Original Article

MOLECULAR METABOLISM

Pancreatic deletion of the interleukin-1 receptor disrupts whole body glucose homeostasis and promotes islet β-cell de-differentiation

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ABSTRACT

Objective: Pancreatic tissue, and islets in particular, are enriched in expression of the interleukin-1 receptor type I (IL-1R). Because of this enrichment, islet β-cells are exquisitely sensitive to the IL-1R ligands IL-1α and IL-1β, suggesting that signaling through this pathway regulates health and function of islet β-cells.

Methods: Herein, we report a targeted deletion of IL-1R in pancreatic tissue (IL-1R$^{Pdx1-/-}$) in C57BL/6J mice and in *db/db* mice on the C57 genetic background. Islet morphology, β-cell transcription factor abundance, and expression of the de-differentiation marker Aldh1a3 were analyzed by immunofluorescent staining. Glucose and insulin tolerance tests were used to examine metabolic status of these genetic manipulations. Glucose-stimulated insulin secretion was evaluated *in vivo* and in isolated islets *ex vivo* by perifusion.

Results: Pancreatic deletion of IL-1R leads to impaired glucose tolerance, a phenotype that is exacerbated by age. Crossing the IL-1R$^{Pdx1-/-}$ with *db/db* mice worsened glucose tolerance without altering body weight. There were no detectable alterations in insulin tolerance between IL-1R$^{Pdx1-/-}$ mice and littermate controls. However, glucose-stimulated insulin secretion was reduced in islets isolated from IL-1R$^{Pdx1-/-}$ relative to control islets. Insulin output *in vivo* after a glucose challenge was also markedly reduced in IL-1R$^{Pdx1-/-}$ mice when compared with littermate controls. Pancreatic islets from IL-1R$^{Pdx1-/-}$ mice displayed elevations in Aldh1a3, a marker of de-differentiation, and reduction in nuclear abundance of the β-cell transcription factor MafA. Nkx6.1 abundance was unaltered.

Conclusions: There is an important physiological role for pancreatic IL-1R to promote glucose homeostasis by suppressing expression of Aldh1a3, sustaining MafA abundance, and supporting glucose-stimulated insulin secretion *in vivo*.

Keywords Cytokine; Glucose homeostasis; Inflammation; Insulin; Islet

1. INTRODUCTION

Pancreatic islet β-cells secrete insulin in response to fuel stimuli, with glucose regarded as the primary trigger for hormone release [1]. The mature, adult β-cell expresses a multitude of transcription factors (TFs) responsible for maintaining a robust insulin secretory phenotype. Representative examples of such regulatory TFs in β-cells include MafA, Nkx6.1, and Pdx-1. Indeed, Pdx-1 is important for pancreas development and remains expressed in pancreatic β-cells to sustain insulin production [2]; reductions in Pdx-1 impair glucose-stimulated insulin secretion [3]. Nkx6.1 is important for suppression of the glucagon gene and together MafA and Nkx6.1 support glucose-stimulated insulin secretion [4,5]. Several of these TFs are either reduced in abundance or in nuclear presence in mouse models of obesity and in humans with obesity and T2D [6–8]. In addition, Aldh1a3 has recently been associated with de-differentiation of β-cells, helping to explain reduced insulin secretion in both rodents and humans [9,10].

Obesity correlates with increased pancreatic islet size [11–13], while inflammation is linked with reductions in β-cell function and mass in both type 1 (T1D) and type 2 diabetes (T2D) [14,15]. A variety of inflammatory stimuli, including free fatty acids and cytokines, have been associated with tissue dysfunction during progression to metabolic disease. For example, elevated levels of interleukin-1β promote key signaling changes within adipose tissue [16], liver [17], and pancreatic islets [18,19]. The cytokines IL-1α and IL-1β signal through the interleukin-1 receptor type I [20]. These two cytokines are opposed by the interleukin-1 receptor antagonist protein, encoded by the IL-1RN gene [21]. The type II interleukin-1 receptor is proposed to function as a decoy receptor, able to bind ligands, but not communicate an

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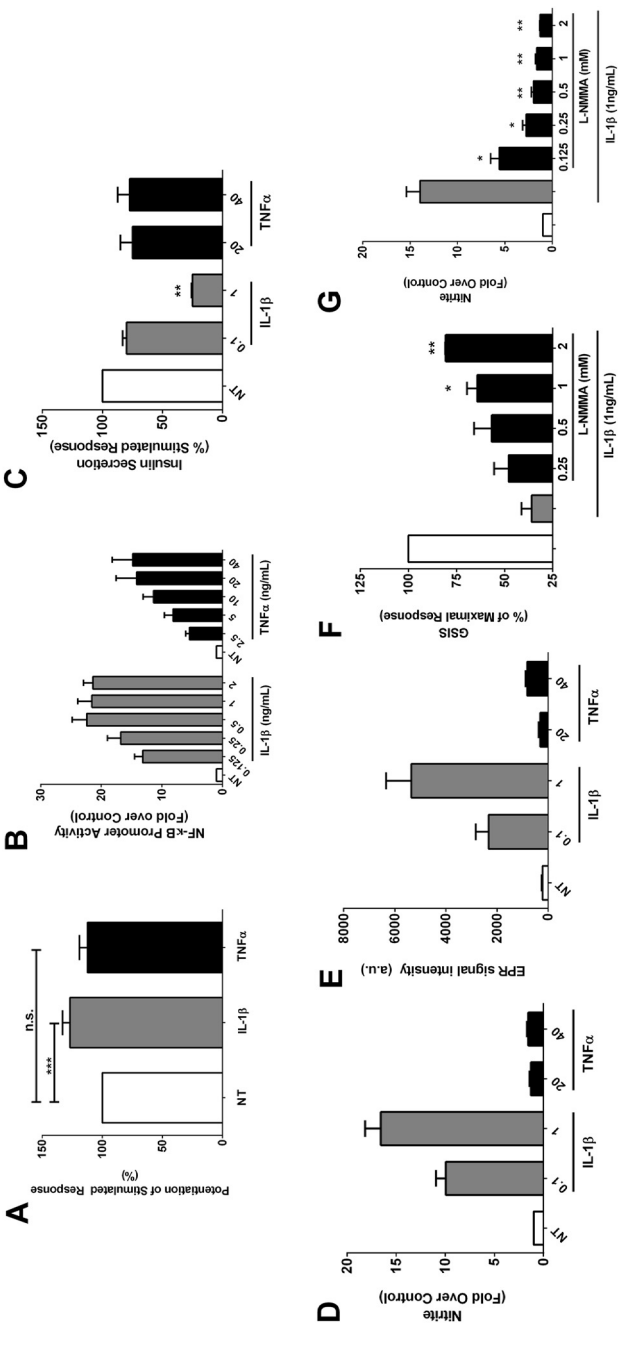

Figure 1: Interleukin-1 signaling in β-cells potentiates insulin secretion acutely but restricts insulin secretion over time, with nitric oxide functioning as a negative feedback signal. (A) Insulin secretion in cultured 832/13 rat β-cells normalized as percent of the maximal stimulated response after no treatment (NT), or 30 min incubations in either 1 ng/mL IL-1β or 40 ng/mL TNF-α (n = 4, some of which were performed in duplicate or triplicate). (B) Dose response of IL-1β and TNF-α on a 5x NF-κB promoter luciferase reporter gene in cultured 832/13 rat β-cells; luciferase output is normalized to total cellular protein and shown as fold over the unstimulated control cells (n = 4). (C) Insulin secretion in cultured 832/13 rat β-cells normalized as percent of the maximal stimulated response after overnight exposure to either 0.1 and 1 ng/mL IL-1β or 20 and 40 ng/mL TNF-α versus cells left untreated (NT; n = 3, some of which were conducted in duplicate). (D) Total nitrite accumulation in the media in response to the indicated doses of IL-1β or TNF-α (n = 3). (E) Electron paramagnetic resonance spin trapping measurements of nitric oxide radical in response to the indicated doses of IL-1β or TNF-α (n = 3). (F) Insulin secretion in cultured 832/13 rat β-cells normalized as percent of the maximal stimulated response to show the marked reduction in insulin secretion by overnight exposure to 1 ng/mL IL-1β versus the dose-dependent recovery of insulin secretion by L-NMMA (n = 3). (G) Fold increase in total nitrite production in 832/13 cells by exposure to 1 ng/mL IL-1β overnight and the dose-dependent reduction by L-NMMA (n = 3). Error bars represent SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., not significant.

