Neutralizing Interleukin-1β (IL-1β) Induces β-Cell Survival by Maintaining PDX1 Protein Nuclear Localization

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The transcription factor PDX1 plays a critical role during β-cell development and in glucose-induced insulin gene transcription in adult β-cells. Acute glucose exposure leads to translocalization of PDX1 to the nucleoplasm, whereas under conditions of oxidative stress, PDX1 shuttles from the nucleus to the cytosol. Here we show that cytosolic PDX1 expression correlated with β-cell failure in diabetes. In isolated islets from patients with type 2 diabetes and from diabetic mice, we found opposite regulation of insulin and PDX1 mRNA; insulin was decreased in diabetes, but PDX1 was increased. This suggests that elevated PDX1 mRNA levels may be insufficient for insulin production in islets from patients with T2DM. In contrast, overexpression of either IL-1 receptor antagonist or shuttling-defective PDX1 restored β-cell survival and function and PDX1 nuclear localization that nuclear localization of PDX1 is essential for a functional β-cell and provides a novel mechanism of the protective effect of IL-1 receptor antagonist on β-cell survival.

New therapies for diabetes that lead to protection of the insulin-producing β-cell are urgently needed. Only when the β-cell compensates for the higher insulin demand during insulin resistance can normoglycemia be maintained. Recent studies suggest that the low grade inflammation in type 2 diabetes mellitus (T2DM) contributes to β-cell failure (1). Especially, interleukin-1β (IL-1β), whose secretion has been postulated from intraislet macrophages (2) and from β-cells themselves when exposed to double-stranded RNA (3), to elevated glucose concentrations (4–6), or to free fatty acids (7), initiates β-cell destruction.

The receptor for IL-1β, IL-1R1, is highly expressed in the β-cell, and more than a hundred expression of IL-1R1 mRNA was observed in islets and in total pancreas, which is significantly higher than in β-cells (7). This may explain the sensitivity of the β-cell to IL-1β. A recent study demonstrated that IL-1β secretion involves caspase 1 activation mediated by the NALP3 inflammasome. The inflammation (e.g. chronic or endogenous stress) increases IL-1β expression in insulins (1, 11). Glucose-induced IL-1β secretion is prevented in NALP3−/− mice, indicating that IL-1β secretion is activated through glucose-induced reactive oxygen species production and oxidative stress (6). The thioredoxin-interacting protein, which has been linked to insulin resistance (12), functions as an activator of NALP3. In line with these data, another recent study shows that thioredoxin-interacting protein is highly increased by elevated glucose in β-cells and that thioredoxin-interacting protein-deficient islets are protected against glucose toxicity (13). Although glucose-induced IL-1β production in the β-cell was not observed in all studies (14), IL-1β expression in islets from patients with T2DM has been confirmed (5, 15), supporting the concept of blocking IL-1β signals as a target for diabetes treatment. Indeed, daily injection of IL-1Ra in mice fed a high fat diet (HFD) improves glycemia, glucose-stimulated insulin secretion, and survival (16); reduces hyperglycemia; and reverses the islet inflammatory phenotype in the GK rat (17). Treatment with an IL-1β antibody also improves glycemic control in diet-induced obesity in mice (18, 19). Results from a clinical study in patients with T2DM showed that IL-1Ra improves glycemic control and β-cell function (20–22). Blocking IL-1β signals reduces expression of inflammatory marker in fat tissue and in islets (16). None of the studies have studied the mechanisms of the protective effect of IL-1Ra at the level of β-cell gene regulation. IL-1β affects expression of the transcription factor PDX1 (pancreatic duodenal homeobox-1, previously called IPF1, IDX1, STFI, or IUF1) (23), a key factor in pancreas development and function. Reduced PDX1 expression levels negatively regulates insulin expression and secretion and predispose islets to apoptosis (24–26). Its homozygous mutations result in...
pancreas agenesis associated with neonatal diabetes (27, 28). PDX1 directly binds to the promoter and controls expression of important β-cell genes, which are vital for β-cell function, such as insulin, Glut2, and glucokinase (27). PDX1 deficiency contributes to impaired proliferation and enhanced apoptosis via transcriptional mechanisms in models of type 2 diabetes, such as Psammomys obesus and the leptin receptor-deficient (Lepr<sup>db/db</sup>) db/db mice (29). Overexpression of PDX1 restores β-cell mass and function, thereby preventing the onset of diabetes in IRS2 knock-out mice, showing the critical role of PDX1 in β-cell survival (30).

Complicated signaling networks control PDX1 regulation, and nucleo-cytoplasmic shuttling plays a major role in the regulation of PDX1 function (31, 32). Well characterized PDX1 nuclear import signal and nuclear export signal (NES) suggest that PDX1 might be regulated at the level of cellular localization (31, 33). Post-translational modification of proteins is the most abundant form of cellular regulation, affecting many cellular signal pathways, including metabolism, growth, differentiation, and apoptosis. In response to acute elevation of glucose and survival factors, such as insulin, PDX1 is phosphorylated and translocates to the nucleus (34, 35). By contrast, stimuli associated with diabetes, such as oxidative stress (31) and free fatty acids (36), cause nuclear exclusion of PDX1 (36). This suggests that cytoplasmic accumulation may represent a mechanism to reduce the nuclear action of PDX1 under pathologic conditions, rather than to promote a specific cytoplasmic function.

We have shown previously that a diet rich in sucrose (“Surwit”; HFD) induces impaired glucose tolerance after 4 weeks of feeding, impaired glycosylation, and hyperglycemia after 12 weeks. These changes in glycemia were accompanied by fluctuations in β-cell mass. Despite the known increase in β-cell apoptosis, the primary increase in β-cell mass up to 4 weeks and subsequent reduction in the HFD-treated mice. IL-1β antagonism by anti-IL-1β antibody treatment, IL-1Ra injections, or endogenous overexpression restored normoglycemia and β-cell function and survival (16, 19, 37), but also changes in the inflammatory state of the fat tissue were involved in the protective effects of IL-1β antagonism. Therefore, we asked how IL-1β signals regulate gene transcription in the β-cell. We tested the effect of IL-1Ra on regulating glucose homeostasis in another animal model, the obese diabetic leptin receptor-deficient Lepr<sup>db/db</sup> mice (db/db). Commonly, we detected that IL-1Ra was able to maintain the cellular localization of PDX1. A diabetic milieu in vitro as well as T2DM in vivo induced a switch of PDX1 from the nucleus to the cytosol, which was accompanied by a loss in β-cell mass and function.

Whether PDX1 is altered in its localization in β-cells during the progression to diabetes and whether these changes may affect β-cell function in different levels was previously unknown. Because PDX1 regulates insulin and specific β-cell genes, altered localization may contribute to β-cell death and loss of function. In the short term, this stressful response may be tolerated, but under chronic situations in T2DM, prolonged stress conditions ultimately affect β-cell survival.

**EXPERIMENTAL PROCEDURES**

**Animals**—Transgenic mice overexpressing IL-1Ra (IL-1Ra-OE) were kindly provided by Dr. Emmet Hirsch (Northwestern University, Evanston, IL) (38). Beginning at 5 weeks of age and continuing for 12 weeks, transgenic animals as well as their wild type littermates were fed a normal diet (WT ND and IL-1Ra-ND) or a high fat/high sucrose diet (WT HFD and IL-1Ra-HFD, Surwit (Research Diets, New Brunswick, NJ), containing 58, 26, and 16% calories from fat, carbohydrate, and protein, respectively (39)) or were treated daily with IL-1Ra from the starting day of the diets (ND+IL-1Ra and HFD+IL-1Ra; Kineret<sup>®</sup>, Amgen (Thousand Oaks, CA), intraperitoneal injection of 10 mg/kg body weight) as described previously (16). Four independent experiments with a total of 16 mice (4 mice/cage) in each group were performed, respectively.

Heterozygous leptin receptor deficient mice on the C57BLKS/J background (Lepr<sup>db/+</sup>, db/) were purchased from Jackson Laboratory. By cross-breeding these mice to C57BL/ 6J-IL-1Ra-overexpressing mice (OE), we obtained diabetic Lepr<sup>db/db</sup> (db/db) and non-diabetic Lepr<sup>db/+</sup> (WT) with endogenous overexpression of IL-1Ra (OE-db/db and OE) as well as their wild type littermates without IL-1Ra as appropriate negative controls. Animals were housed in a temperature-controlled room with a 12-h light/dark cycle and were allowed free access to food and water. All experiments, littermates from the F2 breeding were used. All protocols were approved by the Animal Care and Use Committee at the National Institutes of Health animal care and use section 8 of the German animal protection law.

**Islet Isolation and Culture**—Islets from all groups were isolated as described previously (40). Human islets were isolated from eight pancreases of healthy organ donors and from eight with T2DM at the University of Illinois (Chicago, IL), Lille University, or Pisa University as described previously (41) and cultured in CMRL-1066 medium as described previously (42). Islet purity was greater than 95% as judged by dithizone staining (if this degree of purity was not achieved by routine isolation, islets were hand-picked). Islets were exposed to 5.5, 11.1, 22.2, or 33.3 mM glucose or 5.5 mM plus 2 ng/ml recombinant human IL-1β (R&D Systems, Minneapolis, MN), with or without 500 ng/ml recombinant human IL-1Ra (R&D Systems) or 10 μM JNKi (kindly provided by Xigen S.A., Lausanne, Switzerland) for 72 h.

**Transfections**—At 2 days postisolation and culture on extracellular matrix-coated dishes, isolated islets were exposed to transfection Ca<sup>2+</sup>-KRH medium (4.74 mM KCl, 1.19 mM KH<sub>2</sub>PO<sub>4</sub>, 1.19 mM MgCl<sub>2</sub>6H<sub>2</sub>O, 119 mM NaCl, 2.54 mM CaCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, 10 mM HEPES). After 1 h of incubation, lipoplexes (Lipofectamine2000 (Invitrogen)/DNA ratio 2.5:1, 5 μg of DNA/100 islets) were added to transfect the islets. After an additional 6 h of incubation, CMRL 1066 medium containing 20% FCS and l-glutamine was added to the transfected islets. Efficient transfection was evaluated based on enhanced GFP-positive cells, which resulted in 60% transfection efficiency in...
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β-cells through the whole islets, analyzed by fluorescence and confocal microscopy.

**Glucose-stimulated Insulin Secretion and Insulin Content**—Islets used to perform glucose-stimulated insulin secretion experiments were kept in culture medium on matrix-coated plates derived from bovine corneal endothelial cells (Novamed Ltd., Jerusalem, Israel) for 4 days, allowing the cells to attach to the dishes and spread (43). These experimental conditions allowed direct comparison with our previous studies in human and mouse islets pretreated with IL-1Ra in vitro (4, 16). Thereafter, islets were washed with PBS and extracted with HCl (0.18 N) in 70% ethanol for 24 h at 4 °C. The acid-ethanol extracts were collected for determination of insulin content. Insulin was determined using a human insulin ELISA kit (Alpco).

**β-Cell Mass and Histochemical Analyses**—Pancreatic sections from seven healthy controls and from seven patients with T2DM were obtained from the National Disease Research Interchange (NDRI) and from Kangnam St. Mary’s Hospital (Seoul, Korea) as described (44); approval for the studies was granted by the Kangnam St. Mary’s Hospital and by the Ethical Commission of Bremen University.

Mouse pancreases and sections from isolated islets were obtained as described (16). To determine β-cell mass, 10 sections (spanning the width of the pancreas) were analyzed as described (16). For detection of β-cell apoptosis, insulin TUNEL staining were performed (In Situ Cell Death Detection Kit, TMR Red; Roche Applied Science) (16). Enzymatic digestion studies, after dehydration, sections were blocked in blocking buffer containing 0.2% Tween 20, 3% IgG-free BSA, and 0.5% Triton X-100. Subsequently, all sections were double-stained for insulin and mouse PDX1 (kindly provided by Christopher Wright, Vanderbilt University Medical Center, Nashville, TN). Sections were incubated with rabbit anti-PDX1 (Seoul, Korea) as described (44); approval for the studies was granted by the Kangnam St. Mary’s Hospital and by the Ethical Commission of Bremen University.

**RESULTS**

**High Fat Diet Induces PDX1 Translocalization to the Cytosol and Is Prevented by IL-1Ra**—To look into detailed mechanisms of the loss of function of PDX1 in T2DM, isolated RNA from islets and IL-1Ra treatment and observed its transcription factor localization in islets from HFD animals was compared. Results (Fig. 1A), PDX1 levels were increased 2.8-fold in HFD-fed (Fig. 1B). Also, insulin content from isolated islets markedly decreased in the HFD-fed mice (Fig. 1B), whereas changes did not occur in IL-1Ra-treated animals. Since it has been reported that PDX1 activity is primarily regulated by its subcellular localization (45, 46), we stained pancreatic tissue sections for PDX1 and insulin. Sections from both normal diet groups showed PDX1 immunoreactivity predominantly (95 and 94%) in the nucleus of β-cells (Fig. 1D, b and d). In contrast, the majority (67%) of the β-cells under the high fat diet expressed PDX1 in the cytoplasm (Fig. 1C, f). IL-1Ra treatment inhibited this translocalization to the cytoplasm (30% cytosolic PDX1), and the islets showed more prominent staining in the nucleus (54% nuclear PDX1; Fig. 1D, h).

Western blot analysis from nuclear and cytosolic fractions from isolated islets from WT and IL-1Ra-OE mice fed for 12 weeks with normal chow or HFD confirmed the decrease in nuclear together with the increase in cytosolic PDX1 in islets under the HFD condition, which was prevented by IL-1Ra overexpression (Fig. 1E).

**PDX1 Translocalization to the Cytosol in db/db Mice Is Prevented by IL-1Ra Overexpression**—To investigate whether PDX1 shuttling also occurs in another mouse model, we examined the effects of IL-1Ra in Lepr^db/db^ mice (db/db). By cross-breeding mice, which endogenously overexpress IL-1Ra (OE) to C57BLKS-Lepr^db/db^, we used four groups of mice in the experiments: IL-1Ra-OE-Lepr^db/db^ (db/dbIL-1Ra) and heterozygous Lepr^db/+^, IL-1Ra-OE (db/db IL-1Ra) as well as their littermates without IL-1Ra-overexpression, Lepr^db/db^ (db/db) and Lepr^db/+^ (db/+).

Heterozygous db/+ and db/db IL-1Ra mice showed clear nuclear PDX1 localization independent of their age (84 and 87% for cytosolic and anti-PARP or anti-histone H3 for nuclear extracts.

**Western Blot Analysis**—At the end of the incubation periods, islets were washed in ice-cold PBS and lysed as described (42). Membranes were incubated with rabbit anti-phospho-JNK (Thr183/Tyr185), rabbit anti-histone H3, rabbit anti-PARP, rabbit anti-tubulin, rabbit anti-GAPDH, and rabbit anti-β-actin (Cell Signaling Technology) antibodies, followed by horseradish peroxidase-linked anti-rabbit IgG. Density of the bands was analyzed using DocIT® LS image acquisition 6.6a (UVP BioImaging Systems, Upland, CA).

**Statistical Analysis**—Samples were evaluated in a randomized manner by a single investigator, who was blinded to the treatment conditions. Data are presented as means ± S.E. and were analyzed by paired, Student’s t test or by analysis of variance with a Bonferroni correction for multiple group comparisons.
nuclear PDX1; Fig. 2A, a–f). In contrast, 6-week-old db/db mice showed a clear switch of PDX1 from the nucleus into the cytosol (Fig. 2A, g–i). Littermates, which overexpressed IL-1Ra, were clearly protected from such localization change (Fig. 2A, j–l). The switch in localization was predominant in the 6-week-old mice. With the progression of diabetes in 10-week-old mice, loss of PDX1 expression was observed (only 15% PDX1/H11001 cells; Fig. 2A, m–o). IL-1Ra-OE mice partly restored overall PDX1 expression and its nuclear localization (51% H9252 cells had nuclear and 12% cytosolic PDX1; Fig. 2A, p–r). The localization switch was again confirmed by Western blot analysis in 6-week-old mice. Although heterozygous db/+ IL-1Ra and db/+ mice showed normal glucose tolerance, db/db mice revealed increased fasting glucose levels and impaired glucose tolerance. IL-1Ra overexpression in the db/+ IL-1Ra mice could partially but significantly restore glucose tolerance at all time points during the intraperitoneal glucose tolerance test and decrease fasting glucose levels (supplemental Fig. 1). Glucose-stimulated insulin secretion was completely abolished in the db/db mice but was significantly restored in the db/dbIL-1Ra mice with the significant increase in stimulated insulin secretion and a restoration of the stimulatory index (supplemental Fig. 1, B and C). β-Cell failure seen by the impaired GSIS was also confirmed when we analyzed β-cell mass and survival. β-Cell apoptosis was 4.5-fold.
increased, and β-cell mass was 2.1-fold reduced in the db/db mice. In contrast, β-cell apoptosis was reduced, and β-cell mass was partially restored in the db/db IL-1Ra mice (supplemental Fig. 1, D and E).

**PDX1 Is Localized in the Cytosol in T2DM**—To investigate whether our results in the two diabetic mouse models can be translated into human diabetes, we analyzed PDX1 localization and expression in human T2DM (Fig. 3, A–D) and the effect of IL-1Ra on PDX1 localization in human isolated islets (Fig. 3, F) and in the rat β-cell line INS-1E (Fig. 3, G and H). In most β-cells of the control patients investigated in pancreatic sections (Fig. 3, A (a and b) and B) or in sections from isolated islets (Fig. 3, A (e and f) and C), PDX1 was clearly nuclear (Fig. 3A, b and f). In contrast, PDX1 expression was much weaker in islets from patients with T2DM, and no nuclear PDX1 was observed in (Fig. 3, A (d and h), B, and C). Similar opposite effects in insulin and PDX1 mRNA expression as observed in the HFD mouse model could be observed in human diabetes. Whereas insulin mRNA in islets from patients with T2DM was reduced to 40% of the expression in islets isolated from non-diabetic control patients, PDX1 was 2.8-fold increased (Fig. 3D). This is in
Insulin content from diabetic islets was decreased when compared with islets from control patients (Fig. 3E, p < 0.01). Isolated human islets were treated with increasing glucose concentrations (5.5, 11.1, and 33.3 mM glucose) or 5.5 mM glucose plus 2 ng/ml IL-1β for 72 h. As reported before and in our previous studies (4, 48), under conditions of elevated glucose levels or IL-1β treatment, β-cells failed to adequately increase insulin secretion in response to a glucose challenge. As shown in Fig. 3F(b), PDX1 is localized in the nucleus in insulin-positive β-cells at control conditions at 5.5 mM glucose. Co-exposure of islets to IL-1Ra did not change PDX1 localization (Fig. 3G and H). Western blot analysis of PDX1 of glucose-treated (3 days; 11.1–33.3; G) and IL-1β-treated (2–3 days; 2 ng/ml; H) nuclear and cytosolic cell lysates of the β-cell line INS-1E. GAPDH was used as loading control, and purity of fractions for the cytosolic extracts and full-length PARP for the nuclear extracts in the same membrane after stripping was assessed. One representative blot of three experiments is shown. Densitometry analysis of bands normalized to PARP or GAPDH shows the ratio of nuclear and cytosolic PDX1 expression. Because there were no changes, the 2-day results were not analyzed in H.
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Increasing glucose concentrations to 11.1 (not shown) and 33.3 mM (Fig. 3F) or the addition of IL-1β (Fig. 3F) shifted the PDX1 signal to the cytoplasm, resulting in co-localization of PDX1 with insulin (yellow merged color), whereas co-incubation with IL-1Ra protected from such PDX1 shift; PDX1 remained in the nucleus in the IL-1Ra-treated islets (Fig. 3F, j and l). In order to show a β-cell-specific mechanism, such changes were confirmed by Western blot analysis of glucose- and IL-1β-treated cell lysates of the β-cell line INS-1E. Glucose induced a dose-dependent decline in nuclear PDX1 together with an increase in cytosolic PDX1 expression after 72 h (Fig. 3G). 2 ng/ml IL-1β reduced nuclear and induced cytosolic PDX1 expression after 3 days of exposure but not after 2 days, showing a time-dependent IL-1β effect (Fig. 3H).

IL-1Ra Prevents Prolonged Glucose- and IL-1β-induced JNK Activation—PDX1 nuclear export is induced by oxidative stress through a signaling pathway that involves JNK activity (31, 49, 50). Therefore, we investigated whether JNK also mediates glucose- and IL-1β-induced effects on PDX1 localization by first evaluating whether IL-1Ra regulates JNK activation.

In isolated mouse islets, JNK remained in its activated state over a 3-day period in the presence of elevated glucose concentrations (Fig. 4A, left). In islets from mice that endogenously overexpress IL-1Ra (IL-1Ra-OE), glucose induced transient JNK phosphorylation after 1 day of culture, whereas after 3 days, JNK activation decreased (Fig. 4A, right). We found these findings in isolated human islets. Phosphorylation levels were increased by a 3-day culture in the presence of 2 ng/ml IL-1β (Fig. 4B, a–c) compared with cultures at 5.5 mM glucose. JNK activation ameliorated when IL-1Ra was added (Fig. 4B, d–f).

Because we postulated that IL-1Ra leads to PDX1 export, JNK inhibition was included in this hypothesis, we treated human islets with 22.2 mM glucose or 2 ng/ml IL-1β, with or without the addition of 10 μM JNKi, a small peptide that inhibits JNK activity, as described before (51, 52). We again observed glucose- and cytokine-mediated PDX1 translocalization (Fig. 4C, top, a–c). We not only found cells that co-express insulin and PDX1 in the cytoplasm but found that some cells displayed PDX1 in the nuclear periphery, which has been observed previously in cell lines kept at low glucose concentrations (34). JNK inhibition prevented glucose- and IL-1β-induced PDX1 translocalization and kept the transcription factor in the nucleus in most cells, indicating that the JNK pathway is involved in regulating PDX1 localization (Fig. 4C, bottom, d–f).

β-Cell apoptosis and function were analyzed in the same experiments. The switch of nuclear to cytosolic PDX1 was accompanied by increased β-cell apoptosis. 3-day incubation of human islets with elevated glucose (22.2 mM) or 2 ng/ml IL-1β resulted in a 1.8- and 2.1-fold increase in β-cell apoptosis, and glucose-stimulated insulin secretion was impaired by 75 and 52%, respectively. In contrast, 1 h of pre-exposure and prolonged culture of the islets with 10 μM JNKi or 250 ng/ml IL-1Ra restored β-cell survival (Fig. 4D) and function (Fig. 4E and F).

Mutation of the PDX1 Nuclear Export Signal Restores β-Cell Survival and Function—Because we have observed PDX1 nuclear exclusion in a prodiaabetic milieu, we tested whether maintaining PDX1 localization in the nucleus may restore β-cell function and survival.

PDX1 WT plasmid and a mutant PDX1, in which the NES-like sequence was disrupted by substituting alanine for leucine at positions 91 and 93 and linked to GFP as described before (33, 49), were expressed in human islets by transient plasmid lipofectamine-mediated transfection. Plasmids were kindly provided by Dr. Ingo Leibiger. Transfection efficiency was analyzed immunocytochemically by GFP plasmid overexpression, which showed a ~60% transfection efficiency (supplemental Fig. S2). We also evaluated transfection efficiency by Western blot analysis. Linking PDX1 to GFP resulted in an additional band at 70 kDa, which gave an intensive band in the transfected but not in the GFP-transfected islets (Fig. 5D). Under these conditions, we analyzed β-cell apoptosis and function. Overexpression of WT or NES mutant PDX1 did not result in significant changes of β-cell proliferation or function at 5.5 mM glucose. 3-day exposure to 22.2 mM glucose or 2 ng/ml IL-1β resulted in a 1.9- and 1.7-fold increase in β-cell apoptosis and to a 56 and 68% decrease in glucose-stimulated insulin secretion, respectively, compared with GFP-transfected islets. The NES mutant PDX1 improved glucose-stimulated insulin secretion ameliorated PDX1 shuttling (Fig. 5C, top, a–c) as well as nuclear PDX1 for restoration of β-cell function. When PDX1-WT was overexpressed in human islets, IL-1β and glucose induced shuttling of transfected PDX1 to the cytosol (Fig. 5D), which resulted in a clear decrease in the nuclear/cytosolic PDX1 expression ratio (Fig. 5E). In contrast, PDX1-NES overexpression inhibited such PDX1 shuttling in IL-1β- and glucose-treated islets, and the ratio of nuclear/cytosolic PDX1 expression was significantly increased, compared with PDX1-WT (Fig. 5, D and E).

Immunostaining of PDX1-NES and PDX1-WT confirmed Western blot results (Fig. 5F). PDX1 was localized in the nucleus in insulin-positive β-cells in both WT- and PDX1-NES-transfected islets (Fig. 5F, a, b, g, and h). Although PDX1-WT expression was shifted to the cytosol in response to IL-1β and glucose treatment (Fig. 5F, c–f), shuttling-deficient PDX1-NES remained in the nucleus (Fig. 5F, i–l).

DISCUSSION

Human mutations of PDX1 as well as changes in its transactivation are strongly associated with diabetes (53, 54). In the present study, we show that localization of PDX1 correlates with T2DM, β-cell function, and survival. PDX1 shuttling is one of the mechanisms that may explain the dual role of glucose on β-cell function and survival. Although acute elevated glucose induces β-cell proliferation and insulin secretion, chronically elevated glucose concentrations impair β-cell function, induce apoptosis (40), and thus may accelerate diabetes. Upon acute exposure of β-cells to glucose, PDX1 translocates to the nucleus, leading to insulin gene transcription (34). In contrast,
oxidative stress induces PDX1 shuttling to the cytosol (49) and impairs insulin secretion (55). We show here that in diabetic islets or under conditions of chronic hyperglycemia or IL-1β exposure for 3 days in vitro, conditions of impaired β-cell survival and function shifted PDX1 expression to the cytoplasm. These data show the impact of PDX1 localization in regulating β-cell turnover and function.

Whenever it was shifted to the cytosol, PDX1 showed a weaker staining and signal intensity, which suggests the possibility of PDX1 degradation after translocalization. This is also supported by the observation that overexpression of PDX1-NES, which remains in the nucleus, prevents PDX1 shuttling together with degradation at conditions of chronically elevated glucose. Although transfection could not be achieved in all islet cells, nuclear PDX1 overexpression prevented the deleterious effects of glucose and IL-1β on β-cell survival and function. Previously, we have observed such quantitative decrease in PDX1 induced by elevated glucose concentrations in human and rat islets. At this time, whole islet lysates were used for the analysis, and we did not investigate PDX1 localization. PDX1 decrease was dose-dependent on glucose concentrations (5.5–33.3 mM) (56). In the same previous study, we also show an age-dependent PDX1 decrease in human and rat islets, which was confirmed in pancreatic biopsy samples (57). Such PDX1 decrease correlated with the increased susceptibility to glucose-induced apoptosis and with a decline in

FIGURE 4. IL-1Ra prevents prolonged glucose- and IL-1β-induced JNK-activation. A, isolated islets from mice overexpressing IL-1Ra (IL-1Ra-OE) and wild type littermates were treated for 24–72 h with 5.5 mM glucose. Shown is immunoblotting for phosphorylated JNK and β-actin (loading control). The antibodies were blotted on the same membrane. One representative experiment of three is shown. Shown is densitometry analysis of phospho-JNK bands normalized to β-actin. Islets were cultured in suspension for 24–72 h in 5.5 mM glucose (control), 22.2 or 33.3 mM glucose, or 2 ng/ml IL-1β with or without addition of 500 ng/ml recombinant human IL-1Ra (B) or 10 μM JNKi (C). B, immunoblotting of phosphorylated JNK and β-actin (loading control). The antibodies were blotted on the same membrane. One representative blot of three experiments from three donors is shown. Shown is densitometry analysis of phospho-JNK bands normalized to β-actin. C, after 72 h of treatment, fixed and paraffin-embedded islet sections were double-stained for insulin in green and PDX1 in red and analyzed under the microscope (magnification, ×250; inset magnification, ×1000). Islets were isolated from three different donors, and 3 independent experiments were performed. D and E, for the analysis of β-cell survival and glucose-stimulated insulin secretion, islets were cultured on extracellular matrix-coated dishes and treated for 72 h. D, β-cell apoptosis expressed as a percentage of TUNEL-positive β-cells ± S.E. The mean number of β-cells scored was 2945 ± 218 for each treatment condition in three independent experiments from three different donors. E and F, glucose-stimulated insulin secretion of islets. Stimulatory index (E) denotes the ratio between stimulated (16.7 mM glucose) and basal (2.8 mM glucose) values normalized to insulin content (F) of insulin secretion during successive 1-h incubations. Results are means ± S.E. (error bars) from three independent experiments from three donors. *, p < 0.05 compared with control at 5.5 mM glucose. **, p < 0.05, IL-1Ra- or JNKi-treated islets compared with untreated islets under the same conditions.
Neither Reers et al. (57) nor we were able to show nuclear PDX1 localization. It was suggested that the human pancreas embedding method prevented detection of nuclear PDX1. Here we improved tissue permeabilization and clearly detected nuclear PDX1 even in sections from human autopsy and biopsy, with strong PDX1 signals in the nucleus in non-diabetic control pancreases and a shift of PDX1 to the cytosol in diabetic conditions, a signal that appeared much weaker in sections from patients with T2DM. Together, the data support PDX1 shuttling in response to chronic glucose to the cytosol and its subsequent degradation as one deleterious factor contributing to β-cell failure.

Strategies to block these deleterious effects on the β-cell are needed for a successful diabetes therapy. Blocking IL-1β signals has been suggested as a novel treatment for diabetes. The anti-inflammatory cytokine IL-1Ra prevents glucose-induced apoptosis by blocking proapoptotic IL-1β signaling in vitro (4) and
improves glycemia and β-cell function and survival in vivo (16, 17, 20).

In this study, we provide further mechanisms of the protective effect of IL-1Ra directly on the β-cell transcriptional regulation in C57BL/6J mice fed a high fat/high sucrose diet (Surwit) and in db/db mice, serving as two animal models of T2DM and in human islets exposed to a diabetic milieu. 12 weeks of high fat feeding induced impaired glucose tolerance, which was inhibited in the IL-1Ra-OE mice. Unexpectedly, insulin and PDX1 mRNA levels were oppositely regulated in the HFD-treated mice; insulin was reduced, and PDX1 was significantly increased in islets after 12 weeks of high fat/high sucrose diet. IL-1Ra prevented such changes. Many previous studies have observed no changes or increases in insulin mRNA in response to a high fat diet without increase in sucrose (“Western diet”) in rat or mouse models (e.g. see Refs. 58 and 59). The addition of sucrose to the diet (Surwit) causes β-cell failure together with reduced insulin mRNA in the β-cell (60).

Although our results are in contrast to previous data showing PDX1 down-regulation in response to hyperglycemia in β-cells in culture as well as in type 2 diabetic animal models (e.g. in ZDF rats (61), in P. obesus (29), and in partially pancreatectomized rats (62)), studies in isolated human islets from patients with T2DM show a similar opposite regulation, with reduced insulin mRNA and increased PDX1 mRNA levels compared with islets isolated from non-diabetic controls (47).

PDX1 expression seems to be important for response to a higher insulin demand (e.g. in β-cells and β-cell compensation may occur through increased PDX1 mRNA). But suppression of PDX1 mRNA does not lead to a decrease of insulin in β-cells and activate insulin transcription in the nucleus, where it binds to DNA. 

JNK activity has previously been linked to PDX1 shuttling rather than mRNA expression, which plays an important role under diabetic conditions. Oxidative stress induces PDX1 shuttling between the cytosol and the nucleus and thus causes a severe reduction in PDX1 activity (49). Also, 24-h exposure of rat islets to palmitic acid at elevated glucose concentrations causes PDX1 localization to the cytosol together with a decrease in MafA expression and inhibition of insulin expression (36). In line with these previous observations, we detected PDX1 predominantly expressed in the cytosol in HFD-treated and in hyperglycemic db/db mice. In contrast, in IL-1Ra-treated as well as the normal diet groups, PDX1 was localized in the nucleus. Because IL-1Ra protected from the prodiabetic effect of the diet, we propose that this is a result of maintaining PDX1 functionally in the nucleus.

Homozygous Lepr<sup>db/db</sup> mice (db/db) on the C57BLKS/J background with this depletion in the leptin receptor become obese, hyperglycemic, and hyperinsulinemic within the first month of age. In these mice, IL-1β-mediated innate immunity is augmented, which results from a diabetes-associated loss of IL-1β counterregulation (65). To test the hypothesis that IL-1Ra would prevent diabetes progression in this model of T2DM, we injected db/db mice daily with IL-1Ra or with vehicle from 4 weeks of age on. db/db mice that received IL-1Ra showed improved glucose tolerance during intraperitoneal glucose tolerance test experiments after 2 weeks of treatment as compared with their vehicle-treated littermates (p < 0.05 at time points 30 and 60 min; data not shown). From 3 weeks of treatment on, we did not observe any differences in glucose levels between the two groups anymore. Considering the short half-life of IL-1Ra (6–8 h) and the 10–100-fold excess that is needed to block IL-1β-mediated effects (66), we tested whether constitutive endogenous overexpression of IL-1Ra would improve the outcome of elevated IL-1Ra levels in db/db mice. We could confirm in the db/db mouse model that IL-1Ra overexpression was protective against the development of diabetes and β-cell failure, although the effects of IL-1Ra in db/db mice were quite modest. However, considering the db/db mouse as a model of severe diabetes with rapid development of hyperglycemia, any significant rescue in the model and a combined effect on glucose tolerance, insulin secretion, β-cell mass, and apoptosis confirms a protective effect on the β-cell. This was paralleled by IL-1Ra-induced PDX1 stabilization in the nucleus in the db/db mice. Already at 6 weeks of age, PDX1 was predominantly expressed in the cytosol in the db/db mice, whereas at the age of 4 weeks of age it was strongly decreased. IL-1Ra restored PDX1 localization already after 6 weeks of age.

Activation of JNK is a hallmark in glucose and IL-1β effect on β-cell function and β-cell transcriptional regulation and survival, whereas chronic activation correlates with glucotoxicity. IL-1Ra protected islets from prolonged JNK activation. JNK activity has previously been linked to PDX1 shuttling under conditions of oxidative stress (49) and in prostaglandin E2-induced β-cell dysfunction (32), together with Foxo1 as a key player (31). Foxo1 cellular localization determines PDX1 localization, and both are reversedly expressed. Foxo1 itself is regulated by JNK and AKT activity; JNK induces Foxo1 nuclear import, which leads to PDX1 export, whereas AKT-mediated Foxo1 phosphorylation results in Foxo1 cytoplasmic and PDX1 nuclear localization. Our findings suggest the JNK-PDX1 pathway as a critical signaling network that transduces short term as well as long term glucose stimulation and therefore might partly mediate the dual effect of glucose on β-cell function and survival.

The fact that IL-1Ra potentially prevented hyperglycemia and improved β-cell function favors the critical role of IL-1β signaling in the β-cell not only in a type 1 but also in a type 2 diabetic environment. Our data provide new insights into mechanisms of the protective effect of IL-1Ra on β-cell function and turnover, establish the important role of nuclear PDX1
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localization, and support IL-1Ra as a potential therapy for diabetes.

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Metabolism: Neutralizing Interleukin-1β (IL-1β) Induces β-Cell Survival by Maintaining PDX1 Protein Nuclear Localization

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