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Original Article

Low Concentration of Interleukin-1β Induces FLICE-Inhibitory Protein–Mediated β-Cell Proliferation in Human Pancreatic Islets

Kathrin Maedler,[1] Desiree M. Schumann,[2] Nadine Sauter,[2] Helga Ellingsgaard,[2] Domenico Bosco,[3] Reto Baertschiger,[3] Yoichiro Iwakura,[4] José Oberholzer,[5] Claes B. Wollheim,[6] Benoit R. Gauthier,[6] and Marc Y. Donath[2]

High glucose concentrations have a dual effect on β-cell turnover, inducing proliferation in the short-term and apoptosis in the long-term. Hyperglycemia leads to β-cell production of interleuking (IL)-1β in human pancreatic islets. Fas, a death receptor regulated by IL-1β, is involved in glucose-induced β-cell apoptosis. Fas engagement can be switched from death signal to induction of proliferation when the caspase 8 inhibitor, FLICE-inhibitory protein (FLIP), is active. Here, we show that IL-1β at low concentrations may participate in the mitogenic actions of glucose through the Fas-FLIP pathway. Thus, exposure of human islets to low IL-1β concentrations (0.01–0.02 ng/ml) stimulated proliferation and decreased apoptosis, whereas increasing amounts of IL-1β (2–5 ng/ml) had the reverse effects. A similarly bimodal induction of FLIP, pancreatic duodenal homeobox (PDX)-1, and Pax4 mRNA expression, as well as glucose-stimulated insulin secretion, was observed. In contrast, Fas induction by IL-1β was monophasic. Low IL-1β also induced the IL-1 receptor antagonist (IL-1Ra), suppression of which by RNA interference abrogated the beneficial effects of low IL-1β. The Fas antagonistic antibody ZB4 and small interfering RNA to FLIP prevented low IL-1β–stimulated β-cell proliferation. Consistent with our in vitro results, IL-1β knockout mice displayed glucose intolerance along with a decrease in islet Fas, FLIP, Pax4, and PDX-1 transcripts. These findings indicate that low IL-1β levels positively influence β-cell function and turnover through the Fas-FLIP pathway and that IL-1Ra production prevents harmful effects of high IL-1β concentrations. *Diabetes* 55:2713–2722, 2006

From the [1]Larry L. Hillblom Islet Research Center, University of California, Los Angeles, California; the [2]Division of Endocrinology and Diabetes and Center for Integrative Human Physiology, University Hospital Zurich, Zurich, Switzerland; the [3]Department of Surgery, University Medical Center, Geneva, Switzerland; the [4]Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Tokyo, Japan; the [5]Division of Transplantation, University of Illinois at Chicago, Chicago, Illinois; and the [6]Department of Cell Physiology and Metabolism, University Medical Center, Geneva, Switzerland.

Address correspondence and reprint requests to Marc Y. Donath, MD, Division of Endocrinology and Diabetes, Department of Medicine, University Hospital, CH-8091 Zurich, Switzerland. E-mail: marc.donath@usz.ch.

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FLIP, FLICE-inhibitory protein; GSIS, glucose-stimulated insulin secretion; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; IRS, insulin receptor substrate; KRBB, Krebs-Ringer bicarbonate buffer; PDX, pancreatic duodenal homeobox; rh, recombinant human; siFLIP, siRNA directed to FLIP; siIL-1Ra, siRNA directed to IL-1Ra; siRNA, small interfering RNA; TUNEL, transferase-mediated dUTP nick-end labeling.

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The capacity of the pancreatic β-cell to adaptively increase insulin secretion in response to long-term insulin resistance (for example in obesity) is fundamental to the maintenance of normoglycemia. Failure of such a response results in diabetes (1–8). Changes in glucose concentration are key regulators of β-cell secretory function. Short-term exposure of human islets to increased glucose concentrations will enhance insulin production and β-cell proliferation, while prolonged exposure will have toxic effects leading to impaired insulin secretion and β-cell apoptosis (9–17). Various mechanisms for this glucotoxic effect have been proposed, including advanced glycation end products, reactive oxygen species, impairment of insulin gene transcription, and endoplasmic reticulum stress. Recently, interleukin (IL)-1β was shown to be induced in β-cells of patients with type 2 diabetes, indicating that the cytokine could be an important mediator contributing to glucotoxicity (18). Consistent with the latter, β-cell expression of IL-1β correlates with appearance of hyperglycemia in several animal models of diabetes, including the Psammomys obesus, the OLEFT rat, the GK rat, and the human islet amyloid polypeptide transgenic rat ([18–21] and P.C. Butler, personal communication). IL-1β has been shown to impair insulin release and to induce Fas expression enabling Fas-triggered apoptosis in rodent and human islets (16,22–31). Accordingly, increased glucose concentrations also induce β-cell expression of Fas in vitro and in vivo, which will then be activated by the endogenous Fas ligand (16,28,32,33).

Interestingly, the beneficial short-term effects of high glucose on β-cell function and proliferation are also partly mediated by Fas. Indeed, in the presence of the caspase 8 inhibitor, FLICE-inhibitory protein (FLIP), Fas signaling switches from apoptosis to cell replication (34). Furthermore, we recently demonstrated an additional role for the Fas pathway in regulating insulin production and release (35). Additionally, low concentrations of IL-1β stimulate insulin release in rat islets (36). Since glucose induces IL-1β, we hypothesized that IL-1β may also mediate beneficial effects of glucose. We show that low concentrations of IL-1β induce β-cell proliferation and enhance β-cell secretory function via the Fas-FLIP pathway, an effect facilitated by the concomitant IL-1 receptor agonist (IL-1Ra) production.

RESEARCH DESIGN AND METHODS

Ethical approval for mouse studies was granted by the Zurich Cantonal Animal Experimentation Committee. C57BL/6j wild-type mice were obtained from The Jackson Laboratory (Bar Harbor, ME). IL-1β knockout (IL-1β$^{-/-}$) mice, on a C57BL/6j background, were produced by gene targeting as previously described (37). Animals were housed at 22°C with a 12-h light-dark cycle (lights on at 0700) and allowed free access to water and food.

Intraperitoneal glucose and insulin tolerance tests. Mice were fasted 12 h overnight and injected intraperitoneally with 2 mg/g body wt glucose (40% glucose solution; Laboratorium Dr. G. Bichsel AG, Interlaken, Switzerland) or with 0.75 mU/g recombinant human (rh) insulin (Novo Nordisk, Bagsværd, Denmark) for the glucose or insulin tolerance tests, respectively. Blood samples were obtained from tail-tip bleedings, and blood glucose concentration was measured with a Glucometer (Freestyle, Disetronic Medical Systems, Burgdorf, Switzerland).

Islet isolation and culture. Human islets were isolated from pancreata of nine organ donors at the University of Geneva Medical Center and at the University of Illinois at Chicago. Mouse islets were isolated as previously described (38). The islets were cultured on extracellular matrix–coated plates derived from bovine corneal endothelial cells (Novamed, Jerusalem, Israel), allowing the cells to attach to the dishes and spread. Human islets were cultured in CMRL 1066 medium containing 5.5 mmol/l glucose and mouse islets in RPMI-1640 medium containing 11.1 mmol/l glucose, both supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Invitrogen, Carlsbad, CA), hereafter referred to as culture medium. Two days after plating, when most islets were attached and began to flatten, the medium was changed to culture medium containing 5.5, 11.1, or 33.3 mmol/l glucose with or without 0.01–5 ng/ml rhIL-1β, 500 ng/ml rhIL-1Ra (R&D Systems, Minneapolis, MN), 500 ng/ml antagonistic Fas antibody (ZB4; MBL, Nogoya, Japan), or transfected as described below.

RNA interference. RNAs of 21 nucleotides, designed to target human IL-1Ra (5′AUCUGCAGAGGCCUCCGCAtt3′/5′UGCGGAGGCCUCUGCAGAUtt3′), human FLIP$_{long}$ (silencer predesigned small interfering RNA [siRNA]), and scrambled siRNA were synthesized by Ambion (Austin, TX). siRNA was transfected using SiPortAmine and transfection efficiency estimated with cy3-labeled siRNA using a Silence siRNA Labeling Kit (Ambion), as previously described (39).

β-Cell replication and apoptosis. For β-cell proliferation studies, a monoclonal antibody against the human (Zymed Laboratories, San Francisco, CA) or mouse (Santa Cruz Biotechnology, Santa Cruz, CA) Ki-67 antigen was used. The free 3′-OH strand breaks resulting from DNA degradation were detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique according to the manufacturer's instructions (In Situ Cell Death Detection Kit; Boehringer, Mannheim, Germany) and as previously described in detail (14,18). Thereafter, islets were incubated for 30 min at 37°C with a guinea pig anti-insulin antibody (Dako, Carpinteria, CA), followed by detection using the streptavidin-biotin-peroxidase complex (Zymed) or a fluorescein-conjugated rabbit anti–guinea pig antibody (Dako).

Histochemical analysis. The pancreata were weighed, fixed in formalin, and embedded in paraffin. Ten representative sections from each pancreas (spanning the width of the pancreas) were used in the analysis of β-cell mass. Tissue sections were deparaffinized, rehydrated, and incubated with guinea pig anti-insulin antibody (Dako) followed by detection with a fluorescein-conjugated rabbit anti–guinea pig antibody (Dako). Subsequently, the specimens were labeled for glucagon with mouse anti-glucagon antibody (Dako), followed by detection with donkey anti-mouse Cy3-conjugated antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). β-Cell mass was analyzed using Openlab software. The relative area of β-cells (green fluorescence) was determined by quantification of the cross-sectional β-cell area divided by the cross-sectional area of total tissue. The β-cell mass per pancreas was estimated as the product of the relative cross-sectional area of β-cells per total tissue and the weight of the pancreas. For detection of FLIP expression in β-cells, islets were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, incubated with rabbit anti-FLIP (R&D Systems), followed by detection with donkey anti-rabbit Cy3-conjugated antibody (Jackson). Subsequently, the specimens were stained for insulin as described above.

Western blot analysis. Equivalent amounts of protein from each treatment group were run on 15% SDS polyacrylamide gels. Proteins were electrically transferred to nitrocellulose filters and incubated with rabbit anti–caspase 8 (Stressgen, Victoria, BC, Canada), rabbit anti-human FLIP-long (R&D Systems), rabbit anti–pancreatic duodenal homeobox (PDX)-1 (kindly provided by Stefan Zahn; NovoNordisk, Bagsværd, Denmark), or rabbit anti-actin (Cell Signaling, Beverly, MA) antibodies followed by incubation with horseradish peroxidase–linked anti-rabbit IgG peroxidase-conjugated antibodies (Santa Cruz Biotechnology). Immune complexes were detected by chemiluminescence using LumiGLO (Cell Signaling).

RNA extraction and quantitative RT-PCR. Total RNA was extracted from the cultured islets by using the RNeasy Mini Kit (Qiagen, Basel, Switzerland), and RT-PCR was performed by using the SuperScript Double-Stranded cDNA Synthesis Kit according to the manufacturer's instructions (Life Technologies, Gaithersburg, MD). For quantitative analysis, we used the LightCycler Quantitative PCR System (Roche, Basel, Switzerland) with a commercial kit (LightCycler-DNA Master SYBR Green I; Roche). Mouse primers used were 5′TACGGGGTTTGTGAAAGGAG3′ and 5′CACATCATTCCCCAGGAAAC3′ (insulin), 5′GAGGACCCGTACAGCCTACA3′ and 5′CGTTGTCCCGCTACTAC GTT3′ (PDX-1), 5′CTAAATTTGGTTGCCCCAGA3′ and 5′CTCCCATTATG GAGCCTGAA3′ (FLIP long), 5′GCTCTTTTTGCCTGGGAGATC3′ and 5′CCCG AAGGACTCGATTGATAGA3′ (Pax4), 5′TATCAAGGAGGCCCATTTTG3′ and 5′GGTCAGGGTGCAGTTTGTTT (Fas), and 5′GTGGCAGTGATGGCATGG AC3′ and 5′CAGCACCAGTGGATGCAGGG3′ (glyceraldehyde-3-phosphate dehydrogenase). Human primers used were 5′CCACCTTGGGACCTGTTTAG3′ and 5′TGATGCCAGAGGAAGAGGAG3′ (PDX-1), 5′CCACCGGAATCGGAC TATCTT3′ and 5′TACTGCCCACGCTGGAACTC3′ (Pax4), 5′GAGCAAGC CCCTAGGAATCT3′ and 5′GCCCTGAGTGAGTCTGATCC3′ (FLIP long), 5′CTACCTAGTGTGCGGGGAAC3′ and 5′GCTGGTAGAGGGAGCAGATG3′ (insulin), 5′GCATCTGGACCCTCCTACCT3′ and 5′CAGTCTGGTTCATCCC CATT3′ (Fas), 5′AGAGTCGCGCTGTAAGAAGC3′ and 5′TGGTCTTGTCACT TGGCATC3′ (α-tubulin), 5′AACAGCGACACCCACTCCTC3′ and 5′GGAGG GGAGATTCAGTGTGGT3′ (glyceraldehyde-3-phosphate dehydrogenase), and 5′TACGGGTCCTGGCATCTTGT3′ and 5′CCATTTGTGTTGGGTCCAGC3′ (cyclophilin).

Insulin and IL-1Ra release and insulin content. For acute insulin release in response to glucose, islets were washed and preincubated (30 min) in Krebs-Ringer bicarbonate buffer (KRBB) containing 2.8 mmol/l glucose and 0.5% BSA. KRBB was then replaced by KRBB 2.8 mmol/l glucose for 1 h (basal), followed by an additional 1 h in KRBB 16.7 mmol/l glucose. Islets were extracted with 0.18 N HCl in 70% ethanol for determination of insulin content. To determine the total insulin content of the pancreas, the tissue was homogenized in 1 ml 0.18 N HCl in 70% ethanol and left overnight at 4°C. Insulin was determined using a human insulin radioimmunoassay kit (CIS Bio International, Gif-Sur-Yvette, France), which has similar affinity for both mouse and human insulin. Serum insulin was measured using Luminex technology according to the manufacturer's instructions (Linco Research, St. Charles, MO). IL-1Ra release in the islet supernatant was measured by using human anti–IL-1Ra enzyme-linked immunosorbent assay kits (R&D).

Statistical analysis. Samples were evaluated in a randomized manner by a single investigator (K.M.) who was blinded to the treatment conditions. Data are presented as means ± SE and were analyzed by Student's *t* test or by ANOVA with a Bonferroni correction for multiple-group comparisons.

RESULTS

Low concentrations of IL-1β induce β-cell proliferation, are antiapoptotic, and enhance β-cell secretory function. Human islets were cultured on extracellular matrix–coated plates in the presence of increasing IL1-β concentrations for 4 days. Exposure to 0.02 ng/ml IL-1β induced a twofold increase in β-cell proliferation compared with controls, but exposure to higher concentrations of 2 and 5 ng/ml resulted in an ~1.5-fold decrease (Fig. 1*A* and *B*; Table 1). By contrast, 0.01 ng/ml IL-1β reduced baseline β-cell apoptosis twofold, whereas higher doses of 2 and 5 ng/ml IL-1β increased the apoptosis rate by 2.3- and 3.6-fold, respectively (Fig. 1*A* and *C*; Table 1). Those changes in β-cell turnover were accompanied by a 1.6-fold increase in glucose-stimulated insulin secretion (GSIS) at 0.02 ng/ml IL-1β and a 2.8-fold reduction by 2 ng/ml IL-1β (Fig. 1*D* and *E*). Insulin content of the islets was not significantly affected (data not shown).

Endo- and exogenous modulation of IL-1 signaling by IL-1Ra. We have recently shown expression of IL-1Ra in human β-cells (39). Interestingly, secreted IL-1Ra will antagonize the effect of IL-1β. To highlight the potential cross-talk between these two molecules, we repressed endogenous production IL-1Ra by RNA interference. As previously shown, siRNA directed to IL-1Ra (siIL-1Ra) with a transfection efficiency of ~70% suppressed IL-1Ra

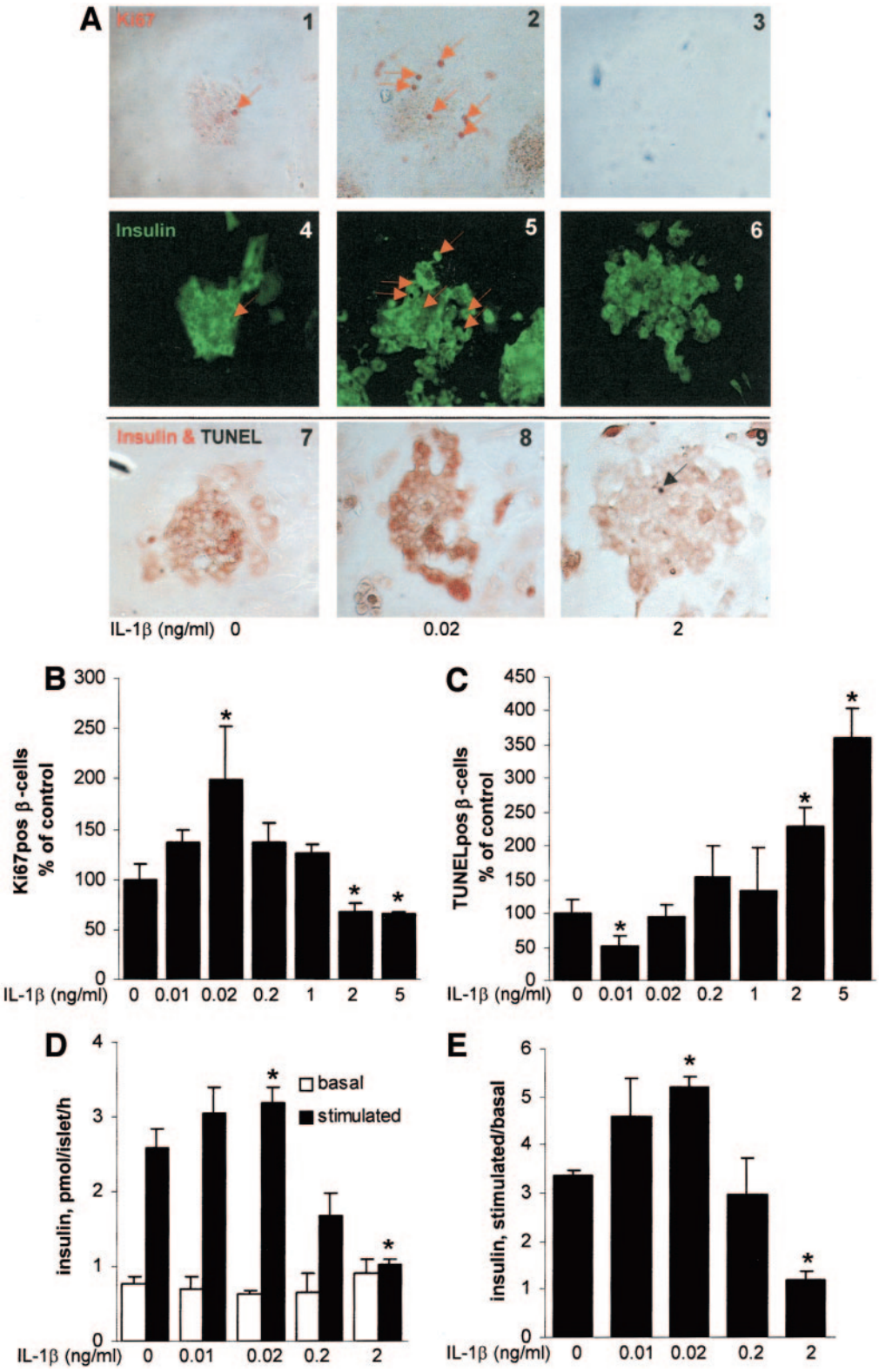

FIG. 1. Low concentrations of IL-1β induce β-cell proliferation, are antiapoptotic, and enhance β-cell secretory function. Human islets were cultured on extracellular matrix–coated dishes for 4 days at 5.5 mmol/l glucose with increasing concentrations of IL-1β. Double immunostaining for β-cell proliferation with anti–Ki-67 in brown (*A1–3*) and anti-insulin in green (*A4–6*) and for β-cell apoptosis with the TUNEL assay in black and anti-insulin in brown (*A7–9*), in control islets (*A1, 4,* and *7*), and in islets treated with 0.02 (*A2, 5,* and *8*) and with 2 (*A3, 6,* and *9*) ng/ml IL-1β. The orange arrows mark β-cells stained positive for Ki-67 and insulin, and the black arrow marks a TUNEL-positive β-cell. Percentage of Ki-67–positive (*B*) and TUNEL-positive (*C*) β-cells normalized to control incubations at 5.5 mmol/l glucose alone (100%; in absolute values: 0.19 ± 0.07% Ki-67–positive β-cells and 0.4 ± 0.05% TUNEL-positive β-cells). The mean number of islets scored was 41 (*B*) and 52 (*C*) for each treatment condition from each donor. Basal and GSIS denote the amount secreted during successive 1-h incubations at 2.8 (basal) and 16.7 (stimulated) mmol/l glucose following the 4-day culture period expressed as secreted insulin (*D*) or stimulatory index (*E*). Islets were isolated from four organ donors. Results are means ± SE. *$P < 0.05$ to untreated controls.

TABLE 1
TUNEL and Ki-67 labeling of β-cell nuclei

IL-1β (ng/ml)	TUNEL			Ki-67		
	Number of cells counted	Number of TUNEL-labeled nuclei	Percent of TUNEL-labeled nuclei	Number of cells counted	Number of Ki-67–labeled nuclei	Percent of Ki-67–labeled nuclei
0	14,800	57	0.3851	21,300	39	0.1831
0.01	8,500	15	0.1765	10,100	20	0.1980
0.02	16,400	50	0.3049	18,600	63	0.3387
0.2	16,900	79	0.4675	18,000	39	0.2167
1	8,100	30	0.3704	10,500	17	0.1619
2	15,500	132	0.8516	22,100	30	0.1357
5	16,900	215	1.2722	19,800	22	0.1111

Human islets were cultured on extracellular matrix–coated dishes for 4 days at 5.5 mmol/l glucose with increasing concentrations of IL-1β and double stained for β-cell apoptosis with the TUNEL assay and for proliferation with anti–Ki-67. Islets were isolated from four organ donors.

mRNA by 69 ± 6% (39). Blockade of endogenous IL-1Ra by siIL-1Ra did not significantly affect baseline β-cell proliferation (Fig. 2*A*) but led to a twofold increase in apoptosis, levels similar to the ones obtained with 2 ng/ml IL-1β (Fig. 2*B*). Addition of 0.02 ng/ml IL-1β failed to increase β-cell proliferation and to further increase β-cell apoptosis (Fig. 2*A* and *B*). In contrast, addition of exogenous rhIL-1Ra (500 ng/ml) increased β-cell proliferation and protected from the deleterious effects of a high concentration of 2 ng/ml IL-1β. Furthermore, the compound protected cells against prolonged exposure to 33.3 mmol/l glucose (Fig. 2*A* and *B*). Similarly, siIL-1Ra completely blocked GSIS in the absence or presence of 0.02 ng/ml IL-1β, whereas exogenous rhIL-1Ra prevented the impairment of GSIS in the presence of 2 ng/ml IL-1β or 33.3 mmol/l glucose (Fig. 2*C* and *D*). Interestingly, the low concentration of 0.02 ng/ml IL-1β induced a 1.8-fold increase in the release of its receptor antagonist IL-1Ra into the culture medium (Fig. 2*E*).

Low-dose IL-1β–induced β-cell proliferation is mediated via the Fas-FLIP pathway and may involve PDX-1 and Pax4. Next, we investigated the underlying mechanisms of the proliferative effect of IL-1β. We hypothesized that the Fas pathway mediates IL-1β–induced β-cell proliferation. To test this hypothesis, first we investigated whether low concentrations of IL-1β are capable of inducing Fas. Exposure of human islets to 0.02 ng/ml IL-1β for 4 days induced Fas expression to levels similar to those observed with higher concentrations of IL-1β and 33.3 mmol/l glucose (Fig. 3*A*). However, low concentrations of IL-1β did not activate caspase 8, the most upstream caspase in the Fas apoptotic pathway, while higher concentrations than 0.02 ng/ml IL-1β cleaved procaspase 8, releasing the active form of the protease (Fig. 3*B*). To examine whether the induction of proliferation by 0.02 ng/ml IL-1β is caused by the interaction of constitutively expressed Fas ligand (16,28) and upregulated Fas, we used the Fas antagonistic antibody ZB4. ZB4 inhibited the proliferative effect of IL-1β (Fig. 3*C*). Since Fas signaling may induce proliferation in the presence of FLIP (34), we analyzed FLIP protein modulation by increasing concentrations of IL-1β. Similar to IL-1β–mediated changes in β-cell proliferation (Fig. 1*B*), low concentrations of IL-1β induced FLIP expression, while higher concentrations were inhibitory (Fig. 3*D* and *E*). Of note, FLIP mRNA expression remained unchanged (data not shown) in agreement with previous studies showing regulation of FLIP only at the protein level (34). The functional role of FLIP in IL-1β–induced β-cell proliferation was then investigated by RNA interference (siRNA directed to FLIP [siFLIP]). siFLIP suppressed FLIP expression in most β-cells (Fig. 3*F*) leading to a 5.2-fold decrease in FLIP protein expression (for representative blot, see Fig. 3*G*). Repression of FLIP resulted in impaired baseline β-cell proliferation and prevented IL-1β–mediated replication, whereas scrambled siRNA had no effect on the number of Ki-67–positive β-cells (Fig. 3*H*). We next investigated the potential implication of the transcription factors PDX-1 and Pax4, known to promote β-cell replication (40–45). Low concentrations of IL-1β stimulated PDX-1 mRNA and protein expression, which were decreased by higher concentrations of IL-1β (Fig. 3*I–K*). Similarly, low concentrations of IL-1β stimulated Pax4 and insulin mRNA expression, which were decreased by higher concentrations (Fig. 3*L* and *M*). However, the stimulation of insulin mRNA by low IL-1β failed to reach statistical significance. Furthermore, Pax4 was stimulated at 12 h but no longer after 4 days.

Impaired glucose tolerance in IL-1β knockout mice. To substantiate the role of IL-1β in normal glucose homeostasis in vivo, glucose tolerance tests were performed in IL-1β knockout (IL-1β$^{-/-}$) mice. As predicted, we found that IL-1β$^{-/-}$ mice displayed glucose intolerance (Fig. 4*A*). Normal sensitivity of the IL-1β$^{-/-}$ mice to injected insulin (Fig. 4*B*) ruled out the possibility that insulin resistance was responsible for the impaired glucose tolerance. Food intake and body weight were similar in IL-1β$^{-/-}$ and wild-type mice (not shown). Analysis of pancreatic tissue of IL-1β$^{-/-}$ mice revealed a normal structure of the islets with no significant decrease in β-cell mass (Fig. 4*C* and *D*). However, insulin mRNA expression was strongly decreased (Fig. 4*E*), although pancreatic insulin content showed no significant changes (5.05 ± 1.04 vs. 4.0 ± 0.7 mol/g insulin per pancreas in normal versus IL-1β$^{-/-}$ mice, respectively). Consistent with our in vitro experiments, Fas, FLIP, PDX-1, and Pax4 were decreased in IL-1β$^{-/-}$ mice (Fig. 4*E*). Islets of IL-1β$^{-/-}$ mice exhibited a strong decrease in baseline β-cell proliferation and GSIS compared with wild-type mice, while apoptosis and insulin content were not significantly different (Fig. 5).

The resistance of IL-1β$^{-/-}$ islets to β-cell glucotoxicity was then studied. Chronic exposure of wild-type mouse islets to 33.3 mmol/l glucose for 4 days impaired β-cell proliferation, induced apoptosis, impaired GSIS, and decreased insulin content (Fig. 5). In contrast, islets of IL-1β$^{-/-}$ mice were not further altered by high glucose

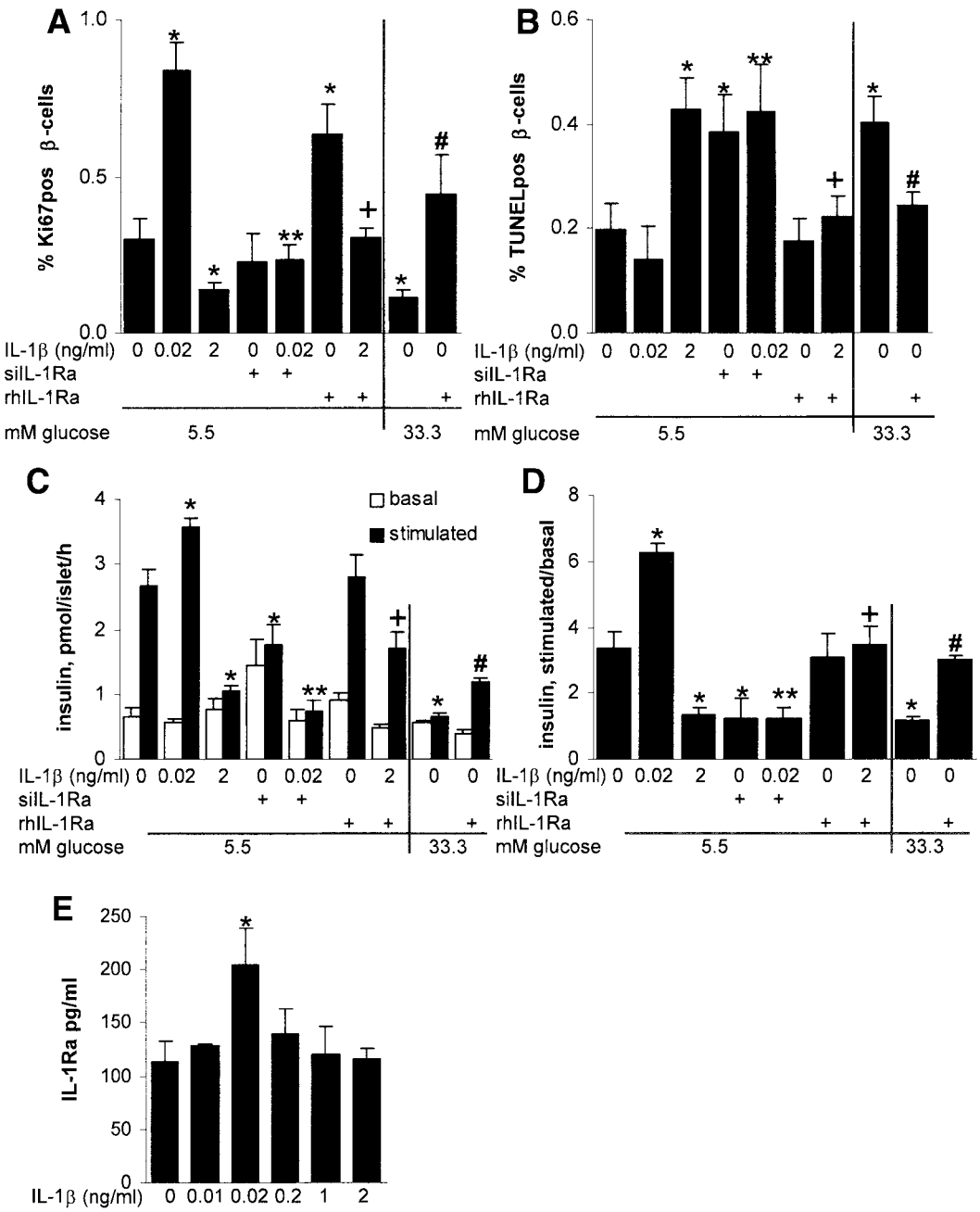

FIG. 2. Endo- and exogenous modulation of IL-1 signaling by IL-1Ra. Human islets were cultured on extracellular matrix–coated dishes for 4 days at 5.5 or 33.3 mmol/l glucose alone or with increasing concentrations of IL-1β or after transfection with 50 nmol/l siIL-1Ra or with addition of 500 ng/ml exogenous rhIL-1Ra. Results are means ± SE of percentage of Ki-67–positive (*A*) and TUNEL-positive (*B*) β-cells. The mean number of islets scored was 45 (*A*) and 43 (*B*) for each treatment condition from each donor. Basal and GSIS denote the amount secreted during successive 1-h incubations at 2.8 (basal) and 16.7 (stimulated) mmol/l glucose following the 4-day culture period expressed as secreted insulin (*C*) or stimulatory index (*D*). *E*: Secretion of IL-1Ra from human islets during 4 days of culture. Data were collected from three tubes per treatment in five separate experiments from five donors. Results are means ± SE. **P* < 0.05 to untreated control. ***P* < 0.05–0.02 ng/ml IL-1β alone. +*P* < 0.05–2 ng/ml IL-1β alone. #*P* < 0.05–33 mmol/l glucose alone.

concentrations (Fig. 5), supporting the concept of IL-1β–mediated glucotoxicity (18). Finally, we have confirmed that glucose regulates IL-1β secretion in mouse islets (2.85 ± 0.8 and 5.68 ± 1.6 pg/ml of IL-1β in islets cultured for 4 days in 11.1 and 33.3 mmol/l glucose, respectively, $n = 5$) as described in human islets (18).

DISCUSSION

The role of IL-1β and other cytokines in the pathogenesis of type 1 diabetes is well established (25,46). More recently, the concept emerged that cytokines may also mediate nutrient-induced β-cell dysfunction during the development of type 2 diabetes (5,6,47,48). Intriguingly, some of these cytokines can be produced by β-cells, including IL-6, IL-1β, IL-1Ra, and pancreatic-derived factor (18,19,39,49–53). The fact that the nonimmune β-cell synthesizes cytokines suggests their implication in islet physiology. Accordingly, we observed beneficial effects of low concentrations of IL-1β on β-cell proliferation, apoptosis, and secretory function.

A low concentration of IL-1β stimulated IL-1Ra, which in turn stimulated β-cell proliferation. Likewise, adenovi-

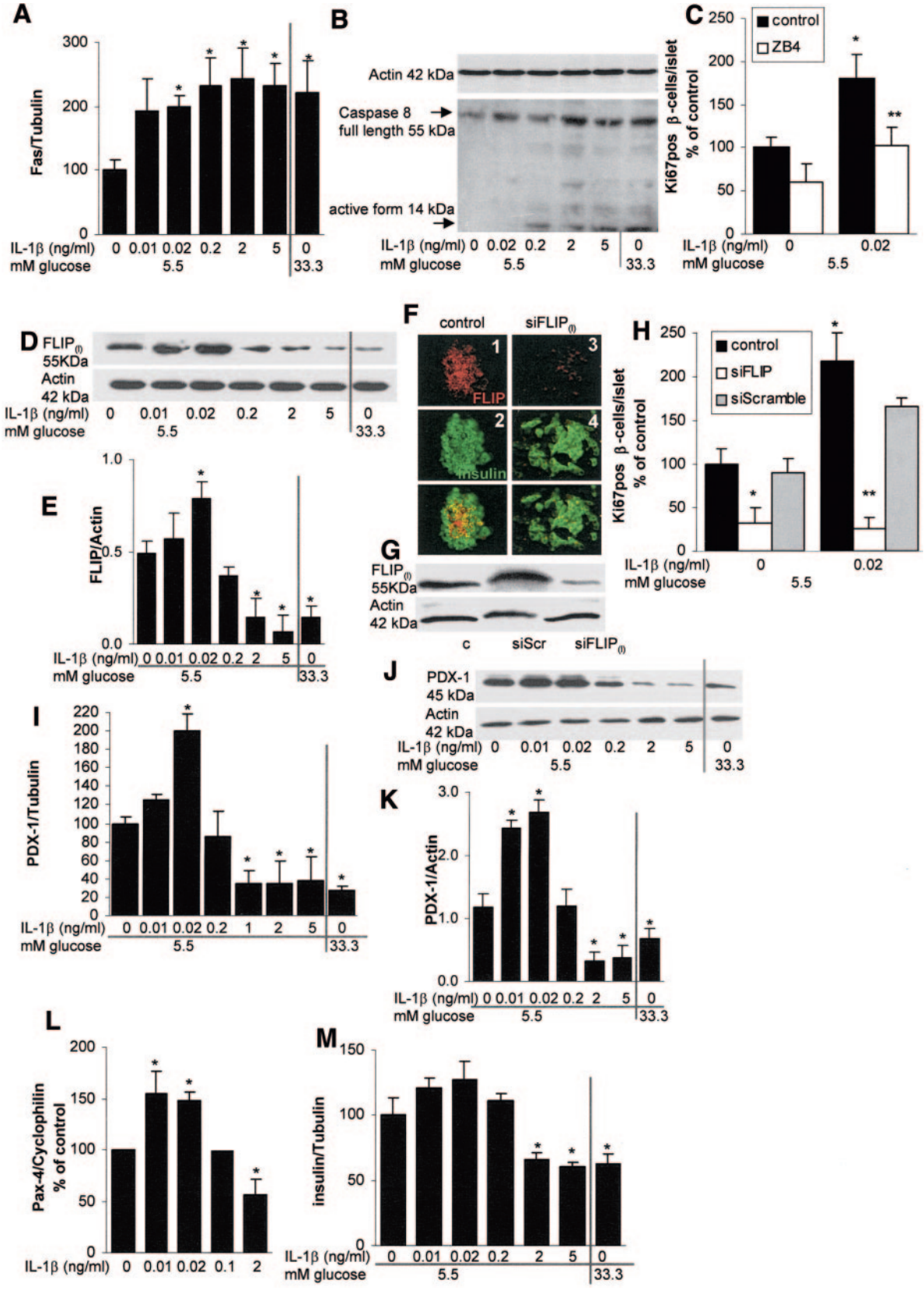

FIG. 3. Low-dose IL-1β–induced β-cell proliferation is mediated via the Fas-FLIP pathway and involves PDX-1 and Pax4. Human islets were cultured on extracellular matrix–coated dishes and exposed for 4 days to increasing IL-1β concentrations or 33.3 mmol/l glucose or 500 ng/ml of the antagonistic anti-Fas antibody ZB4 or 50 nmol/l siRNA to $FLIP_{(long)}$ [$siFLIP_{(l)}$] or to scrambled siRNA (siScr). *A*: Quantitative RT-PCR analysis of Fas expression. *B*: Representative immunoblotting of caspase 8 and actin. For detection of caspase 8, an antibody recognizing full-length (procaspase 8; 55 kDa) and the 14 kDa processed active form of caspase 8 was used. *C*: β-Cell proliferation determined by double staining with anti–Ki-67 and anti-insulin. *D*: Representative immunobloting of $FLIP_{(l)}$ and actin. *E*: The density of expression levels were quantified after scanning and normalized to actin levels. *F*: Double immunostaining of the islets with anti-$FLIP_{(l)}$ (*1* and *3*) and anti-insulin (*2* and *4*) antibodies

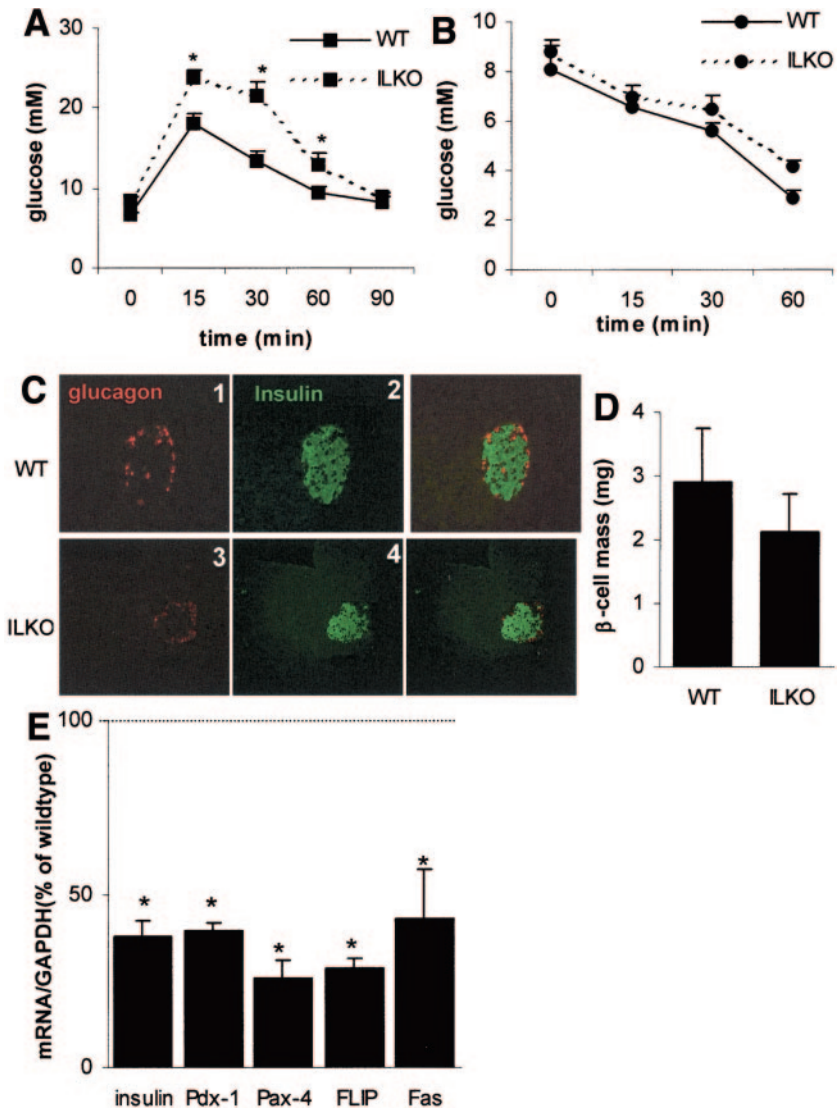

FIG. 4. Impaired glucose tolerance in IL-1β knockout mice. Blood glucose levels following intraperitoneal injection of glucose (*A*) or insulin (*B*) in 3-month-old male IL-1β$^{-/-}$ (ILKO) and wild-type (WT) C57BL/6j mice. *P < 0.05, ILKO vs. C57BL/6j. Data were collected from three separate experiments, each with five animals per group. Double immunostaining for glucagon in red (*1* and *3*) and insulin in green (*2* and *4*) (*C*) and β-cell mass in tissue sections of 4-month-old male wild-type C57BL/6j (*1* and *2*) and IL-1β$^{-/-}$ mice (*3* and *4*) (*D*); n = 5 for each group. *E*: Quantitative RT-PCR detection of insulin, PDX-1, Pax4, FLIP, and Fas mRNA expression. Total RNA was isolated from IL-1β$^{-/-}$ and C57BL/6j islets after 1 day in culture. The level of mRNA expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the results expressed as percentage of wild-type islets mRNA levels; n = 3 for each group of mice islets, each in duplicate. *P < 0.05 to wild-type islets.

ral expression of IL-1Ra increases β-cell replication in rat islets (54). At first sight, these results suggest a signaling function for IL-1Ra. However, this is unlikely since IL-1Ra only has a single IL-1 receptor–binding domain and is therefore unable to recruit the IL-1 receptor accessory protein, the second chain of the receptor complex, which is believed to be necessary for signaling (55). Therefore, the proliferative effects of exogenous IL-1Ra probably result from a restoration of a beneficial ratio of IL-1 to IL-1Ra and not from direct effects of the latter. Corroborating this hypothesis, repression of endogenous IL-1Ra was deleterious, probably due to unbalanced actions of IL-1.

Fas expression on the surface of pancreatic β-cells contributes to cytokine-induced apoptosis (30,56). However, when FLIP is activated, Fas becomes mitogenic (34). We propose that the Fas-FLIP pathway is modulated by IL-1β. This is supported by a simultaneous elevation of Fas and FLIP proteins at low IL-1β concentrations leading to β-cell proliferation. In contrast, higher concentrations of IL-1β decreased FLIP while Fas remained elevated, leading to decreased proliferation and induction of apoptosis. Furthermore, the Fas antagonistic antibody ZB4 and siRNA to FLIP both prevented IL-1β–induced β-cell proliferation.

The physiological importance of IL-1β was also apparent

in control islets (*1* and *2*) and siFLIP$_{(L)}$-treated islets (*3* and *4*). *G*: Representative immunobloting of FLIP$_{(L)}$ and actin. *H*: β-Cell proliferation determined by double staining with anti–Ki-67 and anti-insulin. *I*: Quantitative RT-PCR analysis of PDX-1 expression. *J*: Representative immunobloting of PDX-1 and actin. *K*: The density of expression levels were quantified after scanning and normalized to actin levels. Quantitative RT-PCR analysis of Pax4 in islets exposed for 12 h to IL-1β (*L*) and of insulin in islets exposed for 4 days to IL-1β (*M*). Results are means ± SE. In the LightCycler quantitative PCR system, the levels of Pax4 and insulin expression were normalized against tubulin or cyclophylin and the results were expressed as mRNA levels relative to control incubations. The antibodies were blotted on the same membrane after stripping, and actin was used as loading control. Islets were isolated from six organ donors. *P < 0.05 to untreated control; **P < 0.05–0.02 ng/ml IL-1β.

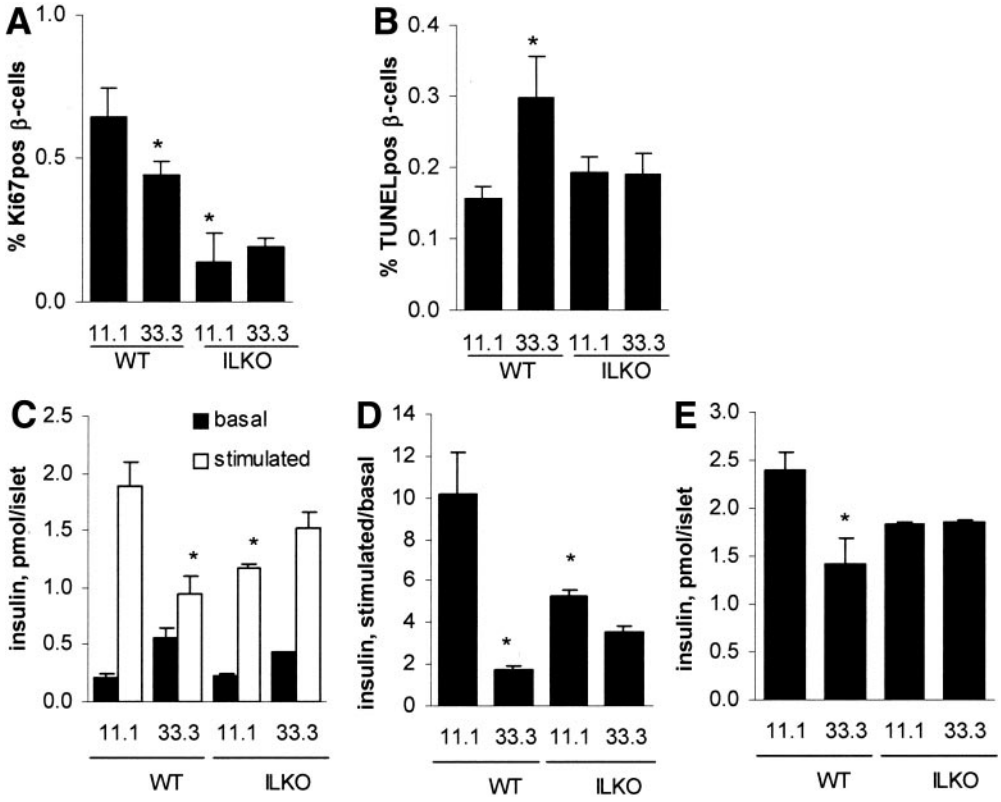

FIG. 5. Resistance of IL-1β$^{-/-}$ islets to glucotoxicity. Isolated islets from male IL-1β$^{-/-}$ (ILKO) and wild-type (WT) C57BL/6j mice were cultured on extracellular matrix–coated dishes for 4 days at 11.1 or 33.3 mmol/l glucose. Results are means ± SE of percentage of Ki-67–positive (*A*) and TUNEL-positive (*B*) β-cells. The mean number of islets scored was 132 (*A*) and 118 (*B*) for each treatment condition in three independent experiments. *C*: Basal and GSIS denote the amount secreted during successive 1-h incubations at 2.8 (basal) and 16.7 (stimulated) mmol/l glucose following the 4-day culture period expressed as secreted insulin (*C*) or stimulatory index (*D*). *E*: Insulin content. Results are means ± SE. Islets were isolated from nine mice for each treatment group in three independent experiments. *$P < 0.05$ to wild-type control.

in IL-1β knockout mice, showing impaired glucose tolerance. Islets of these mice displayed decreased mRNA expression levels of PDX-1, Pax4, insulin, Fas, and FLIP. Of note, these transcripts were enhanced by 0.02 ng/ml IL-1β. At higher concentrations, IL-1β suppressed these factors uncovering its toxic effects. IL-1β may participate in glucotoxicity (18), a concept substantiated by the protection from harmful actions of high glucose of IL-1β knockout mouse islets.

Most factors playing a role in β-cell turnover are also involved in the regulation of β-cell secretory function (57). This is also true for IL-1β, which will have distinct effects depending on the duration of exposure. On the short-term, IL-1β will predominantly influence β-cell secretory function independently of changes in β-cell mass. Indeed, in vitro, a 4-day exposure of human islets to different concentrations of IL-1β led to changes in cell turnover in <1% of β-cells, although β-cell secretory function was almost completely blocked at high concentrations. Nevertheless, this does not mean that prolonged exposure to IL-1β will not affect β-cell mass. Indeed, β-cells remain positive for Ki-67 for ~12 h. In absolute value, 0.02 ng/ml IL-1β increased the number of proliferating β-cells by 0.2%. The human pancreas has between 500,000 and 1,000,000 islets, comprising 500–2,000 β-cells each. Therefore, it can be estimated that low concentrations of IL-1β will increase the number of β-cells by ~4,000,000 per day, leading to a doubling of β-cell mass within 250 days. Such an impressive increase in β-cell mass may occur in vivo over a similar time period (e.g., during obesity [4]). Therefore, the observed differences are within an expected range and support the relevance of the finding.

A key signaling molecule regulating β-cell turnover and function is insulin receptor substrate (IRS)-2 (58). Interestingly, it has been proposed that IL-1β may mediate its deleterious effects via IRS-2 degradation (8). Conversely, it is conceivable that at low concentrations IL-1β also signals via IRS-2. Indeed, while IRS-2 degradation leads to apoptosis, IRS-2 activation enhances β-cell proliferation and function. However, further investigations are required to support this hypothesis.

Cytokines are central in the development of diabetes. However, at low concentrations, IL-1β promotes β-cell function and survival. Therefore, glucose-induced IL-1β may have an important role in the long-term adaptation of the β-cells to hyperglycemia. Short-term exposure to hyperglycemia will induce low levels of IL-1β, inducing IL-1Ra, Fas, and FLIP, leading to decreased apoptosis and enhanced proliferation and function. However, prolonged hyperglycemia will increase the ratio of IL-1β to IL-1Ra, decreasing FLIP and directing Fas to signals deleterious to the cell.

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