PARP-1 and cytokine-mediated β-cell damage: a nick in the Okamoto model?

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TYPE 1 DIABETES MELLITUS (T1DM), a complex genetic disease characterized by the destruction of pancreatic islet β-cells, leads to insulin deficiency and, ultimately, hyperglycemia in inflicted individuals. The etiology stems from a progressive autoimmune assault, in which macrophages and T cells secrete proinflammatory cytokines, such as IL-1β, IFN-γ, and TNF-α, as well as nitric oxide (NO), which provoke β-cell death. The latter situation is further aggravated, as remaining β-cells produce chemokines, thereby attracting more immune cells and, thus, escalating the inflammatory process. Cytokine-mediated cell death proceeds through necrosis or apoptosis and implicates the activation of the Jak/Stat and NF-κB signaling pathways (4). A common downstream target gene of these pathways is inducible NO synthase (iNOS), which generates NO, a major effector of β-cell death along with reactive oxygen species.

Historically, T1DM has been recapitulated in rodents with use of diabetogenic agents, such as streptozotocin (STZ) and alloxan, to destroy the β-cell mass. In particular, STZ is distinctly taken up by β-cells, inducing DNA alkylation and leading to impaired β-cell function and death. In addition, STZ was also found to generate NO species, further deteriorating β-cell survival (7). More than 30 years ago, Okamoto and colleagues (13) demonstrated that STZ and alloxan cause cell death by inducing DNA strand breaks and the activation of poly(ADP)-ribose synthase. This enzyme, also known as poly-(ADP)-ribose polymerase-1 (PARP-1), is activated by DNA breaks and uses NAD^+ to covalently link units of ADP ribose, thereby forming long polymers on nuclear acceptor proteins such as histones. This has been suggested to diminish their affinity for DNA, opening the site of DNA damage and allowing access to the base excision repair machinery (8). Despite this important role in signaling and facilitating DNA repair, the split functional personality of PARP-1 as Mr. Hyde and Dr. Jeckyll is revealed when PARP-1 is hyperactivated. Indeed, in an attempt to replenish the NAD^+ pool that is being drained by the PARP-1, the cellular ATP stores are rapidly exhausted, resulting in energy crisis-induced necrosis. The latter forms the basis of the "Okamoto model," which provides a unifying hypothesis on how hyperactivation of PARP-1 leads to impaired β-cell function (insulin biosynthesis and secretion) and, ultimately, death in response to chemical insults (12, 13). In agreement with this model, several independent studies have shown that mice lacking PARP-1 were resistant to STZ-mediated β-cell death and to development of hyperglycemia (3, 9, 11). In parallel, PARP-1-deficient mice were found to be extremely resistant to LPS-induced endotoxic shock as a consequence of a blunted NF-κB-dependent transcription, leading to inhibition of iNOS, as well as TNF-α and IFN-γ, expression in macrophages (10). Thus, PARP-1 not only mediates the cytotoxic effect of NO, but it also appears to stimulate inflammation by regulating the NF-κB signal transduction pathway.

These compelling data led to a large randomized double-blind, placebo-controlled trial in which pre-T1DM participants recruited from 18 European countries, as well as the United States and Canada, were given the PARP-1 inhibitor nicotinamide or a placebo. After five years of treatment, the incidence of developing T1DM was identical in both groups (5). These studies highlight the complexity of T1DM and the pitfalls in translating lessons learned in animal models of diabetes to human physiology. Indeed, one critical question arises: How well does the STZ-induced β-cell death model mimic T1DM? In fact, hyperactivation of PARP-1 by STZ results in β-cell death through necrosis, while β-cell destruction in T1DM predominantly proceeds through a cytokine-mediated apoptosis process. Interestingly, PARP-1 is cleaved and inactivated by caspase-3 and -7 in the early phase of apoptosis, suggesting that PARP-1 does not participate in this mode of cell death (8). Furthermore, Heller et al. (6), as early as 1995, demonstrated that islets isolated from PARP-1-deficient mice were as sensitive as control islets to cell lysis by high concentrations of NO and reactive oxygen species. These data clearly raise serious doubts about the importance of PARP-1 as a key actor in β-cell destruction in a setting of T1DM. Thus, although the role of PARP-1 in β-cell destruction via necrosis has been irrefutably associated with STZ and in establishing the validity of the Okamoto model, its role in cytokine-mediated cell death remains to be fully explored.

In this issue of the Journal, Andreone et al. (1) address this gap and challenge the Okamoto model by demonstrating that PARP-1 does not participate in the deleterious effect of cytokines on islet function and, potentially, survival. Indeed, Andreone et al. demonstrate that, similar to control islets, PARP-1-deficient islet cells exposed to the proinflammatory cytokines IL-1 and IFN-γ exhibit normal activation of the NF-κB signaling pathway, which leads to induction of iNOS expression and production of NO. Furthermore, deletion of PARP-1 did not alter IL-1/IFN-γ-mediated phosphorylation of Stat-1, indicating that the Jak/Stat signaling pathway implicated in apoptosis is activated by cytokines in PARP-1-deficient islets (4). Using a biochemical assay that measures uptake of a neutral red dye within acidic lysosomes, Andreone et al. show that islets lacking PARP-1 are resistant to cytokine-mediated cytotoxicity, whereas they remain sensitive to the general apoptotic agent staurosporine. As lysosome acidification is an ATP-dependent process, which may be preserved in PARP-1-deficient islets due to sustained levels of ATP, Andreone et al. astutely used the terminal deoxynucleotidyl transferase-medi-

ated dUTP nick-end labeling (TUNEL) assay as an alternative approach to evaluate cell death. The TUNEL assay is particularly useful, as it reveals DNA strand breaks, which are a hallmark of NO-mediated cell death. Consistent with preserved NO production, PARP-1-deficient islets exhibited increased TUNEL-positive cells in the presence of cytokines to levels similar to those of treated control islets. Consequently, glucose-induced insulin secretion was also impaired in PARP-1-lacking islets treated with cytokine. As development of T1DM results from an intricate cross talk between immune cells and islets, Andreone et al. also assessed whether inflammatory cell signaling was impaired in macrophages derived from PARP-deficient mice, as previously reported by others (10). Surprisingly, the NF-κB signaling pathway, as well as downstream iNOS activation and NO production, were unaltered in macrophages derived from PARP-1 knockout mice that were exposed to cytokines or LPS. A potential explanation for discrepancies in results may stem from the fact that previous studies were performed with macrophages exposed in vivo to LPS (10), while Andreone et al. studied naïve macrophages treated in vitro with the endotoxin.

The work by Andreone et al. (1) demonstrates that, in contrast to STZ-mediated cell death and the Okamoto model, PARP-1 does not contribute to cytokine-induced β-cell damage. Furthermore, PARP-1 does not appear to be implicated in the signaling of the inflammatory response by macrophages. These results are consistent with the clinical trial demonstrating that nicotinamide does not improve onset of T1DM, causing a serious "nick" in the Okamoto model. Nonetheless, one question remains to be clearly resolved from the study of Andreone et al.: Does PARP-1 deficiency prevent, beyond doubt, islet cell death? Indeed, DNA damage was increased in islets from STZ-treated PARP-1-deficient mice, yet these animals did not develop hyperglycemia, and islet integrity and function were preserved (11). It is tempting to speculate that PARP-2, which accounts for ~10% of all PARP activity in mammalian cells (8), may provide sufficient residual activity to repair STZ-mediated DNA damage in PARP-1 knockout mice, thereby avoiding β-cell death and development of hyperglycemia. In this context, it is interesting to note that PARP-2 was recently shown to be essential for β-cell function and expansion in response to a high-fat diet (2). Unfortunately, the long-term effect of cytokines on β-cell DNA damage and insulin secretion was not addressed by Andreone et al., as all experiments were performed within 24 h of treatment. A key study that could resolve whether PARP-1 deficiency is an asset in protecting β-cells against a continuous exposure to proinflammatory cytokines would involve breeding of PARP-1-deficient mice to animal models of experimental autoimmune diabetes. This approach could also offer a valuable tool to highlight and resolve potential pitfalls associated with the failure of PARP-1 inhibitors to delay or prevent onset of T1DM in human subjects. Nonetheless, recent findings demonstrating the importance of PARP-2 in β-cell function, combined with the apparent redundancy of PARP-1 in cytokine-mediated β-cell dysfunction, strongly argue against the use of new and more specific PARP inhibitors such as olaparib as novel therapies for the treatment of T1DM.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

N.C.-V. and B.R.G. drafted the manuscript; N.C.-V. and B.R.G. edited and revised the manuscript; N.C.-V. and B.R.G. approved the final version of the manuscript.

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