Deletion of Fas in the pancreatic β-cells leads to enhanced insulin secretion

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Deletion of Fas in the pancreatic β-cells leads to enhanced insulin secretion. Am J Physiol Endocrinol Metab 297: E1304–E1312, 2009. First published September 15, 2009; doi:10.1152/ajpendo.00217.2009.—Fas/Fas ligand belongs to the tumor necrosis factor superfamily of receptors/ligands and is best known for its role in apoptosis. However, recent evidence supports its role in other cellular responses, including proliferation and survival. Although Fas has been implicated as an essential mediator of β-cell death in the pathogenesis of type 1 diabetes, the essential role of Fas specifically in pancreatic β-cells has been found to be controversial. Moreover, the role of Fas on β-cell homeostasis and function is not clear. The objective of this study is to determine the role of Fas specifically in β-cells under both physiological and diabetes models. Mice with Fas deletion specifically in the β-cells were generated using the Cre-loxP system. Cre-mediated Fas deletion was under the control of the rat insulin promoter. Absence of Fas in β-cells leads to complete protection against FasL-induced cell death. However, Fas is not essential in determining β-cell mass or susceptibility to streptozotocin- or HFD-induced diabetes. Importantly, Fas deletion in β-cells leads to increased p65 expression, enhanced glucose tolerance, and glucose-stimulated insulin secretion, with increased exocytosis as manifested by increased changes in membrane capacitance and increased expression of Syntaxin1A, VAMP2, and munc18α. Together, our study shows that Fas in the β-cells indeed plays an essential role in the canonical death receptor-mediated apoptosis but is not essential in regulating β-cell mass or diabetes development. However, β-cell Fas is critical in the regulation of glucose homeostasis through regulation of the exocytosis machinery.

Under basal conditions, β-cells do not constitutively express Fas. However, during diabetes progression, inflammatory cytokines, including IL-1β, induce upregulation of Fas, leading to β-cell loss (20, 21, 32). Nonobese diabetic (NOD) mice homozygous for the lpr (lymphoproliferative) mutation, thus lacking functional Fas, were shown to be protected from diabetes development in vivo (5, 12). Furthermore, adoptive transfer experiments in which splenocytes from NOD donors were transferred into irradiated NOD-lpr/lpr mice showed protection from diabetes (12), which implicated Fas-mediated β-cell apoptosis to be essential for diabetes development in the NOD mice.

Although Fas has been implicated in the pathogenesis of type 1 diabetes, the definitive role of Fas specifically in pancreatic β-cells has been difficult to ascertain in the lpr/lpr mouse model for two major reasons. First, the absence of Fas in the whole body does not provide a clear understanding of the role of Fas specifically in the β-cells; and second, the lymphoproliferative disorder observed in this model fails to provide a true physiological representation of the homeostatic state during diabetes progression in mice.

In addition to the role of apoptosis in disease development, apoptosis is an important mechanism for tissue remodeling during development and adult life. Furthermore, in contrast to the death-inducing role of Fas, increasing evidence suggests that ligation of Fas can also trigger a variety of nonapoptotic cellular processes, including proliferation and survival (7, 28). Thus, although Fas-FasL interactions may induce apoptosis in some cell types under specific conditions, they may also protect cells and regulate tissue regeneration. It appears that Fas functions in a context-dependent manner, and thus its function in specific tissues would be difficult to address in a globally targeted model.

To focus on the role of Fas in the pancreatic β-cells in homeostatic conditions and diabetes development, we targeted Fas specifically in β-cells using the Cre-loxP recombination system. We have shown using this genetic approach that the absence of Fas in pancreatic β-cells leads to protection against FasL-induced apoptosis but does not appear to play an essential role in determining β-cell mass or in providing protection against β-cell apoptosis in the streptozotocin- or high-fat diet (HFD)-induced model of type 1 diabetes and type 2 diabetes, respectively. Rather, loss of Fas in β-cells leads to enhanced function as manifested by improved glucose tolerance and glucose-stimulated insulin secretion due to increased exocytosis of insulin granules.

MATERIALS AND METHODS

Mouse protocol. RIPcre+ m(20) were bred to Fas+/− mice (2, 10) to generate RIPcre+Fas+/− animals, and these mice were inter-

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crossed to generate RIPcre\(^+\), Fas\(^{−/−}\), Fas\(^{+/−}\), and Fas\(^{+/+}\) mice. Mice were maintained on a mixed 129/C57BL/6 background, and only littermates were used as controls. All mice examined were between 8 and 14 wk of age, and both male and female mice were used. Genotyping of the mice was performed by PCR amplification of ear clip DNA, as described previously (10, 35). Mice were maintained on a 12:12-h light-dark cycle with free access to water and standard irradiated rodent chow (5% fat; Harlan Teklad, Indianapolis, IN) and housed in specific pathogen-free barrier facilities at the central animal facility at the Ontario Cancer Institute (Toronto, ON, Canada). Animal experiments were approved by the Ontario Cancer Institute Animal Care Facility.

**Metabolic studies and hormone measurements.** Overnight fasts were 14–16 h in duration. Blood glucose levels were determined from tail venous blood with an automated glucose monitor (One Touch II; Lifescan, Milpitas, CA). Glucose tolerance tests were performed on overnight-fasted animals utilizing a glucose dose of 1 g/kg body wt injected intraperitoneally (ip), and measurements of blood glucose levels were taken at 0, 15, 30, 45, 60, and 120 min after the injection. Insulin tolerance tests were performed utilizing human regular insulin (Novo Nordisk, Toronto, ON, Canada) at a dose of 0.5 U/kg body wt, and blood glucose levels were measured at 0, 15, 30, 45, 60, and 120 min after the injection of insulin. Glucose-stimulated insulin secretion in vivo was performed on overnight-fasted animals after an ip injection of glucose at a dose of 3 g/kg body wt, with tail vein blood collected at 0, 2, and 30 min after the injection. Pancreatic and islet insulin content were determined by acid ethanol extraction and RIA (Linco Research, St. Charles, MO). Serum insulin levels were measured using an enzyme-linked immunosorbent assay kit with a rat insulin/β-cell line (Linco Research). Serum insulin levels were taken at 0, 15, 30, 45, 60, and 120 min after the injection. Glucose-stimulated insulin secretion was determined using an RIA kit (Linco Research). Animals were fasted overnight and were administered by ip injection twice a day, at 9 AM and 5 PM, for 3 days.}

**Insulin and Pdx-1 cDNA isolation.** Total RNA was extracted from isolated islets by TRIzol, following the manufacturer’s protocol (Invitrogen), and treated with RNase-free DNase (Invitrogen). Semiquantitative reverse transcription-PCR (RT-PCR) amplification was performed using a one-step RT-PCR kit (Invitrogen). Insulin and Pdx-1 cDNA were amplified by PCR using specific primers. Densitometric analysis was performed using Image J software. To correct for differences in loading, we corrected densitometric values of insulin and Pdx-1 expression levels with corresponding values of β-actin and calculated the insulin/β-actin and Pdx-1/β-actin ratios.

**Confocal immunofluorescence microscopy.** Isolated islets were trypsinized with 0.05% trypsin-EDTA (1 mg/ml; Gibco) for 10 min in a 37°C incubator. Dispersed β-cells were plated on glass coverslips and kept in a 37°C heated chamber overnight. The following day, the cells were stimulated with mouse IL-1β (5 ng/ml) for 1 h at 37°C. The cells were fixed with 2% paraformaldehyde and rinsed in PBS for 5 min. The β-cells were then blocked with 5% normal goat serum with 0.1% saponin for 1 h at room temperature. After the cells were washed with PBS, they were incubated with primary antibodies against insulin and p65 for 1 h at 1:100 and 1:50 dilution, respectively, followed by appropriate fluorochrome secondary antibodies. The coverslips were mounted on slides and examined using a laser-scanning confocal imaging system (LSM510) equipped with the LSM software version 5.00 (Carl Zeiss, Oberkochen, Germany).

**Islet morphology, immunohistochemistry, and immunofluorescence.** Pancreatic tissue was fixed for 24 h in 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Samples were dehydrated and prepared as paraffin blocks. Seven-micrometer-thick sections were obtained at 150-µm intervals on at least three levels and stained with insulin, Ki-67 (DAKO), glucagon (NovoCastra Laboratories), and synaptophin (Boehringer Mannheim). Total islet area and number as well as total pancreatic area were determined on synaptophysin-stained sections, as described previously (17). Total islet area was calculated per total pancreatic area, and total islet number was counted per pancreatic section. Immunofluorescent-stained sections were visualized using a Zeiss inverted fluorescent microscope.

**Islet perifusion secretory assay.** Batches of 50 islets on average were placed in 37°C perfusion chambers and perfused at a flow rate of 1 ml/min with a Krebs-Ringer bicarbonate buffer. Islets were initially equilibrated for 30 min in Krebs-Ringer bicarbonate HEPES buffer supplemented with 2.8 mmol/l glucose. They were then stimulated with 2.8 mmol/l glucose for 10 min, which was followed by stimulation with 16.7 mmol/l glucose for 40 min. After 40 min with 16.7 mmol/l glucose alone, the islets were subjected to an additional 40-min stimulation period with 30 mmol/l KCl. Fractions were collected for insulin determination using an RIA kit (Linco Research). At the end of each perifusion, islets were collected to assess insulin content. Results are presented as insulin secreted normalized to total insulin content.

**Capacitance measurements.** Cells were patch-clamped in conventional whole cell configuration at 33–34°C. β-Cells were identified by cell size (>4 picofarad (pF)) and by their Na\(^+\) current inactivation properties. Capacitance measurements were performed using EPC-9 amplifier and Pulse software from HEKA Electronik (Lambrecht, Germany). Patch pipettes had typical resistances of 3–6 MΩ. Islet viability was assessed using trypan-stained isolated islets to generate single-cell suspensions and performing annexin V/7-amino-actinomycin D (7-AAD) binding assay. Cells were resuspended with annexin V binding buffer and incubated with 1 µl of annexin V/100 µl of binding buffer and 0.5 µm of 7-AAD at room temperature for 15 min. Cells were washed again with annexin V binding buffer. The percentage of 7-AAD\(^+\) and Annexin V\(^+\) cells was determined by flow cytometry with a FACS Calibur (Becton Dickinson) and analyzed using CELLQuest software (Becton Dickinson).

**Western blotting.** Islets, hypothalamus, liver, muscle, and fat were isolated, and protein lysates were obtained as described previously (36). The lysates were separated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis and then immunoblotted with antibodies to c-FLIP (Alexis Biochemicals, San Diego, CA), pancreas duodenum homeobox-1 (Pdx-1; Chemicon, Temecula, CA), Fas, NF-κB p65 (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-NF-κB p65 (Cell Signaling Technology, Toronto, ON, Canada), syntaxin 1A (Sigma), VAMP2, and munc18a (BD Transduction Laboratories, San Jose, CA). Western blot signal densities were analyzed using Image J software. Protein levels were normalized with α-tubulin and expressed in arbitrary units relative to littermate control levels.

**Reverse transcription-PCR.** mRNA was extracted from isolated islets by TRIZol, following the manufacturer’s protocol (Invitrogen), and treated with RNase-free DNase (Invitrogen). Semiquantitative reverse transcription-PCR (RT-PCR) amplification was performed using a one-step RT-PCR kit (Invitrogen). Insulin and Pdx-1 cDNA were amplified by PCR using specific primers. Densitometric analysis was performed using Image J software. To correct for differences in

**Administration of exendin-4.** Synthetic exendin-4 (Ex-4) (Sigma) was dissolved in PBS at a concentration of 8 nmol/µl. Ex-4 was administered by ip injection twice a day, at 9 AM and 5 PM, for 3 consecutive days at a dose of 24 nmol/kg body wt. PBS-injected RIPcre\(^+\), Fas\(^{+/−}\), and Fas\(^{+/+}\) mice were used as controls.
analysis of cell proliferation, pancreatic sections were stained for Ki-67. Ki-67-positive cells were analyzed as a percentage of total islet cells.

**Streptozotocin protocol.** Multiple low doses of streptozotocin (MLDS) (40 mg/kg body wt) were injected into mice for 5 consecutive days, as described previously (17). Blood glucose was measured weekly after ip MLDS injection.

**HFD-induced diabetes.** RIPcre<sup>Fas<sup>+</sup></sup> mice did not exhibit any differences in weight compared with wild-type control mice, suggesting that hypothalamic function was not affected in the mutant mice (Fig. 1B). Consistent with the absence of Fas in the β-cells, islets of RIPcre<sup>Fas<sup>+</sup></sup> mice were protected from FasL-induced islet cell killing, showing the lack of functional Fas in the islets of these mice (Fig. 1C). We also performed a ceramide-induced killing assay in isolated islets, because this compound has been shown to induce cell death using the Fas-mediated pathway (11). Indeed, islets of RIPcre<sup>Fas<sup>+</sup></sup> mice were protected from ceramide-induced killing compared with those of control mice (Fig. 1D), further supporting that Fas is absent in the islets of RIPcre<sup>Fas<sup>+</sup></sup> mice.

**RESULTS**

**Generation of RIPcre<sup>Fas<sup>+</sup></sup> mice.** Mice lacking Fas in the pancreatic β-cells were generated by breeding animals harboring the Fas gene flanked by loxP sites (Fas<sup>fl/fl</sup>) (2, 10) to mice expressing the Cre transgene under the control of the rat insulin promoter (RIPcre<sup>+</sup>) (30). We observed efficient deletion of Fas specifically in the pancreatic β-cells, as confirmed by Western blotting of lysates from isolated islets. The expression of Fas in the hypothalamus, liver, muscle, and fat was unaffected (Fig. 1A). RIPcre<sup>Fas<sup>+</sup></sup> mice did not exhibit any differences in weight compared with wild-type control mice, suggesting that hypothalamic function was not affected in the mutant mice (Fig. 1B). Consistent with the absence of Fas in the β-cells, islets of RIPcre<sup>Fas<sup>+</sup></sup> mice were protected from FasL-induced islet cell killing, showing the lack of functional Fas in the islets of these mice (Fig. 1C). We also performed a ceramide-induced killing assay in isolated islets, because this compound has been shown to induce cell death using the Fas-mediated pathway (11). Indeed, islets of RIPcre<sup>Fas<sup>+</sup></sup> mice were protected from ceramide-induced killing compared with those of control mice (Fig. 1D), further supporting that Fas is absent in the islets of RIPcre<sup>Fas<sup>+</sup></sup> mice.

**Fig. 1. Efficient deletion of Fas in RIPcre<sup>Fas<sup>+</sup></sup> mice.** A: representative Western blots showing absence of Fas expression in isolated islets (I) and intact Fas expression in the hypothalamus (H), liver (L), muscle (M), and fat (F) tissues of RIPcre<sup>Fas<sup>+</sup></sup> mice (n = 5/genotype). *P < 0.01. B: weights of RIPcre<sup>Fas<sup>+</sup></sup> mice are similar to control mice at 8 wk of age (n = 5/genotype). Results represent means ± SE. C: FasL-mediated killing assay shows that islets of RIPcre<sup>Fas<sup>+</sup></sup> mice are protected from β-cell death (n = 5/genotype). D: ceramide-killing assay showing protection from β-cell destruction in islets of RIPcre<sup>Fas<sup>+</sup></sup> animals (n = 5/genotype). +, +/+ = RIPcre<sup>Fas<sup>+</sup></sup> fl/fl; +, −/+ = RIPcre<sup>Fas<sup>+</sup></sup> Fl/fl; +, −/− = RIPcre<sup>Fas<sup>+</sup></sup> fl/fl. 7-AAD, annexin V/7-amino-actinomycin D.
RIPcre-Fas<sup>fl/fl</sup> mice and their littermate controls (Fig. 2D), which suggests that the enhanced glucose tolerance was attributed to Fas deletion in the β-cells in RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> mice rather than to changes in peripheral insulin sensitivity. In keeping with the enhanced glucose tolerance in RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> mice, insulin secretion in response to an ip glucose challenge was increased in RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> mice compared with control mice in vivo (Fig. 2E). We then proceeded to examine individual islets to assess glucose-stimulated insulin secretion in vitro by perifusion. Our results showed that islets from RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> mice exhibited increased glucose-stimulated insulin secretion compared with littermate RIPcre<sup+</sup>Fas<sup>/H11001</sup>/H11001<sup>/H11001</sup> controls in response to high glucose (16.7 mM) and KCl (Fig. 3, A and B). Furthermore, the RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> mice had similar islet insulin content (Fig. 3C) and total pancreatic insulin content (Fig. 3D) compared with those of the control mice.

Increased exocytosis of insulin in RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> islets. To determine whether the increased insulin release upon glucose and KCl stimulation in the RIPcre<sup>+</sup>Fas<sup>fl/fl</sup> islets correlated with an increase in the exocytosis of insulin granules from individual β-cells, we measured using patch-clamp capacitance measurements (Fig. 4A). The first two depolarization pulses, which determine the initial readily releasable pool of insulin granules,
Fig. 4. Fas deletion in the pancreatic β-cells leads to increased exocytosis of insulin. A train of ten 500-ms depolarization pulses was applied to the β-cells, and changes in cell membrane capacitance (ΔCm) were measured. ΔCm density [femtofarad/picofarad (fF/pF)] is the capacitance change normalized to cell size. A: representative capacitance traces recorded from RIPcreFas+/+ and RIPcreFas−/− β-cells. The inset shows the electrical depolarization protocol. B: summary of the cumulative increases in cell capacitance for each depolarizing pulse. C: summary of ΔCm evoked by the first 2 pulses (pulses 1–2), the last 8 pulses (pulses 9–10), and all 10 depolarization pulses (pulses 1–10). n = 19 for RIPcreFas+/+ β-cells; n = 23 for RIPcreFas−/− β-cells. *P < 0.05; **P < 0.01. D: representative Ca2+ currents evoked by the 1st pulse during a train of 10 depolarizations. Currents associated with the capacitance currents evoked by the 1st pulse during a train of 10 depolarizations and found no differences between the two groups (Fig. 4, D and E). We also assessed for key proteins involved in the exocytosis of insulin granules, including plasma membrane syntaxin 1A, vesicle-associated membrane protein 2 (VAMP2), and exocytosis-priming protein munc18a. Expression levels of these proteins by Western blot were increased in the RIPcreFas−/− mice compared with control mice (Fig. 4F), which may facilitate the enhanced insulin secretion observed in these mice.

Fas deletion in β-cells has no effect on islet mass or glucagon-like peptide 1-mediated β-cell proliferation. β-Cell mass is dictated by a dynamic balance between neogenesis from a precursor cell, proliferation of preexisting β-cells, and apoptosis (3). The Fas/FasL system has been implicated in maintaining tissue homeostasis (7, 28, 33). However, the role of Fas specifically in β-cell homeostasis was unclear. To evaluate whether Fas deletion in pancreatic β-cells has an effect on β-cell growth and mass determination under basal conditions, we examined pancreatic islet morphology. RIPcreFas−/− mice showed similar total islet area/total pancreatic area and islet number/pancreatic section compared with their controls (Fig. 5, A and B). These results indicate that Fas deletion does not have any significant effect on β-cell homeostasis. Furthermore, islet architecture was intact in islets of RIPcreFas−/− mice, suggesting that Fas is not required.

resulted in an increased change in membrane capacitance in the RIPcreFas+/+ β-cells [12.73 ± 2.24 femtofarad (fF)/pF] compared with control cells (4.64 ± 0.71 fF/pF). Similarly, the incremental and cumulative increases in cell membrane capacitance between the third and tenth pulses, which measure the rate of insulin granule mobilization to the releasable pool, were also increased in the RIPcreFas+/+ β-cells (28.49 ± 2.77 fF/pF in RIPcreFas+/+ β-cells vs. 19.99 ± 1.96 fF/pF in wild-type cells; Fig. 4, B and C). We analyzed whether there were any differences in voltage-dependent Ca2+ magnitude between the mutant and wild-type mice by the first pulse during a train of 10 depolarizations and found no differences between the two groups (Fig. 4, D and E). We also assessed for key proteins involved in the exocytosis of insulin granules, including plasma membrane syntaxin 1A, vesicle-associated membrane protein 2 (VAMP2), and exocytosis-priming protein munc18a. Expression levels of these proteins by Western blot were increased in the RIPcreFas−/− mice compared with control mice.
for the maintenance of normal β- and α-cell distribution (Fig. 5C).

To assess the role of Fas in β-cell proliferation, we administered Ex-4, a long-acting glucagon-like peptide 1 (GLP-1) agonist, into the mice for 3 consecutive days and stained the pancreatic sections for a proliferation marker, Ki-67 (9). Our data show that stained the pancreatic sections for a proliferation marker, administered Ex-4, a long-acting glucagon-like peptide 1 (Fig. 5C). Ki-67 positivity was calculated as %total islet/pancreatic area in the mutant mice (n = 4/genotype). Original magnification ×20. *P < 0.05. All mice were 8–14 wk of age. +/+ = RIPcre+; +/− = RIPcre−/+; −/− = RIPcre−−. Results represent means ± SE.

Fig. 5. Islet morphometry in RIPcre+ mice. A: no difference was observed in total islet/pancreatic area in RIPcre+ compared with controls, as calculated on synaptophysin-stained pancreatic sections (n ≥ 4/genotype). B: islet number was analyzed per pancreatic section, since pancreatic area was similar between RIPcre+ and control mice (n ≥ 4/genotype). C: pancreatic section stained with insulin (red) and glucagon (green) revealed intact islet architecture in the mutant mice (n = 3/genotype). Original magnification ×20. D: Ki-67-stained pancreatic sections showing the increase in β-cell proliferation following exendin-4 (Ex-4) administration. Ki-67 positivity was calculated as %total islet cells (n = 4/group). Original magnification ×40. *P < 0.05. Western blots and RT-PCR results show that c-FLIP expression remained unchanged in RIPcre+ mice compared with controls, as assessed by RT-PCR (Fig. 5D). Finally, we examined c-FLIP (cellular FLICE-inhibitory protein) expression in isolated islets, because c-FLIP is a critical component of the death-inducing signaling complex, which is formed upon ligation of Fas (29, 31). Western blot analyses showed that c-FLIP expression remained unchanged in RIPcre+ mice (Fig. 6A), consistent with the absence of changes in β-cell mass in the mutant mice compared with control mice.

RIPcre+ mice are not protected from streptozotocin- or HFD-induced diabetes. To examine whether Fas deletion in the β-cells would provide protection against β-cell apoptosis, we used two experimental diabetes models to recapitulate type 1 and type 2 diabetes pathogeneses in the mice. Experimental type 1 diabetes was induced by MLDS (17, 19), whereas a HFD was used to induce type 2 diabetes in the mice. Our results showed that the RIPcre+ mice were not protected from the development of diabetes following MLDS. Both mutant and wild-type mice with STZ injections showed a similar rise in blood glucose levels. Furthermore, body weight, fasting serum insulin levels, and islet area were similar between the genotypes (Fig. 7, A–D), suggesting that Fas is not an essential mediator of β-cell death in the MLDS model of...
type 1 diabetes. When the mice were subjected to a HFD for 12wk, there were no differences between the weights (Fig. 7E) and random blood glucose levels (Fig. 7F) of the RIPcre^Fas^+/+ and wild-type mice. Glucose tolerance tests after 12wk on HFD also showed no difference between the two genotypes (Fig. 7G), which suggests that β-cell Fas deletion cannot protect mice against type 2 diabetes development.

Fig. 6. p65 (NF-κB), pancreas duodenum homeobox-1 (Pdx-1), insulin, and cellular FLICE inhibitory protein (c-FLIP) expression in islets of RIPcre^Fas^+/+ mice. A: Western blots of lysates of isolated islets showing no changes in Pdx-1 protein expression and increased phospho-p65 and total p65 protein levels in islets of RIPcre^Fas^+/+ compared with control mice (n = 6/genotype). c-FLIP expression in islets was similar between RIPcre^Fas^+/+ and control mice (n = 4/genotype). *P < 0.05. B: RT-PCR showing increased insulin and unchanged Pdx-1 transcript levels in isolated islets of RIPcre^Fas^+/+ compared with wild-type mice (n = 5/genotype). *P < 0.001. C: confocal immunofluorescence microscopy stained for insulin (red) and p65 (green) to detect the localization of p65 in the β-cells under basal conditions and following IL-1β stimulation for 1h. Original magnification x63. All mice were 8–14wk of age. *P < 0.05, +, +/+ = RIPcre^Fas^+/+; +, −/− = RIPcre^Fas^−/−. Results represent means ± SE.

DISCUSSION

The Fas/FasL system plays a pivotal physiological role in the regulation of apoptotic processes, including cytotoxic T cell-mediated tissue destruction, immune privilege, and tumor surveillance (reviewed in Ref. 16). Thus, the Fas/FasL system was appreciated mainly for its role in cell death. However, increasing evidence shows that activation of Fas can also result in nonapoptotic responses, including cell proliferation. For example, liver cells die through Fas-mediated apoptosis during viral hepatitis and liver cirrhosis, but Fas is also involved in liver regeneration subsequent to partial hepatectomy (7, 28). Furthermore, it has previously been reported that, although central nervous system-derived tumor cells die by Fas-mediated apoptosis, Fas ligation in neurons may also accelerate functional recovery from experimental sciatic nerve crush injury in mice (6). Thus, accumulating data suggest a physiological role for Fas in both apoptotic and nonapoptotic activities in a context- and tissue-dependent manner.

Previous studies using the mouse model with the lpr mutation, and therefore lacking functional Fas, suggest the importance of Fas in maintaining tissue homeostasis, particularly in the immune system, since these mice present with lymphadenopathy and splenomegaly due to the accumulation of immune cells (33). However, elucidating the function of Fas in the pancreatic β-cells, the focus of our study, would be difficult to address in a globally targeted model such as the lpr mutation model. We circumvented these confounders by targeting Fas specifically in the pancreatic β-cells using the Cre-loxP system.

Our results show that Fas deletion in the β-cells leads to a significant enhancement in β-cell function. Phospho-p65 expression was increased in the islets of the RIPcre^Fas^+/+ mice. The increase in activated p65 and nuclear localization in the Fas-deficient β-cells are in keeping with previous studies, which show activated NF-κB to be necessary in regulating insulin secretion (26). Our results show no difference in Pdx-1 levels in the mutant mice compared with control mice. However, there was an increase in insulin gene expression despite the lack of difference in insulin protein content between the RIPcre^Fas^+/+ and wild-type mice. The mechanisms behind these discrepant findings are not clear.

Caspase-8 is a critical component of Fas-mediated cellular apoptosis because caspase-8 deletion in various tissues, including β-cells, has been shown to be protective against Fas-induced cell death (14, 18). Similarly, Fas deficiency also led to complete protection against FasL-induced cell death, confirming Fas and caspase-8 to play an essential role in the canonical death receptor-mediated apoptosis. Interestingly, caspase-8 has also been reported to play a critical role in regulating β-cell survival and maintaining β-cell mass under physiological conditions (18). As a result, β-cell caspase-8-deficient mice develop progressive hyperglycemia and a decline in β-cell mass with aging, resulting in a defect in glucose-stimulated insulin secretion in vivo. However, despite the decreased insulin secretion in the whole mouse, the individual islets of the β-cell caspase-8 mutant mice demonstrate increased insulin secretion upon glucose stimulation (18). These results on insulin secretion are reminiscent of our data presented in the current study on Fas-deficient β-cells, which suggest that Fas and caspase-8 may cooperate and function in
a similar molecular pathway that regulates insulin secretion. In contrast, the prosurvival effects of caspase-8 on the β-cells during homeostasis are not observed in the absence of Fas, which suggests that the homeostatic function of caspase-8 in β-cells does not appear to depend on Fas.

Fas was initially thought to be critical in β-cell apoptosis in type 1 diabetes since NOD mice homozygous for the lpr mutation were protected from diabetes development (5, 12). However, this is most likely a result of their dysregulated immune system from the lpr mutation rather than the role of Fas in β-cell destruction per se (34). In keeping with this notion, NOD-lpr/lpr islets transplanted into normal female NOD mice did not give protection from diabetes development (1). Therefore, our results are consistent with studies that show only a minor role for Fas in β-cell apoptosis in type 1 diabetes models (1, 2). Other reports have shown that Fas is required for the initiation of autoimmunity (insulitis) in the NOD mouse model and not in the destruction of the β-cells and in the progression of the disease per se (25, 34). However, in these studies, Fas was not targeted specifically in the β-cells, but rather the lpr/lpr model (1) or anti-FasL antibody administration (25) was employed in the NOD model. Due to the presence of Fas and FasL on both the β-cells and the autoreactive T cells, in addition to other immune cells these studies are not definitive in elucidating the role of Fas specifically in the pancreatic β-cells in type 1 diabetes pathogenesis.

Our findings on the role of Fas on β-cell function differ from that published by another group (31). The phenotypes of the mice examined in the two studies are diametrically opposite, including the results of glucose tolerance tests and insulin secretion tests. Although the explanation for these significant differences is not readily apparent, one major difference to note is the genetic differences of the mice used. The mice used in our present study had β-cell-specific Fas deletion and were on a mixed 129J-C57BL/6 background, whereas some data presented in the study by Schumann et al. (31) were obtained from mice homozygous of the lpr mutation, which lack functional Fas in the whole body and display the lymphoproliferative disorder across various genetic backgrounds, including MRL and B6 (35).

In summary, our genetic approach to elucidate the role of Fas in β-cells shows that Fas deletion in the pancreatic β-cells leads to protection against FasL-induced cell death; however, Fas is not an essential mediator of β-cell death in the MLDS or HFD diabetes model or in β-cell homeostasis. Importantly, absence of Fas in β-cells leads to enhanced function, as evidenced by improved glucose tolerance and glucose-stimulated insulin secretion both in vivo and in vitro. Further studies on the NOD model would better delineate the role of Fas in the pancreatic β-cells during progression of type 1 diabetes.
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