. doi:10.1172/JCI119175
8. Delaunay F, Khan A, Cintra A, Davani B, Ling ZC, Andersson A, et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. J Clin Invest (1997) 100:2094–8. doi:10.1172/JCI119743
9. Drachenberg CR, Klassen DK, Weir MR, Wiland A, Fink JC, Bartlett ST, et al. Islet cell damage associated with tacrolimus and cyclosporine: morphological
features in pancreas allograft biopsies and clinical correlation. *Transplantation* (1999) 68:396–402. doi:10.1097/00007890-199908150-00012
10. Hult M, Ortsater H, Schuster G, Graessler F, Beckers J, Adamski J, et al. Short-term glucocorticoid treatment increases insulin secretion in islets derived from lean mice through multiple pathways and mechanisms. *Mol Cell Endocrinol* (2009) 301:109–16. doi:10.1016/j.mce.2008.09.038
11. Lund T, Fosby B, Korsgren O, Scholz H, Foss A. Glucocorticoids reduce pro-inflammatory cytokines and tissue factor in vitro and improve function of transplanted human islets in vivo. *Transpl Int* (2008) 21:669–78. doi:10.1111/j.1399-2227.2008.00664.x
12. Buchwald F, Bocca N, Marzorati S, Hochhaus G, Bodor N, Stabler C, et al. Feasibility of localized immunosuppression: 1. exploratory studies with glucocorticoids in a biohybrid device designed for cell transplantation. *Pharmacoe* (2010) 65:421–8.
13. Rafacho A, Marroqui L, Taboga SR, Abrantes JL, Silveira LR, Boschero AC, et al. Glucocorticoids in vivo induce both insulin hypersecretion and enhanced glucocorticoid sensitivity of stimulus-secretion coupling in isolated rat islets. *Endocrinology* (2010) 151:85–95. doi:10.1210/en.2009-0704
14. Turban S, Liu X, Ramage L, Webster SP, Walker BR, Dunbar DR, et al. Optimal elevation of beta-cell 11beta-hydroxysteroid dehydrogenase type 1 is a compensatory mechanism that prevents high-fat diet-induced beta-cell failure. *Diabetes* (2012) 61:642–65. doi:10.2337/db11-1054
15. Eshes JA, Ellingsgaard H, Boni-Schnetzer M, Donath MY. Pancreatic islet inflammation in type 2 diabetes: from alpha and beta cell compensation to dysfunction. *Arch Physiol Biochem* (2009) 115:240–7. doi:10.1080/1388024080258779
16. King A. The use of animal models in diabetes research. *Br J Pharmacol* (2012) 166:877–94. doi:10.1111/j.1476-5381.2012.01911.x
17. Kopp JL, Dubois CL, Scharff AE, Hao E, Shih HP, Seymour PA, et al. S≡C≡C ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* (2011) 138:653–65. doi:10.1242/dev.05649
18. Xu X, D’Hoker J, Stange G, Bonné S, De Leu N, Xiao X, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* (2008) 132:197–207. doi:10.1016/j.cell.2007.12.015
19. Mensah-Brown E, Shahin A, Parekh K, Hakim AA, Shamisi MA, Hsu DK, et al. Functional capacity of macrophages determines the induction of type 1 diabetes. *Ann N Y Acad Sci* (2006) 1084:49–57. doi:10.1196/annals.1372.014
20. Grimbler-Bleyer Y, Saadoun D, Bayens A, Billiard F, Goldstein JD, Grégoire S, et al. Pathogenic T cells have a paradoxical protective effect in murine autoimmune diabetes by boosting Tregs. *J Clin Invest* (2010) 120:4558–68. doi:10.1172/JCI42945
21. Herold KC, Bloch TN, Vens V, Sun Q. Diabetes induced with low doses of streptozotocin is mediated by V beta 8.2+ T-cells. *Diabetes* (1995) 44:354–9. doi:10.2337/diabetes.44.3.354
22. Elias D, Prigozin H, Polak N, Rapoport M, Lohse AW, Cohen IR. Autoimmunity to the beta-cell toxin STZ. Immunity to the 60-kDa heat shock protein and to insulin. *Diabetes* (1994) 43:992–8. doi:10.2337/db4a.43.8.992
23. Kwok NS, Lee SH, Chou CS, Kho T, Lee HS. Nitric oxide generation from streptozotocin. *FASEB J* (1994) 8:529–33.
24. Reddy SI, Wu D, Elliott RB. Low dose streptozotocin causes diabetes in severe combined immunodeficient (SCID) mice without immune cell infiltration of the pancreatic islets. *Autoimmunity* (1995) 20:83–92. doi:10.3109/08916939509001931
25. Radomski MW, Palmer RM, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci U S A* (1990) 87:10043–7. doi:10.1073/pnas.87.24.10043
26. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* (2004) 429:41–6. doi:10.1038/nature02520
27. Jonas JC, Sharma A, Hasenkamp W, Ikhoua H, Patané G, Laybutt R, et al. Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. *J Biol Chem* (1999) 274:14112–21. doi:10.1074/jbc.274.20.14112
28. Yang I, Zhang W, Jiang W, Sun X, Han Y, Ding M, et al. P21-cip-overexpression in the mouse beta cells leads to the improved recovery from streptozotocin-induced diabetes. *PLoS One* (2009) 4(12):e8344. doi:10.1371/journal.pone.0008344
29. Liu K, Paterson AJ, Chin E, Kudlow JE. Glucocorticoids stimulate protein modification by O-linked GlcNAc in pancreatic beta cells: linkage of O-linked GlcNAc to beta cell death. *Proc Natl Acad Sci U S A* (2000) 97:2820–5. doi:10.1073/pnas.97.6.2820
30. Kurren MO, Pakala SV, Hanson HL, Katz JD. Beta cell apoptosis in T cell-mediated autoimmune diabetes. *Proc Natl Acad Sci U S A* (1997) 94:213–8. doi:10.1073/pnas.94.1.213
31. Kraaij MD, van der Kooij SW, Reinders ME, Koekkoek K, Rabelink TJ, van Kooten C, et al. Dexamethasone increases ROS production and T cell suppressive capacity by anti-inflammatory macrophages. *Mol Immunol* (2011) 49:549–57. doi:10.1016/j.molimm.2011.10.002
32. Ono M, Yaguchi H, Ohkura N, Kitabayashi I, Nagamura Y, Nomura T, et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runxl. *Nature* (2007) 446:889–95. doi:10.1038/nature05673
33. Schmid J, Ludwig B, Schally AV, Steffen A, Ziegler CG, Block NL, et al. Modulation of pancreatic islets-stress axis by hypothalamic releasing hormones and 11beta-hydroxysteroid dehydrogenase. *Proc Natl Acad Sci U S A* (2011) 108:13722–7. doi:10.1073/pnas.1109651080

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