β Cell-Specific Deletion of the IL-1 Receptor Antagonist Impairs β Cell Proliferation and Insulin Secretion

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Highlights

- β cells are the main source of mouse islet IL-1Ra expression

- Deletion of IL-1Ra in β cells impairs insulin secretion and β cell proliferation

- β cell-specific IL-1Ra knockout inhibits Kir6.2 and proliferation gene expression

- IL-1Ra protects from IL-1-mediated β cell dysfunction via the E2F1-Kir6.2 pathway

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In Brief

In pancreatic islets of patients with type 2 diabetes, β cell expression of the IL-1 receptor antagonist (IL-1Ra) is decreased. Böni-Schnetzler et al. show that deletion of β cell-derived, but not of myeloid cell-derived, IL-1Ra impairs glucose homeostasis, β cell proliferation, and insulin secretion, partly via E2F1-regulated Kir6.2 expression.

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**β Cell-Specific Deletion of the IL-1 Receptor Antagonist Impairs β Cell Proliferation and Insulin Secretion**

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**SUMMARY**

Interleukin-1 receptor antagonist (IL-1Ra) is elevated in the circulation during obesity and type 2 diabetes (T2D) but is decreased in islets from patients with T2D. The protective role of local IL-1Ra was investigated in pancreatic islet β cell (βIL-1Ra)-specific versus myeloid-cell (myeloIL-1Ra)-specific IL-1Ra knockout (KO) mice. Deletion of IL-1Ra in β cells, but not in myeloid cells, resulted in diminished islet IL-1Ra expression. Myeloid cells were not the main source of circulating IL-1Ra in obesity. βIL-1Ra KO mice had impaired insulin secretion, reduced β cell proliferation, and decreased expression of islet proliferation genes, along with impaired glucose tolerance. The key cell-cycle regulator E2F1 partly reversed IL-1β-mediated inhibition of potassium channel Kir6.2 expression and rescued impaired insulin secretion in IL-1Ra knockout islets. Our findings provide evidence for the importance of β cell-derived IL-1Ra for the local defense of β cells to maintain normal function and proliferation.

**INTRODUCTION**

Metabolic diseases, including type 2 diabetes (T2D), are associated with inflammatory processes characterized by changes in cytokines and immune cells in pancreatic islets and insulin-sensitive tissues (Donath and Shoelson, 2011; Hotamisligil et al., 1993; Wellen and Hotamisligil, 2005). The prototypical cytokine interleukin (IL)-1β, which is a master regulator of pathogen-induced and sterile inflammation (Dinarello, 2013), has a dual role in metabolism. Acutely, it participates in glucose control and contributes to postprandial insulin secretion (Dror et al., 2017; Hajmrle et al., 2016), while chronically, it is implicated in β cell dysfunction, insulin resistance, and cardiovascular disease, a major complication in T2D (Donath and Shoelson, 2011; Herder et al., 2015; Masters et al., 2011; Ridker et al., 2017; Stienstra et al., 2010; Vandannagars et al., 2011; Wen et al., 2011). Clinical studies in patients with T2D using IL-1 antagonists showed improved insulin secretion and glycemia pointing to the therapeutic potential of IL-1 blockade in T2D (Herder et al., 2015; Larsen et al., 2007).

Human and rodent pancreatic islets locally express various IL-1 family members, including IL-1β, IL-1α, IL-1 receptor antagonist (IL-1Ra), and the major signaling receptor IL-1 receptor type 1 (IL-1R1). Of note, insulin-producing β cells have the most abundant expression of IL-1R1 compared to other tissues (Benner et al., 2014; Böni-Schnetzler et al., 2009). The genes of the agonistic IL-1R1 ligands IL-1β (Il1b) and IL-1α (Il1a), and of the IL-1Ra (Il1rn), are typically coexpressed, and IL-1Ra expression is induced by all signals known to trigger IL-1 production and also by IL-1 itself (Arend et al., 1998; Böni-Schnetzler et al., 2008). IL-1 agonists and the IL-1 antagonist bind to the IL-1R1 receptor with similar affinities. While IL-1 binding induces receptor signaling through additional binding to the IL-1 receptor accessory protein, IL-1Ra solely binds the IL-1R1 and, thus, acts as a competitive inhibitor. Therefore, the balance between IL-1 agonists and IL-1Ra at the IL-1 receptor site is critical in determining responses in target cells (Palomo et al., 2015). Systemic IL-1Ra has a short half-life (Cawthorne et al., 2011) indicating that local IL-1 regulatory systems within tissues may be in place and may limit IL-1 action in target tissues.

Circulating IL-1Ra in humans and rodents is upregulated in obesity and correlates with insulin resistance (Herder et al., 2009, 2015; Juge-Aubry et al., 2003; Somm et al., 2006). Further, elevated circulating IL-1Ra in humans is associated with an increased risk to develop T2D, and prospective studies show a sharp increase in circulating IL-1Ra prior to the onset of the disease (Grossmann et al., 2015; Herder et al., 2009). This may reflect the body’s reaction to counter-regulate and limit the enhanced inflammation in vain. Staining of IL-1Ra in pancreas sections is reduced in islets of patients with T2D, compared to non-diabetic individuals (Maedler et al., 2004), and glucotoxicity...
To investigate the source and the role of local IL-1Ra on islet function and glucose homeostasis, we produced and characterized mouse islets using qPCR (Figure 1A). The gene of the IL-1R1 (Il1r1) was robustly expressed (7.5 ± 0.3 ct values above the assay cutoff), while gene expression of the decoy receptor IL-1R2 (Il1r2) was barely detectable (1.2 ± 0.2 ct values above cutoff). Genes encoding the agonistic IL-1 receptor (IL-1R) ligands IL-1β (Il1b) and IL-1α (Il1a) and the antagonist IL-1Ra (Il1rn) were expressed at 3.5 ± 0.3, 1.8 ± 0.3, and 3.6 ± 0.6 ct values above cutoff, respectively. To determine the cellular source of Il1m expression, we dispersed islets into single cells and sorted them into endocrine, pan-CD45+ immune, and CD31+ endothelial cells to measure Il1m gene expression (Figure 1B). Il1m expression was only detected in the endocrine and immune cell pools, and expression was similar in both cell types, while it was not detectable in CD31+ endothelial cells. Since islets contain only few CD45+ cells (Figure 1C; Dalmas et al., 2017), islet IL-1Ra most likely stems from endocrine cells. Islets with α cell ablation (Thorel et al., 2011; Traub et al., 2017) have similar Il1m mRNA levels compared to islets from control mice (Figure 1D), pointing to islet β cells as the leading source of islet Il1m expression. To validate this conclusion, we generated β cell-specific IL-Ra knockout (Il1r1 KO) and myeloid cell-specific IL-Ra knockout (myeloIl1r1 KO) mice (see production of mice in Figure S1A), and Cre-negative mice served as respective littermate, wild-type (WT) controls. Islets isolated from Il1r1 KO mice displayed strongly diminished Il1m mRNA expression (Figure 1E) and IL-1Ra protein release (Figure 1F), compared to islets from...
control mice, while KO of Il1rn in the myeloid cells did not alter islet Il1rn expression (Figure 1H). Expression of Il1b, Il1a, Il1r1, and Il1r2 was not changed in islets from both mouse genotypes compared to that from controls (Figures 1E and 1H). Thus, the ratio of Il1b/Il1rn expression is only increased in islets from jIl1Ra KO mice. IL-1Ra secretion from peritoneal macrophages of myelol-1Ra KO mice was strongly diminished relative to control macrophages (Figure 1I), while secretion from jIl1-1Ra KO peri-
toneal macrophages was unchanges (Figure 1G). Reduced Il1rn expression in cultured islets from jIl1-1Ra KO mice relative to WT islets was maintained when Il1rn was induced with IL-1β (Figure 1J), while Il1r1 expression remained unchanged (Figure 1K). Ins2 gene expression was lower in islets from jIl1Ra KO than from WT mice, and the addition of IL-1β strongly inhibited Ins2 and stimulated inflammation genes in both genotypes to a similar extent (Figure 1L; Figures S1B–S1E). Since Ins2 promoter-driven

**p < 0.05; **p < 0.01.

See also Figure S2.

**β Cell-Specific IL-1Ra KO Reduces Glucose-Stimulated Insulin Secretion In Vivo and in Isolated Islets**

(A) Weight gain of jIl1-1Ra KO and control mice (n = 19 WT and 13 KO). (B and C) The ipGTTs of 12-week-old chow-fed jIl1-1Ra KO and WT mice; (B) blood glucose and (C) plasma insulin (n = 8 WT and 10 KO). (D and E) The ipGTTs of 12-week-old RIPcre+/− mice and RIPcre−/− littermates; (D) blood glucose and (E) plasma insulin (n = 8 WT and 10 KO). (F) Insulin secretion in ipGTT at 8, 12, 24, and 52 weeks of age; area under the curve (AUC). (G) Weight gain of chow-fed myelol-1Ra KO and WT mice (n = 10 WT and 10 KO). (H and I) The ipGTTs of 12-week-old chow-fed myelol-1Ra KO and control mice; (H) blood glucose and (I) plasma insulin (n = 16 WT and 17 KO). (J) Glucose-stimulated insulin secretion (GSIS) of islets from 12-week-old WT and jIl1-1Ra KO mice at 2.8 mM (open bars) and 16.7 mM glucose (closed bars) expressed as percentage of the insulin content of the respective islet culture (n = 30–36, 5 isolations). (K) Glucose- and GLP-1-stimulated insulin secretion of islets from WT and jIl1-1Ra KO mice (n = 15, 3 isolations). (L) Insulin content per 25 islets (n = 41–43, 5 isolations). (M) GSIS of islets from 12-week-old WT and myelol-1-1Ra KO mice at 2.8 mM (open bars) and at 16.7 mM glucose (closed bars) expressed as percentage of insulin content of the respective islet culture (n = 10–11, 2 isolations). (N) Glucose- and GLP-1-stimulated insulin secretion of islets from wt and myelol-1Ra ko mice (n = 15, 3 isolations). (O) Insulin content per 25 islets (n = 15, 3 isolations). n indicates the number of mice or biological replicates, and error bars represent SEM. p < 0.05; **p < 0.01.

See also Figure S2.

**β Cell-Specific IL-1Ra KO, but Not myelol-1Ra KO Mice, Have Reduced Insulin Secretion**

Next, we determined whether IL-1Ra deficiency in β or myeloid cells has a functional consequence in chow-fed mice. Body weight gain and plasma glucose in intraperitoneal glucose tolerance tests (ipGTTs) were not different in jIl1-1Ra and myelol-1Ra KO mice compared to the respective litter-

mate control mice (Figures 2A, 2B, 2G, and 2H). However, deletion of IL-1Ra in β cells resulted in significantly reduced insulin secretion (Figure 2G) compared to that in controls.
Figure 3. Obese βIL-1Ra KO Mice Have Reduced Glucose-Stimulated Insulin Secretion and Impaired Glucose Tolerance

(A) Weight gain of βIL-1Ra KO and WT control mice (n = 19 WT and 13 KO).

(B and C) The ipGTTs of 8-week-old βIL-1Ra KO and WT mice fed an HFD for 4 weeks; (B) blood glucose (n = 24 WT and 22 KO) and (C) plasma insulin (n = 24 WT and 19 KO).

(D) The ipITT of 8-week-old HFD βIL-1Ra KO mice (n = 10 WT and 11 KO).

(E) Insulin secretion of βIL-1Ra KO and WT mice in ipGTT after 4, 8, and 20 weeks of HFD feeding expressed as AUC.

(F and G) The ipGTTs of 8-week-old RIPcre+/− mice and RIPcre+/− WT mice; (F) blood glucose (n = 11 WT and 11 RIPcre+/−) and (G) plasma insulin (n = 11 WT and 11 RIPcre+/−).

(H) The ipITT of 8-week-old RIPcre+/− mice (n = 11 WT and 11 RIPcre+/−).

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RIPre-only control experiments with RIP Cre<sup>+/−</sup> and RIP Cre<sup>−/−</sup> mouse littermates revealed no difference in ipGTT and insulin secretion (Figures 2D and 2E). Diminished insulin release in male βIL-1Ra KO in ipGTT was also observed in aged mice (Figure 2F; Figures S2A and S2B) and in female mice (Figure S2C). Insulin tolerance as determined by intraperitoneal insulin tolerance tests (ipITTs) was not altered in βIL-1Ra KO mice (Figure S2D). The myeloIL-1Ra KO mice displayed no change in insulin secretion after a glucose bolus compared to control mice at 12 weeks (Figures 2H and 2I) and 20 weeks (data not shown) of age.

Next, we investigated β cell function in isolated islets from βIL-1Ra KO and myeloIL-1Ra and their respective littermate WT mice. We measured glucose-stimulated insulin secretion (GSIS) in isolated islets by sequential incubation at a low-glucose (2.8 mM, 1 hr) concentration followed by a high-glucose (16.8 mM, 1 hr) concentration (Figures 2J–2O; Figures S2E–S2H). GSIS of βIL-1Ra KO islets was significantly reduced relative to WT islets (Figure 2J). This was accentuated by the addition of GLP-1, together with a high-glucose concentration to further boost insulin release (Figure 2K; Figure S2F). Islet insulin content was slightly elevated in islets of βIL-1Ra KO mice, further pointing to an insulin secretory defect (Figure 2L). Islets from myeloIL-1Ra KO mice showed no difference in GSIS with or without GLP-1 and insulin content compared to control islets (Figures 2M–2O; Figures S2G and S2H). Therefore, islet local β cell-derived IL-1Ra is functional, and its KO leads to impaired insulin secretion in vivo and in vitro.

Obese βIL-1Ra KO Mice Have Reduced Insulin Secretion and Impaired Glucose Tolerance

Next, we increased the metabolic burden by high-fat-diet (HFD) feeding of βIL-1Ra KO and myeloIL-1Ra KO mice and their respective littermate controls. Body weight gain in mice of both genotypes was not different when compared to their respective controls (Figures 3A and 3I). βIL-1Ra KO mice had a markedly reduced insulin secretion along with impaired glucose tolerance after 4 weeks of HFD feeding (Figures 3B and 3C), while insulin sensitivity was not different (Figure 3D). Insulin secretion was also decreased during ipGTT after 8 and 20 weeks of HFD feeding in KO mice (Figure 3E; Figure S3A). Compared to controls, HFD-fed RIPcre-only mice had identical ipGTT, insulin, and ipITT (Figures 3F–3H; Figures S3B–S3D). Obese myeloIL-1Ra KO mice had similar insulin secretion and glucose tolerance between genotypes (Figures 3J and 3K) and marginally improved insulin sensitivity relative to controls (Figure 3L).

Knockdown of islet Il1rn expression in islets of obese βIL-1Ra KO mice persisted upon HFD feeding for 8 and 20 weeks (Figure 3M). Plasma IL-1Ra was not different between obese βIL-1Ra KO and control mice (Figure 3N), corroborating local islet Il1rn deficiency in these mice. Next, we examined insulin secretion in vivo (Figures 3O–3T). Like in vivo, we found diminished GSIS in islets from obese βIL-1Ra KO mice fed an HFD for 8 and 20 weeks relative to islets from respective control mice (Figures 3Q and 3R). Further, there was less insulin release into the culture medium (Figures 3S and 3T) and a lower insulin content in islets from obese βIL-1Ra KO mice after 8 weeks of HFD (Figures 3O and 3P).

Next, we examined whether β cell-derived IL-1Ra is protective in a rodent model of T2D (Mu et al., 2006). We induced hyperglycemia by treating HFD-fed mice with a single low dose of streptozotocin (stz) to induce partial β cell destruction. βIL-1Ra KO mice had higher fasting blood glucose (Figure 4A) along with lower fasting (Figure 4B) and glucose-stimulated plasma insulin (Figure 4C) than littermate controls. In contrast, stz caused no difference in fasting glucose between HFD-fed myeloIL-1Ra KO and littermate controls (Figure 4D). Altogether, this points to a protective role of β cell-derived IL-1Ra in this model of T2D.

Therefore, also in obese mice, only KO of IL-1Ra in islet β cells, but not in myeloid cells, impaired glucose homeostasis and reduced insulin secretion in vivo and in vitro.

Myeloid Cells Are Not the Main Source of Increased Plasma IL-1Ra in Obesity

Plasma IL-1Ra concentration rises and correlates with increasing obesity (Somm et al., 2006), along with induced Il1rn expression in liver and adipose tissue (Juge-Aubry et al., 2003). To determine whether myeloid cells are the source of elevated circulating IL-1Ra in obesity, we compared plasma IL-1Ra levels of obese myeloIL-1Ra mice to those of respective controls. After 16 weeks of HFD (age, 20 weeks), the plasma IL-1Ra concentration was similar in both genotypes (Figure 4E), despite massively reduced IL-1Ra secretion in peritoneal macrophages from HFD-fed myeloIL-1Ra KO mice (Figure 4F). At the age of 52 weeks, plasma IL-1Ra was lower in obese myeloIL-1Ra KO mice compared to their littermate controls (Figure 4E), suggesting a role for...
Figure 4. IL-1Ra Protects from Diabetes Induction and Myeloid Cells Are Not the Main Source of Increased Plasma IL-1Ra in Obesity
(A) Fasting blood glucose of HFD-fed βIL-1Ra KO and littermate control mice at the indicated day after single low-dose streptozotocin (stz) treatment (11 WT, 11 KO).
(B and C) Shown here: (B) Fasting insulin 18 days after stz treatment and (C) glucose-stimulated insulin secretion (AUC).
(D) Fasting blood glucose of HFD-fed myeloIL-1Ra KO and littermate control mice after single low-dose sstz treatment (7 WT, 9 KO).
(E) Plasma IL-1Rs of HFD-fed 20- and 52-week-old myeloIL-1Ra KO mice and WT littermates (20 WT, 24–26 KO).
(F) IL-1Ra secretion from peritoneal macrophages of HFD-fed WT and myeloIL-1Ra KO mice (n = 7 each).

(legend continued on next page)
myeloid cell-derived IL-1Ra only at later obesity stages. We measured Il1rn gene expression in metabolic tissues that up-regulate Il1rn expression and accumulate myeloid cells during obesity. At the ages of 17 and 52 weeks, we observed reduced Il1rn expression in adipose tissue of HFD-fed myeloIL-1Ra KO mice compared to that of control mice, and there was an overall age-related increase of Il1rn expression in fat (Figure 4G). Liver Il1rn expression was higher in myeloIL-1Ra KO mice than in controls at 17 weeks of age and dropped at 52 weeks of age in both genotypes (Figure 4H). This points to compensatory non-myeloid regulation of Il1rn, which was lost at later time points. Importantly, despite lower plasma IL-1Ra in 52-week-old myeloIL-1Ra KO mice, we did not observe differences in fasting glucose, ipGTT, insulin secretion, and ipITT upon aging (Figures 4J–4L).

We conclude that circulating IL-1Ra may originate from different cell sources during the development of obesity with dispensable contribution from myeloid cells. The reduced plasma IL-1Ra in 52-week-old myeloIL-1Ra KO mice in the face of unchanged plasma glucose and insulin, suggests that obesity-induced plasma IL-1Ra has little influence on islet function and further supports the critical role of islet local IL-1Ra for β cell function.

**jIL-1Ra KO Mice Have an Altered Islet Morphology with a Higher Proportion of Small Islets**

Next, we investigated islet morphology in 4- and 12-week-old chow-fed and in 12- and 25-week-old HFD-fed jIL-1Ra KO mice (Figure 5A). In all 4 groups, the mean pancreas weight and the average number of islets per section were not different between WT and jIL-1Ra KO mice (Figures S4A–S4H), except for 4-week-old chow-fed jIL-1Ra mice, which had slightly more islets compared to controls (Figure S4A). Insulin-positive area was reduced in 4- and 12-week-old chow-fed jIL-1Ra KO mice.
mice relative to littermate controls (Figure 5B). We next grouped the islets into four size classes and observed that, at 4 weeks of age, more than 50% of the islets were in the smallest size group 1 (≤1,000 μm²), with a decline in 12-week-old mice (Figures 5C and 5D). A comparison of WT and KO mice revealed a larger proportion of the small islet size group 1 in IL-1Ra KO mice than in WT mice, along with a lower proportion of islets in the size category 2 (1,000 μm² to 5,000 μm²; Figures 5C and 5D). 12-week-old HFD-fed mice had a similar islet size and similar area distributions compared to chow-fed mice of the same age (Figures 5E and 5F). At 25 weeks of age, the islet area of HFD-fed WT and IL-1Ra KO mice was similarly increased (Figure 5G). However, there was still a higher proportion of small islets (<1,000 μm²) and fewer large islets (size group, 3; 5,000–15,000 μm²) in IL-1Ra KO mice than in controls (Figure 5G). Altogether, this shows that KO of IL1rn in β cells leads to an altered islet morphology, characterized by an increased proportion of small islets, suggesting an inhibition of age- and HFD-induced islet expansion.

**Islets of βIL-1Ra KO Mice Have Reduced β Cell Proliferation**

Islets from IL-1Ra KO mice express an increased ratio of IL-1 agonists (II1b and II1a) to antagonist (II1rn) (Figure 1E; Figure S5A), most likely leading to higher IL-1 activity in β cells. Inhibition of β cell identity genes, stimulation of INOS, cyto- and chemokines, decreased proliferation, and increased apoptosis have been associated with IL-1β-mediated β cell demise in cultured islets [Bendtzen et al., 1986; Böni-Schnetzler et al., 2008; Corbett et al., 1993; Dohnath et al., 2013; Eguchi et al., 2012; Maedler et al., 2002; Nordmann et al., 2017]. To investigate which pathway may be altered in vivo by IL-1Ra KO in β cells, we screened candidate genes for changes in ex vivo isolated islet from chow- or HFD-fed IL-1Ra KO mice relative to respective controls (chow, Figures 6A–6D; HFD, Figures S6B–S5D). There was no difference in expression of Pdx1, proglucagon (Gcg), Glut2 (Slc2a2), or hormone-processing enzymes PC1/3 and PC2 (Pcsk1 and Pcsk2) between genotypes. Ins2 and Foxo1, a key factor in β cell dedifferentiation [Talchai et al., 2012], were slightly increased in islets from

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**Figure 6. Ex Vivo Isolated Islets of βIL-1Ra KO Mice Express Reduced Proliferation Markers**

(A) Endocrine cell gene expression of ex vivo isolated islets from 12-week-old chow-fed βIL-1Ra KO mice and WT littermates (n = 9–12).

(B) Inflammation genes of chow-fed mice (n = 9–12).

(C) Proliferation genes Mki67, Ccn2, Ccnd1, and E2f1 and apoptosis marker Casp3 of chow-fed mice (n = 9–12).

(D) Proliferation genes in cultured islets from chow-fed βIL-1Ra KO and littermate mice (n = 13).

( E) Triple-fluorescence staining of dispersed islets. Blue indicates DAPI+; green indicates insulin+ (ins+); pink indicates KI67+ and DAPI+ (arrows).

(F) Percent KI67+ and ins+ double-positive nuclei per total ins+ cells in dispersed islets of 4-week-old chow-fed mice (for WT, 4,965 ins+ cells from 4 mice were scored; for KO, 6,614 ins+ cells from 4 mice were scored).

(G) Percent KI67+ and ins+ double-positive nuclei per total ins+ cells in dispersed islets of 13-week-old chow-fed mice (for WT, 4,581 ins+ cells from 8 mice were scored; for KO, 4,120 ins+ cells from 7 mice were scored).

(H) KI67+ and ins+ positive nuclei per islet from pancreas sections of 4-week-old chow-fed mice, IL-1Ra KO and littermate mice (n = 13).

(I) KI67+ and ins+ nuclei per islet area (WT: n = 1,401 islets from 7 mice; KO: n = 1,731 islets from 7 mice).

J indicates the number of biological replicates for gene expression data from 3 separate islet isolations and the number of islets in KI67 histology stainings. Error bars represent SEM. **p < 0.01; ***p < 0.001. See also Figure S5.
chow-fed βIL-1Ra KO mice and unchanged in islets from HFD-fed βIL-1Ra KO mice (Figure 6A; Figure S5B). Pan-macrophage marker Emr1 (F4/80) was not changed between genotypes. Proinflammatory Cxcl1 and iIl6 gene expression levels were decreased in islets isolated from chow-fed βIL-1Ra KO mice compared to islets from WT mice, whereas in islets from HFD-fed mice there was no difference in expression between genotypes (Figure 6B; Figure S5C). Nos2 (encoding iNOS) was decreased in islets from chow- and HFD-fed βIL-1Ra KO mice. Reduced expression of proinflammatory markers in islets of chow-fed βIL-1Ra KO mice may be a consequence of the altered islet morphology observed in βIL-1Ra KO mice. Indeed, a pool of small islets from normal C57BL6/N mice expressed lower proinflammatory and immune cell markers than a pool of large islets (Figure S5E). Interestingly, the cell-cycle genes Mki67 (Ki67), Ccna2 (cyclin A2), and Ccnd1 (cyclin D1) (Figure 6D; Figure S5D) and the key cell-cycle regulator E2f1 were suppressed in ex vivo and in cultured islets from βIL-1Ra KO mice (Figures 6C and 6D). The apoptosis marker Casp3 was not increased in islets from chow-fed βIL-1Ra KO mice and slightly decreased in islets from HFD-fed βIL-1Ra KO mice (Figure S5D). Proliferation and endocrine genes also depended on size of the islets (Figures S5F and S5G).

We next evaluated β cell proliferation using Ki67 as a marker and quantified the number of Ki67+ nuclei among insulin+ cells (Ki67+/ins+/DAPI+) in dispersed islets from WT and βIL-1Ra KO mice (illustration in Figure 6E). Islet cells from 4-week-old βIL-1Ra KO mice had significantly decreased numbers of Ki67+/ins+ nuclei relative to control cells (Figure 6F). At 13 weeks of age, β cell proliferation had strongly declined, and no difference between genotypes was measurable (Figure 6G). Confirming data were obtained by staining of pancreas sections (representative staining is in Figure S5H). At 4 weeks of age, βIL-1Ra KO mice had significantly fewer Ki67+/ins+ nuclei per islet (Figure 6H) and per islet area (Figure 6I) compared to controls. Finally, we could not observe an increase in apoptotic β cells (as determined by TUNEL staining) in βIL-1Ra KO mice, but due to the scarcity of events (1 to 3 TUNEL+ cells per pancreas section), accurate quantification was not possible. To conclude, lack of islet local IL-1Ra neither increased inflammation markers nor inhibited β cell identity gene expression, but it reduced proliferation gene expression and β cell proliferation.

**IL-1β Impairs β Cells Partly via E2F1**

The only known biological function of IL-1Ra is the competitive inhibition of IL-1 signaling. To examine whether IL-1β, indeed, inhibits islet proliferation gene expression, we treated cultured islets from C57BL6/N mice with or without IL-1β for 24 hr. IL-1β treatment did not affect expression of Il1r1, while Il1m was increased, and Mki67, Ccna2, Ccnd1, and E2f1 were strongly reduced (Figure 7A). Expression levels of Ins2 and Pdx1 were suppressed by IL-1β, and proinflammatory genes strongly increased (Figures S6A and S6B). The transcription factor E2F1 not only is a key regulator of cell-cycle genes (Wu et al., 2001) but also targets the ATP-sensitive inward rectifier potassium channel Kir6.2, which regulates insulin secretion in β cells (Annicotte et al., 2008; Fajas et al., 2004; Grouwels et al., 2010). To examine whether E2F1 plays a role in IL-1β-mediated suppression of proliferation genes and impairment of β cell function, we overexpressed mouse E2F1 using adenoviruses expressing either E2F1 and GFP (Ad E2F1) or GFP only (Ad empty) (Figure 7B; Figure S6C). E2F1 overexpression was similar with or without IL-1β (Figure 7B). Ad E2F1 ameliorated IL-1β-mediated inhibition of Mki67 and Kir6.2 expression (Figures 7C and 7D; Figures S7E and S7F), while there was no rescue of Ccnd1 and Ccnar2 or a significant change of Il1m expression by E2F1 (Figures S7A–S7D and S7G). Next, we measured GSIS in islets infected with Ad empty or Ad E2F1. IL-1β treatment decreased GSIS, while E2F1 overexpression partly rescued IL-1β-mediated inhibition of GSIS (Figure 7E). Islets from βIL-1Ra KO mice expressed less Kir6.2 compared to WT islets, and in vitro IL-1β treatment led to a further decrease in islets from both genotypes (Figure 7F). Most importantly, E2F1 overexpression rescued the impaired insulin secretion observed in islets isolated from βIL-1Ra KO mice relative to controls (Figures 7G and 7H; Figure S7H).

Altogether, IL-1β repressed the expression of Mki67 and of the key channel protein Kir6.2, partly via E2F1. Further, E2F1 overexpression rescued glucose-stimulated insulin secretion in islets from βIL-1Ra KO mice. This suggests that IL-1Ra protects β cells from IL-1-mediated inhibition of insulin secretion via the E2F1-Kir6.2 pathway.

**DISCUSSION**

In the present study, we demonstrate that β cell-derived IL-1Ra is part of an islet local IL-1 defense system and plays an important role for β cell replication and insulin secretion. KO of IL-1Ra in β cells of normal and obese mice led to diminished glucose-stimulated insulin secretion in vivo and in vitro, to impaired glucose tolerance in obese mice, to an increased proportion of smaller islets, and to reduced β cell proliferation. Further, we identified a link between IL-1β-mediated inhibition of proliferation genes; β cell function; and the transcription factor E2F1, which regulates expression of Kir6.2, the potassium channel subunit involved in insulin secretion.

The high IL-1R1 expression level on islet β cells (Benner et al., 2014; Böni-Schnetzler et al., 2009) and the known adverse effects of IL-1β on β cell function and survival (Bendtzen et al., 1986; Corbett and McDaniell, 1995; Maedler et al., 2002) hint at the need for counterregulatory mechanisms within islets. By analysis of fluorescence-activated cell sorting (FACS)-isolated islet cells and deletion of IL-1Ra in β cells, we identified β cells as the main source of functional IL-1Ra in mouse islets. IL-1Ra was previously also localized to human β cells using electron microscopy staining (Maedler et al., 2004). βIL-1Ra KO mice displayed a changed balance of IL-1 agonists to antagonist in favor of the agonists in islets and, thus, may serve as an in vivo model for chronic, unopposed IL-1 signaling within islets. Further, since IL-1Ra is diminished in islets of subjects with T2D along with increased IL-1β, the βIL-1Ra KO mouse mimics aspects of islet pathology in human T2D.

To avoid Cre model artifacts, we used a RIPcre mouse without mini growth hormone gene insertion and which does not have its own phenotype (Alejandro et al., 2011; Girard et al., 2009; Herrera, 2000; Vetterli et al., 2012) and performed Cre-only control...
experiments with littermate RIPcre+/− and WT RIPcre+/− mice fed a chow diet or an HFD. Further, we did not detect Il1rn expression in brain subregions that could be targeted by the RIP promoter. To limit phenotypic variability, we used for all expression in brain subregions that could be targeted by the RIP promoter. To limit phenotypic variability, we used for all experiments wild-type littermate mice infected with Ad empty or Ad E2F1 (Ad E2F1) and treated with or without 1 ng/mL IL-1β for 24 hr (n = 6–7).

**Figure 7. IL-1β-Mediated Impairment of β Cell Function Is Partly Rescued by E2F1**

(A) Gene expression in islets isolated from C57BL/6N mice and cultured on ECM-coated dishes for 2 days and treated for the following 24 hr with or without 1 ng/mL mouse recombinant IL-1β (n = 13–16).
(B) Overexpression of E2F1 in isolated islets transduced with empty adenovirus (Ad empty) or mouse E2F1 (Ad E2F1) and treated with or without 1 ng/mL IL-1β for 24 hr (n = 6–7).
(C and D) Mean percent inhibition of (C) Mki67 and (D) Kir6.2 gene expression by IL-1β relative to untreated islets transduced with Ad empty or Ad E2F1 (n = 5 experiments, paired t test).

(E) GSIS of islets from C57BL/6N mice infected with Ad empty or Ad E2F1 and treated with or without 1 ng/mL IL-1β for 24 hr (n = 24, 4 isolations) and expressed as percentage of insulin secretion at 2.8 mM glucose.
(F) Kir6.2 expression in islets from IL-1Ra KO and littermate WT mice cultured for 2 days and treated for 24 hr with or without 1 ng/mL IL-1β (n = 14–15).
(G) GSIS of islets from IL-1Ra KO and littermate mice infected with Ad empty or Ad E2F1 (n = 16–18).
(H) 24-hr insulin release in βIL-1RA KO and littermate mice infected with Ad empty or Ad E2F1 (n = 16–18).

If not otherwise indicated, n indicates the number of biological replicates from 3 islet isolations, and error bars represent SEM. *p < 0.05; **p < 0.01; ***p < 0.001. See also Figures S6 and S7.

The main consequence of IL-1Ra KO in islet β cells was reduced insulin secretion and an increased susceptibility to diabetes induction upon treatment with a single low dose of stz. This may stem from a combination of deficient GSIS, decreased islet area with more small islets, and reduced β cell proliferation. Effects on islet size were also reported in a genetic model where diminished IL-1β, due to the lack of the inflammasome component NLRP3, led to an increased islet area "Youm et al., 2011." This points to a critical role of IL-1 family cytokines in islet development and adaptation to obesity.

The impaired islet phenotype upon lack of β cell-derived IL-1Ra appears to be at odds with our recent observation that postprandial macrophage-derived IL-1β acts as an insulin secretagogue in a rodent fasting-refeeding model "Dror et al., 2017." A major difference between this model and our β cell-specific IL-1Ra KO model is the acute versus long-term unopposed action of IL-1β. The chronic model resulted in altered islet morphology, reduced protection from stz, reduced expression of proliferation gene, and impaired β cell function. Further, chronic stimulation of insulin secretion may exhaust β cells. Such dual behavior characterizes inflammatory processes in general; it is often beneficial in the short term, restoring homeostasis, but deleterious in a chronic setting.

A candidate gene approach to investigate the underlying mechanism for the islet phenotype of βIL-1RA KO mice revealed no major changes in endocrine-cell-specific genes or induction
of a broad inflammatory response in KO mice but revealed diminished expression of proliferation genes along with reduced Ki67+ staining in β cells. Furthermore, we observed that IL-1β treatment of islets from normal mice inhibited the expression of cyclin kinase genes, Mki67 and E2f1. While Ki67 (a product of Mki67) is one of the most frequently used proliferation markers, its critical biological function in the dispersion of mitotic chromosomes was only recently uncovered (Cuylen et al., 2016). The E2F1 transcription factor is a general key cell-cycle regulator (Wu et al., 2001), and in β cells, it regulates insulin secretion by directly targeting the promoter of potassium channel subunit Kir6.2 (Annicotte et al., 2009; Grouvels et al., 2010). Here, we uncovered a link between E2F1 and the inhibition of GSIS and proliferation gene expression by IL-1β. Overexpression of E2F1 partly prevented the inhibition of Mki67 and Kir6.2 expression by IL-1β and partially reversed the negative effects of IL-1β on GSIS. Importantly, E2F1 overexpression rescued the reduced GSIS of islets with β cell-specific IL-1β KO. Altogether, this suggests that the observed reduction of proliferation genes in mice lacking IL-1Ra in β cells impacts β cell proliferation and function, partly via the E2F1-Kir6.2 pathway. Therefore, part of the beneficial effect of IL-1 antagonism in the treatment of T2D may occur via targeting IL-1 effects on proliferation genes and thereby may improve β cell turnover and function.

**EXPERIMENTAL PROCEDURES**

**Experimental Animals**

To induce ablation of IL-1Ra selectively in pancreatic β cells, C57BL/6 RIP Cre<sup>+</sup> (Herrera, 2000) were crossed with C57BL/6 mice homozygous for a floxed exon 2 Itim gene (Lamacchia et al., 2010) to obtain RIP Cre<sup>+/−</sup> Itim<sup>fl/fl</sup> mice (Il1rn<sup>-/-</sup> RipCre<sup>−/−</sup>) and littermate RIP Cre<sup>−/−</sup> Itim<sup>fl/fl</sup> control mice. Myeloid-specific IL-1Ra KO mice were produced by crossing C57BL/6 Lyz2Cre<sup>−/−</sup> mice (Claussen et al., 1999) with Itim<sup>fl/fl</sup> mice to obtain Lyz2 Cre<sup>−/−</sup> Itim<sup>fl/fl</sup> (myeloid-L1Ra KO) and littermate Lyz2 Cre<sup>-/−</sup> Itim<sup>fl/fl</sup> control mice. GluDTR mice with β cell ablation were described previously (Thorel et al., 2011; Traub et al., 2017). If not indicated otherwise, islet isolations were done with 9- to 12-week-old male C57BL6/6N mice from our own breeding. For all experiments, littermate control mice and two or more separate cohorts were used. High-fat-diet (D12321, Research Diets, New Brunswick, NJ, USA) feeding was started at the age of 4 weeks, and for the HFD-single-dose zst model (Mu et al., 2006), HFD-feeding was started at 5 weeks. For the latter model, 8-week-old mice were treated with a single intraperitoneal injection of stz (Sigma-Aldrich) at 130 mg/kg (dissolved in citrate buffer), and blood glucose was determined after 6 hr of fasting at 1, 2, and 3 weeks after stz injection. All animal experiments were done according to the Swiss veterinary law and institutional guidelines and upon approval by Swiss authorities.

**Glucose and Insulin Tolerance Tests**

For ipGTTs, male mice at indicated ages were fasted for 6 hr in the morning and intraperitoneally injected with 2 g/kg of glucose. Blood glucose levels were measured using a glucometer (Freestyle; Abbott Diabetes Care, Alameda, CA, USA), and blood was collected at the indicated time points for later plasma insulin determination. For ipGTts, mice were fasted 3 hr in the morning before administration of 1 U/kg insulin (Novo Nordisk, Bagsvaerd, Denmark) followed by blood glucose measurements.

**Mouse Islet Isolation**

Mouse pancreatic islets were isolated from male mice as described previously (Dalmas et al., 2017). Islets were handpicked, and 4 hr after isolation, islets were collected for RNA extractions (ex vivo isolated islets) or cultured on extra-cellular matrix (ECM)-coated 24-well plates (Novamed, Jerusalem, Israel) in mouse islet media (RPMI-1640 containing 11.1 mM glucose, 100 U/mL peni-cillin, 100 µg/mL streptomycin, 2 mM glutamax, 50 µg/mL gentamycin, 0.25 µg/mL amphotericin B and 1% fetal calf serum (FCS); Gibco, ThermoFisher Scientific). For the comparison of large and small islets, islets pooled from 10 mice were handpicked and arbitrarily grouped into small, medium, and large islets by experienced personnel. The small and large fractions were cultured and compared.

**FACS of Mouse Islet Cells**

Handpicked islets from 6 to 12 male C57BL/6 mice per sort were pooled, incubated overnight at 37 °C, and dispensed with a 0.0125% tropin-EDTA (Gibco) for 5 min at 37 °C, washed with cold FACS buffer (PBS with 0.5% BSA and 5 mM EDTA), and resuspended in FACS buffer. After 15-min incubation with an Fc blocker (93; eBioscience), islet cells were stained with APC anti-CD31 (R&D; eBioscience) and PEcy7 anti-CD45 (30-F11; eBioscience) or isotypes for 30 min at 4 °C. DAPI+ and doublet cells were excluded from all analysis. Islet cells were sorted with a FACS ARIA III cell sorter (BD Biosciences) using FACS Diva software (BD Biosciences) and used for total RNA extraction (PuraLink RNA Micro Kit, Invitrogen).

**Peritoneal Macrophage Isolation**

Peritoneal macrophages were isolated by perfusing the peritoneum with 5 mL PBS containing 1% FCS. The lavage was filtered through a 70-µm cell strainer (BD Biosciences), cells were pelleted, and 2 × 10<sup>6</sup> cells per well were cultured in 48-well plates (TPP) in RPMI containing 10% FBS for 24 hr. The non-adherent cells were removed, and fresh culture medium was added. 16 hr later, supernatants were collected, centrifuged (at 4 °C, 2000 x g) for 5 min, and stored at −80 °C.

**GSIS Assay**

25 islets per well were seeded in 24-well ECM-coated plates in quintuplicates for 40 hr in RPMI-1640 containing mouse islet medium. Islets were pre-incubated for 30 min in modified Krebs-Ringer bicarbonate buffer (KRb; 115 mM NaCl, 4.7 mM KCl, 2.6 mM CaCl<sub>2</sub> 2H<sub>2</sub>O, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub> 7H<sub>2</sub>O, 10 mM HEPES, 0.5% BSA [pH 7.4]) containing 2.8 mM glucose. KRb was then replaced by KRb 2.8 mM glucose for 1 hr (basal insulin release), followed by 1 hr in KRb (16.7 mM glucose) (stimulated insulin release) with or without 100 nM GLP-1(7-36) amide ( Bachem, Bubendorf, Switzerland). Insulin content was harvested by extraction with 0.18 N HCl in 70% ECH (Insulin concentrations were determined using a mouse insulin ultrasensitive mouse/rat insulin kit (Meso Scale Discovery, Rockville, MD, USA).

**IL-1β Stimulation of Cultured Mouse Islets**

80 islets per well were allowed to attach for 2 days on 24-well ECM-coated plates. Medium with or without 1 ng/mL of mouse recombinant IL-1β (Bio-Techne, Zug, Switzerland) was added for 24 hr. Cells were washed 3× with PBS and harvested for RNA extraction.

**Transduction of Islets with E2F1-GFP Adenoviruses**

Islets were infected from either C57BL6/N or Il1rn<sup>−/-</sup> KO and littermate control mice as described earlier and immediately infected with adenoviruses expressing mouse E2F1 and GFP (Ad-GFP-m-E2F1 and AD-257649, Vector Bioslabs) or an empty virus (AdGFP, Vector Bioslabs) with an MOI of 50. After 24 hr, virus containing medium was removed, and islets were distributed into 24-well ECM-coated plates (20 islets per well for GSIS and 80 islets per well for RNA extraction) and cultured for 72 hr in mouse islet medium. Insulin release was visualized by inspection of GFP in live cells. Islets were then treated with or without 1 ng/mL IL-1β for 24 hr. Medium was collected to determine the 24-hr insulin release, and islets were subjected to GSIS (as described earlier) or RNA extraction.

**RNA Extraction and qPCR Using TaqMan or SYBR Green Assays**

Total RNA of mouse islets or organs was extracted using the NucleoSpin RNA II Kit (Machery Nagel, Düren, Germany) or the RNAeasy Lipid Tissue Mini Kit (Qiagen) for fat tissue. cDNA was prepared with random hexamers and Superscript II (Invitrogen) according to the supplier’s instructions. For qPCR, TaqMan assays (Applied Biosystems) or SYBR Green assays with GoTaq Polymerase (Promega) were used with the Applied Biosystems 7500 Real-Time
DECLARATION OF INTERESTS

M.Y.D. is listed as the inventor on a patent filed in 2003 for the use of an IL-1 receptor antagonist for the treatment of or prophylaxis for type 2 diabetes (WO2004002512 A1).

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