OBJECTIVE—Cytokines contribute to β-cell destruction in type 1 diabetes. Endoplasmic reticulum (ER) stress-mediated apoptosis has been proposed as a mechanism for β-cell death. We tested whether ER stress was necessary for cytokine-induced β-cell death and also whether ER stress gene activation was present in β-cells of the NOD mouse model of type 1 diabetes.

RESEARCH DESIGN AND METHODS—INS-1 β-cells or rat islets were treated with the chemical chaperone phenyl butyric acid (PBA) and exposed or not to interleukin (IL)-1β and γ-interferon (IFN-γ). Small interfering RNA (siRNA) was used to silence C/EBP homologous protein (CHOP) expression in INS-1 β-cells. Additionally, the role of ER stress in lipid-induced cell death was assessed.

RESULTS—Cytokines and palmitate triggered ER stress in β-cells as evidenced by increased phosphorylation of PKR-like ER kinase (PERK), eukaryotic initiation factor (EIF2)α, and Jun NH<sub>2</sub>-terminal kinase (JNK) and increased expression of activating transcription factor (ATF)4 and CHOP. PBA treatment attenuated ER stress, but JNK phosphorylation was reduced only in response to palmitate, not in response to cytokines. PBA had no effect on cytokine-induced cell death but was associated with protection against palmitate-induced cell death. Similarly, siRNA-mediated reduction in CHOP expression protected against palmitate- but not against cytokine-induced cell death. In NOD islets, mRNA levels of several ER stress genes were reduced (ATF4, BiP [binding protein], GRP94 [glucose regulated protein 94], p58, and XBP-1 [X-box binding protein 1] splicing) or unchanged (CHOP [binding protein], GRP94 [glucose regulated protein 94]) in comparison, we also assessed the role of ER stress in cytokine-induced cell death. For pancreatic islets depleted in CHOP are partially protected against NO-induced cell death (6). These correlations led us to investigate the role of ER stress in cytokine-induced β-cell death. Diabetes 57:3034–3044, 2008

Recent studies have proposed that cytokine-induced β-cell death involves endoplasmic reticulum (ER) stress (6–8). The ER stress response is an evolutionarily conserved, adaptive program triggered by accumulation of unfolded protein in the lumen of the ER (9,10). Upon initiation of ER stress, cells activate intracellular signaling pathways that transmit information from the ER to the cytoplasm and nucleus, known as the unfolded protein response (UPR). The UPR includes transient attenuation of de novo protein synthesis that limits protein load on the ER. This is followed by transcriptional activation of genes encoding ER chaperones to increase protein folding capacity in the ER and proteins involved in ER-associated degradation to eliminate misfolded proteins by the ubiquitin-proteasome system. When functions of the ER are severely or irreversibly impaired, the UPR activates apoptosis. Signaling from the ER is initiated by ER-localized stress sensors PKR-like ER kinase (PERK), activating transcription factor (ATF)6, and inositol-requiring (IRE)1, which remain inactive under nonstress conditions due to association of their ER luminal domains with binding protein (BiP) (11). Upon accumulation of unfolded protein, BiP dissociates from the sensor proteins, leading to their activation. PERK activation by phosphorylation leads to phosphorylation of the eukaryotic initiation factor (EIF2)α, which in turn leads to translational attenuation and induction of ATF4 (12). ER stress can lead to apoptosis by various pathways, including via ATF4 transcriptional activation of the gene for C/EBP homologous protein (CHOP) (13–15) and activation of Jun NH<sub>2</sub>-terminal kinase (JNK) and caspase-12 (9,10).

The importance of ER stress signaling in regulating β-cell function and survival was first demonstrated in PERK-deficient mice (16) and in mice with a mutation in the EIF2α phosphorylation site (Ser51Ala) (17,18). Other experiments in the Akita mouse have shown that β-cell apoptosis and diabetes could be delayed by inhibiting CHOP induction (14). That ER stress is at least partially required for lipoapoptosis in β-cells was suggested by the recent finding that MIN6 cells overexpressing BiP are partially protected against apoptosis induced by the saturated fatty acid palmitate (19). Furthermore, increased ER stress gene expression has been observed in islets of db/db mice (19) and humans with type 2 diabetes (19,20). The relevance of ER stress signaling to β-cell destruction in type 1 diabetes was suggested in studies showing that treatment of β-cells with cytokines or NO leads to increased expression of ATF4 and CHOP (6–8) and that pancreatic islets depleted in CHOP are partially protected against NO-induced cell death (6). These correlations led us to investigate the role of ER stress in cytokine-induced β-cell death and the development of type 1 diabetes. For comparison, we also assessed the role of ER stress in lipid-induced β-cell death.
**RESEARCH DESIGN AND METHODS**

**Cell culture.** INS-1 cells were passaged in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 0.2 mmol/l glutamine, 10 mmol/l HEPES, 10% heat-inactivated FBS, 100 units/ml penicillin, 1 mmol/l sodium pyruvate, and 50 mmol/l 2-mercaptoethanol. MIN6 cells were passaged in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) containing 25 mmol/l glucose, 10 mmol/l HEPES, 10% FCS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded at either 1–1.5 × 10⁵ in 0.5 ml per well in a 24-well plate or at 5 × 10⁶ in 2 ml per well in a 6-well plate. Cells were pretreated with the chemical chaperones phenyl butyric acid (PBA) (2.5 mmol/l, Sigma, St. Louis, MO) or trimethylamine N-oxide (TMAO) (100 µmol/l) for 24 h. Cells were then treated with PBA or TMAO in combination with 50 units/ml IL-1β and 100 units/ml IFN-γ (R&D Systems, Minneapolis, MN) or 0.4 mmol/l palmitate coupled to 0.92% BSA (palmitate to BSA molar ratio 2:1) (Sigma). Cell death was measured with an ELISA kit (Cell Death Detection ELISA; Roche Diagnostics, Castle Hill, Australia) (21). NO was measured in culture media using Griess reagent.

**Rat islet isolation and culture.** Islets were isolated from male Wistar rats (175–200 g) by pancreatic digestion with liberase RI (Roche Diagnostics). Islets were further separated with a Ficoll-Paque PLUS gradient (GE Healthcare Bio-Sciences, Uppsala, Sweden) and handpicked under a stereomicroscope. Procedures were approved by the Garvan Institute/St. Vincent’s Hospital Animal Experimentation Ethics Committee following guidelines issued by the National Health and Medical Research Council of Australia.

**Islets were cultured for 3–4 days at 37°C in RPMI-1640 medium supplemented with 0.2 mmol/l glutamine, 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Islets with central necrosis were discarded, and those of similar size were then pretreated with 2.5 mmol/l PBA for 24 h. Islets were then treated for 16 h with 2.5 mmol/l PBA in combination with 50 units/ml IL-1β and 100 units/ml IFN-γ. Cell death was assessed as above, and RNA was extracted for RT-PCR analysis.

**Plasmid construction and transfection.** Rat BIP cDNA was amplified by PCR, cloned into the Gateway donor vector pDONR221, and then subcloned into the Gateway expression vector pcDNA-DEST40 (Invitrogen). Two days before transfection, INS-1 cells were seeded at 3 × 10⁵ cells per well in a 12-well plate. BIP-DEST40 or control pmaxGFP (green fluorescent protein) was transfected into INS-1 cells using DharmaFECT Transfection Reagent (Dharmacon, Lafayette, CO). Forty eight hours later, nontargeting siRNA were transfected into INS-1 cells using Lipofectamine 2000 (Invitrogen) with 0.2 mmol/l glutamine, 10% heat-inactivated FBS, 100 units/ml penicillin, 10% FCS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded at either 1–1.5 × 10⁵ in 0.5 ml per well in a 24-well plate or at 5 × 10⁶ in 2 ml per well in a 6-well plate. Cells were pretreated with the chemical chaperones phenyl butyric acid (PBA) (2.5 mmol/l, Sigma, St. Louis, MO) or trimethylamine N-oxide (TMAO) (100 µmol/l) for 24 h. Cells were then treated with PBA or TMAO in combination with 50 units/ml IL-1β and 100 units/ml IFN-γ (R&D Systems, Minneapolis, MN) or 0.4 mmol/l palmitate coupled to 0.92% BSA (palmitate to BSA molar ratio 2:1) (Sigma). Cell death was measured with an ELISA kit (Cell Death Detection ELISA; Roche Diagnostics, Castle Hill, Australia) (21). NO was measured in culture media using Griess reagent.

**RESULTS**

**PBA attenuates cytokine-induced ER stress in INS-1 cells.** We first tested whether PBA was capable of attenuating ER stress induced by cytokines in INS-1 cells. PBA acts as a chemical chaperone to reduce the load of unfolded proteins in the ER by improving folding capacity and trafficking of mutant proteins out of the ER (24). In control (non–PBA-treated) INS-1 cells, IL-1β + IFN-γ treatment induced ER stress after both 6 and 24 h, as evidenced by increased phosphorylation of PERK (Fig. 1A and B) and EIF2α (Fig. 1A and C) and increased protein expression (Fig. 1A and C) and reduced protein expression (Fig. 1A and D and E) and mRNA levels (Fig. 1F and G) of ATF4 and CHOP. In PBA-treated INS-1 cells, ER stress due to IL-1β + IFN-γ was attenuated, as indicated by reduced PERK (Fig. 1A and B) and EIF2α phosphorylation (Fig. 1A and C) and reduced protein expression (Fig. 1A, D, and E) and mRNA levels (Fig. 1F and G) of ATF4 and CHOP.

**PBA does not protect against cytokine-induced cell death in INS-1 cells.** We tested the sensitivity of PBA-treated INS-1 cells to cytokine-mediated cytotoxicity. In control (non–PBA-treated) INS-1 cells, IL-1β + IFN-γ treatment led to a significant increase in cell death after both 6 and 24 h (Fig. 1H). However, PBA treatment did not protect INS-1 cells from cytokine-induced cell death; IL-1β + IFN-γ-induced cell death was similar in control and PBA-treated cells after both 6 and 24 h (Fig. 1H). Thus, PBA-treated cells retain their sensitivity to IL-1β + IFN-γ cytotoxicity despite the attenuation of ER stress, suggesting that ER stress induced by cytokines is not required for β-cell death.

**PBA protects against palmitate-induced cell death and ER stress in INS-1 cells.** We also tested the sensitivity of PBA-treated INS-1 cells to lipotoxicity induced by the saturated fatty acid palmitate. In control INS-1 cells, treatment with 0.4 mmol/l palmitate for 6 h led to a significant increase in cell death (Fig. 2F). Palmitate treatment of control INS-1 cells was also associated with induction of ER stress, as indicated by increased phosphorylation of PERK (Fig. 2A and B) and EIF2α (Fig. 2A and C) and increased expression of ATF4 (Fig. 2A and D) and CHOP (Fig. 2A and E). In PBA-treated INS-1 cells, palmitate-induced ER stress was attenuated, as indicated by reduced PERK (Fig. 2A and B) and EIF2α (Fig. 2A and C) phosphorylation and decreased expression of ATF4 (Fig. 2A and D) and CHOP (Fig. 2A and E). Strikingly, this was associated with a complete block of palmitate-induced cell death in PBA-treated INS-1 cells (Fig. 2F). Thus, PBA-treated cells associated selective resistance to the toxic effects of palmitate. These data suggest that,
Unlike cytokines, ER stress is required for lipid-induced cell death in β-cells.

We further assessed the role of ER stress in β-cell death using another chemical chaperone, trimethylamine oxide (TMAO). Treatment of INS-1 cells with TMAO reduced ER stress, as indicated by decreased
ATF4 and CHOP protein expression in palmitate- (Supplementary Fig. 1A and B) and cytokine-treated (Supplementary Fig. 2A and B) INS-1 cells. This was associated with reduced cell death in palmitate-treated cells (Supplementary Fig. 1C), whereas cytokine-induced cell death was not affected (Supplementary Fig. 2C). Thus, the data using TMAO support the suggestion that ER stress is required for lipid- but not for cytokine-induced cell death. We also examined cleaved caspase-3 expression under these experimental conditions. Cleaved caspase-3 expression was induced in both palmitate- (Supplementary Fig. 3A) and cytokine-treated (Supplementary Fig. 3B) INS-1 cells. In PBA- and TMAO-treated INS-1 cells, cleaved caspase-3 expression was reduced in response to palmitate (Supplementary Fig. 3A) and not in response to cytokines (Supplementary Fig. 3B).

PBA treatment does not lower cytokine-induced iNOS expression or NO production in INS-1 cells. To check that the ability of PBA to attenuate cytokine-induced ER stress was not due to lower NO production, we measured iNOS expression and NO production after cytokine exposure in control and PBA-treated β-cells. Treatment of INS-1 cells with IL-1β + IFN-γ led to an increase in iNOS expression (Fig. 3B) and stimulated production of NO (Fig. 3A). With PBA treatment, cytokine-induced iNOS expression and NO production were not
When INS-1 cells were treated with palmitate, we found no induction of iNOS expression and no stimulation of NO production (data not shown). PBA treatment lowers palmitate-induced XBP1 splicing, whereas cytokines do not activate XBP1 in INS-1 cells.

We next examined ER stress signaling downstream of IRE1 by assessing XBP1 splicing. Palmitate treatment led to an increase in the spliced form of XBP1 mRNA in INS-1 cells (Fig. 4A), indicating activation. In contrast, cytokine treatment was associated with reduced XBP1 splicing in INS-1 cells (Fig. 4B), suggesting reduced signaling via this arm of the ER stress response. In PBA-treated cells, palmitate activation of XBP1 was attenuated, as indicated by reduced mRNA levels of the spliced form (Fig. 4A). PBA lowers JNK phosphorylation in response to palmitate but not in response to cytokines. We next examined JNK phosphorylation (activation), which can also be stimulated by ER stress downstream of IRE1 (25). Increased phosphorylation of JNK1 (46 kDa) and JNK2 (54 kDa) was observed in both palmitate- (Fig. 5A–C) and cytokine-treated (Fig. 5D–F) INS-1 cells. PBA treatment inhibited (Fig. 3A and B). When INS-1 cells were treated with palmitate, we found no induction of iNOS expression and no stimulation of NO production (data not shown).
lowered JNK1/2 phosphorylation in response to palmitate (Fig. 5A–C), suggesting that ER stress is required for palmitate-induced JNK activation. In contrast, cytokine-induced JNK1/2 phosphorylation was not significantly altered by PBA treatment (Fig. 5D–F), suggesting that ER stress is not required for cytokine-induced JNK activation. Thus, ER stress-independent activation of JNK may provide a mechanism for the inability of PBA to protect against cytokine-induced cell death.

PBA does not protect against cytokine-induced cell death in primary islets. We next tested in rat islets whether attenuation of ER stress with PBA altered the sensitivity of primary β-cells to cytokine-induced cell death. In control rat islets, IL-1β + IFN-γ treatment for 16 h led to a significant increase in cell death (Fig. 6A), as well as increased ATF4 and CHOP mRNA levels (Fig. 6B), suggestive of UPR activation. In PBA-treated islets, exposure to cytokines led to significantly reduced ATF4 and CHOP mRNA levels compared with cytokine-treated control islets (Fig. 6B). However, in PBA-treated islets, cytokine-induced cell death was similar to that in control islets (Fig. 6A). Thus, the PBA-mediated reduction in UPR activation had no effect on cytokine-induced cell death in primary islets. These data are in accordance with the results obtained in INS-1 cells and confirm the suggestion that ER stress is not required for cytokine-induced β-cell death in islets.

BIP overexpression does not protect INS-1 cells from cytokine-induced cell death. The overexpression of the ER chaperone BiP attenuates ER stress in β-cells (19), as it does in other cell types (11,13,26). BiP acts as an ER resident molecular chaperone by enhancing protein folding and maintaining PERK, ATF6, and IRE1 in their inactive states (11). We previously demonstrated that lipid-induced cell death was significantly reduced in BiP-overexpressing β-cells (19). Here, we tested whether BiP overexpression influenced cytokine-induced toxicity in β-cells. INS-1 cells were transfected with an expression vector encoding BiP or GFP (Fig. 7A) and then cultured in the absence or presence of cytokines. Cell death induced by IL-1β + IFN-γ was similar in GFP- and BiP-overexpressing INS-1 cells (Fig. 7B), despite lowered CHOP expression in BiP-overexpressing cells (Fig. 7C). Similarly, in studies with MIN6 cells, overexpressing murine BiP had no influence on cytokine-induced cell death (data not shown). These data indicate that BiP-overexpressing β-cells retain their sensitivity to the toxic effects of cytokines.

siRNA-mediated silencing of CHOP expression in β-cells protects against palmitate- but not against cytokine-induced cell death. In a previous study (6), a modest reduction in cell death induced by cytokines was observed in islets from CHOP-deficient mice. To assess the
role of CHOP as a potential mediator of β-cell death, INS-1 cells were transfected with CHOP siRNA or control siRNA. CHOP siRNA transfection led to reduced CHOP expression in INS-1 cells exposed to palmitate (Fig. 8A and B) or cytokines (Fig. 8E and F) compared with control siRNA-transfected cells. This resulted in protection against palmitate-induced cell death (Fig. 8D) and reduced cleaved caspase-3 expression in response to palmitate (Fig. 8A and C). In contrast, siRNA-mediated reduction of CHOP expression had no effect on cell death (Fig. 8H) or cleaved caspase-3 expression (Fig. 8E and G) induced by cytokines. This evidence suggests that CHOP expression is necessary for lipid- but not for cytokine-induced β-cell death.

To further assess the role of CHOP as a potential mediator of cytokine-induced β-cell death, MIN6 cells were transfected with CHOP, CHOPΔBR (dominant-negative mutant), and GFP constructs (Supplementary Fig. 4A). By immunostaining, we confirmed that overexpressed CHOP was localized in the nucleus of transfected cells (not shown). Furthermore, induction of CHOP target genes Gadd34 and Car6 in CHOP-overexpressing cells was blocked in cells cotransfected with CHOP and CHOPΔBR (Supplementary Fig. 4B). In tissues other than β-cells, overexpression of CHOP is sufficient to induce cell death (15,27–30). Here, under basal conditions, the rate of cell death in CHOP-overexpressing MIN6 cells was not different compared with control GFP-expressing MIN6 cells.
BSA (0.2% infection reagent) and treated with 0.4 mmol/l palmitate coupled to 0.92% siRNA or negative control nontargeting siRNA using DharmaFECT Transfection Reagent and subsequently treated with 0.4 mmol/l palmitate in each group. *P < 0.05, **P < 0.01 versus untreated control siRNA. Results are means ± SEM, n = 3–5 independent determinations per group. *P < 0.05, **P < 0.01 for pre-diabetic and diabetic NOD mice versus control C57BL/6J mice at the same time point. †P < 0.05, ††P < 0.001 for diabetic NOD mice versus pre-diabetic NOD mice.

**Fig. 8.** siRNA-mediated silencing of CHOP expression in INS-1 cells

INS-1 cells were transfected with CHOP ON-TARGET SMARTpool siRNA or negative control nontargeting siRNA using DharmaFECT Transfection Reagent and treated with 0.4 mmol/l palmitate coupled to 0.92% BSA (A–D) or IL-1β (50 units/ml) and IFN-γ (100 units/ml) (E–H). A and E: Western blot analysis of CHOP and cleaved caspase-3. β-Actin served as a loading control. CHOP (B and F) and cleaved caspase-3 bands (C and G) were quantified by densitometry and are expressed as fold change compared with untreated control siRNA. Results are means ± SEM, n = 5–6 in each group. *P < 0.05, **P < 0.01 versus treated control siRNA. D and H: Cell death was measured using a cell death detection ELISA. Results are means ± SEM, n = 6–7 in each group. **P < 0.01 versus palmitate-treated control siRNA.

**Table 1** Changes to mRNA levels of genes involved in ER stress in islets isolated from pre-diabetic and diabetic NOD mice

| Gene     | C57BL/6J Pre-diabetic | C57BL/6J Diabetic | NOD Pre-diabetic | NOD Diabetic |
|----------|-----------------------|------------------|------------------|-------------|
| ATF4     | 100 ± 2 82 ± 10       | 100 ± 13 62 ± 2* | 100 ± 2 82 ± 10  | 100 ± 13 62 ± 2* |
| BIP      | 100 ± 3 84 ± 5*       | 100 ± 2 87 ± 1** | 100 ± 3 84 ± 5*  | 100 ± 2 87 ± 1** |
| GRP94    | 100 ± 2 82 ± 4**      | 100 ± 2 87 ± 1** | 100 ± 2 82 ± 4** | 100 ± 2 87 ± 1** |
| CHOP     | 100 ± 4 115 ± 17      | 100 ± 13 124 ± 8 | 100 ± 4 115 ± 17 | 100 ± 13 124 ± 8 |
| Edem1    | 100 ± 2 90 ± 3        | 100 ± 10 90 ± 2  | 100 ± 2 90 ± 3  | 100 ± 10 90 ± 2  |
| p58      | 100 ± 4 78 ± 5*       | 100 ± 10 59 ± 2**| 100 ± 4 78 ± 5* | 100 ± 10 59 ± 2**|
| XBP1s    | 100 ± 3 77 ± 8*       | 100 ± 4 69 ± 2** | 100 ± 3 77 ± 8* | 100 ± 4 69 ± 2** |
| Fas      | 100 ± 6 180 ± 29*     | 100 ± 4 931 ± 93***| 100 ± 6 180 ± 29* | 100 ± 4 931 ± 93***|
| MCP-1    | 100 ± 6 372 ± 50***   | 100 ± 18 5437 ± 284*** | 100 ± 6 372 ± 50*** | 100 ± 18 5437 ± 284***|

Data are means ± SEM and are expressed as a percent of mRNA levels in islets isolated from control C57BL/6J mice at the same time. n = 3–5 independent determinations per group. *P < 0.05, **P < 0.01, ***P < 0.001 for pre-diabetic and diabetic NOD mice versus control C57BL/6J mice at the same time point. †P < 0.05, ††P < 0.001 for diabetic NOD mice versus pre-diabetic NOD mice.

**Absence of ER stress gene activation in islets from NOD mice.** Finally, we assessed whether ER stress was present in islets of NOD mice. mRNA levels were compared in islets isolated from non–diabetes-prone C57BL/6J mice and pre-diabetic and diabetic NOD mice. Blood glucose levels were 5.3 ± 0.4 mmol/l in pre-diabetic NOD mice and 13.4 ± 1.7 mmol/l in diabetic NOD mice. Using real-time RT-PCR, we determined that the expression of several ER stress genes was either downregulated or unchanged in NOD islets compared with control C57BL/6J islets. Islet mRNA levels were reduced for BiP, GRP94, and ATF4 in diabetic NOD mice (Table 1). p58 mRNA levels were reduced in islets of pre-diabetic NOD mice and were further reduced in islets of diabetic NOD mice (P < 0.05). CHOP and Edem1 mRNA levels were unchanged in islets of NOD mice both before and after the onset of diabetes compared with their control C57BL/6J mice (Table 1). In contrast, Fas and monocyte chemoattractant protein-1 mRNA levels were significantly increased in islets of pre-diabetic and diabetic NOD mice, indicative of the autoimmune and inflammatory insult. Thus, in contrast to db/db mice (19,31) and humans with type 2 diabetes (19,20), there was no increase in ER stress gene expression in islets of NOD mice.

**Discussion**

The mechanisms by which cytokines stimulate pancreatic β-cell death have been the subject of much attention because of their potential relevance to type 1 diabetes
ROLE OF ENDOPLASMIC RETICULUM STRESS IN β-CELL DEATH

(1–5). Previous studies have proposed a role of ER stress in cytokine-mediated cytotoxicity in β-cells (6–8). Here, we tested whether ER stress makes a necessary contribution to cytokine-induced β-cell death and whether it occurs in islets of the premier animal model of type 1 diabetes, the NOD mouse. Consistent with previous studies (6–8), we show that cytokines activate the UPR in clonal β-cells and cultured rodent islets. For the first time, we show that cytokines lead to increased phosphorylation of PERK and thus activation of this ER stress sensor pathway. This is accompanied by increased phosphorylation of EIF2α and transcriptional activation of ATF4 and CHOP. Importantly, we show that this activation of the UPR is not necessary for the associated β-cell death. Chemical and molecular approaches to increase the chaperone capacity of the ER failed to confer protection to the toxic effects of cytokines, suggesting an ER stress–independent mechanism of cell death. Furthermore, inhibition of CHOP had no influence over cytokine-mediated β-cell death. The relevance of this to type 1 diabetes is demonstrated by the fact that expressions of ER stress–responsive proteins are either reduced or not changed, rather than increased, in islets of NOD mice.

Importantly, our studies strengthen the case for ER stress, providing a mechanism for lipotoxicity and the reduced β-cell mass that characterizes type 2 diabetes (35,32). This is based on the observations that lipotoxicity was significantly reduced by CHOP knock down and by the treatments of PBA and TMAO, chemical chaperones that enhance ER functional capacity. This is consistent with our previous studies indicating that BiP overexpression partially protects against the effects of lipid overexposure (19). With ER stress potentially also providing a link between obesity and insulin resistance in liver and fat, our studies, together with others (8,19,20,24,33,34), suggest that ER stress may act as a common mechanism for β-cell failure and defective insulin signaling. The findings highlight the potential for increasing chaperone capacity of the ER as a therapeutic approach for type 2 diabetes treatment by preventing β-cell lipotoxicity and peripheral insulin resistance.

There are several differences in cytokine and palmitate signaling that may contribute to the divergent β-cell responses to ER stress (5). Whereas cytokines induce nuclear factor-κB activation, NO production, downregulation of the sarcoendoplasmic reticulum pump Ca2+ ATPase 2b (SERC2b), and ER Ca2+ depletion (7,8), palmitate induces ER stress via nuclear factor-κB, NO–, and ER Ca2+– independent mechanisms (8,34). Furthermore, unlike palmitate (8,19), cytokines fail to activate ATF6 in β-cells (7), and, indeed, the presence of IFN-γ within the inflammatory milieu may actually lower the expression of ATF6/XBP1-targeted genes (chaperones) and thus ER stress defenses (35). We confirmed this in our own studies, finding significantly reduced BiP and p58 mRNA levels in 24-h IL-1β + IFN-γ–treated INS-1 cells (data not shown). There are also subtle differences in the way palmitate and cytokines regulate CHOP transcription (36). Moreover, prolonged phosphorylation of EIF2α leads to marked potentiation of the deleterious effects of fatty acids but not cytokines (37). Other studies have demonstrated that UPR activation may in fact inhibit cytokine signaling (38). Recently, cytokine-induced cell death and UPR activation were dissociated on the basis that IL-1 failed to stimulate caspase-3 activity (39).

Interestingly, BiP overexpression in NIT-1 insulinoma cells was shown to lower cytokine-mediated cytotoxicity (40), in opposition to our findings in INS-1 and MIN6 cells. However, it is important to note that in the studies with NIT-1 cells, BiP overexpression through unknown mechanisms led to lowered cytokine-induced NO production and increased superoxide dismutase activity (40). Thus, the mechanism by which BiP overexpression protects NIT-1 cells may be secondary to effects of lowering NO and/or increasing capacity of the cell to cope with reactive oxygen species rather than to attenuation of ER stress. In contrast, neither BiP overexpression (data not shown) nor PBA treatment (Fig. 3) lowered cytokine-induced NO production or iNOS expression in our studies.

In the present study, we provide evidence of differential signaling mechanisms for the activation of JNK by palmitate and cytokines. The ability of palmitate to increase XBP1 splicing and of PBA to lower palmitate-induced JNK1/2 phosphorylation is consistent with the possibility that ER stress is necessary for palmitate-induced JNK activation. On the other hand, the failure of cytokines to activate XBP1 and of PBA to reverse cytokine-induced JNK1/2 phosphorylation suggests that cytokine-mediated activation of JNK occurs independently of ER stress. Several previous studies have highlighted the importance of JNK protein kinases in cytokine- (41,42) and palmitate-induced (43) cell death and in T-cell–mediated killing of β-cells in NOD mice (44).

Our data suggesting a CHOP-independent mechanism of cytokine-induced β-cell death are at variance with prior studies by Oyadomari et al. (6) that apportioned CHOP with a modest contribution. This apparent discrepancy might relate to important differences in experimental design. Given that β-cells appear especially sensitive to disruption of ER stress signaling (16,17,45), it is possible that developmental changes and alterations in cell differentiation in CHOP (−/−) mice (15,46,47) may influence outcomes from experiments using their islets ex vivo. In contrast, our experiments were performed in otherwise normal β-cells without influence of potential changes in cell development or differentiation, with similar results found in pure β-cell populations of INS-1 and MIN6 cells.

We conclude that although both cytokines and palmitate induce ER stress in cultured β-cells, UPR activation is selectively necessary for lipotoxicity and not for cytokine-induced β-cell death. Furthermore, we did not find evidence of ER stress in islets of NOD mice, and it was not found in islets of humans with type 1 diabetes either (20). On the other hand, islets from type 2 diabetic patients may be susceptible to ER stress induction after metabolic challenge (48), thus underlining the potential importance of ER stress and UPR activation in the development of type 2 diabetes (19,20). These data support the view that different mechanisms are responsible for β-cell death in type 1 and type 2 diabetes.

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