Cytokine-mediated β-cell damage in PARP-1-deficient islets

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1Department of Pediatrics, Saint Louis University, St. Louis, Missouri; 2Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama; 3Edward A. Doisy Department of Biochemistry, Saint Louis University, St. Louis, Missouri; and 4Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin

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Andreon T, Meares GP, Hughes KJ, Hansen PA, Corbett JA. Cytokine-mediated β-cell damage in PARP-1-deficient islets. Am J Physiol Endocrinol Metab 303: E172–E179, 2012. First published April 24, 2012; doi:10.1152/ajpendo.00055.2012.—Poly(ADP-ribose) polymerase (PARP) is an abundant nuclear protein that is activated by DNA damage; once active, it modifies nuclear proteins through attachment of poly(ADP-ribose) units derived from β-nicotinamide adenine dinucleotide (NAD+). In mice, the deletion of PARP-1 attenuates tissue injury in a number of animal models of human disease, including streptozotocin-induced diabetes. Also, inflammatory cell signaling and inflammatory gene expression are attenuated in macrophages isolated from endotoxin-treated PARP-1-deficient mice. In this study, the effects of PARP-1 deletion on cytokine-mediated β-cell damage and macrophage activation were evaluated. There are no defects in inflammatory mediator signaling or inflammatory gene expression in macrophages and islets isolated from PARP-1-deficient mice. While PARP-1 deficiency protects islets against cytokine-induced islet cell death as measured by biochemical assays of membrane polarization, the genetic absence of PARP-1 does not affect cytokine-induced inhibition of insulin secretion or cytokine-induced DNA damage in islets. While PARP-1 deficiency appears to provide protection from cell death, it fails to provide protection against the inhibitory actions of cytokines on insulin secretion or the damaging actions on islet DNA integrity.

insulin; nitric oxide; poly(ADP-ribose) polymerase

POLY(ADP)-RIBOSE POLYMERASE (PARP) is a highly abundant nuclear protein that is activated by DNA nicks and breaks. PARP uses β-nicotinamide adenine dinucleotide (NAD) as a substrate for the addition of poly(ADP-ribose) to acceptor proteins (for review see Refs. 55 and 61). PARP scans DNA, identifies DNA rupture, and participates in base-excision repair by metabolizing NAD+ into branched polymers of ADP ribose that are then transferred to nuclear binding proteins such as DNA polymerase I and II, Ca2+·Mg2+ endonuclease, histones, chromatin binding proteins, and PARP-1 itself (55, 61). It has been hypothesized that poly(ADP) ribosylation modifies genomic proteins proximal to DNA breaks and facilitates the opening of chromatin, which is required for the recruitment of repair enzymes (67). While this mechanism of action has been challenged by evidence that DNA repair can occur in PARP-1-deficient (PARP-1–/–) mice (64), the functions performed by PARP do not exist in a solitary enzyme. The PARP family of enzymes is encoded at several gene loci, all of which contain the ADP ribosylation function at the COOH end of the protein molecule. There are a number of isoforms of PARP; PARP-1 (26), short PARP (56), tankyrase (60), PARP-2 (1, 4), vault PARP (33), and PARP-10 (29) (70). With the discovery of multiple isoforms of PARP has come an increase in the variety of nuclear processes catalyzed by the enzyme family, including the regulation of genomic stability, gene expression, gene transcription and amplification, cellular differentiation, malignant transformation, and DNA replication, in addition to the participation of these enzymes in many physiological and developmental pathways (45, 55, 59, 61).

Despite the theoretically beneficial effects of the activation of the PARP family of enzymes in the repair of DNA nicks and breaks, PARP-1 may also mediate cell death through the depletion of cellular energy stores (NAD+ and the subsequent depletion of ATP), as proposed in the Okamoto model >25 years ago (46, 68, 69). Consistent with the Okamoto model, mice lacking PARP-1 are resistant to diabetes induced by a single bolus injection of streptozotocin (STZ) (9, 43, 50). STZ is a β-cell toxin that induces DNA damage through alkylation (38, 65). While inhibition of PARP-1 (49) protects islets against free radical- and cytokine-mediated islet death (2, 52, 57), these inhibitors, as well as PARP-1 deficiency, have also been associated with attenuation of cytokine and endotoxin signaling, as evidenced by reduced NF-κB activation and subsequent expression of inflammatory gene targets, such as inducible nitric oxide (NO) synthase (iNOS) (3, 18, 37, 48).

Glucose stimulates insulin secretion by β-cells through its oxidation to CO2, with the subsequent accumulation of ATP to levels sufficient to stimulate Ca2+-dependent exocytosis (28). Treatment of rat islets with the macrophage-derived cytokine IL-1 or mouse and human islets with IL-1 and IFN-γ results in an inhibition of glucose-stimulated insulin secretion that is mediated by the expression of iNOS and the subsequent production of micromolar levels of NO (11, 12, 14, 19). NO inhibits insulin secretion by attenuating the oxidation of glucose to CO2 and, thereby, reducing cellular levels of ATP. NO also induces DNA damage through direct strand breaks and base modification and has been shown to inhibit the activity of enzymes that participate in the repair of damaged DNA (15, 17, 27, 30, 31).

The purpose of this study was to determine if PARP-1 participates in the destructive effects of cytokines on islet function and viability. To address this question, the actions of IL-1 + IFN-γ on iNOS expression, NO production, insulin secretion, DNA damage, and cell viability were examined in islets isolated from wild-type and PARP-1–/– mice. Since NF-κB activation and iNOS expression are impaired in macrophages isolated from PARP-1–/– mice treated with endotoxin (47, 48), the effects of endotoxin and double-stranded (ds) RNA on macrophage iNOS expression were also evaluated. We show that PARP-1 deficiency does not modify iNOS expression, NO production, or inflammatory effector signaling.
in peritoneal macrophages and β-cells isolated from PARP-1−/− mice. While PARP-1 deficiency appears to provide protection from cytokine-mediated islet cell death, as determined by biochemical assays of membrane polarization, under conditions of iNOS expression and NO production, the absence of PARP-1 does not prevent the inhibitory actions of cytokines on insulin secretion or the induction of DNA damage.

MATERIALS AND METHODS

Materials and animals. Wild-type (PARP-1+/+) and PARP-1−/− mice were obtained from Wang and colleagues and are described elsewhere (64). Collagenase type XI, polynosinic:polycytidylic acid [poly(IC)], and LPS were purchased from Sigma Chemical (St. Louis, MO); CMRL-1066 and DMEM tissue culture medium, L-glutamine, albumin, pH 7.4) containing 3 mM D-glucose. Groups of 15 islets were cultured as indicated.

Islet isolation and culture. Pancreatic islets were isolated from male and female PARP-1+/+ and PARP-1−/− mice by collagenase digestion, as previously described (32, 36). After isolation, islets were cultured overnight in complete CMRL (CMRL-1066 containing 2 mM L-glutamine, 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin) under an atmosphere of 95% air-5% CO2 at 37°C. For each experiment, islets were washed with complete CMRL, counted, and then cultured for an additional 3 h. Experiments were initiated by the addition of cytokines [IL-1 (15 U/ml) and IFN-γ (150 U/ml)], and the islets were cultured as indicated.

Peritoneal macrophage isolation and culture. Peritoneal exudate cells were isolated from PARP-1+/+ and PARP-1−/− mice by lavage, as previously described (41). After isolation, 4 × 107 cells in 400 µl of complete CMRL were incubated at 37°C under an atmosphere of 95% air-5% CO2 for 3 h. Cells were washed with complete CMRL to remove nonadherent cells before treatment with LPS (10 µg/ml), poly(IC) (50 µg/ml), and 150 U/ml IFN-γ.

Insulin secretion. Mouse islets (120 islets/ml complete CMRL) were cultured in the presence or absence IL-1 and IFN-γ and NMMA for 24 h. The islets were washed three times in Krebs-Ringer bicarbonate buffer (25 mM HEPES, 115 mM NaCl, 24 mM NaHCO3, 5 mM KCl, 1 mM MgCl2, 2.5 mM CaCl2, and 0.1% bovine serum albumin, pH 7.4) containing 3 mM d-glucose. Groups of 15 islets were counted into 10 × 75-mm borosilicate test tubes and preincubated for 30 min at 37°C with shaking in 200 µl of the same buffer. The preincubation buffer was removed, and glucose-stimulated insulin secretion was initiated by the addition of 200 µl of Krebs-Ringer bicarbonate buffer containing 3 or 20 mM d-glucose. Islets were then incubated at 37°C for 45 min, the incubation buffer was removed, and insulin content was determined by radioimmunoassay at the Diabetes Research and Training Center at Washington University School of Medicine (St. Louis, MO).

Animal authorization. The experiments were performed in accordance with the provisions of the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and as described in the animal use protocols approved by the Saint Louis University and the University of Alabama at Birmingham Institutional Animal Care and Use Committees.

Research and Training Center at Washington University School of Medicine (St. Louis, MO).

Results

The presence of functional PARP-1 is not required for iNOS expression and NO production. Since NO is a primary mediator of β-cell damage in response to cytokines (5, 14, 19) and previous studies suggest that iNOS expression and NO production are attenuated in PARP-1−/− cells (39, 47), the effects of cytokine and endotoxin treatment on macrophage (Fig. 1) and islet (Fig. 2) iNOS expression and NO production were examined. Peritoneal macrophages derived from PARP-1+/+ and PARP-1−/− mice respond in a similar manner to LPS + IFN-γ treatment, with an increase in the production of nitrite (Fig. 1A) and the expression of iNOS (data not shown). This is consistent with a requirement for two proinflammatory signals to activate macrophages, as LPS or IFN-γ alone does not stimulate nitrite production by mouse macrophages (Fig. 1A). In addition to the bacterial product LPS, the viral dsRNA mimic poly(IC), in the presence of IFN-γ, also stimulates iNOS expression and nitrite formation by macrophages (20). Similar to the findings using LPS, there is no difference in the induction of iNOS or production of NO in response to polyIC and IFN-γ by macrophages derived from PARP-1+/+ and PARP-1−/− mice (Fig. 1, B and C). These findings indicate that macrophage expression of iNOS and production of NO are not dependent on PARP-1 activity.
 PARP-1-INDEPENDENT INHIBITION OF β-CELL FUNCTION

Fig. 1. Induction of inducible nitric oxide (iNOS) in macrophages isolated from wild-type and poly(ADP-ribose polymerase (PARP)-deficient (PARP-1+/+ and PARP-1−/−) mice. Peritoneal macrophages harvested from PARP-1+/+ and PARP-1−/− mice were treated for 24 h with LPS, synthetic double-stranded RNA (polynosinic:polycytidylic acid [poly(IC)]), and IFN-γ. Supernatants were harvested, and nitrite accumulation was determined by the Griess assay (A and B) and iNOS expression was determined by Western blot analysis of the cells (C). PARP-1 levels were determined by Western blot analysis and are shown as a control for PARP-1 deficiency. Results are representative of 3 independent experiments.

Fig. 2. iNOS induction and NO production by islets isolated from PARP-1+/+ and PARP-1−/− mice. Mouse islets (120 per 400 µl of complete CMRL) were treated for 24 h with IL-1 and murine IFN-γ. Supernatants were harvested, and nitrite production was determined by the Griess assay (A) and iNOS expression was evaluated by Western blot analysis of the islets (B). PARP-1 levels were determined by Western blot analysis and are shown as a control for PARP-1 deficiency. Results are representative of 3 independent experiments.

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PARP-1 deficiency and islet cell viability. PARP-1–/– mice are protected from the development of diabetes induced by a single bolus injection of STZ, and this protection is associated with the preservation of β-cell mass (9, 43, 50). In mice, diabetes induced by a single bolus injection of STZ is mediated by the selective uptake of STZ in β-cells, followed by the induction of DNA alkylation and strand breaks (38, 66). STZ also liberates NO during its decomposition, resulting in an impairment of β-cell oxidative metabolism (63). Since cytokine-induced DNA damage and β-cell death are mediated by β-cell production of NO (10, 62) and PARP-1 deficiency protects β-cells from STZ-mediated death (9, 43, 50), the effects of PARP-1 deficiency on cytokine-mediated β-cell damage were examined. Consistent with a number of previous studies (10, 42, 62), treatment of islets isolated from PARP-1+/+ mice for 24 h with IL-1 + IFN-γ results in a 25% loss of islet viability (Fig. 4A), as determined using neutral red dye assay. The loss of viability occurs under conditions in which the cytokines stimulate ~10-fold increase in NO production (Fig. 4B). Islets from PARP-1+/+ mice produce NO to levels that are comparable to those produced by islets from PARP-1+/+ mice in response to IL-1 + IFN-γ (Fig. 4B); however, the islets from PARP-1–/– mice appear to be protected against the toxic actions of the cytokine combination (Fig. 4A). This protection from cell death is not universal, as islets from PARP-1+/+ and PARP-1–/– mice are equally sensitive to the effects of staurosporine, a classical inducer of apoptosis (Fig. 4A).

Insulin secretion and PARP-1 deficiency. While islets from PARP-1–/– mice appear to be protected from the toxic actions of cytokines, even under conditions where they produce micromolar levels of NO, the inhibitory actions of these cytokines on glucose-stimulated insulin secretion are unchanged. Treatment of islets isolated from PARP-1–/– mice for 24 h with IL-1 + IFN-γ results in an inhibition of insulin secretion to levels similar in magnitude to the inhibitory actions of these cytokines on insulin secretion by islets isolated from PARP-1+/+ mice (Fig. 5). The cytokine treatment inhibits the secretion of insulin from islets isolated from PARP-1+/+ and PARP-1–/– mice by 70%, and the NOS inhibitor NMMA prevents this inhibition.

Cytokines induce DNA damage in islet cells isolated from PARP-1–/– mice. Since islets from PARP-1–/– mice appear to be protected from cytokine-induced death (Fig. 4), yet they are sensitive to cytokine-mediated inhibition of insulin secretion (Fig. 5), we examined effects of cytokine treatment on islet cell viability by a second assay, TUNEL (Fig. 6). While commonly used as an assay of apoptosis, TUNEL detects DNA strand breaks. For these experiments, isolated islets were treated with IL-1 + IFN-γ for 24 h and then dispersed into individual cells. The islet cells were centrifuged onto slides, and DNA integrity was evaluated by TUNEL (green), β-cells were identified by immunohistochemical staining for insulin (red), and nuclei were identified by DAPI staining (blue). As shown in Fig. 6A, cytokine treatment induces DNA damage in insulin-containing cells from PARP-1–/– and PARP-1+/+ islets. The damaging actions of IL-1 + IFN-γ treatment on islet cell DNA integrity are prevented by NMMA, indicating that DNA damage is
mediated by NO. Quantification of these data reveals no differences in the levels of DNA damage observed in insulin-containing islet cells from PARP-1<sup>+/+</sup> and PARP-1<sup>−/−</sup> mice, as IL-1 + IFN-γ stimulates a nearly eightfold increase in the number of TUNEL-positive cells, in the presence or absence of PARP-1 (Fig. 6B). Western blot analysis was used to confirm the absence of PARP-1 in islets isolated from PARP-1<sup>−/−</sup> mice (Fig. 6C).

**DISCUSSION**

In the early 1980s, the Okamoto model was proposed to explain damage and death of pancreatic β-cells during the development of diabetes. This model, updated in recent reviews (46), posits that extensive DNA damage leads to the consumption of NAD<sup>+</sup> and ATP, which results in the depletion of energy stores and necrotic cell death. Central to this model is the hyperactivation of PARP-1. In response to high levels of DNA damage, PARP-1 is recruited to DNA, where its catalytic activity is dramatically enhanced, resulting in NAD<sup>+</sup>-dependent polymerization of ADP-ribose conjugated to proteins. These poly(ADP)-ribose units are then rapidly degraded by enzymatic cleavage in an ATP-dependent manner, causing the depletion of cellular NAD<sup>+</sup> and ATP and cell death by necrosis (71, 72). Chemotherapeutic agents and chemical toxins, such as the diabetogenic agent STZ, are known to induce DNA damage to levels sufficient to hyperactivate PARP-1 and cause PARP-1-dependent cell death (68, 69). While these studies provide evidence in support of the Okamoto model of β-cell death in response to chemical toxins and DNA-damaging agents (46), the role of PARP-1 in cytokine-mediated β-cell damage has yet to be fully explored.

NO, produced following iNOS expression, is the primary mediator of cytokine-induced β-cell damage. Produced in micromolar levels by β-cells following cytokine treatment, NO inhibits the mitochondrial oxidation of glucose to CO<sub>2</sub>, glucose-induced insulin secretion, and protein synthesis and induces DNA damage (14, 19). When supplied exogenously using chemical donors such as 3-morpholinosydnonimine, NO induces PARP-1-dependent DNA damage and death of islet cells and insulinoma cells (23, 34). While the effects of PARP-1 inhibition/deletion on NO donor-mediated damage have been examined, the role of PARP-1 in cytokine-mediated β-cell damage has not been explored. In this study, the role of PARP-1 in cytokine-induced β-cell damage was examined using islets and macrophages isolated from PARP-1<sup>+/+</sup> and PARP-1<sup>−/−</sup> mice. Because previous studies suggest that in-

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**Fig. 5.** Effects of cytokines on glucose-stimulated insulin secretion. Islets (120 per 400 μl of complete CMRL), isolated from PARP-1<sup>+/+</sup> and PARP-1<sup>−/−</sup> mice, were treated for 24 h with IL-1, IFN-γ, and N<sup>ω</sup>-monomethyl-L-arginine (NMMA). Islets were harvested, and insulin secretion in response to 45 min of incubation with 3 or 20 mM glucose was determined. Results are averages ± SE of 3 independent experiments. *Significantly different from control (*P < 0.05). There are no statistically significant differences between PARP-1<sup>+/+</sup> and PARP-1<sup>−/−</sup> mice.

**Fig. 6.** Effects of cytokines on islet cell DNA damage. Islets were isolated from PARP-1<sup>+/+</sup> and PARP-1<sup>−/−</sup> mice and were treated for 24 h with a combination of cytokines (IL-1 (15 U/ml) + IFN-γ (150 U/ml)) with or without NMMA. Islets were dispersed into individual cells, and integrity of islet cell DNA was examined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining (green), insulin-containing β-cells by immunocytochemistry (red), and nuclei by 4′,6-diamidino-2-phenylindole (DAPI) staining (blue) (A). Number of insulin-containing cells with DNA damage was quantified by microscopy (B). As a control, PARP-1 levels were examined by Western blot analysis (C). Results are averages ± SE of 3 independent experiments. *Significantly different from control (*P < 0.05). There are no statistically significant differences between PARP-1<sup>+/+</sup> and PARP-1<sup>−/−</sup> mice.
flammatory mediator signaling is attenuated in macrophages isolated from PARP-1-/- mice (3, 39, 48), we initiated these studies by examining the effects of cytokines, endotoxin, and dsRNA on the activation of signaling cascades responsible for inflammatory gene expression (iNOS) in macrophages and islets. The stimulatory actions of LPS, poly(IC), and IFN-γ on transcription factor (NF-κB or Stat-1 activation), iNOS expression, and NO production by macrophages or islets were not modified in macrophages or islets isolated from PARP-1-/- mice. Thus cytokine- and pathogen-activated molecular pattern signaling [LPS and poly(IC)] is not defective in macrophages or islets isolated from PARP-1-/- mice.

Since PARP-1 is activated in response to DNA damage (e.g., single-strand breaks, double-strand breaks, and DNA deamination) and NO is known to induce islet cell DNA damage (23, 31), the effects of the endogenous production of NO on islet cell viability were examined using islets isolated from PARP-1+/+ and PARP-1-/- mice. Consistent with previous studies (10, 14, 62), 24-h incubation with IL-1 + IFN-γ results in ∼10-fold increase in NO production by islets isolated from PARP-1+/+ mice, and this correlates with a 25% reduction in islet cell viability. In contrast, islets isolated from PARP-1-/- mice appear to be resistant to the loss of viability in response to IL-1 + IFN-γ treatment, even though they produce NO to levels equivalent to those produced by islets isolated from PARP-1+/+ mice. While previous studies show that NO is the primary mediator of cytokine-induced islet damage (10, 14, 62), these findings could suggest that NO is not responsible for the loss of islet cell viability under these conditions.

The protective effect of PARP-1 deficiency on islet cell viability is not congruent with the lack of protection against the inhibitory actions of cytokines on insulin secretion or the damaging actions of cytokines on DNA integrity. This somewhat paradoxical finding may be due to the relative levels of PARP-1 activation (physiological or hyperactivation), which are proportional to the level of DNA damage. In response to IL-1 + IFN-γ-induced NO production, there is a physiological activation of PARP-1 that occurs in response to NO-mediated DNA damage. This level of activation is in contrast to the hyperactivation of PARP-1 that is commonly observed in response to high levels of DNA damage, such as that induced by STZ (8, 46, 71). The result of this physiological PARP-1 activation in response to cytokine treatment is a reduction in NAD+ and ATP levels that prevents the uptake of the neutral red dye into islet cells (7). Consistent with this interpretation, Bolaffi et al. (6) showed that IL-1 reduces rat islet NAD+ levels by 50% and that this effect is prevented by NMMA. Furthermore, the neutral red dye assay is based on the accumulation of this dye in acidic cellular compartments (such as lysosomes), and acidification is based on the ATPase activity; thus the neutral red dye assay is an indirect assay of cellular ATP levels (54). In its absence, this physiological activation of PARP-1 does not take place, thus maintaining ATP at levels sufficient to support the acidification of cellular compartments, allowing for the sequestration of neutral red dye. While these results are suggestive of viable cells that may be resistant to the destructive effects of cytokines, the absence of PARP-1 does not provide protection against the inhibitory actions of IL-1 + IFN-γ on glucose-stimulated insulin secretion or the induction of DNA damage. In contrast, iNOS inhibition prevents the loss of cell viability, as well as the damaging actions of cytokine treatment on insulin secretion and DNA integrity (Figs. 5 and 6).

In contrast to the effects of cytokine-induced NO production, STZ or prolonged incubations with high (millimolar) concentrations of NO donors cause hyperactivation of PARP-1, large (severalfold) reductions in cellular NAD+ and ATP levels, and PARP-1-dependent islet cell death (23, 69). Importantly, a number of studies support different modes of cell death under these conditions. We have shown that short exposure (24- and 48-h incubation) of islets to IL-1 results in low levels of islet cell death that are associated with the NO-dependent release of high-mobility group box-1 protein (62) and necrotic cell death. Studies have shown that PARP-1 overactivation is also associated with the translocation of high-mobility group box-1 protein from the nucleus to the cytoplasm, where it can be released in response to necrotic cell death (13). Recently, we identified the forkhead transcription factor FoxO1 and the NAD+-dependent deacetylase Sirt-1 as central players controlling β-cell fate in response to cytokine treatment (25). In an NO-dependent manner, cytokines stimulate FoxO1 nuclear localization, and when Sirt-1 is active, FoxO1 is deacetylated and stimulates the expression of protective molecules such as GADD45α (25). Under conditions in which Sirt-1 is less active, FoxO1-dependent expression of GADD45α is attenuated, and the expression of proapoptotic genes, such as the Bel-2 scavenger Puma, is enhanced (25). There also appears to be a regulatory association between PARP-1 and Sirt-1. In response to hyperactivation of PARP-1, the activity of Sirt-1 is attenuated due to NAD+ depletion (51). PARP-1 activity can also be enhanced by acetylation, and Sirt-1 can physically associate with and deacetylate PARP-1, resulting in an attenuation of PARP-1 activity (53). A physiological activation of PARP-1 by NO, produced following short (18–24 h) exposures to cytokines, would be consistent with previous findings (51, 53), as this activation would take place under conditions in which Sirt-1 is also active, and through its deacetylase activity Sirt-1 would further limit the extent of PARP-1 activation.

Overall, these findings suggest that the amount of NO, length of exposure, and extent of DNA damage determine whether PARP-1 participates in cytokine- and NO-dependent β-cell damage. When supplied exogenously for long periods (24 h) and at high (millimolar) concentrations, there is extensive DNA damage, hyperactivation of PARP-1, and PARP-1-dependent necrosis (23, 55, 72). Furthermore, hyperactivation of PARP-1 causes the depletion of cellular levels of ATP, leading to PARP-1-dependent activation of AMP kinase (24, 55). NO, produced exogenously following short (0.5–3 h) exposures to chemical donors or endogenously following cytokine treatment, activates AMP kinase in β-cells; however, this activation is not PARP-1-dependent. NO activates AMP kinase by a pathway that requires the unfolded protein response transducer inositol-requiring enzyme-1α (44). Additional evidence supporting the PARP-1-independent nature of AMP kinase activation by NO includes the absence of ADP-ribosyl polymer formation in cytokine-treated (24 h) or NO-treated (0.5–3 h) islets. In contrast, hydrogen peroxide, which activates AMP kinase in a PARP-1-dependent manner, stimulates the formation of ADP-ribosyl polymers (unpublished observations). These findings provide further evidence that cytokines and cytokine-induced NO production do not stimulate hyper-
activation of PARP-1, unlike the effects of STZ or other β-cell toxins, which induce high levels of DNA damage in β-cells.

The Okamoto model is an attractive hypothesis to explain β-cell death in response to agents that cause severe DNA damage. This model is consistent with a role for PARP-1 in the loss of β-cell mass and the subsequent development of diabetes in mice treated with a bolus of STZ (46, 69). The model has also been extremely useful in identifying potential pathways that may mediate the loss of β-cell viability and function; however, it seems to fall short of explaining the mechanisms responsible for the loss of β-cell viability and function in response to cytokine treatment. While PARP-1 is likely activated in response to cytokines, this is physiologically activation associated with NO-mediated DNA damage. This activation causes loss of cellular NAD+ and ATP to levels that result in what appears to be a loss of viability using biochemical assays of cell death. While cytokines do not appear to reduce the viability of PARP-1−/− islets when examined using this biochemical assay, PARP-1 deficiency does not protect against the induction of DNA damage or the inhibition of glucose-induced insulin secretion in cytokine-treated islets. Unlike the role of PARP-1 in STZ-induced diabetes, these findings suggest that cytokine-induced, NO-dependent β-cell damage is not mediated by the hyperactivation of PARP-1. In contrast, β-cell function and fate in response to cytokines appear to be regulated by NO-dependent activation of FoxO1 and the control of FoxO1 transcriptional activity by Sirt1 (25).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.A., K.J.H., P.A.H., and J.A.C. are responsible for conception and design of the research; T.A., G.P.M., and P.A.H. performed the experiments; T.A., G.P.M., K.J.H., P.A.H., and J.A.C. analyzed the data; T.A., G.P.M., K.J.H., P.A.H., and J.A.C. interpreted the results of the experiments; T.A., G.P.M., and J.A.C. prepared the figures; T.A. and J.A.C. drafted the manuscript; T.A., G.P.M., K.J.H., P.A.H., and J.A.C. edited and revised the manuscript; T.A., G.P.M., K.J.H., P.A.H., and J.A.C. approved the final version of the manuscript.

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