Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozocin

VOLKER BURKART[1], ZHAO-QI WANG[2], JÜRGEN RADONS[1], BIRGIT HELLER[1], ZDENKO HERCEG[2], LAURA STINGL[3], ERWIN F. WAGNER[3] & HUBERT KOLB[1]

[1] *Diabetes Research Institute at the Heinrich Heine University Düsseldorf, Auf´ m Hennekamp 65, D-40225 Düsseldorf, Germany*

[2] *International Agency for Research on Cancer (IARC), 150 Cours Albert Thomas, F-69008 Lyon, France*

[3] *Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria*

V.B. and Z.-Q. W. contributed equally to this work.

Correspondence should be addressed to V.B.; e-mail: burkart@dfi.uni-duesseldorf.de

Human type 1 diabetes results from the selective destruction of insulin-producing pancreatic beta cells during islet inflammation. Cytokines and reactive radicals released during this process contribute to beta-cell death. Here we show that mice with a disrupted gene coding for poly (ADP-ribose) polymerase (*PARP*$^{-/-}$ mice) are completely resistant to the development of diabetes induced by the beta-cell toxin streptozocin. The mice remained normoglycemic and maintained normal levels of total pancreatic insulin content and normal islet ultrastructure. Cultivated *PARP*$^{-/-}$ islet cells resisted streptozocin-induced lysis and maintained intracellular NAD$^+$ levels. Our results identify NAD$^+$ depletion caused by PARP activation as the dominant metabolic event in islet-cell destruction, and provide information for the development of strategies to prevent the progression or manifestation of the disease in individuals at risk of developing type 1 diabetes.

The determining event in the pathogenesis of type 1 diabetes is the destruction of insulin-producing pancreatic beta cells. There is strong evidence that the progressive reduction of the beta-cell mass is the result of a chronic autoimmune reaction[1]. During this process, islet-infiltrating immune cells[2,3], islet capillary endothelial cells[4] and the beta cell itself[5] are able to release cytotoxic mediators. Cytokines, and in particular nitric oxide (NO), are potent beta-cell toxic effector molecules[6,7]. The reactive radical NO mediates its deleterious effect mainly through the induction of widespread DNA strand breaks[8]. This initial damage presumably triggers a chain of events terminating in the death of the beta cell.

Diabetes induced in rodents by the beta-cell toxin streptozocin (SZ) has been used extensively as animal model to study the mechanisms involved in the destruction of pancreatic beta cells[9]. SZ is taken up by the pancreatic beta cell through the glucose transporter GLUT-2 (ref. 10). This substance decomposes intracellularly, and causes damage to DNA either by alkylation[11] or by the generation of NO (refs. 12,13). The appearance of DNA strand breaks leads to the activation of the abundant nuclear enzyme PARP, which synthesizes large amounts of the (ADP-ribose) polymer, using NAD$^+$ as a substrate[11]. As a consequence of PARP activation, the cellular concentration of NAD$^+$ may then decrease to very low levels, which is thought to abrogate the ability of the cell to generate sufficient energy and, finally, to lead to cell death[14].

Here we analyzed the proposed role of PARP activation in pancreatic islet-cell death and diabetes development in an animal model of type 1 diabetes experimentally induced by SZ. Complete protection from diabetes was found in mice lacking the intact PARP gene. The mice remained normoglycemic and their beta cells showed preserved structure and function. The protection from diabetes occurred at the level of the beta cells, as isolated islet cells lacking PARP maintained their NAD$^+$ levels and viability after SZ exposure *in vitro*, although the extent of primary DNA damage was unaffected by the PARP status.

Effect of PARP deficiency on SZ-induced diabetes

Wild-type mice (*PARP*$^{+/+}$) that received a single injection of SZ on day 0 (160 mg per kg body weight) had transient hypoglycemia on day 1 (Fig. 1*a*), indicative of insulin release from acutely damaged beta cells. After 2 days of treatment, they developed severe hyperglycemia, with blood glucose levels exceeding 15 mmol/l (Fig. 1*a*, days 2 and 3). Nicotinamide, a pleiotropic B vitamin, suppresses SZ toxicity[15] and prevents beta-cell destruction in autoimmune-diabetes-prone non-obese diabetic mice[14]. Both actions may be related to the fact that nicotinamide is an inhibitor of PARP (ref. 16). Mice pretreated with nicotinamide were completely protected from SZ-induced hyperglycemia (Fig. 1*a*). To determine whether the hyperglycemia is mediated by PARP, we repeated this experiment in mice with homozygous (*PARP*$^{-/-}$) and heterozygous (*PARP*$^{+/-}$) PARP deficiencies. These mice were generated by gene-targeting technology and had a genetic background of 129SV × C57BL/6 (ref. 17). To exclude possible effects that might result from strain differences in SZ susceptibility[18], mice with the three genotypes (*PARP*$^{+/+}$, *PARP*$^{+/-}$ and *PARP*$^{-/-}$) from the same litter or the same family were used for all *in vivo* and *in vitro* experiments. Mice were injected with SZ at a dose of 160 mg per kg body weight, and their blood glucose levels were monitored for 3 days. This treatment induced neither an initial decrease nor

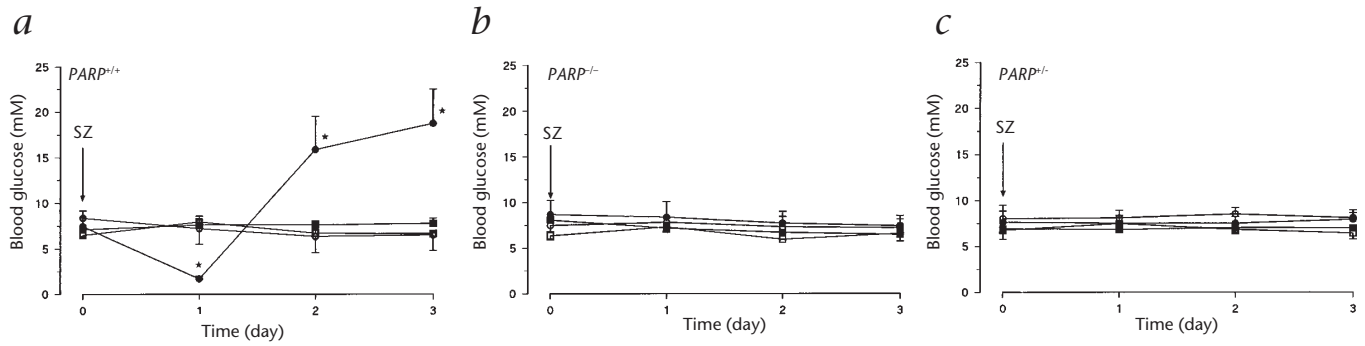

Fig. 1 Analysis of blood glucose levels after the administration of SZ in *PARP*$^{+/+}$ (***a***), *PARP*$^{-/-}$ (***b***) and *PARP*$^{+/-}$ (***c***) mice. On day 0, groups of three to eight mice of each genotype received a single injection of SZ (160 mg per kg body weight; ●) or an equal corresponding volume of sodium citrate buffer alone (○). Nicotinamide was administered 6 h before the SZ injection (■) or the mock treatment (□). Data represent mean (s.d. of blood glucose concentrations from two independent experiments. *, $P < 0.01$.

any later increase of blood glucose levels in mice with a homozygous PARP defect (*PARP*$^{-/-}$) compared with levels in wild-type mice (Fig. 1*a* and *b*). All mice remained normoglycemic throughout the observation period. Moreover, even at a dose of 240 mg per kg body weight, SZ was unable to cause hyperglycemia (data not shown). Mice with heterozygous PARP defect (*PARP*$^{+/-}$) had resistance to SZ-induced hyperglycemia identical to that in *PARP*$^{-/-}$ mice (Fig. 1*c*). Follow-up of the mice for up to 5 days confirmed hyperglycemia in *PARP*$^{+/+}$ mice and continuous normoglycemia in *PARP*$^{-/-}$ and *PARP*$^{+/-}$ mice (data not shown).

Preservation of islet ultrastructure and function

To determine the basis for this resistance to diabetes development, we assessed the morphology of the pancreatic islets. Histological analysis of pancreata from untreated *PARP*$^{+/+}$, *PARP*$^{-/-}$ and *PARP*$^{+/-}$ mice demonstrated normal structure and distribution of islets in all mice (not shown), and showed no difference in the percentage of the islet area occupied by insulin containing cells (about 70% of total islet area; Fig. 2*a*). This was confirmed by similar amounts of insulin (about 6 µg per 100 mg pancreas weight) extracted from total pancreata of *PARP*$^{+/+}$, *PARP*$^{-/-}$ and *PARP*$^{+/-}$ mice (Fig. 2*b*), indicating that PARP deficiency did not affect beta-cell growth or differentiation. After SZ administration, a substantial loss of insulin-positive cells was seen in the islets of wild-type mice (Fig. 2*c*). In contrast, the islet structure of *PARP*$^{-/-}$ mice was well-preserved, with a typical central insulin-positive area, similar to the islets of untreated controls (Fig. 2*d*) and of SZ-treated *PARP*$^{+/-}$ mice (data not shown). Morphometric evaluation of pancreatic sections from SZ-treated wild-type mice demonstrated a decrease in the insulin-positive islet area from 67.6 ± 11.8% to 23.5 ± 12.6% ($P < 0.001$; Fig. 2*a*). The resistance to diabetes induction in *PARP*$^{-/-}$ mice was also confirmed by the analysis of pancreatic insulin content (Fig. 2*b*). In *PARP*$^{+/+}$ mice there was an almost complete loss of insulin 3 days after SZ treatment (<0.5 µg per 100 mg pancreas weight compared with 5.9 ± 2.2 µg per 100 mg pancreas weight of untreated controls), whereas the pancreatic insulin content was only slightly decreased in *PARP*$^{-/-}$ mice (4.1 ± 1.9 µg per 100 mg pancreas weight, not significant compared with untreated mice) and in *PARP*$^{+/-}$ mice (3.4 ± 0.6 µg per 100 mg pancreas weight; $P < 0.01$ compared with untreated mice). Both values remained significantly higher than those of SZ-treated wild-type mice ($P < 0.01$). The morphometric data and analysis of pancreatic insulin content provide unequivocal evidence that the resistance to diabetes induction in *PARP*$^{-/-}$ or *PARP*$^{+/-}$ mice is because of improved survival and preserved function of beta cells.

Increased resistance of islet cells to SZ-induced damage

To test directly whether beta-cell destruction is due to the activation of PARP in these cells, we isolated primary pancreatic islet cells from mice of the three different genotypes (*PARP*$^{+/+}$, *PARP*$^{-/-}$ and *PARP*$^{+/-}$) and incubated them for 18 hours in the presence of

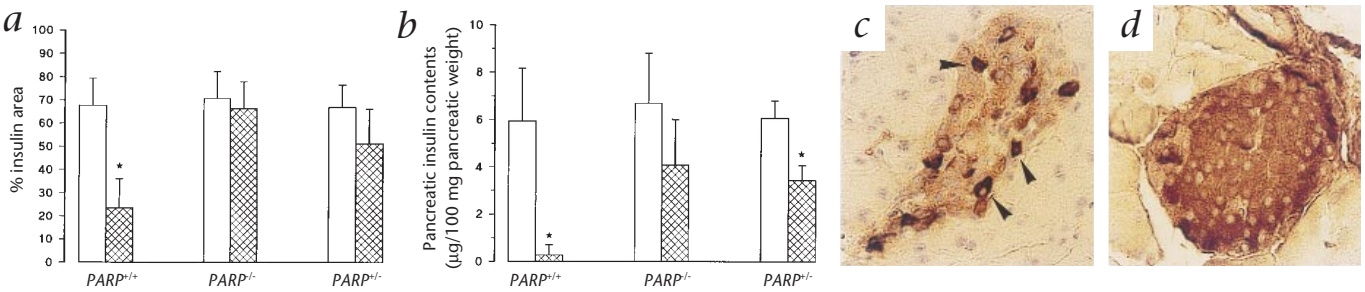

Fig. 2 Insulin levels in the pancreata of SZ-treated *PARP*$^{+/+}$, *PARP*$^{-/-}$ and *PARP*$^{+/-}$ mice. Pancreata were excised 3 days after mock treatment (□) or SZ treatment (▨). ***a***, The proportion of the islet area containing insulin-positive cells was quantified by morphometry from thin sections of paraffin embedded tissue. From each experimental group, six to eight pancreas sections with a total number of 30–80 islets were evaluated. ***b***, In parallel, insulin was extracted from the pancreata and the total insulin levels were determined by radioimmunoassay. Data represent mean (s.d. of determinations from three to five mice. *, $P < 0.001$. *c* and d, Immunohistochemical staining of pancreas sections from SZ-treated *PARP*$^{+/+}$ mice demonstrated an almost complete loss of insulin-positive beta cells (only a few cells stained brown; ***c***, arrowheads), whereas islets of SZ treated *PARP*$^{-/-}$ mice showed a well-preserved central area homogeneously occupied by insulin-containing cells (***d***). Original magnification, ×360.

Table SZ-induced lysis and NAD⁺ depletion in *PARP*$^{+/+}$, *PARP*$^{+/-}$ and *PARP*$^{-/-}$ islet cells

Islet cell genotype	SZ [a]	Lysis[b] (%)	NAD⁺ contents (pmol/10³ cells)	
PARP$^{+/+}$	-	7.9 ± 2.0	0.193 ± 0.075	(100.0 %)
	+	69.7 ± 5.8[c]	0.061 ± 0.012[c]	(31.6 %)
PARP$^{+/-}$	-	7.4 ± 0.7	0.109 ± 0.016	(100.0 %)
	+	35.8 ± 2.2[d]	0.104 ± 0.003[d]	(95.4 %)
PARP$^{-/-}$	-	6.9 ± 1.0	0.145 ± 0.019	(100.0 %)
	+	28.0 ± 5.8[d]	0.143 ± 0.013[d]	(98.6 %)

Pancreatic islet cells from *PARP*$^{+/+}$, *PARP*$^{+/-}$ and *PARP*$^{-/-}$ mice were isolated and exposed to SZ, and islet cell lysis and NAD⁺ levels were determined. Data represent means (s.d. from three to five experiments. [a]SZ concentrations: 1.2 mM for cell lysis; 2 mM for NAD⁺ determination. [b]Lysis was determined by the trypan blue exclusion assay. [c]$P < 0.001$ compared with untreated *PARP*$^{+/+}$ islet cells. [d]$P < 0.001$ compared with *PARP*$^{+/+}$ islet cells exposed to SZ.

various doses of SZ. Cell death was determined by staining them with a combination of CAM and EthD-1, which allows the simultaneous examination of membrane integrity and morphologic changes of the nuclear chromatin distribution[19], or by the trypan blue exclusion method, allowing the detection of membrane damage[20] (Fig. 3).

As expected, staining with CAM/EthD-1 showed that after 18 hours of exposure to 1.2 mM SZ, most of the wild-type *PARP*$^{+/+}$ cells were dead, as determined by the loss of calcein retention and the appearance of red nuclear fluorescence (Fig. 3*a* and *d*). The dying cells showed signs of disintegration, as seen in necrosis, whereas no morphological signs of apoptosis, such as chromatin condensation, nuclear fragmentation or membrane blebbing, could be detected. In contrast, there was no obvious reduction in the viability of the *PARP*$^{+/-}$ cells (Fig. 3*b* and *e*) or of the *PARP*$^{-/-}$ cells (Fig. 3*c* and *f*). Quantification of cell death showed that even in the presence of 1.2 mM SZ, only 16–19 % of the *PARP*$^{+/-}$ and *PARP*$^{-/-}$ cells lost the bright, uniform, green fluorescence indicative of living cells (Fig. 3*g*). In contrast, there was a dose-dependent increase of cell death in wild-type *PARP*$^{+/+}$ cells. At a concentration of 1.2 mM SZ, 69.0 ± 2.8% of the cells were dead, showing red nuclear fluorescence and a loss of calcein retention ($P < 0.005$ compared with *PARP*$^{+/-}$ and *PARP*$^{-/-}$ samples; Fig. 3*g*). Parallel analysis with trypan blue yielded comparable results (Fig. 3*h*). In SZ-exposed *PARP*$^{+/+}$ islet cells, dose-dependent lysis was observed, reaching 63.8 ± 12.7 % at 1.2 mM SZ. *PARP*$^{-/-}$ and *PARP*$^{+/-}$ islet cells were almost completely resistant to the lytic effects of the lower doses of SZ and showed only small amounts of lysis at 1.2 mM SZ (24.7 ± 13.3% and 26.1 ± 2.3%, respectively; $P < 0.01$ compared with *PARP*$^{+/+}$ samples; Fig. 3*h*). These results indicate that pancreatic islet cells are sensitive to SZ-induced toxicity and that PARP activation is required for islet-cell destruction.

SZ is taken up by the beta cell through GLUT-2 (ref. 10), and a differential expression of this gene on the cell surface could influence the sensitivity of islet cells. To exclude the possibility that the difference of *PARP*$^{+/+}$ and *PARP*$^{-/-}$ mice to SZ toxicity is due to different levels of GLUT-2 expression in these mice, we analyzed the expression of GLUT-2 mRNA in *PARP*$^{+/+}$ and *PARP*$^{-/-}$ islets by RT–PCR. Evaluation of the signals obtained after electrophoretic separation of the specific DNA fragments (Fig. 4) demonstrated no difference in GLUT-2 mRNA expression. This indicates that the mechanism of SZ uptake into the cell is not altered by the lack of the intact PARP gene or by the genetic alterations introduced during gene targeting.

SZ induces DNA damage rapidly after uptake[11,13]. We therefore tested for DNA fragmentation by labeling genomic DNA with digoxigenin-conjugated dUTP, and found substantial DNA fragmentation in cells from *PARP*$^{+/+}$ mice within 1 hour of incubation with SZ (Fig. 5). DNA fragmentation was also seen in SZ-exposed cells from *PARP*$^{-/-}$ mice, as indicated by the loss of intact DNA at the top of the gel and the concomitant appearance of DNA fragments in the lower part of the gel. The appearance of DNA fragments is 'ladder-like' because DNA breakage is mostly internucleosomal not only in apoptosis but also in radical-induced cell necrosis[8]. The identical expression of GLUT-2 and

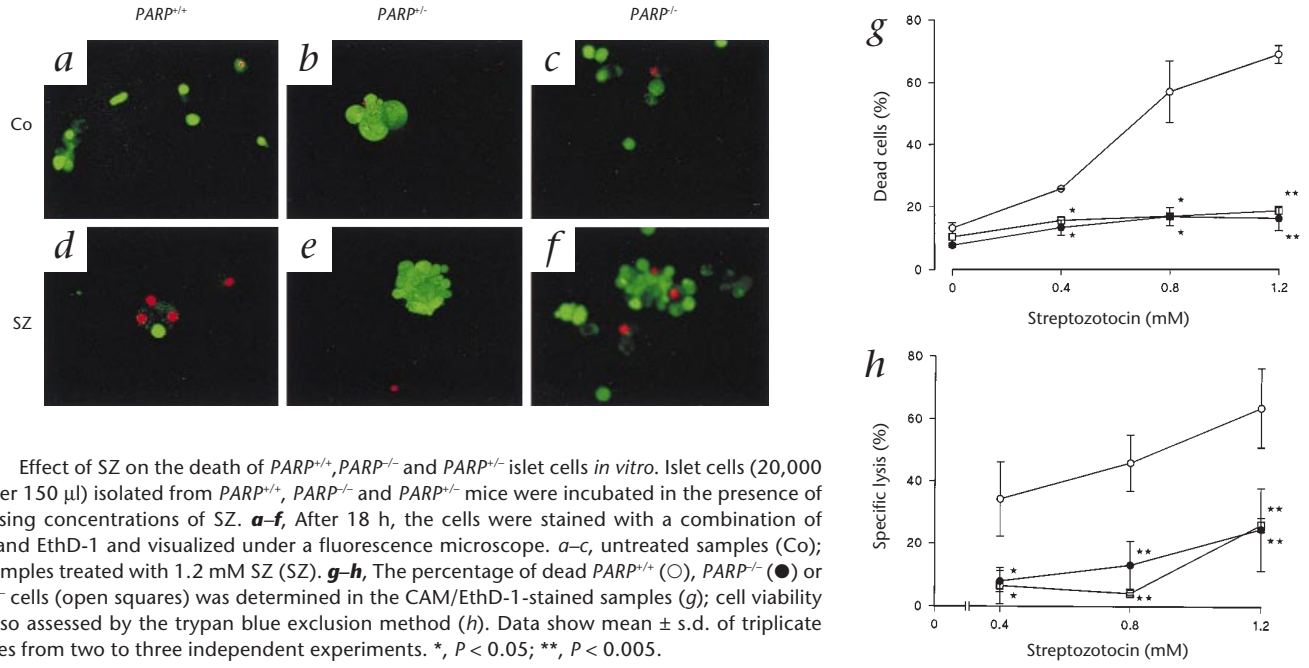

Fig. 3 Effect of SZ on the death of *PARP*$^{+/+}$,*PARP*$^{-/-}$ and *PARP*$^{+/-}$ islet cells *in vitro*. Islet cells (20,000 cells per 150 µl) isolated from *PARP*$^{+/+}$, *PARP*$^{-/-}$ and *PARP*$^{+/-}$ mice were incubated in the presence of increasing concentrations of SZ. ***a–f***, After 18 h, the cells were stained with a combination of CAM and EthD-1 and visualized under a fluorescence microscope. *a–c*, untreated samples (Co); *d–f*, samples treated with 1.2 mM SZ (SZ). ***g–h***, The percentage of dead *PARP*$^{+/+}$ (○), *PARP*$^{-/-}$ (●) or *PARP*$^{+/-}$ cells (open squares) was determined in the CAM/EthD-1-stained samples (*g*); cell viability was also assessed by the trypan blue exclusion method (*h*). Data show mean ± s.d. of triplicate samples from two to three independent experiments. *, $P < 0.05$; **, $P < 0.005$.

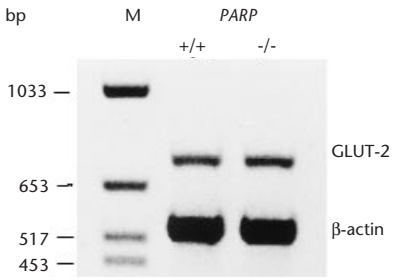

Fig. 4 Expression of GLUT-2 mRNA in *PARP*$^{+/+}$ and *PARP*$^{-/-}$ islets. RT–PCR and electrophoresis yielded identical signals for GLUT-2 in islets isolated from *PARP*$^{+/+}$ and *PARP*$^{-/-}$ mice, indicating an identical level of GLUT-2 expression in the islets of the two genotypes. β-actin served as internal control for the RNA preparation and the PCR reaction.

the same DNA fragmentation pattern observed in *PARP*$^{+/+}$ and *PARP*$^{-/-}$ islets indicate that the lack of PARP does not prevent SZ from entering cells and damaging DNA.

Because DNA breaks can activate PARP to form poly(ADP-ribose) using NAD^+, we determined the PARP activity by measuring intracellular NAD^+ levels in SZ-treated islet cells (Table). The NAD^+ levels of untreated islets were in a range of 0.109–0.193 pmol/10^3 cells (not statistically significant). After SZ treatment, the NAD^+ levels in islet cells isolated from wild-type mice decreased from 0.193 ± 0.075 pmol/10^3 cells to almost the detection limit within 1 hour (0.061 ± 0.012 pmol/10^3 cells; $P < 0.001$). In contrast, NAD^+ levels were almost completely preserved in *PARP*$^{-/-}$ cells as well as in *PARP*$^{+/-}$ cells (less than 5 % reduction for both cell types; Table). The lack of NAD^+ depletion in heterozygous *PARP*$^{+/-}$ cells indicates little PARP activity in these cells. This result correlates well with the observed diabetes resistance in heterozygous *PARP*$^{+/-}$ mice (Fig. 1). To determine whether the expression of PARP in these cells is altered, we analyzed lysates of islets of the three genotypes by western blot. PARP protein expression was only found in *PARP*$^{+/+}$ islets, whereas in both *PARP*$^{+/-}$ and *PARP*$^{-/-}$ islets, no PARP protein was detectable (Fig. 6).

Discussion

The data demonstrate resistance to SZ-induced diabetes development in mice with deficient PARP activity. The diabetes-protective action of PARP deficiency seems to occur at the level of insulin-producing beta cells of pancreatic islets. This conclusion is derived from our observation that isolated islet cells with PARP defect are resistant to SZ cytolytic activity in tissue culture.

The studies *in vitro* further provide information on the putative mechanism of PARP function in the pathogenesis of type 1 diabetes. PARP-dependent cell lysis was associated with substantial 'draining' of the NAD^+ 'pool'. PARP-mediated NAD^+ depletion has also been observed in islet cells[21,22] and also in other non-endocrine cell types[23–25] after exposure to reactive oxygen intermediates and NO. These studies indicate that PARP activation causes a rapid reduction of intracellular NAD^+ concentrations below an essential threshold, leading to cell death. Our data here show a significant reduction of NAD^+ levels only in SZ-treated islet cells with the *PARP*$^{+/+}$ genotype, which correlates with beta-cell death. Therefore, NAD^+ depletion due to PARP activation represents a principal event in SZ-induced islet cell death.

A decrease in intracellular NAD^+ levels causes depletion of the ATP 'pool' (ref. 26), a mechanism that can kill various cell types. Low levels of ATP enable cells to undergo necrosis[19,27]. Consistent with these findings, our results here on SZ-induced islet-cell death demonstrated obvious signs of necrosis, as evidenced by increased membrane leakage and lack of chromatin condensation. There was concomitant DNA breakage due to the DNA-damaging properties of SZ. Our findings are in agreement with reports suggesting that PARP deficiency does not interfere with apoptotic cell death, although PARP is universally cleaved during apoptosis, and in some cell types, transient PARP activity is detected in the early phase of apoptosis[28,29]. Cells isolated from *PARP*$^{-/-}$ mice have normal apoptotic responses[30], indicating that PARP is dispensable for most apoptotic processes. In another animal model of PARP deficiency[31], splenocytes and fibroblasts died faster after irradiation and treatment with an alkylating agent.

An unexpected finding of our study was that *PARP*$^{+/-}$ mice and islet cells were resistant to SZ-induced diabetes development and lysis *in vitro*, respectively. Indeed, despite heterozygosity for the intact *PARP* gene, islet cells with this genotype do not express any detectable amounts of PARP protein. Deficient PARP activity in *PARP*$^{+/-}$ mice is not limited to pancreatic islets but has also been reported for the brain tissue of *PARP*$^{+/-}$ mice; heterozygous *PARP*$^{+/-}$ mice are resistant to ischemic treatment, a phenotype similar to that of *PARP*$^{-/-}$ mice[23,32]. In contrast, *PARP*$^{+/+}$ as well as *PARP*$^{+/-}$ fibroblasts exposed to the DNA-damaging agent N-methyl-N-nitro-N-nitrosoguanine form high amounts of poly(ADP-ribose), indicating the presence of active PARP protein in the heterozygous fibroblast cells as well[17]. Moreover, embryonic fibroblasts isolated from *PARP*$^{+/-}$ mice express large amounts of PARP protein, similar to levels found in wild-type *PARP*$^{+/+}$ cells[30]. The results of our studies and these observations indicate a tissue- and/or cell-type-specific regulation of PARP protein expression in a genotype with heterozygous PARP defect. These differences may contribute to the differential susceptibility of the various *PARP*$^{+/-}$ cell and tissue types towards DNA damaging agents. However, the mechanism for this differential regulation of PARP expression is unknown.

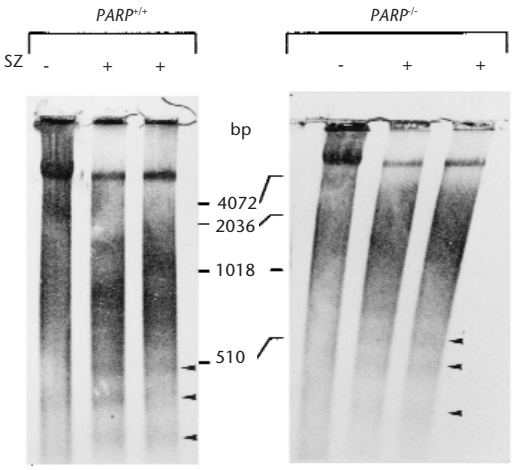

Fig. 5 SZ-induced DNA fragmentation in *PARP*$^{+/+}$ and *PARP*$^{-/-}$ cells. Splenic lymphocytes from *PARP*$^{+/+}$ and *PARP*$^{-/-}$ mice were incubated with or without 2 mM SZ for 1 h. After electrophoretic separation and immunoblotting, DNA from SZ-exposed cells shows fragmentation (arrowheads). Treatment with SZ is shown in duplicate to demonstrate reproducibility. Middle column, positions of molecular size standards, in base pairs (bp).

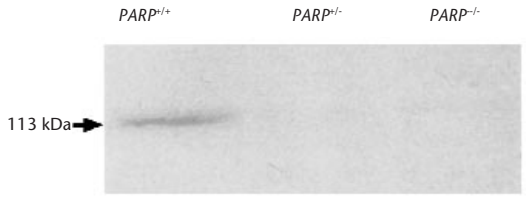

Fig. 6 Western blot analysis of PARP protein expression in *PARP*$^{+/+}$, *PARP*$^{+/-}$ and *PARP*$^{-/-}$ islets with a polyclonal antiserum. PARP was found only in samples derived from wild-type *PARP*$^{+/+}$ islets; samples of *PARP*$^{+/-}$ or *PARP*$^{-/-}$ islets did not produce detectable PARP protein.

Although PARP-deficient islet cells proved to be resistant to SZ-induced cell death *in vitro*, there was some lysis at very high SZ concentrations (Fig. 3), indicating the existence of a PARP-independent pathway of cell death, induced only by very high doses of SZ. *In vivo*, diabetogenic doses of SZ did not induce this PARP-independent pathway, as *PARP*$^{-/-}$ mice beta cells survived with improved function. The mechanism of PARP-independent toxicity with very high SZ doses *in vitro* may involve the inhibition of glucose and glutamine oxidation[33], the reduction of mitochondrial dehydrogenase activity[34] or the alkylation of essential glycolytic or mitochondrial enzyme systems[35].

Here we have identified PARP activity as an essential downstream executor in the development of SZ induced diabetes, and our results indicate that the repression of PARP expression or activation is able to preserve the functional activity of islet cells. Our findings also relate to beta-cell destruction in other models of human type 1 diabetes, as DNA damage and PARP activation have been reported consistently to occur in islet cells exposed to inflammatory stress[8,36]. Our observations also indicate that the prevention of a considerable loss of NAD^+ is essential for the survival of beta cells and for the protection from diabetes in a mouse model of human type 1 diabetes. After damage to nuclear DNA, a primary target of inflammatory mediators, excessive PARP activation is sufficient to reduce the NAD^+ content of the islet cells to lethal levels. Our proof of the chief role of PARP activation in the induction of beta-cell death and type 1 diabetes will be useful for the development of strategies to prevent this disease.

Methods

Mice. Mice lacking PARP were generated by homologous recombination. The generation and genotyping of the mice have been described in detail[17]. The mice used were derived from a mixed genetic background (129/SV × C57BL/6), and were from the same litter or the same family for each experiment.

Diabetes model. Hyperglycemia was induced in mice 10 weeks of age with a single intraperitoneal injection of streptozocin (160 or 240 mg per kg body weight; Boehringer), freshly dissolved in sodium citrate buffer. Mice in the control group received a corresponding volume of sodium citrate buffer alone. Nicotinamide (500 mg per kg body weight, Serva, Heidelberg, Germany) was administered intraperitoneally 6 h before the SZ injection. On days 0, 1, 2 and 3, blood was drawn from the tail vein and blood glucose concentrations were determined using the hexokinase method[37].

Immunohistochemistry. Three days after SZ administration, thin sections (5 μm in thickness) were prepared from paraffin-embedded pancreatic tissue. For insulin staining, anti-porcine insulin serum from guinea pig (Dako, Hamburg, Germany) was used as primary antibody and the Vectastain ABC Kit with anti-guinea pig IgG (Camon, Wiesbaden, Germany) as detection system. The percentage of insulin-positive cells in the islet area was determined morphometrically using the interactive image analysis system IBAS2 (Contron/Zeiss, Cologne, Germany) with the integrated image analysis software package KS 400.

Pancreatic insulin contents. Whole pancreata were removed from the mice and insulin was extracted by mechanical homogenization of the organ in the presence of 5 ml acid alcohol (ethanol (96%):H_3PO_4 (1 M):distilled water = 19:2:1 volume/volume). After 18 h of incubation at 4 °C, insulin was quantified in the supernatants of the samples by radioimmunoassay (Linco Research, St. Charles, Missouri) for mouse insulin.

Isolation and culture of islet cells and lymphocytes. Pancreatic islets were isolated from mice 4–6 months of age by collagenase digestion and were dispersed into single cells by gentle trypsin treatment as described[38]. Islet cells were cultivated for 18 h at 37 °C in 5 % CO_2 with or without SZ (at various concentrations) in culture medium RPMI 1640 (Sigma) with 10 % fetal calf serum (Sigma) and supplements as described[38]. Single-cell suspensions of splenocytes were prepared from freshly isolated spleens. Adherent macrophages were allowed to attach to plastic Petri dishes for1 h at 37 °C in 5 % CO_2 and were thereby removed from the cell suspension. Erythrocytes were lysed by incubation for 15–30 min in lysis buffer (10 mM $KHCO_3$, 155 mM NH_4Cl and 0.1 mM EDTA, pH 7.4).

DNA fragmentation analysis. DNA was isolated by salt–chloroform extraction from spleen lymphocytes. Isolated DNA was labelled by *in situ* nick translation using digoxigenin-conjugated dUTP (DIG DNA labeling kit; Boehringer). DNA samples (3.5 μg) were electrophoresed in a 1.5% agarose gel and then transferred onto nylon membranes (Hybond-N; Amersham). DIG-labeled hybrids were detected with an anti-DIG-alkaline phosphatase conjugate and nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate as substrates (DIG DNA detection kit; Boehringer).

Intracellular NAD^+ levels. NAD^+ levels were measured in untreated or SZ-exposed (for 1 h at 2mM) *PARP*$^{+/+}$, *PARP*$^{+/-}$ and *PARP*$^{-/-}$ islet cells by an enzymatic cycling method using alcohol dehydrogenase (E.C. 1.1.1.1) from *Saccharomyces cerevisiae*[21] (Boehringer).

Expression of PARP protein. Islets isolated from *PARP*$^{+/+}$, *PARP*$^{+/-}$ and *PARP*$^{-/-}$ mice were lysed in modified RIPA buffer, and 50 μg protein per lane were separated by 10% SDS–PAGE, transferred to nitrocellulose membrane and hybridized with polyclonal antiserum against PARP, Vic-5 (Boehringer). The proteins were visualized with peroxidase-conjugated antibody against rabbit IgG (Boehringer) followed by chemiluminescence detection (Boehringer).

Expression of GLUT-2 mRNA. Total RNA was prepared from 800 freshly isolated islets of *PARP*$^{+/+}$ and *PARP*$^{-/-}$ mice by phenol–chloroform extraction. Determination and quantification of specific mRNA was done by reverse transcription–polymerase chain reaction (RT–PCR). Specific primers for GLUT-2 (provided by H. Gleichmann, Düsseldorf) were 5′–ATGTCAGAAGACAAGATCACCGGA–3′ and 3′–CCTACTGGACGGATTTTGGCTC–5′. After a total of 30 cycles, the products were separated by electrophoresis. GLUT-2 cDNA signals were analyzed using a Lumi-Imager™ (Boehringer) with β-actin as internal reference.

Determination of cell death. Cell lysis was assessed by the trypan blue exclusion assay[20], as studies have shown that this assay yields results identical to those obtained either by determining the release of intracellular radiolabel or by electron microscopy[38]. Specific islet cell lysis was calculated as described[38]. Spontaneous lysis never exceeded 18% of the total cell number. In parallel, islet cells were stained with 2 μM calcein-acetoxymethylester (CAM; Molecular Probes, Eugene, Oregon) and 4 μM ethidium homodimer (EthD-1; Molecular Probes, Eugene, Oregon) and analyzed under the fluorescence microscope for calcein retention and EthD-1 exclusion. Necrotic cells were stained fluorescence red in their nuclei; living cells had uniform green fluorescence. Each experiment was done in triplicate, and 100–200 cells were evaluated microscopically in each sample.

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