Original Article

TIMP-1 Transgenic Mice Recover From Diabetes Induced by Multiple Low-Dose Streptozotocin

Hongwei Jiang,[1,2] Hanyu Zhu,[1] Xiangmei Chen,[1] Youming Peng,[2] Jianzhong Wang,[1] Fuyou Liu,[2] Suozhu Shi,[1] Bo Fu,[1] Yang Lu,[1] Quan Hong,[1] Zhe Feng,[1] Kai Hou,[1] Xuefeng Sun,[1] Guangyan Cai,[1] Xueguang Zhang,[1] and Yuansheng Xie[1]

Type 1 diabetes results from autoimmune destruction of the insulin-producing β-cells of pancreatic islets, of which the capacity for self-replication in the adult is too limited to restore following extensive tissue injury. Tissue inhibitor of metalloproteinase (TIMP)-1 inhibits matrix metalloproteinase activity and regulates proliferation and apoptosis of a variety of cells types, depending on the context. Here, we show that overexpression of human TIMP-1 in pancreatic β-cells of transgenic mice counteracts the cytotoxicity and insulitis induced by multiple low-dose streptozotocin (MLDS). Nontransgenic mice developed severe hyperglycemia, hypoinsulinemia, and insulitis 2 weeks after streptozotocin administration and died within 17 weeks. However, MLDS-treated transgenic mice gradually normalized the metabolic parameters and survived. β-Cell mass increased in parallel as a result of enhancement of β-cell replication. Thus, our results have demonstrated for the first time that overexpression of TIMP-1 in β-cells enhances the replication of pancreatic islets β-cells and counteracts type 1 diabetes, indicating that the *TIMP-1* gene may be a potential target to prevent, or even reverse, type 1 diabetes. *Diabetes* 56:49–56, 2007

β-Cell mass regulation represents a critical issue for understanding diabetes, a disease characterized by a near-absolute (type 1) or relative (type 2) deficiency in the number of pancreatic β-cells (1). The low capacity for self-replication in the adult is too limited to result in a significant regeneration following extensive tissue injury (2). Recent studies have demonstrated that it is feasible to regenerate and expand the β-cell mass by the application of hormones and growth factors (3–8). The mechanisms by which the targeted proteins have brought about islet mass expansion are quite distinct. These mechanisms include accelerated replication of preexisting β-cells, an augmentation of islet neogenesis, an increase in β-cell size (hypertrophy), and a reduction in the rate of β-cell death (3–8).

Tissue inhibitor of metalloproteinase (TIMP)-1 is one representative of the natural matrix metalloproteinase (MMP) inhibitor family, which is known to possess a broad range of biological activities, including inhibition of MMP activity, regulation of proliferation and apoptosis of a variety of cell types, and, depending on the context, differential regulation of angiogenic and inflammatory responses (9,10).

TIMP-1 was first described as proteins potentiating the Epo effect on erythroid progenitor cell proliferation and differentiation (11–13). The cell growth-promoting effects of TIMP-1 have been then extended to a wide range of cells, including keratinocytes, chondrocytes, fibroblasts, epithelial and endothelial cells, lymphoid and myeloid cells, and in pathological conditions in scleroderma fibroblasts, hepatoma, breast carcinomas, and human osteosarcoma cells (14–18). In a very interesting manner, TIMP-1 protein accumulation has been reported in the nucleus of human gingival fibroblasts (Gin-1 cells) and reached a maximum at the phase S of the cell cycle (19). In addition, TIMP-1 expression is associated with resistance to apoptosis of a wide range of cells including Burkitt's lymphoma cell lines, normal tonsillar B-cells, human granulocytes, normal capillary endothelial cells, rat mesangial cells, MCF10A human breast epithelial cells, and Hodgkin/Reed-Sternberg cells (20–26). However, TIMP-1 has also been described to inhibit proliferation and induce apoptosis (27–29).

It is interesting that we discovered that TIMP-1 transgenic mice, which were generated as previously described (30), are resistant to diabetes induced by streptozotocin. This result agrees with the earlier report of Han et al. (31), which proved that TIMP-1 prevented cytokine-induced apoptosis and cytokine-mediated inhibition of glucose-stimulated insulin secretion in rat islets and β-cells in vitro. In addition, it has been recently reported (32,33) that MMP was involved in high fat–induced diabetes in female ZDF rats and that deficiency of TIMP-3 was involved in the pathogenesis of diabetes and vascular inflammation of insulin receptor haploinsufficient mice. These findings let us hypothesize that overexpression of TIMP-1 in pancreatic β-cells may counteract the cytotoxicity or even reverse diabetes induced by multiple low-dose streptozotocin (MLDS).

In this study, we investigated the influence of overex-

From the [1]Department of Nephrology, Kidney Center and Key Lab of the People's Liberation Army (PLA), General Hospital of PLA, Beijing, China; and the [2]Department of Nephrology, 2nd Xiangya Hospital, Institute of Nephrology, Central South University, Changsha, Hunan Province, China.

Address correspondence and reprint requests to Xiangmei Chen, MD, Department of Nephrology, Kidney Center and Key Lab of PLA, General Hospital of PLA, Fuxing Road 28, Beijing 100853, P.R. China. E-mail: xmchen@public.bta.net.cn.

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H.J. and H.Z. contributed equally to this work.

BrdU, 5-bromo-2-deoxyuridine; HBSS, Hanks' buffered saline solution; H&E, hematoxyline & eosin; MLDS, multiple low-dose streptozotocin; MMP, matrix metalloproteinase; RT, reverse transcription; TIMP, tissue inhibitor of metalloproteinase; TUNEL, transferase biotin-dUTP nick-end labeling.

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pression of TIMP-1 on diabetes induced by MLDS. We found that MLDS-treated transgenic mice overexpressing TIMP-1 in pancreatic islets cells can counteract cytotoxicity and insulitis, increase the replication of endocrine pancreas after extensive destruction, and recover from MLDS-induced diabetes.

RESEACH DESIGN AND METHODS

All animal procedures were performed according to approved protocols and in accordance with recommendations for the proper care and use of laboratory animals by the Experimental Animal Committee of General Hospital of PLA. We used human TIMP-1 transgenic mice that were generated with an NIH (National Institutes of Health) Swiss mouse strain (Harlan, Indianapolis, IN), as previously described (30). Briefly, the primers used in cloning human TIMP-1 were based on published sequence (GeneBank accession no. NM_003254), and the plasmid pcDNA3.1–TIMP-1 was constructed. The transgenic mice were established through standard pronuclear microinjection technology. The transgenic mice were then used to backcross onto the NIH Swiss mice, and the fifth-generation mice without transgene were used as the control mice in this study.

Isolation of pancreatic islets. Mouse pancreatic islets were isolated by collagenase digestion followed by separation on Ficoll-Conray gradients (34). Briefly, the pancreas was injected through the pancreatic duct with 2 mg/ml collagenase (Sigma) in Hanks' buffered saline solution (HBSS), removed, incubated at 37°C for 20 min, and then passed through a 500-μm wire nylon mesh. The digested pancreas was rinsed with HBSS, and the islets were separated by density gradient in the Ficoll-Conray device (Sigma). After several washes with HBSS, islets were manually picked using a Pasteur pipette with the aid of a dissecting microscope.

RNA isolation, reverse transcription, and PCR. After isolation, islets were aliquoted and stored at −70°C until RNA was isolated. Total RNA was isolated by the guanidine thiocyanate method (35). First-strand cDNA was synthesized by reverse transcription (RT) of 4 μg total islet RNA using the RT-PCR kit (TaKaRa Biomedicals). RT reaction solution (1 μl) was used in a total volume of 25 μl in the PCR for human TIMP-1.

Northern blot analysis. Expression of the human TIMP-1 transgene in the pancreas was assessed through Northern blotting of total RNA isolated from whole pancreas by the guanidine thiocyanate method (35). After separation on a 1% formaldehyde-agarose gel, 10 μg total RNA was transferred onto a nylon membrane and hybridized with a random prime ^{32}P-labeled human TIMP-1 cDNA probe (30). The blot was stripped and rehybridized with a control probe for glyceraldehyde-3-phosphate dehydrogenase.

Western blot analysis. Mouse pancreas tissue lysate was prepared by homogenization in modified radioimmunopreceipitation assay buffer (50 mmol/l Tris-HCl, pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% SDS, 1 mmol/l sodium ethylene diamine tetra acetate, 1 mmol/l phenylmethylsulfonyl flouride, 5 μg/ml aprotinin, and 5 μg/ml leupeptin). Tissue and cell debris was removed by centrifugation at 13,000*g* at 4°C for 20 min (34). Protein concentration was determined using the MicroBCA assay (Pierce). Blots were incubated with primary antibodies against human TIMP-1 (Santa Cruz Biotechnology, Santa Cruz, CA) and actin (Santa Cruz Biotechnology). The signals were visualized by the enhanced chemiluminescence system (Santa Cruz Biotechnology).

Blood glucose and insulin. Blood glucose levels were measured from whole venous blood isolated from the tail vein using Sure Step (LifeScan; Johnson & Johnson). Insulin was measured in serum samples by a radioimmunoassay using a rat insulin standard (Linco, St. Charles, MO). Blood was drawn from the retroorbital sinus of anesthetized animals. All assays were carried out in duplicate. Each value was the mean of two independent determinations.

Glucose tolerance test. Male mice, aged 6–8 weeks, were fasted 6 h and blood glucose levels measured as described above. A 20% glucose solution was intraperitoneally injected (1 g/kg body wt), and blood glucose was measured at 0, 30, 60, 90, and 120 min, respectively.

Insulin tolerance test. Mice were fasted for 2 h with free access to water. Each mouse then received an intraperitoneal injection of insulin (1 unit/kg body wt). Plasma glucose levels were measured as described above.

Induction of diabetes and glucose monitoring. The mice were intraperitoneally injected with streptozotocin (40 mg streptozotocin/kg body wt) for 5 consecutive days. Tail vein glucose was measured as described above.

Histology and immunohistochemistry. The pancreata were put in formaldehyde for 4 h, dehydrated, and embedded in paraffin. Sections were stained for insulin to evaluate β-cell mass. Four sections (4 μm), spaced at least 80 μm apart, were analyzed from each animal using Image-Pro Plus software (version 5.0.1; Media Cybernetics). We studied four to five animals of each group investigated. Samples were dehydrated and prepared as paraffin blocks and stained with hematoxylin & eosin (H&E). For detection of insulin and glucagons, guinea pig anti-porcine insulin antibody and rabbit anti-human glucagon antibody (Zymed Laboratories, South San Francisco, CA) were used, respectively. We calculated β-cell mass by multiplying the percentage of surveyed pancreatic area occupied by β-cells by the total pancreatic weight.

To prepare the specimen for terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling (TUNEL) staining, paraffin sections were dewaxed and equilibrated in PBS (pH 7.4), followed by incubation in 20 μg proteinase K/μl in 10 mmol/l Tris-HCl (pH 7.5) for 15 min at 37°C. Following washing, the reaction mixture containing terminal deoxynucleotidyl transferase, labeled nucleotides, and DNA polymerase was then applied to sections in a humidified chamber for 90 min at 37°C, according to the manufacturer's instructions (Roche Molecular Biochemicals, Mannheim, Germany).

To measure the percentage of β-cell proliferation, 5-bromo-2-deoxyuridine (BrdU) (60 mg/kg body wt; Sigma) was intraperitoneally injected twice daily after the last dose of streptozotocin (7,36). The mice were killed at the end of the chasing (7 days). Sections (4 μm) were double stained for β-cells, with an anti-insulin antibody, and for BrdU, with a mouse anti-BrdU monoclonal antibody (Roche). Secondary antibodies directly coupled to tetramethyl rhodamine isothiocyanate or fluorescein isothiocyanate were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). BrdU incorporation was scored in ~500 insulin-expressing cells and in 1,000 cells that could be unequivocally located within the lumen of a pancreatic duct but were not part of an islet per sample. Data are expressed as the percentage of BrdU-positive β-cell nuclei per total number of β-cell nuclei per day or as the percentage of BrdU-positive cell nuclei per total number of duct cell nuclei per day (37). In addition, we measured the number of extra-islet β-cell singlets and doublets and normalized the number by the area of pancreas examined. For measurements of duct-associated β-cells, we scored insulin-positive cells that could be unequivocally located within the lumen of a pancreatic duct and were not part of an islet. The number was normalized by section area. At least four independent sections were scored for each of five animals of each genotype.

To determine the severity of insulitis, pancreatic islets from four parallel sections of different cut levels were analyzed per mouse at 11 (n = 3), 14 (n = 4), and 17 days (n = 3) post-MLDS, respectively. The number of islets was counted by one investigator in a blind fashion. The degree of insulitis was classified into four categories (38): 0, normal, no inflammation; 1, periductal lymphocyte infiltrate and lymphocytic infiltrate at the ductal pole of the islet only; 2, peri-insulitis, lymphocytic infiltrate surrounding but not invading the islets; 3, insulitis, lymphocytic infiltrate invading the islets; and 4, severe insulitis, massive lymphocytic infiltrate with islet destruction.

Statistical analysis. Results are expressed as means ± SE. We analyzed the data by Student's t test and Mann-Whitney U test. Significance was defined as $P < 0.05$.

RESULTS

Expression of human TIMP-1 mRNA and protein in transgenic mice and the influence on glucose homeostasis. The pancreata of transgenic mice showed high levels of human TIMP-1 mRNA and protein, which was detected by Northern blot (Fig. 1*A*) and Western blot (Fig. 1*B*), respectively. Furthermore, high levels of human TIMP-1 mRNA was observed in islets of transgenic mice (Fig. 1*C*). Islets from transgenic mice and nontransgenic mice did not show significant differences in islet glucagon immunoreactivity (transgenic mice 13.8 ± 1.2% [n = 5] vs. nontransgenic mice 12.2 ± 1.2% [n = 5]; $P > 0.05$) (Fig. 1*D*). Basal blood glucose and serum insulin levels were not different between transgenic (plasma glucose level 6.8 ± 0.25 mmol/l [n = 12] and serum insulin level 1.08 ± 0.20 ng/ml [n = 5]) and nontransgenic mice (7.6 ± 0.37 mmol/l [n = 12] and 1.11 ± 0.19 ng/ml [n = 5], respectively) in the 6-h fasting state. An intraperitoneal glucose tolerance test was then performed, and similar curves were obtained for transgenic and nontransgenic mice at 6 weeks of age. After the administration of glucose, plasma glucose levels were maximal at 30 min (Fig. 1*E*). Moreover, the results of the intraperitoneal insulin tolerance tests did not show a significant difference at different times indicated between transgenic and nontransgenic mice (Fig. 1*F*).

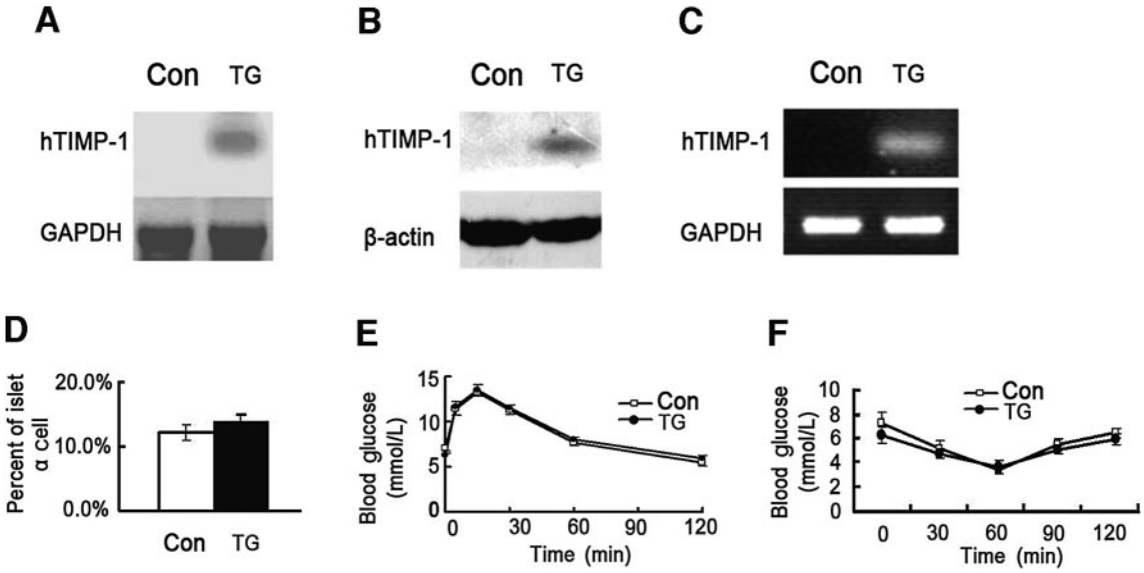

FIG. 1. Expression of human TIMP-1 mRNA and protein in transgenic mice and its influence on glucose homeostasis. We detected human TIMP-1 by Northern blotting (*A*) and Western blotting (*B*) of pancreas of transgenic and nontransgenic mice. Transgenic mice express human TIMP-1 in pancreas, while nontransgenic mice do not express human TIMP-1 in pancreas. *C*: We detected mRNA of human TIMP-1 in islets of transgenic mice and nontransgenic mice by RT-PCR. *D*: Percent of islet α-cell. We performed immunostaining of pancreatic section from nontransgenic and transgenic mice to detect glucagons and insulin, and no difference of glucagons between transgenic and nontransgenic mice was measured. *E*: Intraperitoneal glucose tolerance test. *F*: Intraperitoneal insulin tolerance test. There was no difference in blood glucose concentration at different indicated times postglucose and -insulin injection between transgenic and nontransgenic mice. Con, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hTIMP-1, human TIMP-1; TG, transgenic.

Counteraction of diabetes alterations induced by streptozotocin in transgenic mice. To induce diabetic hyperglycemia, streptozotocin was administered at 40 mg/kg body wt to 6-week-old TIMP-1 transgenic mice and nontransgenic mice for 5 consecutive days, with tail vein glucose level monitored for up to 40 weeks. Blood glucose of nontransgenic mice was higher than that of transgenic mice 4 days after MLDS treatment ($P < 0.05$). Two weeks after MLDS, all nontransgenic mice developed severe hyperglycemia (24.5 ± 1.6 mmol/l), polydipsia, and polyphagia, while transgenic mice showed mild hyperglycemia (13.0 ± 1.3 mmol/l; $P < 0.0001$) (Fig. 2*A*). Blood glucose of transgenic mice slowly increased to 24.4 ± 1.6 mmol/l 12 weeks after MLDS, but 58% of them returned to normal levels (<13.9 mmol/l) 22 weeks after MLDS (Fig. 2*B*). All nontransgenic hyperglycemic mice died within 17 weeks,

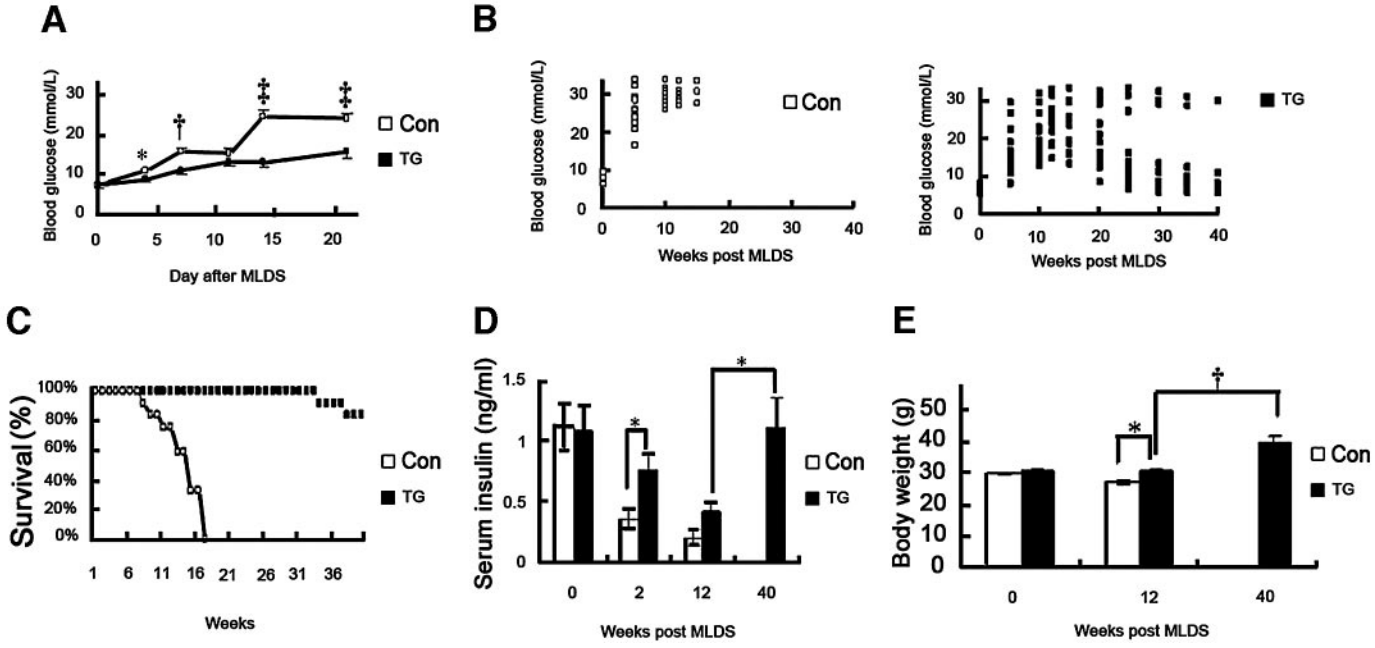

FIG. 2. Comparison of metabolic parameters and survival rate between transgenic and nontransgenic mice after MLDS injection. *A*: Blood glucose levels before and after MLDS treatment of mice after 3 weeks. Glucose concentration was determined as indicated in RESEARCH DESIGN AND METHODS. The actual values of individual nontransgenic (*B*, *left*) and transgenic (*B*, *right*) mice at weeks 0, 5, 10, 12, 15, 20, 25, 30, 35, and 40 after MLDS administration are indicated. *C*: Percent survival of nontransgenic ($n = 12$) and transgenic ($n = 12$) mice after streptozotocin treatment. *D*: Serum insulin of transgenic and nontransgenic mice before and after MLDS administration. *E*: Body weight of mice *$P < 0.05$; †$P < 0.01$; ‡$P < 0.0001$. Con, control; TG, transgenic.

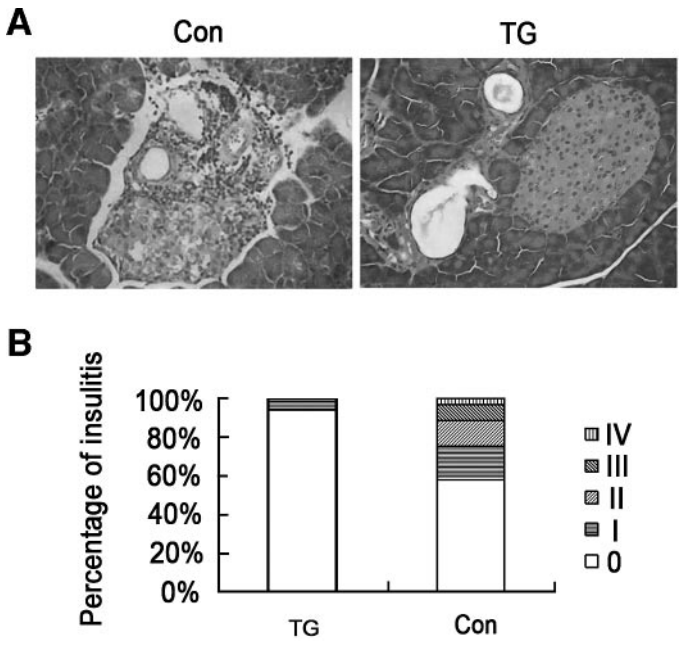

FIG. 3. Lymphocyte infiltration in the islets post-MLDS. H&E-stained islets 14 days post-MLDS showed a near absence of lymphocyte infiltration in the islets of transgenic mice (*A*, *right*) and a presence of lymphocyte infiltration in the islets of nontransgenic mice (*A*, *left*). MLDS-treated transgenic mice had a significantly higher percentage of noninfiltrated islets than that of the nontransgenic mice ($P = 0.000$) (*B*). Con, control; TG, transgenic.

whereas 83% of transgenic mice survived 40 weeks after MLDS injection (Fig. 2*C*). The levels of serum insulin of both transgenic and nontransgenic mice decreased 2 weeks after MLDS treatment, while the serum insulin level of transgenic mice was not only significantly higher than that of nontransgenic mice ($P < 0.05$) but restored to a normal level 40 weeks after MLDS (Fig. 2*D*). Body weight of hyperglycemic transgenic mice did not increase until 12 weeks after MLDS but was significantly greater than that of nontransgenic mice (Fig. 2*E*).

Insulitis and loss of insulin-producing cells in islets of mice post-MLDS administration. The results of H&E staining disclosed that 2 weeks after MLDS injection, ~40% of the islets of nontransgenic mice showed different degrees of insulitis (from grades 1–4) (Fig. 3*A*) but few islets in transgenic mice showed lymphocyte infiltration (11, 14, and 17 days post-MLDS) (Fig. 3*A*). MLDS-treated transgenic mice had a significantly higher percentage of noninfiltrated islets compared with that of the nontransgenic mice ($P < 0.0001$) (Fig. 3*B*). These data indicated that overexpression of TIMP-1 in transgenic mice almost completely prevented insulitis induced by MLDS.

Analysis of insulin staining showed that β-cell mass decreased in both transgenic and nontransgenic mice after MLDS (Fig. 4*A* and *B*) compared with those without MLDS administration (Fig. 4*A* and *B*), but the remaining insulin-positive islet cell mass was significantly higher in transgenic mice than in nontransgenic mice 12 weeks after MLDS and restored to normal 40 weeks after MLDS in transgenic mice ($P < 0.05$; Fig. 4*A*–*C*).

TUNEL assay and BrdU labeling after MLDS injections. To study whether TIMP-1 could prevent β-cells from undergoing MLDS-induced apoptosis in vivo, TUNEL analysis was performed. Although few apoptotic β-cells were observed in either transgenic or nontransgenic islets before MLDS treatment, the number of TUNEL-positive

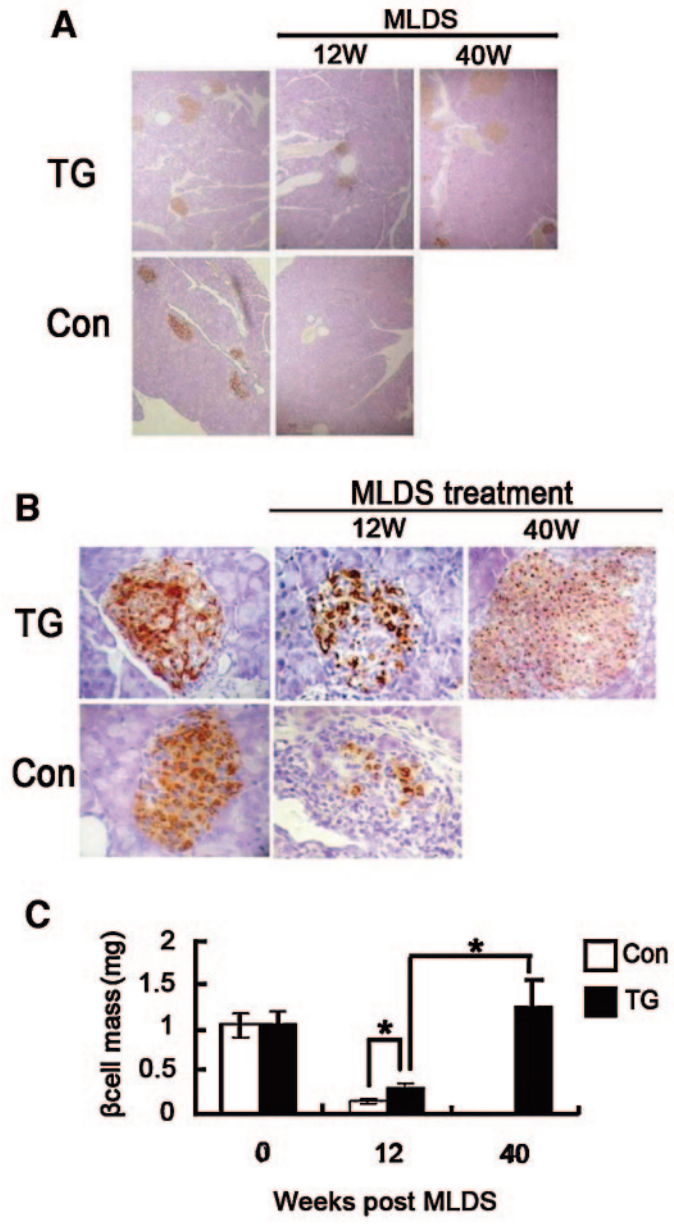

FIG. 4. Immunohistochemical analysis of insulin expression in islets from transgenic and nontransgenic mice. *A* and *B*: Highly reduced insulin-positive cells were seen in transgenic mice and nontransgenic mice 12 weeks post-MLDS. *C*: MLDS-treated transgenic mice had higher β-cell mass than MLDS-treated nontransgenic mice 12 weeks post-MLDS and increased significantly 40 weeks post-MLDS. *$P < 0.05$. Con, control; TG, transgenic.

cells apparently increased 1 week after MLDS treatment in both groups of mice, with the percentage of apoptotic cells significantly lower in islets of transgenic mice (1.7 ± 0.2%) than in islets of nontransgenic mice (2.7 ± 0.3%; $P < 0.05$) (Fig. 5*A* and *B*). In addition, our data also show that one single high-dose streptozotocin (150 mg/kg body wt) could induce massive apoptosis of islet cells in both transgenic and nontransgenic mice, with the percentage of apoptotic β-cells significantly lower in transgenic mice (11.2 ± 1.6%) than in nontransgenic mice (17.6 ± 2.1%; $P < 0.05$) (Fig. 5*B*). These results suggest that TIMP-1 expression partially protected β-cells from apoptosis induced by streptozotocin. Nevertheless, soon after MLDS treatment, transgenic mice also lost a significant number of β-cells, which led to the development of hypoinsulinemia and hyperglycemia.

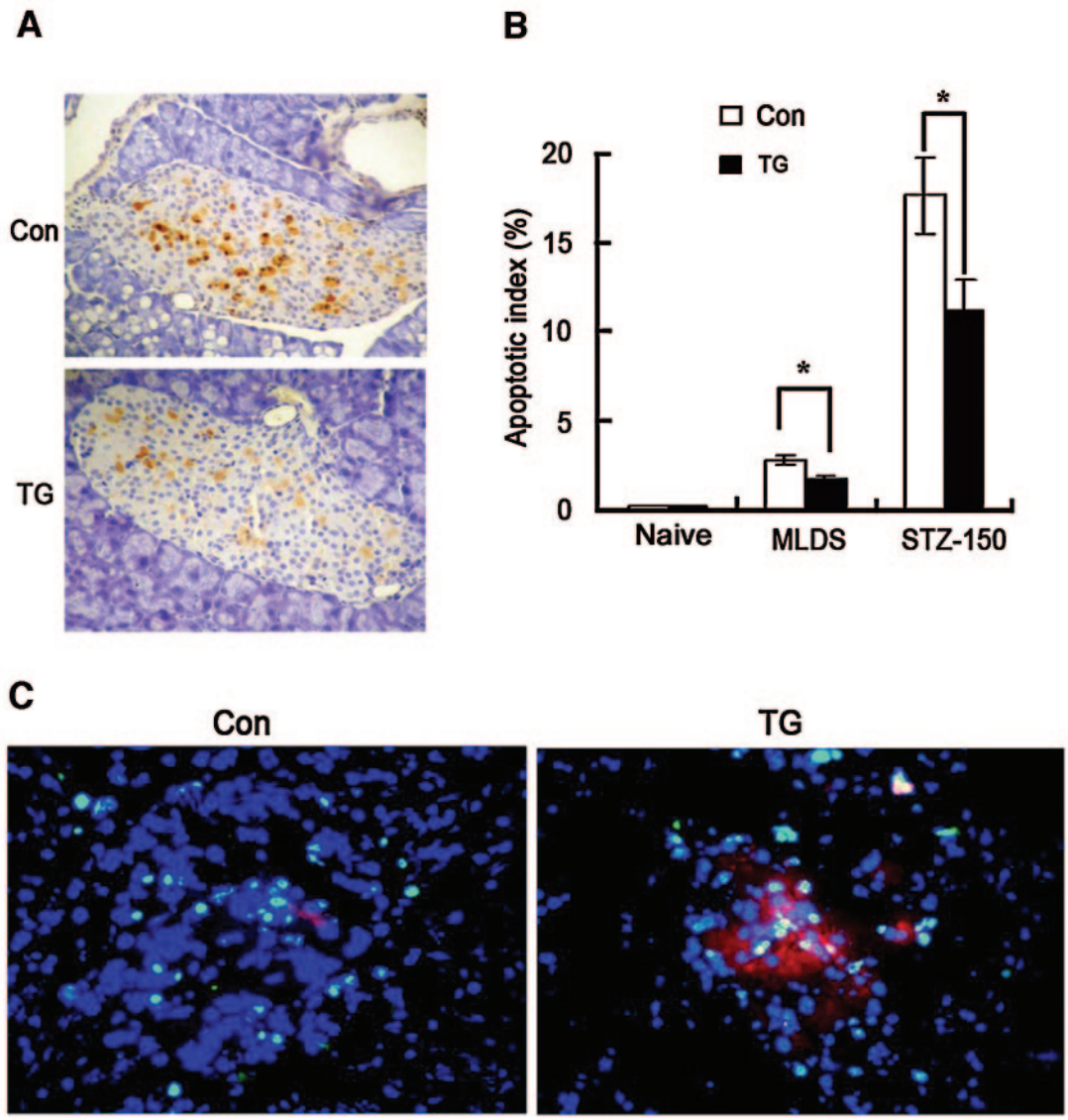

FIG. 5. TUNEL assay and BrdU labeling after MLDS injections. *A*: TUNEL-positive cells in transgenic (*bottom*) and nontransgenic mice (*top*) after a single high dose of streptozotocin. *B*: Percentage of apoptotic cells was significantly lower in islets of transgenic mice than in islets of nontransgenic mice after MLDS or a single high dose of streptozotocin. *C*: Double immunostaining for BrdU (green) and insulin (red) in pancreas from nontransgenic and transgenic mice 7 days after the last dose of streptozotocin, counterstained with HO342 (blue). *$P < 0.05$. Con, control; TG, transgenic.

Furthermore, to examine whether overexpression of TIMP-1 promotes β-cells proliferation, we performed immunohistochemical analysis of BrdU to evaluate duplication of β-cells (Fig. 5*C*).

The ratio of BrdU-positive cells to insulin-positive cells showed no difference between naive transgenic and nontransgenic mice; however, after MLDS injections, proliferating β-cells (BrdU and insulin double-positive cells) were significantly increased in transgenic mice compared with nontransgenic littermates (Fig. 6*A*). Influence of TIMP-1 on neogenesis of β-cell was also explored by counting BrdU-positive cells in ducts, β-cells abutting the lumen of pancreatic ducts, and β-cell singlets/doublets. Our results show that there were no significant differences between transgenic and nontransgenic mice in BrdU-positive duct cells (Fig. 6*B*), insulin-positive duct cells (Fig. 6*C*), and β-cell singlets/doublets (Fig. 6*D*), either before or after MLDS treatment. The results also show that only very few duct cells display double stainings of BrdU and insulin (<0.1%) in the experiments (data not shown).

DISCUSSION

In this study, we used transgenic mice to examine the influence of expression of human TIMP-1 in pancreas on diabetes induced by MLDS. The results our study show that transgenic mice expressing TIMP-1 in pancreas present high levels of immunoreactive TIMP-1 and that islet architecture and function is unaffected in transgenic mice compared with those of controls, indicating that the expression of the TIMP-1 did not alter the whole-body glucose homeostasis, which is consistent with the results of Perez et al. (39). MLDS-treated nontransgenic mice showed different degrees of insulitis, but islets from transgenic mice had little lymphocytic infiltration. Moreover, a significant reduction in β-cell apoptosis was noted in islets of transgenic mice after MLDS injection, indicating that TIMP-1 production by transgenic pancreas partially protected islet β-cells from undergoing apoptosis induced by MLDS. Furthermore, the MLDS-treated transgenic mice were hyperglycemic for the first 22 weeks, indicating loss

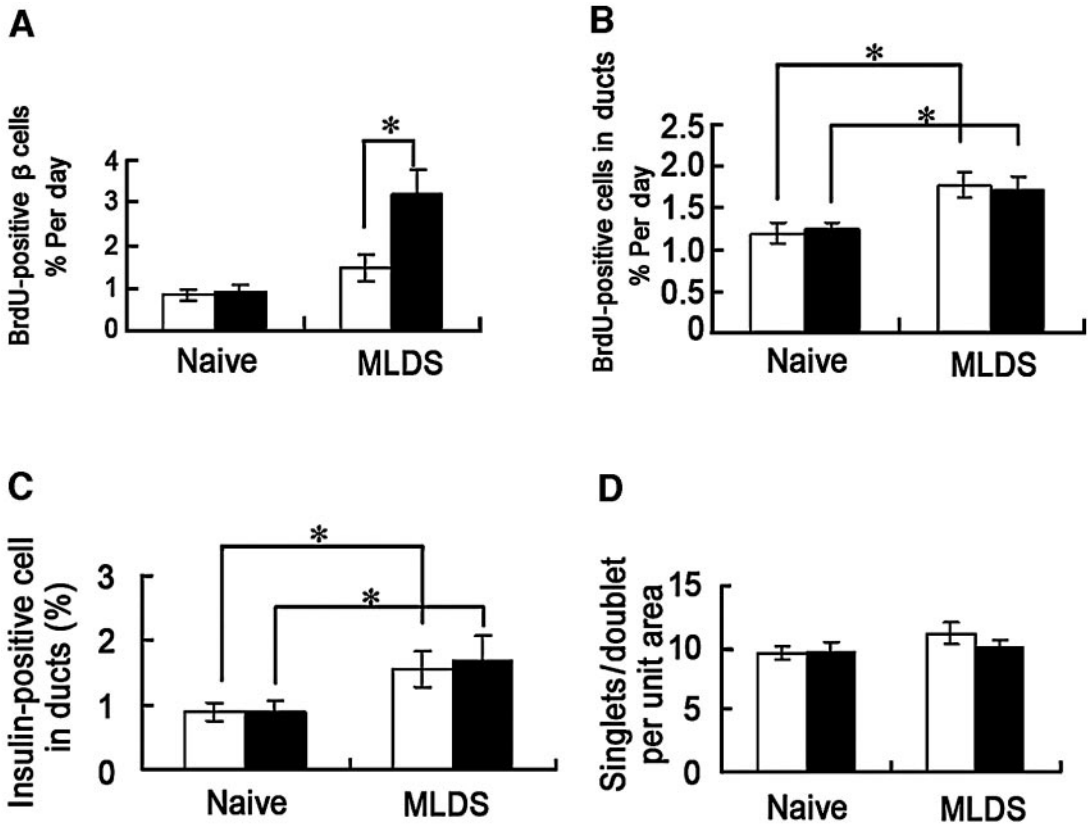

FIG. 6. Proliferation of β-cells in transgenic (■) and nontransgenic (□) mice. *A*: Percent of BrdU insulin double-stained cells in insulin-positive cells per day in naive and MLDS-treated transgenic ($n = 5$) and nontransgenic ($n = 5$) mice. *B*: Duct cell proliferation. We measured the percentage of BrdU-positive cells in ducts of transgenic and nontransgenic mice before and after MLDS treatment. *C*: Duct-associated β-cells. We scored insulin-positive cells that could be unequivocally located within the lumen of a pancreatic duct and were not part of an islet. *D*: β-Cell singlets/doublets. We measured the number of extra-islet β-cell singlets/doublets and normalized the number by the area of pancreas examined. *$P < 0.05$.

of β-cell mass. The fact that most (75%) of these mice recovered from MLDS-induced alterations agrees with the increase of islet β-cell replication observed in these mice. Thus, our results have demonstrated that TIMP-1 restores the β-cell mass and normoinsulinemia when TIMP-1 was overexpressed in pancreatic β-cells in stress conditions leading to β-cell destruction.

It is well established that TIMP-1 exhibits an antiapoptotic effect. The finding that β-cell survival effect induced by TIMP-1 in vivo, which appears to play an important role in ameliorating the hyperglycemia induced by single high-dose streptozotocin or MLDS, agrees with the result in vitro (34). Basically, two different mechanisms of TIMP-1 suppression of apoptosis have been proposed. One pathway is linked to the anti-MMP activity of TIMP-1, and the other is MMP independent. The involvement of MMP inhibition in the antiapoptotic activity of TIMPs could be explained through prevention of matrix degradation, which is known to induce cell death by anoikis, and further mechanisms could be implicated such as shedding or proteolytic cleavage of receptors and ligands involved in cell survival and/or growth. However, such activities appeared unrelated to TIMP-1 function as an MMP inhibitor in most cases. MMP9 has previously been shown to be upregulated in pancreas tissue from patients with acute or chronic pancreatitis and was suggested to exacerbate diabetes by cleaving insulin (40,41). However, the insignificant effect of genetic MMP9 ablation on diabetes development argues against a crucial role for MMP9 as a cause of streptozotocin-induced diabetes (42). In addition, doxycycline, which is widely used as an MMP inhibitor, has no effect on hyperglycemia induced by MLDS in our research (data not shown), as well as in a previous report (43). These results agree with the research of Han et al. (31) in which TIMP-1 protection of β-cells from cytotoxicity induced by cytokines did not depend on inhibiting MMP. Collectively, these findings and our observation do not support the notion that beneficial effects of TIMP-1 on diabetes induced by MLDS depend on inhibiting MMP.

Injection of MLDS leads to type 1 diabetes with or without insulitis (44). Although islets from nontransgenic mice had various degrees of insulitis, little lymphocytic infiltration was detected in TIMP-1 transgenic islets. The reason that TIMP-1 expression in β-cells attenuated insulitis development induced by MLDS may be that MLDS induced only mild apoptosis in transgenic mice leading to slight insulitis, while massive apoptosis and/or necrosis led to extensive insulitis in nontransgenic mice. Apoptosis of β-cells has been implicated in the initiation of type 1 diabetes through antigen cross-presentation mechanisms that lead to β-cell–specific T-cell activation. Recently, Liadis et al. (45) demonstrated that lymphocyte infiltration of the pancreatic islets was completely absent in MLDS-treated caspase 3 knockout (caspse$3^{-/-}$) mice, suggesting that diminishment of β-cell apoptosis may be an important mechanism for TIMP-1 mitigating insulitis induced by MLDS. Of course, an alternative possible mechanism, e.g.,

direct regulation of the autoimmune reaction by overexpression of TIMP-1 (22), cannot be completely ruled out.

It appears that the β-cell population has a very limited potential to expand in the absence of major external stimuli, which is probably due to the limited replication capacity of β-cells and due to the fact that neogenesis from precursor cells is not readily reactivated. Yet, under certain conditions when major external stimuli are applied, there can be a quite vigorous regenerative expansion of the β-cell mass (1). In many cell types, TIMP-1 has been shown to play a role in cell proliferation and differentiation in physiological and pathophysiological states (12–18). Our results show that islet replication rate was significantly higher in transgenic mice than in nontransgenic mice after MLDS treatment. In addition, most diabetic transgenic mice restored normal blood glucose, serum insulin levels, and islet β-cell mass. The results of this study and those of previous findings suggest that TIMP-1 may play an important role in the increased β-cell proliferation of MLDS-treated transgenic mice.

In conclusion, our results have demonstrated for the first time that overexpression of TIMP-1 can counteract the cytotoxicity and insulitis induced by MLDS, enhance the proliferation of β-cells in vivo after extensive destruction, normalize blood glucose, and prolong the survival of transgenic mice, indicating that TIMP-1 may be a potential target for ameliorating, or even reversing, type 1 diabetes.

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