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Original Research Communication

Transgenic Expression of Antioxidant Protein Thioredoxin in Pancreatic β Cells Prevents Progression of Type 2 Diabetes Mellitus

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ABSTRACT

The authors previously established a transgenic mouse line in the type 1 diabetes model, NOD mouse, in which thioredoxin (TRX), a redox protein, is overexpressed in pancreatic β cells, and found that TRX overexpression slows the progression of type 1 diabetes. Recent reports on type 2 diabetes suggest that oxidative stress also degrades the function of β cells. To elucidate whether TRX overexpression can prevent progressive β cell failure from oxidative stress in type 2 diabetes, the authors transferred the TRX transgene from the NOD mouse onto a mouse model of type 2 diabetes, the *db/db* mouse. The progression of hyperglycemia and the reduction of body weight gain and insulin content of the *db/db* mouse were significantly suppressed by the TRX expression. Furthermore, TRX suppressed the reduction of Pdx-1 and MafA expression in the β cells, which may be one of the cellular mechanisms for protecting β cells from losing their insulin-secreting capacity. These results showed that TRX can protect β cells from destruction not only in type 1 but also in type 2 diabetes, and that they provide evidence that oxidative stress plays a crucial role in the deterioration of β cell function during the progression of type 2 diabetes. *Antioxid. Redox Signal.* 10, 43–49.

INTRODUCTION

Prolonged exposure to elevated glucose levels after meals, referred to as *glucose toxicity*, has an adverse impact on cells and tissues. Hyperglycemia can be deleterious, especially for β cell function, and contributes to the progressive impairment of insulin secretion. Thus, during the progression of type 2 diabetes, glucose toxicity is an important factor in progressive β cell failure and the development of overt diabetes (20). Evidence that chronic hyperglycemia is associated with oxidative stress is available from many studies in humans with the disease, in whom markers for oxidative stress, measured by a wide variety of methods, are elevated. The harmful effects of oxidative stress are considered especially relevant for β cells, because these cells express only low levels of antioxidant enzymes, including superoxide dismutase (SOD), which converts superoxide radicals to H_2O_2, catalase and cellular glutathione peroxidase (Gpx-1), which detoxify H_2O_2, and thioredoxin (TRX) (9). Thus, the production of increased reactive oxidative intermediates (ROIs) in the face of low antioxidant defenses could result in the accumulation of ROIs and cause oxidative stress in β cells. In fact, in an animal model of type 2 diabetes, high glucose concentrations caused an increase in the intracellular peroxide level in the islets. Elevated ROIs affect the function and survival of β cells through the direct oxidization of cellular macromolecules, such as DNA and lipids, and through the activation of stress-sensitive signaling pathways. Although convincing data from human trials is lacking, antioxidant drugs can protect β cell function in rodent models of type 2 diabetes. Overexpressing antioxidant enzymes such as SOD, catalase,

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and Gpx-1 provides rodent β-cell lines with various degrees of protection from exogenous oxidants and inflammatory cytokines (17, 24). Although these data suggest that hyperglycemia causes β cell damage through oxidative stress, there has been no direct evidence for it *in vivo*.

TRX is a low-molecular-mass redox-active protein found in both prokaryotic and eukaryotic cells (8). Various stressors, including viral infection, ischemic insult, UV light, X-ray irradiation, and hydrogen peroxide, can induce the expression of TRX (8, 18). The most distinctive role of TRX is its repair of proteins oxidized by reactive oxidative intermediates (ROIs). TRX is also implicated in the intracellular signaling that protects cells from oxidative stress and apoptosis through the regulation of a set of transcription factors, including NF-κB, activator protein-1 (AP-1), cAMP response element-binding protein (CREB), polyomavirus enhancer-binding protein 2/core-binding factor (PEBP2/CBF), estrogen receptor, glucocorticoid receptor, and p53 (18, 25). In addition, TRX has a chaperon-like activity and regulates apoptosis signal-regulating kinase-1 (ASK-1) (18, 25).

Type 1 diabetes mellitus is caused by the autoimmune destruction of insulin-producing pancreatic β cells. We previously produced transgenic mice overexpressing TRX in pancreatic β cells on the genetic background of the non-obese diabetic (NOD) mouse, an animal model of type 1 diabetes mellitus. Analysis of these mice showed that TRX overexpression markedly reduced the incidence of diabetes, although the development of insulitis was not prevented (9). This result suggested that the autoimmune destruction of pancreatic β cells in type 1 diabetes is at least partially mediated by ROIs and that this mechanism can be blocked by TRX.

Recent reports revealed that Txnip/TBP2, a negative regulator of TRX (22), is increased in the pancreas of diabetic animals (17). Txnip exerts a proapoptotic effect, at least in part by inhibiting the activity of thioredoxin and inducing oxidative stress. Thus, hyperglycemia reduces TRX activity by increasing Txnip levels, which leads to an increase in oxidative stress in pancreatic β cells, and it is suggested that progression of type 2 diabetes and deterioration of insulin secretary response evoked by glucose toxicity may be caused by the reduction of TRX activity in pancreatic β cells.

In this report, we transferred the β cell-specific TRX expression to a mouse model of type 2 diabetes, the C57BL/KsJ-*db/db* mouse, and examined the effects of the TRX expression on the progression of diabetes in this mouse. We found that TRX expression suppressed the progressive impairment of β cell function, suggesting that hyperglycemia causes β cell damage through oxidative stress.

MATERIALS AND METHODS

Animals

Human TRX gene (mouse homologue of Thioredoxin 1) was isolated from human T cell line (23). Transgenic NOD mouse lines expressing human TRX under the human insulin promoter were reported previously (9). One of these lines (Line 21), which expresses high levels of TRX in the islets, was backcrossed with C57BL/KsJ-*db/+* mice (CLEA Japan, Tokyo, Japan). In the N5 generation, the mice with the most enriched C57BL/KsJ-type microsatellite markers were selected using the speed congenic approach, as described elsewhere (26). A male mouse was found to have C57BL/KsJ-type microsatellite markers on all chromosomes, and was used to produce the next generation. The resulting TRX-transgenic progeny from the N6–N7 generations were crossed to C57BL/KsJ-*db/+* mice to generate TRX-transgenic C57BL/KsJ-*db/db* mice (*db/db*-TRX(+)). Their littermates (*db/db*-TRX(−), *db/+*-TRX(+), and *db/+*-TRX(−)) served as controls. Mice were kept under specific pathogen-free conditions in the animal facility of Osaka University Graduate School of Medicine. The genotype of the TRX transgene was determined by PCR analysis using genomic DNA prepared from tail biopsies as the template, as described (2). The expression of TRX was confirmed by RT-PCR of the pancreas and Western blot analysis of the isolated islets in the *db/+*-TRX(+) mouse (data not shown).

Measurement of insulin content and serum insulin concentration

Pancreata were removed from mice, weighed, and homogenized in an acid-ethanol solution. The homogenate was spun in a centrifuge, and the supernatant was assayed for insulin by ELISA (Insulin ELISA kit, Mercodia, Uppsala, Sweden). Its protein concentration was measured by the Bradford method using protein assay kit according to the manufacturer's instruction (BioRad, Hercules, CA) and used to normalize the insulin content. Blood samples from fasting mice were obtained from the tail vein. Blood glucose levels were determined using Glutest-Ace (Sanwa Kagaku, Nagoya, Japan), and the serum insulin concentration was measured by ELISA (Mercodia).

Histological analysis

The pancreas was removed from each mouse, fixed in 10% formaldehyde, and embedded in paraffin. Thin sections at five levels, 100 μm apart, were cut for staining with hematoxylin-eosin and observed by light microscopy. For insulin immunohistochemical staining, deparaffinized and dehydrated sections were incubated with 10% normal swine serum in PBS to block nonspecific staining, and then incubated with diluted guinea pig anti-human insulin antibody (Oriental Yeast Company, Tokyo, Japan). Negative controls were incubated with 10% normal swine serum without the first antibody. The sections were then incubated with swine polyclonal anti-rabbit immunoglobulin/biotin (Dakocytomation, Glostrup, Denmark), and then with diluted peroxidase-conjugated streptavidin (Dakocytomation). After incubation with a DAB solution (20 mg of 3,3′-diaminobenzidine tetrahydrochloride and 20 μl of 30% hydrogen peroxidase in 100 ml PBS), the sections were dehydrated and examined.

For frozen sections, pancreatic tissue was embedded in OTC compound (Tissue-TEK, Miles, Elkhart, IN) and frozen in liquid nitrogen. Ten-micrometer-thick frozen sections were cut with a cryostat, placed on slides, and fixed in 4% paraformaldehyde for 10 min. The sections were then rinsed in PBS, incubated for 5 min in 1% Triton X-100, and after a second rinse, incubated in the appropriate blocking serum. The sections were incubated for 60 min at room temperature with the first antibody, washed with PBS, and then incubated for 60 min at room temperature with the fluorescein-conjugated second antibody. The first antibodies were guinea pig anti-porcine insulin antibody (Dakocytomation), rabbit anti-mouse Pdx-1 antibody

(Transgenic Co., Kumamoto, Japan), and rabbit anti-MafA (Bethyl, Montgomery, TX). The second antibodies were Alexa Fluor 488-conjugated anti-guinea pig IgG, Alexa Fluor 488-conjugated anti-rabbit IgG, and Alexa Fluor 594-conjugated anti-guinea pig IgG (Molecular Probes, Eugene, OR). After the sections were washed, specimens were observed with a fluorescence microscope (Olympus, Tokyo, Japan).

Statistical analysis

Results are presented as the mean ± SD. Differences in body weight, serum insulin concentration, and insulin content were analyzed using the Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Changes in body weight, blood glucose levels, and plasma insulin levels

In the *db/db*-TRX(−) mice, body weight rapidly increased until 15 weeks of age, when the rate of weight gain began a gradual decrease (Fig. 1). The body weight gain of *db/db*-TRX(+) male mice was always higher than that of the *db/db*-TRX(−) males (Fig. 1A). The average weight of the *db/db*-TRX(+) mice was significantly higher than that of the *db/db*-TRX(−) mice in the males after 20 weeks of age and in the females at 30 weeks of age, when the body weight of the nontransgenic mice began to decrease (Fig. 1A and B). No difference in body weight was observed between *db/+*-TRX(−) and *db/+*-TRX(+) mice.

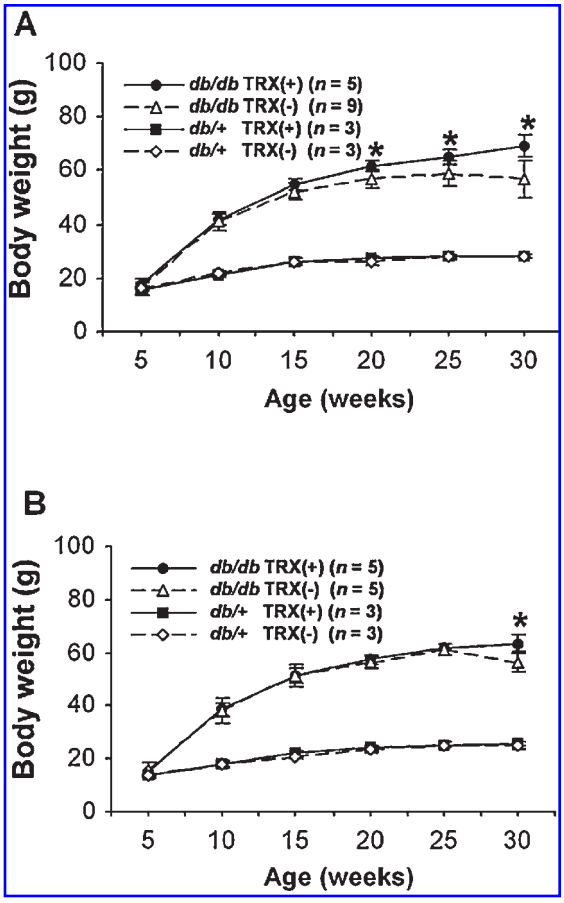

FIG. 1. Changes in body weight. Body weight was monitored for *db/db*-TRX(−), *db/db*-TRX(+), *db/+*-TRX(−), and *db/+*-TRX(+) male (**A**) and female (**B**) mice in the N8 generation from 5 to 30 weeks of age. Values are means ± SD. *$p < 0.05$, *db/db*-TRX(+) vs. *db/db*-TRX(−) mice.

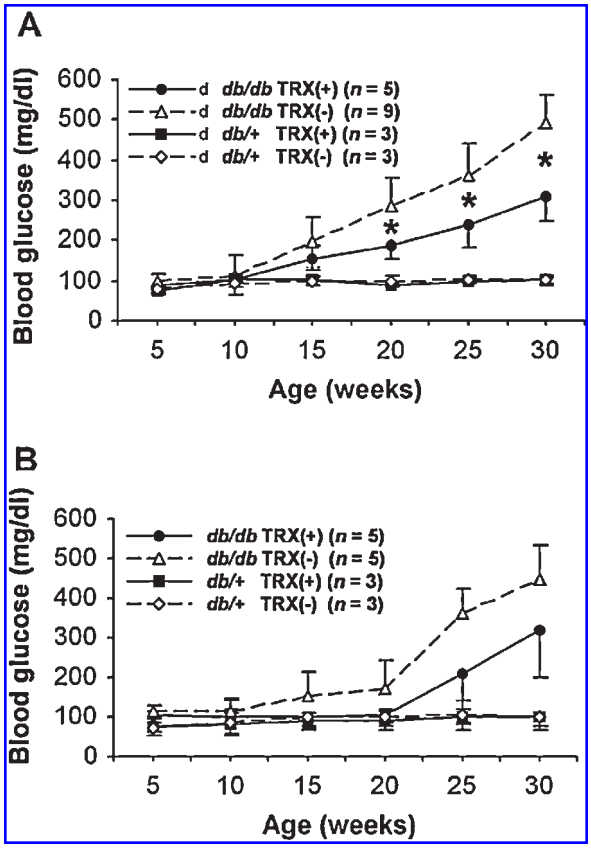

FIG. 2. Changes in blood glucose levels. Blood glucose levels were monitored for *db/db*-TRX(−), *db/db*-TRX(+), *db/+*-TRX(−), and *db/+*-TRX(+) male (**A**) and female (**B**) mice in the N8 generation from 5 to 30 weeks of age. Blood glucose levels of *db/db*-TRX(+) mice were always lower than those of *db/db*-TRX(−) mice. Values are means ± SD. *$p < 0.05$, *db/db*-TRX(+) vs. *db/db*-TRX(−) mice.

Blood glucose levels gradually increased in an age-dependent manner in both male and female *db/db* mice, although the onset of diabetes was earlier in the males (Fig. 2). TRX expression lowered blood glucose levels or retarded their elevation. In *db/+* mice, blood glucose levels did not change significantly during the observation period, regardless of TRX transgene expression. The plasma insulin concentration was monitored at 30 weeks of age (Fig. 3). It was significantly higher in male and female TRX transgenic mice than in their nontransgenic littermates.

The results using male siblings of the N7 generation [*db/db*-TRX(+) and *db/db*-TRX(−); $n = 5$ each] at 15 weeks of age were consistent with the above observations. The average body weight of the *db/db*-TRX(+) mice was significantly higher than

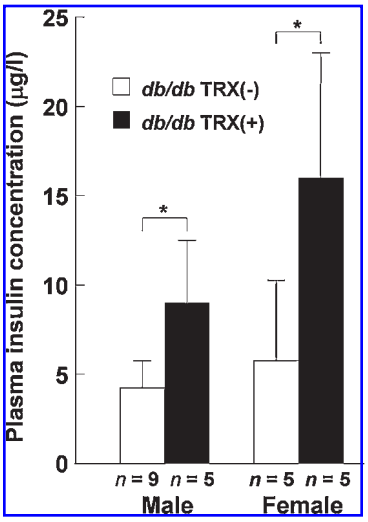

FIG. 3. Comparison of plasma insulin levels. Plasma insulin concentration was measured for *db/db*-TRX(−) and *db/db*-TRX(+) male and female mice in the N8 generation at 30 weeks of age. *$p < 0.05$, *db/db*-TRX(+) vs. *db/db*-TRX(−) mice.

that of the *db/db*-TRX(−) mice [48.4 ± 2.1 and 43.8 ± 2.2 g for *db/db*-TRX(+) and *db/db*-TRX(−) mice, respectively; $p < 0.05$]. The blood glucose levels were significantly lower in the *db/db*-TRX(+) mice than in the *db/db*-TRX(−) mice [247 ± 73 and 390 ± 64 mg/dl for *db/db*-TRX(+) and *db/db*-TRX(−) mice, respectively; $p < 0.05$]. The plasma insulin levels of *db/db*-TRX(+) mice were also significantly higher than those of the *db/db*-TRX(−) mice [7.0 ± 0.9 and 3.4 ± 1.0 ng/ml for *db/db*-TRX(+) and *db/db*-TRX(−) mice, respectively; $p < 0.05$].

Histological analysis of the pancreatic islets

Histological analysis of the pancreas of 16-week-old male mice showed only a few small islets in the *db/db*-TRX (−) pancreas, compared with the pancreas of *db/+* mice (Fig. 4). In contrast, islet hyperplasia was often observed in the pancreas of *db/db*-TRX(+) mice. Moreover, cystic lesions observed in the pancreas of *db/db*-TRX(−) mice were less prominent in *db/db*-TRX(+) mice. Immunohistochemical staining showed that only a subset of the islets in the *db/db*-TRX(−) pancreas stained with the anti-insulin antibody. Obviously more insulin-positive cells were observed in the *db/db*-TRX(+), although the anti-insulin antibody labeling was heterogeneous, and weaker than that in islets of the *db/+* mice.

Pdx-1 and MafA protein staining in islets

Pdx-1 is an essential transcription factor for pancreatic development. It also plays an important role in the maintenance of β cell function. Recently, Pdx-1 protein was reported to translocate from the nucleus to the cytoplasm in response to oxidative stress in a pancreatic β cell line (13). Therefore, if TRX exerts its protective effects against β cell destruction in *db/db* mice through the reduction of oxidative stress, TRX expression could affect the location of Pdx-1 in the β cells of these mice. We therefore used immunohistochemistry to label Pdx-1 in the pancreatic islets of TRX-transgenic and nontransgenic *db/db* mice (Fig. 5). In the *db/+* control mice, Pdx-1 was located in the nucleus even at 30 weeks of age (Fig. 5D), but in 16-week-old *db/db*-TRX(−) mice, Pdx-1 was detected in the cytoplasm of the islet cells (Fig. 5F), and in 30-week-old mice, it was hardly detectable in any of the islet cells (Fig. 5H). In contrast, in 16-week-old *db/db*-TRX(+) mice, most islet cells retained the Pdx-1 protein in the nucleus (Fig. 5J), and nuclear localization persisted in most of the Pdx-1-positive islet cells at 30 weeks (Fig. 5L).

MafA is a transcription factor that binds to the RIPE-3b1 site of the insulin gene, and its levels decrease via a post-translational mechanism in glucotoxic β cells (5). Thus, MafA is another candidate for involvement in the pancreatic β cell dysfunction evoked by oxidative stress. In *db/db*-TRX(−) mice at 30 weeks of age, MafA immunoreactivity was not detected in the nuclei of the islet cells (Fig. 6H). In contrast, MafA immunoreactivity was detected in the islet cells of the *db/db*-TRX(+) mice (Fig. 6K). Note, though, that most of the insulin-positive cells were also MafA-positive in both the TRX-transgenic and -nontransgenic mice (Figs. 6I and L), suggesting MafA plays an essential role in the maintenance of insulin production.

Insulin content of pancreata

The above observations from immunostaining suggested that TRX expression protected the transcription of the insulin gene from being downregulated by oxidative stress. To further confirm this, the insulin content of the pancreas of the male mice was measured. The pancreatic insulin content of *db/+* mice at 30 weeks of age was 142 ± 17 ng/mg pancreas ($n = 3$). In *db/db*-TRX(+) mice, it was lower than this value, but was still ~1.5-fold higher at 30 weeks of age than in *db/db*-TRX(−) mice ($p < 0.05$) (Fig. 7). Thus, although TRX gene could partially restore the insulin content, it could not prevent the decrease of insulin content of the db/db mouse.

To confirm the above observation, male siblings from the N7 generation (*db/db*-TRX(+) and *db/db*-TRX (−); $n = 5$ each) were examined at 15 weeks of age. The pancreatic insulin content of the *db/db*-TRX(+) mice was significantly higher than that of TRX(−) mice, even at this age (Fig. 7).

DISCUSSION

Several reports show that an antioxidant drug can prevent the progression of type 2 diabetes in the *db/db* mouse model (12, 13). However, because the effects of antioxidant drugs are not organ-specific, it was possible that the drugs worked by ameliorating insulin resistance rather than by protecting the β cells (1, 2). Therefore, it has not been clear whether oxidative stress affects the progression of type 2 diabetes through the injury of β cells. On the other hand, *in vitro* studies showed that the overexpression of an antioxidant enzyme protects β cell lines from oxidative stress (20, 21). Therefore, if oxidative stress on β cells plays a major role in the progression of type 2 diabetes, β cell-specific expression of antioxidant enzymes should ameliorate

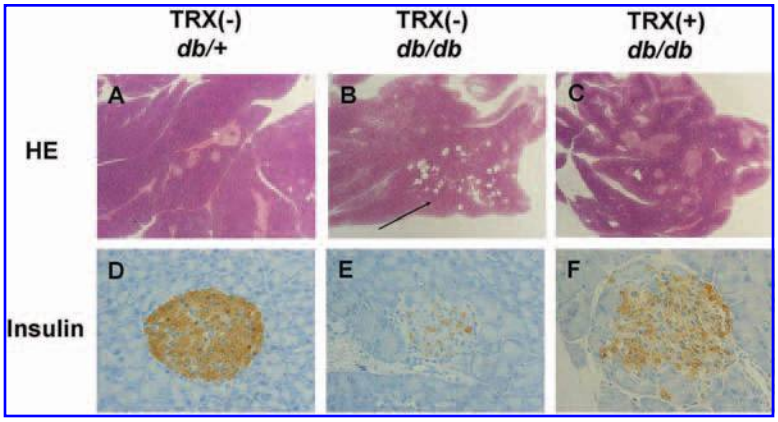

FIG. 4. Histological analysis of pancreatic sections. Sections of pancreas from 16-week-old *db/+*-TRX(−), *db/db*-TRX(−), and *db/db*-TRX(+) mice stained with hematoxylin-eosin (HE) (**A–C**) or immunostained with an anti-insulin antibody (**D–F**). The cystic lesions and small islets observed in the *db/db*-TRX(−) sections (**B**, *arrow*) were less prominent in the *db/db*-TRX(+) sections (**C**). More insulin-positive cells were observed in the *db/db*-TRX(+) islets (**F**) than in the *db/db*-TRX(−) islets (**E**).

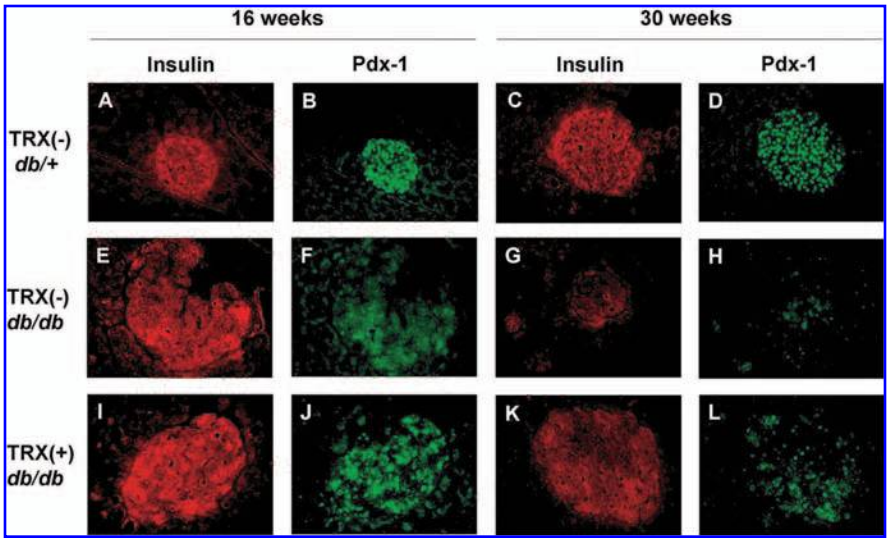

FIG. 5. Immunofluorescence analysis of Pdx-1 expression in islets. Pancreas sections of *db/+*-TRX(−) (**A–D**), *db/db*-TRX(−) (**E–H**), and *db/db*-TRX(+) (**I–L**) mice at 16 (*left*) and 30 (*right*) weeks of age were stained with anti-insulin (*red*) and anti-Pdx1 (*green*) antibodies. In the 16-week-old *db/db*-TRX(+) mice, most islet cells relegated Pdx-1 protein to their nucleus (**J**). In contrast, Pdx-1 was mostly detected in the cytoplasm of the islet cells in *db/db*-TRX(−) mice of the same age (**F**).

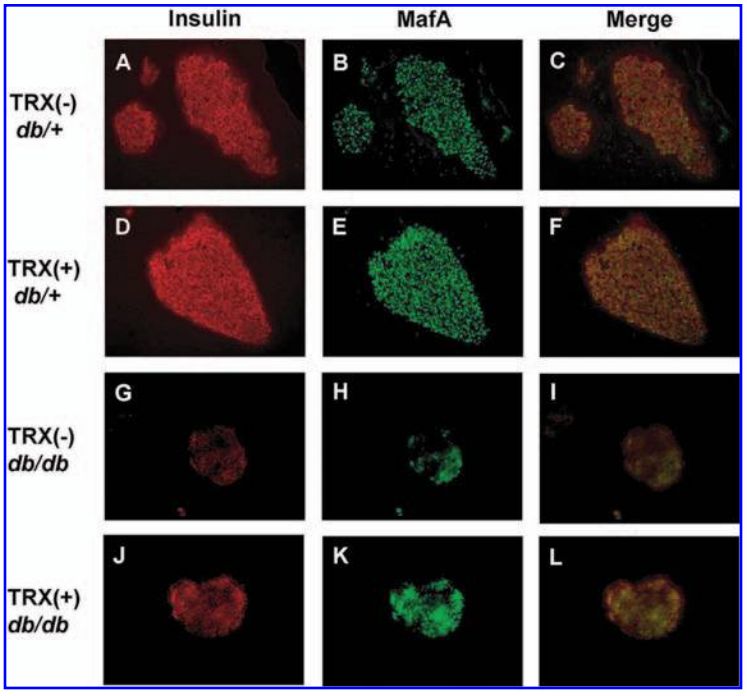

FIG. 6. Immunofluorescence analysis of MafA expression in islets. Pancreas sections of *db/+*-TRX(−) (**A–C**), *db/+*-TRX(+) (**D–F**), *db/db*-TRX(−) (**G–I**), and *db/db*-TRX(+) (**J–L**) mice at 30 weeks of age were stained with anti-insulin (*red*) and anti-MafA (*green*) antibodies. The islets of the *db/db*-TRX(+) mice were clearly stained with the anti-MafA antibody (**K**) in contrast to the weak staining of the islets of *db/db*-TRX(−) mice (**H**).

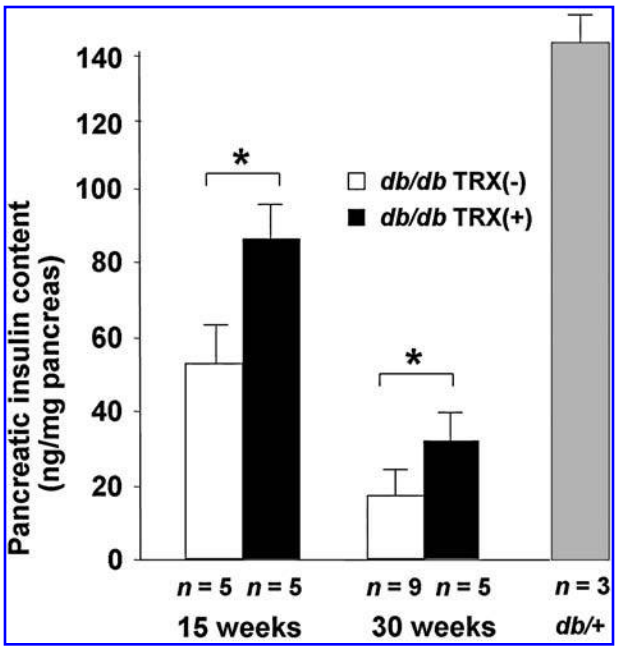

FIG. 7. Insulin content of the pancreas. The pancreatic insulin content was measured for *db/db*-TRX(+) and *db/db*-TRX(−) male mice in the N7 generation at 15 weeks of age and in *db/db*-TRX(+), *db/db*-TRX(−), and db/+ male mice in the N8 generation at 30 weeks of age. Values are mean ± SD. $^*p < 0.05$.

the disease progression in this mouse model. Our present study confirmed this hypothesis by showing that the transgenic overexpression of TRX in pancreatic β cells ameliorated glucose intolerance and helped to preserve β cell function in the *db/db* mouse.

We previously produced three transgenic NOD mouse lines in which the human TRX gene was expressed with the regulatory sequence of the human insulin gene. Among these, Line 21 produced the highest levels of TRX protein, exclusively in the pancreatic islets, and without any toxic effects on pancreatic β cell functions, including glucose-induced insulin secretion (9). In this transgenic mouse line, the expression of TRX in β cells successfully protected β cells from destruction by the autoimmune mechanism. Therefore, we used Line 21 as the parental line in crosses over several generations with *db/+* mice on the C57BL/KsJ background, to establish mice with β cell-specific TRX expression on the *db/db*-C57BL/KsJ genetic background. In these mice, as in the NOD mice, the exogenous TRX protected the β cells from injury. Thus, our results indicate that the levels of exogenous TRX obtained in transgenic mice not only protected β cells from oxidative stress caused by the autoimmune mechanism during the progression of type 1 diabetes, but also suggested to protect them against the oxidative stress induced by glucose toxicity during the progression of type 2 diabetes.

Pdx-1 is a homeodomain-containing transcription factor that plays an essential role in both insulin gene regulation and pancreatic development. Several lines of evidence have shown that the reduced insulin gene expression observed as a result of glucose toxicity is at least partly due to decreased Pdx-1 transcriptional activity (4, 6, 12, 14, 19, 20, 24). Here, we found that the nuclear Pdx protein levels in β cells were reduced during oxidative stress evoked by chronic hyperglycemia, resulting in the deterioration of the insulin secretory capacity of the β cells. In TRX transgenic *db/db* mice, Pdx-1 expression was maintained at a higher level and was readily detectable in islet cell nuclei compared with the nontransgenic *db/db* mice (Fig. 5). Similar phenomena were observed in *db/db* mice treated with antioxidant therapy (20). Thus, the overexpression of TRX supported the expression of Pdx-1 in the nuclei of β cells, which may be one of the cellular mechanisms for protecting β cells from losing their insulin-secreting capacity.

MafA is another transcription factor that is crucial for regulating the insulin gene. Harmon *et al*. recently reported that oxidative stress mediates the post-translational loss of MafA protein in glucotoxic HIT-T15 cells, leading to reduced insulin gene expression; they also showed that an antioxidant, NAC, could prevent the loss of MafA (5). Likewise, Kitamura *et al*. showed that MafA levels were decreased in the pancreatic β cells of a diabetes model mouse (15). In accord with these results, we observed that the MafA protein was lost from the β cells of *db/db* mice, and that TRX overexpression in β cells substantially prevented this loss. Therefore, the regulation of MafA expression may be another cellular mechanism for preserving the insulin-secreting function of β cells.

The hyperinsulinemia seen in young *db/db* mice may be an adaptive response to insulin resistance. There are several lines of evidence showing that the long-term hypersecretion of insulin in response to glucose may lead to enhanced production of ROSs. The DBA/2 mouse is a model of pancreatic islet susceptibility. Unlike the resistant C57BL/6 mouse strain, the DBA/2 mouse islets fail when stressed with insulin resistance or when exposed to chronic high glucose concentrations. Kooptiwut *et al*. showed that the DBA/2 mouse has increased glucose-mediated insulin secretion from a very early age, which is associated with higher glycolysis and glucose oxidation, and thus suggests a link between insulin hypersecretion and subsequent β cell failure (16). Furthermore, Fridlyand and Philipson showed that the same pathway used in the activation of glucose-dependent insulin secretion can dramatically enhance ROS production and manifestation of oxidative stress and, possibly, apoptosis (3). Thus, hypersecretion of insulin leads to ROS production and accelerates β cell failure. Recently, Ivarsson *et al.* also reported that the overexpression of TRX in β cells suppressed insulin exocytosis by inhibiting the NADPH effect (11). Therefore, it is possible that TRX exerts its protective effect in part by suppressing insulin hypersecretion in response to hyperglycemia and ROS production in response to glucose toxicity in the early phase of β cell failure.

Our previous and present studies show that the overexpression of TRX protects β cells and ameliorates the progression of both types 1 and 2 diabetes. In other words, TRX can protect the pancreatic β cells from the oxidative stress triggered by the immune response and by glucose toxicity. Thus, oxidative stress may be a common factor in the progression of both forms of diabetes. It is interesting to note that type 1 and type 2 diabetes have some susceptibility genes in common (10). These genes influence the susceptibility of pancreatic β cells to oxidative stress. The analysis of these common susceptibility genes will contribute to a better understanding of β cell failure during the progression of diabetes.

Our present study suggests that the administration of drugs that help protect β cells from oxidative stress should be an effective treatment for suppressing the progression of diabetes from the insulin-resistance stage to the overt diabetes stage. There are several agents, such as geranylgeranylacetone (GGA), that are known to induce the TRX expression (7). Thus, therapy with increased TRX expression as its target is a promising novel strategy for preventing both type 1 and type 2 diabetes mellitus.

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ABBREVIATIONS

Gpx-1, cellular glutathione peroxidase; Pdx-1, pancreatic-duodenal homeobox-1; ROIs, reactive oxidative intermediates; SOD, superoxide dismutase; TRX, thioredoxin.

REFERENCES

1. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 23: 599–622, 2002.
2. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 52: 1–8, 2003.
3. Fridlyand LE and Philipson LH. Does the glucose-dependent insulin secretion mechanism itself cause oxidative stress in pancreatic beta-cells? *Diabetes* 53: 1942–1948, 2004.
4. Harmon JS, Gleason CE, Tanaka Y, Oseid EA, Hunter–Berger KK, and Robertson RP. *In vivo* prevention of hyperglycemia also prevents glucotoxic effects on PDX-1 and insulin gene expression. *Diabetes* 48: 1995–2000, 1999.
5. Harmon JS, Stein R, and Robertson RP. Oxidative stress-mediated, post-translational loss of MafA protein as a contributing mechanism to loss of insulin gene expression in glucotoxic beta cells. *J Biol Chem* 280: 11107–11113, 2005.
6. Harmon JS, Tanaka Y, Olson LK, and Robertson RP. Reconstitution of glucotoxic HIT-T15 cells with somatostatin transcription factor-1 partially restores insulin promoter activity. *Diabetes* 47: 900–904, 1998.
7. Hirota K, Nakamura H, Masutani H, and Yodoi J. Thioredoxin superfamily and thioredoxin-inducing agents. *Ann NY Acad Sci* 957: 189–199, 2002.
8. Holmgren A. Thioredoxin. *Annu Rev Biochem* 54: 237–271, 1985.
9. Hotta M, Tashiro F, Ikegami H, Niwa H, Ogihara T, Yodoi J, and Miyazaki J. Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J Exp Med* 188: 1445–1451, 1998.
10. Ikegami H, Fujisawa T, and Ogihara T. Mouse models of type 1 and type 2 diabetes derived from the same closed colony: genetic susceptibility shared between two types of diabetes. *ILAR J/National Research Council, Institute of Laboratory Animal Resources* 45: 268–277, 2004.
11. Ivarsson R, Quintens R, Dejonghe S, Tsukamoto K, in 't Veld P, Renstrom E, and Schuit FC. Redox control of exocytosis: regulatory role of NADPH, thioredoxin, and glutaredoxin. *Diabetes* 54: 2132–2142, 2005.
12. Kajimoto Y and Kaneto H. Role of oxidative stress in pancreatic beta-cell dysfunction. *Ann NY Acad Sci* 52: 168–176, 2003.
13. Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, and Hori M. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 48: 2398–2406, 1999.
14. Kawamori D, Kajimoto Y, Kaneto H, Umayahara Y, Fujitani Y, Miyatsuka T, Watada H, Leibiger IB, Yamasaki Y, and Hori M. Oxidative stress induces nucleo-cytoplasmic translocation of pancreatic transcription factor PDX-1 through activation of c-Jun NH(2)-terminal kinase. *Diabetes* 48: 2896–2904, 1999.
15. Kitamura YI, Kitamura T, Kruse JP, Raum JC, Stein R, Gu W, and Accili D. FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. *Cell Metab* 2: 153–163, 2005.
16. Kooptiwut S, Kebede M, Zraika S, Visinoni S, Aston–Mourney K, Favaloro J, Tikellis C, Thomas MC, Forbes JM, Cooper ME, Dunlop M, Proietto J, and Andrikopoulos S. High glucose-induced impairment in insulin secretion is associated with reduction in islet glucokinase in a mouse model of susceptibility to islet dysfunction. *J Mol Endocrinol* 35: 39–48, 2005.
17. Minn AH, Hafele C, and Shalev A. Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. *Endocrinology* 146: 2397–2405, 2005.
18. Nakamura H, Nakamura K, and Yodoi J. Redox regulation of cellular activation. *Annu Rev Immunol* 15: 351–369, 1997.
19. Olson LK, Sharma A, Peshavaria M, Wright CV, Towle HC, Rodertson RP, and Stein R. Reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to a supraphysiologic glucose concentration is associated with loss of STF-1 transcription factor expression. *Proc Natl Acad Sci USA* 92: 9127–9131, 1995.
20. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem* 279: 42351–42354, 2004.
21. Robertson RP, Harmon J, Tran PO, and Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 53: S119–S124, 2004.
22. Schulze PC, Yoshioka J, Takahashi T, He Z, King GL, and Lee RT. Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein. *J Biol Chem* 279: 30369–30374, 2004.
23. Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, Brown N, Arai K, Yokota T, and Wakasugi H. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO J* 8: 757–764, 1989.
24. Tanaka Y, Gleason CE, Tran PO, Harmon JS, and Robertson RP. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci USA* 47: 10857–10862, 1998.
25. Watson WH, Yang X, Choi YE, Jones DP, and Kehrer JP. Thioredoxin and its role in toxicology. *Toxicol Sci* 78: 3–14, 2004.
26. Yamamoto T, Yamato E, Tashiro F, Sato T, Noso S, Ikegami H, Tamura S, Yanagawa Y, and Miyazaki JI. Development of autoimmune diabetes in glutamic acid decarboxylase 65 (GAD65) knockout NOD mice. *Diabetologia* 47: 221–224, 2004.

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2. E. Reich, A. Tamary, R. Vogt Sionov, D. Melloul. 2012. Involvement of thioredoxin-interacting protein (TXNIP) in glucocorticoid-mediated beta cell death. *Diabetologia* . [CrossRef]
3. Soon Lim, Hiroki Ashida, Rie Watanabe, Koji Inai, Yun-Soo Kim, Keiko Mukougawa, Hirokazu Fukuda, Ken-ichi Tomizawa, Kei-ichi Ushiyama, Hiroshi Asao, Masahiro Tamoi, Hiroshi Masutani, Shigeru Shigeoka, Junji Yodoi, Akiho Yokota. 2011. Production of biologically active human thioredoxin 1 protein in lettuce chloroplasts. *Plant Molecular Biology* **76**:3-5, 335-344. [CrossRef]
4. Yan Hang, Roland Stein. 2011. MafA and MafB activity in pancreatic # cells. *Trends in Endocrinology & Metabolism* . [CrossRef]
5. Xinghua Cheng , Richard C.M. Siow , Giovanni E. Mann . 2011. Impaired Redox Signaling and Antioxidant Gene Expression in Endothelial Cells in Diabetes: A Role for Mitochondria and the Nuclear Factor-E2-Related Factor 2-Kelch-Like ECH-Associated Protein 1 Defense Pathway. *Antioxidants & Redox Signaling* **14**:3, 469-487. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Corinne Leloup , Louis Casteilla , Audrey Carrière , Anne Galinier , Alexandre Benani , Lionel Carneiro , Luc Pénicaud . 2011. Balancing Mitochondrial Redox Signaling: A Key Point in Metabolic Regulation. *Antioxidants & Redox Signaling* **14**:3, 519-530. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Hiroshi Masutani, Eiji Yoshihara, So Masaki, Zhe Chen, Junji Yodoi. 2011. Thioredoxin binding protein (TBP)-2/Txnip and #-arrestin proteins in cancer and diabetes mellitus. *Journal of Clinical Biochemistry and Nutrition* . [CrossRef]
8. Li Kong, Xiaohong Zhou, Feng Li, Juni Yodoi, James McGinnis, Wei Cao. 2010. Neuroprotective effect of overexpression of thioredoxin on photoreceptor degeneration in Tubby mice. *Neurobiology of Disease* **38**:3, 446-455. [CrossRef]
9. Rachel L. Fogle, Christopher J. Lynch, Mary Palopoli, Gina Deiter, Bruce A. Stanley, Thomas C. Vary. 2010. Impact of Chronic Alcohol Ingestion on Cardiac Muscle Protein Expression. *Alcoholism: Clinical and Experimental Research* no-no. [CrossRef]
10. Hiroshi Ikegami, Masaya Ono, Tomomi Fujisawa, Yoshihisa Hiromine, Yumiko Kawabata, Eiji Yamato. 2008. Molecular Scanning of the Gene for Thioredoxin, an Antioxidative and Antiapoptotic Protein, and Genetic Susceptibility to Type 1 Diabetes. *Annals of the New York Academy of Sciences* **1150**:1, 103-105. [CrossRef]
11. Benbo Song, Donalyn Scheuner, David Ron, Subramaniam Pennathur, Randal J. Kaufman. 2008. Chop deletion reduces oxidative stress, improves # cell function, and promotes cell survival in multiple mouse models of diabetes. *Journal of Clinical Investigation* **118**:10, 3378-3389. [CrossRef]