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# Diabetes and Tumor Formation in Transgenic Mice Expressing Reg I

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**To examine the effect of overexpressed regenerating gene (Reg) I on pancreatic β-cells, we generated transgenic mice expressing Reg I in islets (Reg-Tg mice). Three lines of Reg-Tg mice were established. In line-1 Reg-Tg mice, the expression level of Reg I mRNA in islets was 7 times higher than those in lines 2 and 3 of Reg-Tg mice, and line 1 mice developed diabetes by apoptosis of β-cells, as well as various malignant tumors. In addition to the decrease in β-cells, compensatory islet regeneration and proliferation of ductal epithelial cells were observed in line-1 Reg-Tg mice. Because Reg I protein was secreted primarily into pancreatic ducts from acinar cells, it may primarily stimulate the proliferation of ductal epithelial cells, and not β-cells, and their differentiation into islets. Moreover, the tumor-promoting activity of Reg I protein should be considered for its possible clinical applications.** © 2000 Academic Press

***Key Words:*** **transgenic mouse; pancreatic islet; Reg I; apoptosis; regeneration; neoplasm.**

Regenerating gene I (Reg I) is a member of the Reg family and was isolated by differential screening of a cDNA library prepared from rat regenerating pancreas (1). Reg I expression is detected primarily in the pancreas and at lower levels in the stomach (2), duodenum, small intestine (3), gall bladder (4), kidney (2), cornea and conjunctiva (5). In normal adult pancreas, Reg I is primarily expressed in acinar cells (6), but neither in islet cells (1, 2) nor in ductal cells (7). Reg I expression in the pancreas increases in several animal models of islet regeneration, including animals subjected to 90% pancreactomy + nicotinamide injection (1, 8), resection of implanted solid insulinoma (9), and wrapping of the splenic lobe of the pancreas (10, 11). Reg I expression is also induced after acute pancreatitis (6, 12), during active diabetogenesis in nonobese diabetic (NOD) mice as a defense mechanism (13), during embryogenesis (14, 15), in malignant neoplasm (2, 7), and by some growth factors (16), whereas it decreases with aging (3) and through β cell differentiation (17). Reg I is mitogenic to β cells and ductal cells (18–20) and the administration of recombinant Reg I protein ameliorates surgical diabetes (20) and Type 1 diabetes in NOD mice (21).

To examine whether overexpressed Reg I increases β cell mass, we generated transgenic mice expressing Reg I in pancreatic islets (Reg-Tg mice). Although the insulin promoter has frequently been used for transgene expression in islets, there is the possibility that β cells are nonspecifically destroyed by the transgenic overexpression of an exogenous protein in these cells (22). To circumvent this issue, we used the rat glucagon promoter for transgenic expression in Reg-Tg mice. Thus, we were able to investigate the direct effects of paracrine Reg I on β cells *in vivo.*

## MATERIALS AND METHODS

*Cloning of mouse Reg I cDNA and transgene construct.* mRNA extracted from a mouse pancreas with ISOGEN (Nippon Gene, Tokyo, Japan) was reverse-transcribed to cDNA with a Super Script II first-strand synthesis kit (Gibco BRL, Rockville, MD). Mouse Reg I cDNA (0.5 Kb) was amplified using the first-strand cDNA as a template with 0.025 U/ml of Ampli Taq (Perkin Elmer-Roche, Branchburg, NJ), and cloned in pCR II vector (Invitrogen, Carlsbad, CA). The nucleotide sequence of the PCR product was identical to the reported sequence of mouse Reg I cDNA (GenBank Accession No. D14010). The expression vector including the rat glucagon promoter (0.9 Kb) for transgenic mice was constructed previously (22), and mouse Reg I cDNA was inserted into its cloning site flanking the exon-intron organization and a polyadenylation signal of the rabbit β-globin gene. An *Xho* I fragment of this vector excluding plasmid-derived sequences was used as the transgene.

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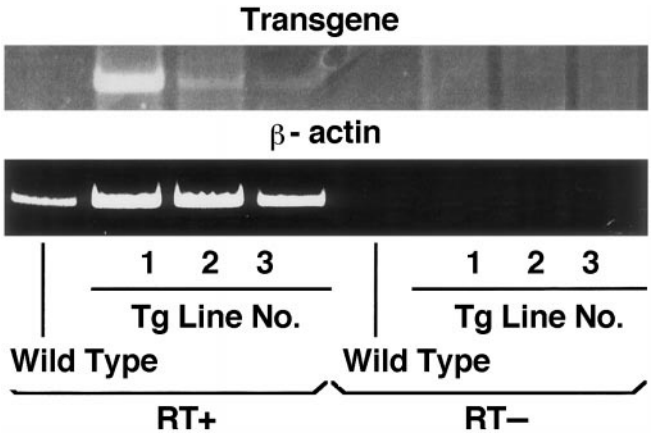


**FIG. 1.** Semiquantitative analysis of transgene expression in pancreatic islets by RT-PCR. Islets obtained from five mice of 20–24 weeks of age in the same line were used in one lot for RNA extraction. Transgene expression levels standardized by $\beta$-actin expression levels are 1.4, 0.2, and 0.2 in Reg-Tg mice of lines 1–3, respectively. RT, reverse transcriptase.

*Generation of transgenic mice.* The transgene was microinjected into the male pronuclei of fertilized eggs obtained from superovulated BDF1 (C57BL/6 x DBA2 F1) female mice crossed with males of the same strain. Injected eggs were implanted into the oviducts of pseudopregnant female mice and allowed to develop (23). DNA was extracted from tail snips of live offspring by the proteinase K/SDS method. The integration of the transgene into the mouse genome was detected by PCR and Southern blot analysis. The copy numbers of integrated transgenes were determined from the intensity of each radioactive band in Southern blot analysis compared with indicator bands of 1, 10, and 100 copies of the transgene. F1 transgenic progeny were bred by crossing transgenic founder mice with BDF1 mice, and used for a long-term observation of phenotypes and for repeating crossing with BDF1. F2 transgenic mice of 20 to 40 weeks of age were used with sex-matched nontransgenic littermates (wild-type mice) in this study. Body weight was determined every week. Mice with blood glucose concentrations of more than 25 mM were diagnosed as diabetic animals.

*Transgene expression in pancreatic islets.* From mouse islets collected by the collagenase method (24), total RNA was extracted with ISOGEN (Nippon Gene). After DNase treatment, 3 $\mu$g of total RNA were reverse-transcribed to cDNA with a Super Script II first-strand synthesis kit (Gibco BRL). Target sequences were amplified using the first-strand cDNA as a template with 0.025 U/$\mu$l of Ampli Taq Gold (Perkin Elmer-Roche). After the initial denaturation at 94°C for 9 min, one cycle of 94°C for 1 min, 60°C for 1 min, and 72°C for 3 min was repeated 20, 25, 30, and 35 times, respectively. During cycles 20 to 35, the range in which the amount of PCR products increases linearly was ascertained by polyacrylamide gel electrophoresis. On the basis of this linearity, the expression levels of target genes were semiquantitatively determined with a computer program (Adobe Photoshop 4.0J; Adobe Systems, Inc., San Jose, CA), by which the amount of each PCR product was measured as the product of the area and the mean intensity of its band after ethidium bromide staining. The Reg I expression level was standardized by the $\beta$-actin expression level. Because Reg I mRNA derived from the transgene includes sequences of exons 2 and 3 of the rabbit $\beta$-globin gene, its expression could be discriminated from endogenous Reg I mRNA by RT-PCR with a primer set on exons 2 and 3 of the rabbit $\beta$-globin gene.

*Histopathological examination of pancreatic islets.* Mouse pancreas was stained with hematoxylin and eosin (HE), aldehyde fuchsin for $\beta$ cells, and by Grimelius' method for $\alpha$ cells. For immunohistochemistry, guinea-pig polyclonal antibodies against porcine insulin and glucagon (DAKO Japan, Kyoto, Japan), and a mouse monoclonal antibody (IgG2a) against proliferating cell nuclear antigen (PCNA; Oncogene Science Inc., Uniondale, NY) were used. The quantitative analysis of the islet-cell area was carried out as previously described (25). In brief, islet area, $\alpha$-cell area, and $\beta$-cell area were identified by HE staining, glucagon immunostaining, and insulin immunostaining, respectively. Because the results obtained from staining with aldehyde fuchsin and by the Grimelius' method were identical to those from the immunohistochemistry for $\beta$ and $\alpha$ cells, respectively, these data are not shown in this paper.

*Determination of glucose and insulin concentrations.* The glucose tolerance test, the insulin tolerance test, and the determination of blood glucose and serum insulin concentrations were carried out as previously described (22, 25). For the determination of insulin content in the whole pancreas, each mouse pancreas was homogenized in 4 ml of ice-cold acid-ethanol solution (26), and insulin was extracted at 4°C overnight. After centrifugation at 2000*g* at 4°C for 30 min, the supernatant was neutralized and diluted with PBS. Insulin concentrations were assayed in 1000- or 10,000-fold diluted samples. Using mouse islets in primary culture, insulin secretion was measured. Five islets isolated by the collagenase method (24) were pre-

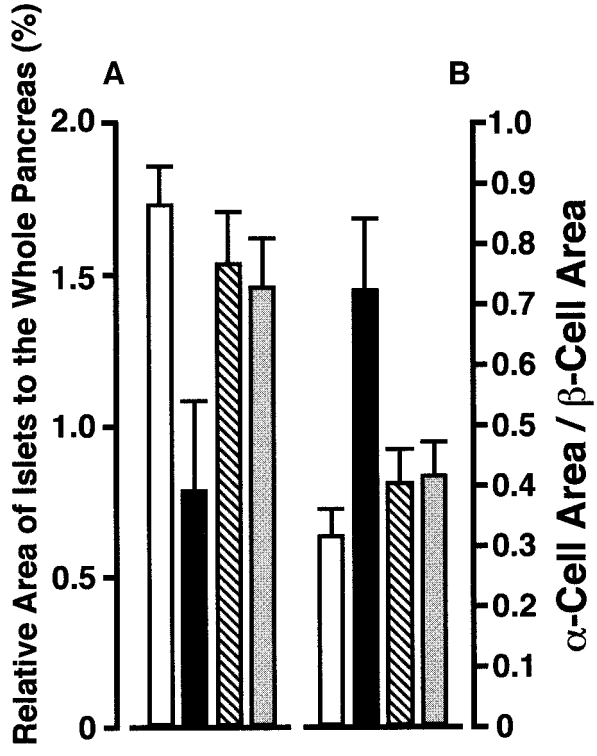


**FIG. 2.** Morphological analysis of pancreatic islets. Using HE and immunostaining of pancreata from mice of 20–28 weeks of age, areas of islets, $\alpha$ cells, $\beta$ cells, and the whole pancreas were determined ($n = 6$ each). Open columns, wild-type mice; solid columns, line 1 Reg-Tg mice; hatched columns, line 2 Reg-Tg mice; dotted columns, line 3 Reg-Tg mice. In line-1 Reg-Tg mice, the islet area relative to the whole area of the pancreas decreased ($P < 0.05$) because of the reduced area of $\beta$ cells (A). Because the $\alpha$-cell area did not decrease, the ratio of the $\alpha$-cell area to the $\beta$-cell area increased ($P < 0.05$) in line-1 Reg-Tg mice (B).

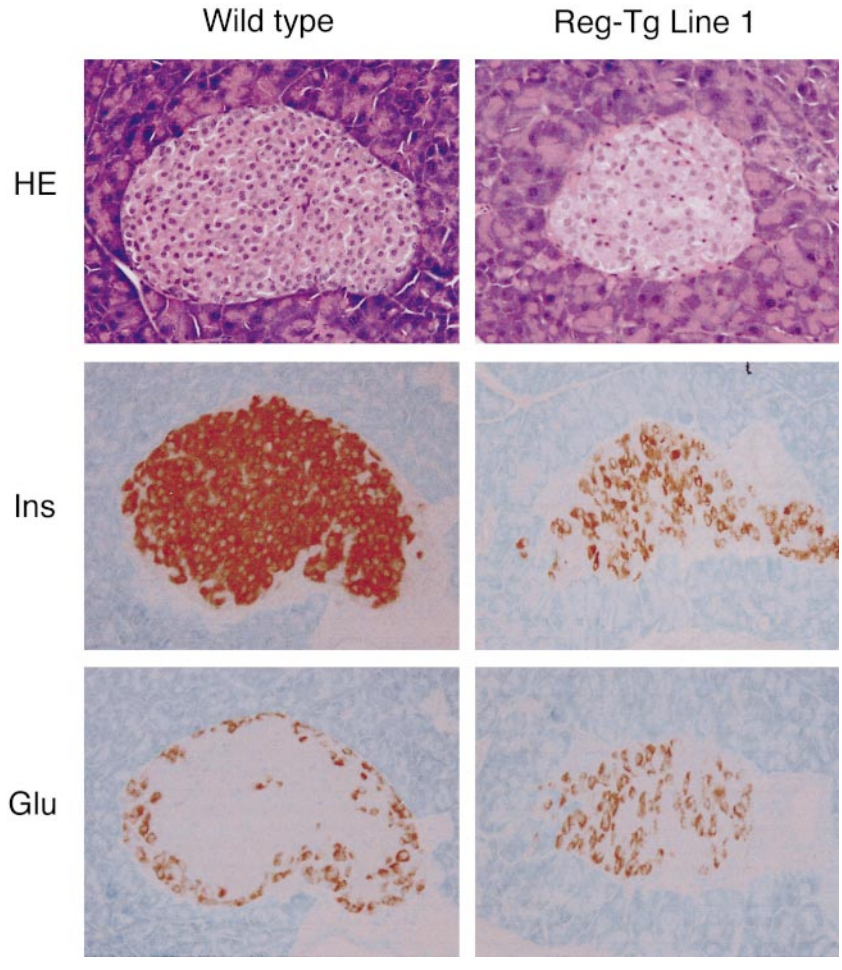


**FIG. 3.** Histological examination of pancreatic islets. Pancreata from mice of 20–28 weeks of age were stained with HE and immunohistochemistry for insulin (Ins) and glucagon (Glu). In line-1 Reg-Tg mice, the number of $\beta$ cells decreased, whereas the number of $\alpha$ cells did not change. The amount of insulin granules in each $\beta$ cell also appears to be smaller in line-1 Reg-Tg mice than in wild-type mice. Magnification, $\times$200.

incubated in 1 ml of modified KRB containing 115 mM NaCl, 5 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM Na2$HPO_4$, 24 mM $NaHCO_3$, 10 mM HEPES/NaOH (pH 7.4), and 0.3% BSA with 2.8 mM glucose at 37°C in an atmosphere of humidified air:$CO_2$ (95:5) for 60 min, and stimulated by an increase in glucose concentration of 16.7 mM.

*Detection of apoptosis.* To detect apoptotic cells in pancreatic islets, the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) method was utilized. Paraffin sections of mouse pancreas were dewaxed by heating at 60°C followed by washing in xylene, rehydrated through a graded series of ethanols and water, and incubated with 20 $\mu$g/ml of proteinase K in 10 mM Tris–Cl (pH 7.4) at 37°C for 30 min. After washing with PBS, the sections were incubated with TdT from calf thymus and fluorescein-labeled dUTP (Boehringer Mannheim, Mannheim, Germany), and the fluorescein incorporated into DNA cleavage sites was detected by Fab fragments of anti-fluorescein sheep antibodies conjugated with alkaline phosphatase (Boehringer Mannheim) and its substrates: nitro blue tetrazolium (Wako, Osaka, Japan) and 5-bromo-4-chloro-3-indolylphosphate (Wako). For a positive control, sections were treated with 1 mg/ml of DNase I (Takara Shuzo, Tokyo, Japan) in 30 mM Tris–Cl (pH 7.5), 4 mM $MgCl_2$, and 0.1 mM dithiothreitol for 10 min just before TdT reaction, to induce DNA strand breaks.

*Statistical analysis.* All data are presented as means ± SEM. For comparison of two means, Student's unpaired *t* test was used. For comparison of two ratios (Q1, Q2), a normal distribution curve with a mean of Q1-Q2 and a variance of Q1(1-Q1)/n1 + Q2(1-Q2)/n2 was used. Probability (*P*) of less than 0.05 was considered statistically significant.

## RESULTS

*Generation of Reg-Tg mice.* Three Reg-Tg founder mice were obtained. The transgene copy numbers of Reg-Tg mice of lines 1–3 were 5, 2, and 1, respectively. The percentages of Reg-Tg mice (66/199; 35%) in F1 mice at the age of 5 weeks were 46% (16/35) in line 1, 50% (7/14) in line 2, 56% (18/32) in line 3, and 51% (41/81) in total, according to Mendel's law, which indicates that overexpressed Reg I protein does not affect the survival rate until 5 weeks of age including embryonic stages. The body weight, measured weekly, was similar in all lines of Reg-Tg mice and wild-type mice, at least up to 40 weeks of age.

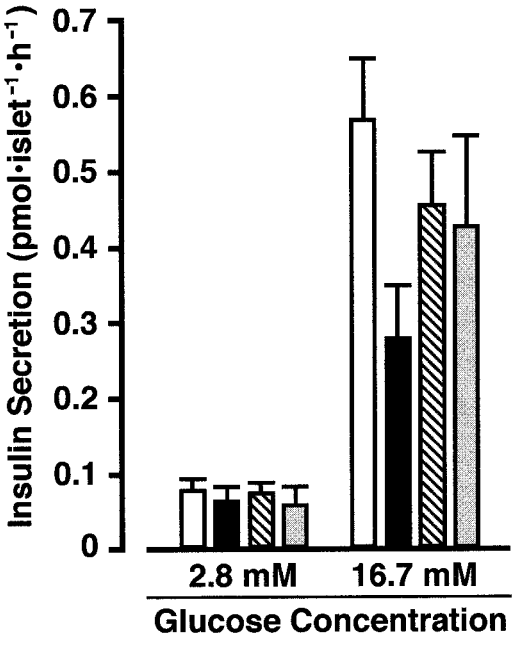


**FIG. 4.** Insulin secretion from isolated pancreatic islets. Pancreatic islets were obtained from mice of 20–28 weeks of age ($n = 6$ each). Open columns, wild-type mice; solid columns, line 1 Reg-Tg mice; hatched columns, line 2 Reg-Tg mice; dotted columns, line 3 Reg-Tg mice. In line-1 Reg-Tg mice, the insulin secretion in response to high glucose concentration (16.7 mM) was significantly ($P < 0.05$) smaller than that of wild-type mice.

*Transgene expression in pancreatic islets.* In all transgenic lines, transgene expression was confirmed by RT-PCR (Fig. 1). The expression levels of the transgene (i.e., the amount of PCR products) in three lines of Reg-Tg mice were correlated with their copy numbers with a high correlation coefficient of 0.9, and the transgene expression level of Reg-Tg mice of line 1 (copy number: 5) was 7 times higher than those of lines 2 (copy number: 2) and 3 (copy number: 1). Transgene expression was not detected in wild-type mice.

*Phenotypes of Reg-Tg mice.* Eighty-one F1 progeny of Reg-Tg mice were used for a long-term observation of phenotypes. Line-1 Reg-Tg mice with the highest copy number of 5 developed diabetes. The percentage of diabetic mice in line 1 was 38% (6/16), and the average age of onset was 23.0 ± 1.3 weeks. By crossing male and female line-1 mice, homozygous Reg-Tg mice of line 1 were generated, and these animals developed diabetes significantly earlier (8.0 ± 2.0 weeks) at a higher rate (100%; 3/3) than heterozygotes of line 1 ($P < 0.01$ and 0.05, respectively). Nondiabetic F2 mice of line 1 also showed impaired glucose tolerance (data not shown). Reg-Tg mice of lines 2 and 3 did not develop diabetes or impaired glucose tolerance.

Out of 16 F1 Reg-Tg mice of line 1, 6 died of diabetes before 50 weeks of age, and another 6 died of malignant

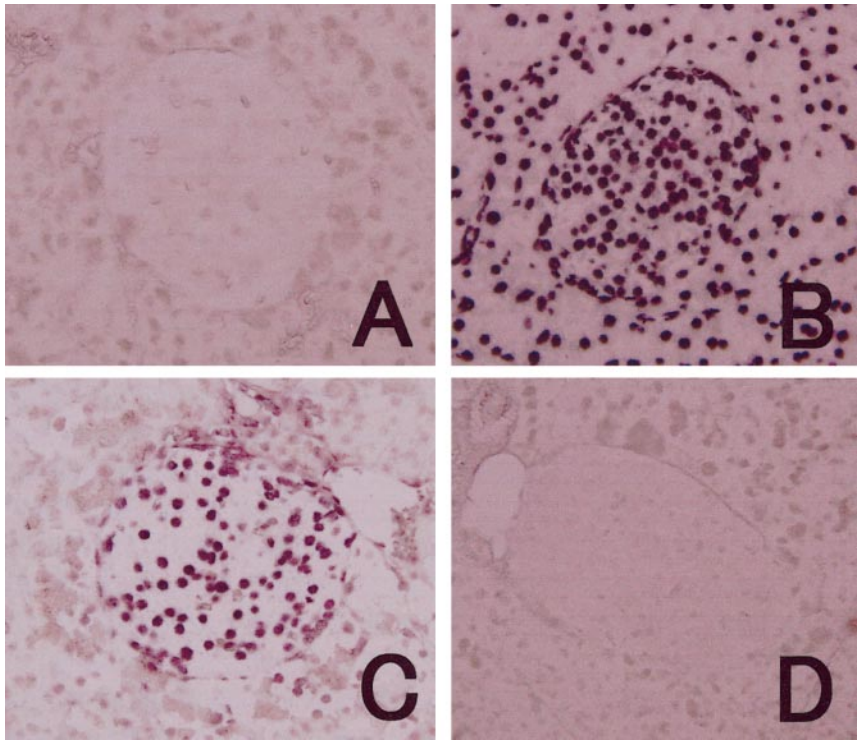


**FIG. 5.** Detection of apoptotic cells in pancreatic islets by the TUNEL method. Each figure is representative of 3 similar observations, using pancreata obtained from 3 mice of 20–28 weeks of age. (A) Line-1 Reg-Tg mouse without TdT treatment as a negative control. (B) Wild-type mouse with DNase treatment as a positive control. (C) Line-1 Reg-Tg mouse. (D) Wild-type mouse. Magnification, ×200.

neoplasms at the age of 50–100 weeks. Wild-type mice and all F1 Reg-Tg mice of lines 2 and 3 survived over 100 weeks of age.

*Pathogenesis of diabetes.* In HE staining, the islet area relative to the whole area of the pancreas in line-1 Reg-Tg mice was significantly smaller than that of wild-type mice (Fig. 2A). In immunohistochemical staining, $\beta$- and $\alpha$-cell areas were determined relative to the whole area of the pancreas. In wild-type mice, $\beta$- and $\alpha$-cell areas were 1.34 ± 0.21% and 0.43 ± 0.06%, respectively ($n = 6$). In line-1 Reg-Tg mice, the $\beta$-cell area (0.54 ± 0.15%; $n = 6$) significantly ($P < 0.05$) decreased, while the $\alpha$-cell area (0.39 ± 0.08%; $n = 6$) did not change (Fig. 3), resulting in an increase in the ratio of the $\alpha$-cell area to the $\beta$-cell area (Fig. 2B). In Reg-Tg mice of lines 2 and 3, the relative islet area to the whole pancreas, the $\beta$- and $\alpha$-cell areas, and the ratio of the $\alpha$-cell area to the $\beta$-cell area were similar to those of wild-type mice.

The insulin content of line-1 Reg-Tg mice (11.4 ± 3.2 mg/pancreas; $n = 7$) was significantly ($P < 0.05$) smaller than that of wild-type mice (25.6 ± 4.6 mg/pancreas; $n = 7$), whereas the insulin contents of Reg-Tg mice of line 2 (20.9 ± 3.0 mg/pancreas; $n = 7$) and line 3 (22.3 ± 5.1 mg/pancreas; $n = 7$) were similar to that of wild-type mice. In line-1 Reg-Tg mice, the insulin secretion from primary cultured islets in response to high glucose concentration (16.7 mM) was significantly ($P < 0.05$) smaller than that of wild-type mice (Fig. 4). In Reg-Tg mice of lines 2 and 3, the response of insulin secretion to high glucose showed intermediate values.

To examine whether the number of $\beta$ cells decreased by apoptosis, the TUNEL method was used. In line-1 Reg-Tg mice, many apoptotic cells were detected in islets (Fig. 5C), whereas no apoptotic cell was detected in Reg-Tg mice of lines 2 and 3 (data not shown) and wild-type mice (Fig. 5D).

To rule out the possibility that Reg I secreted from transgenic islets induces insulin resistance of various organs and subsequently leads to diabetes, insulin tolerance tests were carried out. Reg-Tg mice of all three lines and wild-type mice showed similar decreasing curves in blood glucose concentrations after insulin injection of 0.75 mU/g body weight (data not shown).

As an indicator of the amount of exocrine pancreas, the weight of the whole pancreas was determined. In Reg-Tg mice of lines 1, 2, and 3 of 20–24 weeks of age, the weight of the pancreas was 7.5 ± 0.6, 7.9 ± 0.5, and 7.5 ± 0.6 mg/g of body weight, respectively ($n = 8$), and was similar to that of wild-type mice (7.7 ± 0.3 mg/g of body weight, $n = 10$).

*Regeneration of pancreatic islets.* In addition to $\beta$ cell apoptosis, compensatory islet regeneration was observed in line-1 Reg-Tg mice (Fig. 6). Regenerating islets were often adjacent to pancreatic ducts (Fig. 6A) and cell division of islet cells was ascertained (Fig. 6B). The appearance of insulin-producing epithelial cells in pancreatic ducts (Fig. 6C) is one piece of evidence for islet neogenesis. Furthermore, PCNA-positive cells were detected both in islets (Fig. 6D) and in pancreatic ducts (Fig. 6E). Especially, PCNA-positive ductal cells were more striking in Reg-Tg mice compared with other mouse models of islet regeneration such as streptozotocin treatment (data not shown). In Reg-Tg mice of lines 2 and 3 and wild-type mice, these findings were not observed, including dividing islet cells, insulin-producing epithelial cells in pancreatic ducts, or PCNA-positive cells in pancreatic islets and ducts.

*Tumor formation.* Histopathological examination revealed that the 6 tumors found in F1 Reg-Tg mice of line 1 were the following: pancreatic cancer, hepatocellular carcinoma, uterine leiomyosarcoma, ovarian adenocarcinoma, cervical lymphoma, and lachrymal gland adenocarcinoma, respectively (Figs. 7A–7F). Because pancreatic cancer was suspected to be of islet-cell origin, immunostaining for several markers of neuroendocrine tumors was performed. However, this carcinoma was poorly differentiated and did not produce insulin, glucagon, neuron-specific enolase, synaptophysin, or chromogranin.

## DISCUSSION

Reg-Tg mice of lines 2 and 3 showed a modest decrease in insulin secretion in response to high glucose, and most pathological changes were observed only in line-1 Reg-Tg mice. Because the transgene expression level in Reg-Tg mice of line 1 is 7 times higher than in lines 2 and 3, the pathological changes observed in line-1 Reg-Tg mice were associated with the Reg I expression level. To increase transgenic Reg I expression, homozygous Reg-Tg mice of line 1 were generated. Indeed, all homozygotes developed diabetes significantly earlier than heterozygotes. In heterozygous Reg-Tg mice of line 1, only 38% developed diabetes and the nondiabetic remainder showed impaired glucose tolerance. The reason why only some of the heterozygotes of line 1 was affected with diabetes is unclear. It may be due to differences in the genetic background of individual transgenic mice. Because BDF1, a hybrid strain of C57BL6 and DBA2, was used to generate transgenic mice and for crossing with transgenic mice, the genetic background of transgenic offspring varied between C57BL/6 and DBA2 mouse strains.

Because the possibility of insulin resistance induced by Reg I secreted into blood from transgenic islets was ruled out by the result of the insulin tolerance test, the cause of diabetes in the Reg-Tg mice of line 1 is obviously an insufficient secretion of insulin from $\beta$ cells.

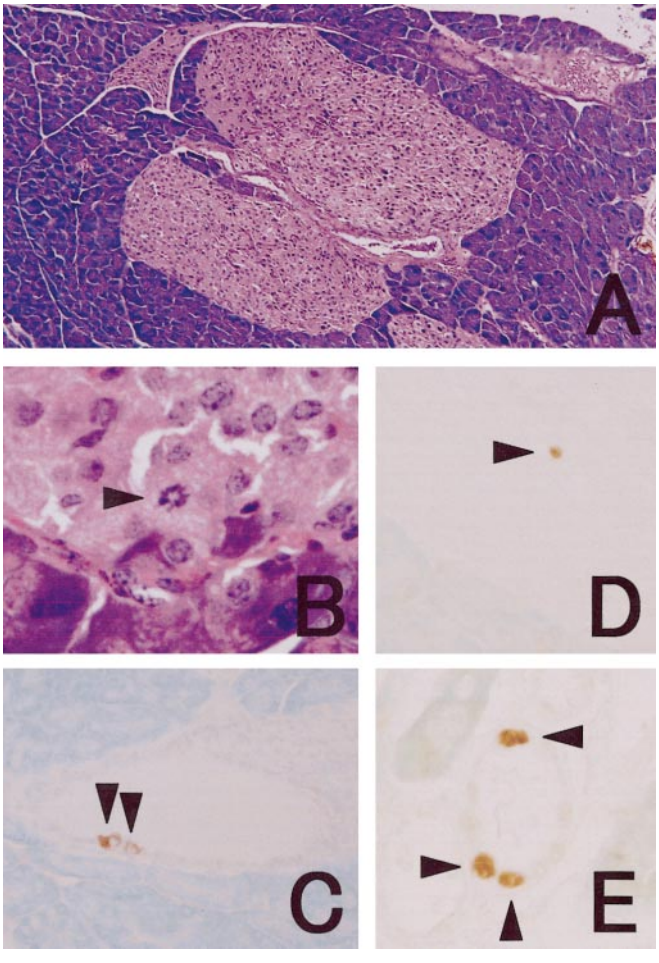


**FIG. 6.** Compensatory regeneration of pancreatic islets. Pancreata from line-1 Reg-Tg mice at the age of 20–28 weeks were used. (A) Regenerating islets adjacent to pancreatic ducts. HE staining. Magnification, ×100. (B) Cell division in an islet. HE staining. Magnification, ×600. (C) Insulin-producing epithelial cells in a pancreatic duct. Immunostaining for insulin. Magnification, ×200. (D) A PCNA-positive islet cell. Immunostaining for PCNA. Magnification, ×400. (E) PCNA-positive epithelial cells in a pancreatic duct. Magnification, ×600.

Morphologically, in line-1 Reg-Tg mice, the islet area, relative to the whole area of the pancreas, decreased by the reduction of the $\beta$-cell area, and not the $\alpha$-cell area. Physiologically, the insulin content of the pancreas and the insulin secretion in response to high glucose both decreased in line-1 Reg-Tg mice. The decrease in the response of insulin secretion to high glucose can be explained, at least in part, by the decreased ratio of $\beta$ cells in each islet of Reg-Tg mice of line 1.

Although the transgenic expression of Reg I by the glucagon promoter may be a burden to $\alpha$ cells, the number of $\alpha$ cells did not change, whereas the number of $\beta$ cells decreased due to apoptosis. The weight of the whole pancreas did not change in Reg-Tg mice of all lines. These findings suggest that $\beta$ cells were specifically injured by overexpressed Reg I, but $\alpha$ cells and exocrine cells were not. In addition to the decrease in the number of $\beta$ cells, the amount of insulin granules in each $\beta$ cell of Reg-Tg mice of line 1 appears to be smaller than that of wild-type mice. Therefore, overexpressed Reg I may also inhibit normal differentiation and function of pancreatic $\beta$ cells. Reg I has been considered to be a growth factor, and in general, growth factors protect cells from apoptosis. However, like Reg I against $\beta$ cells in Reg-Tg mice of line 1, many growth factors have also been reported to induce apoptosis against some cultured cells. For example, EGF acts against MDA-M$\beta$ 468 cells (breast cancer) (27) and A431 cells (epidermoid carcinoma) (28), heparin-binding EGF-like growth factor acts against 32D cells (hematopoietic cell) (29), hepatocyte growth factor (HGF) acts against Sarcoma 180 cells (acites tumor) and Meth A cells (fibrosarcoma) (30), and nerve growth factor acts against oligodendrocytes (31). Moreover, some growth factors were reported to inhibit normal cell differentiation (32). To our knowledge, the harmful effect of overexpressed Reg I against $\beta$ cells *in vivo* was first reported by our laboratory and we are now investigating its precise mechanism. Recently, it was reported that 300–1000 nM of Reg I induce apoptosis of RINm5F cells (a rat insulinoma cell line) *in vitro* (33).

Concurrently with apoptosis of $\beta$ cells, compensatory islet regeneration was also observed in line-1 Reg-Tg mice. Reg I may show a dual action on $\beta$ cells: proliferation of immature $\beta$ cells and harmful effects on mature $\beta$ cells, as well as interferon-$\gamma$ (IFN-$\gamma$) (22). PCNA, a marker for proliferating cells, is striking in epithelial cells of pancreatic ducts. However, the dilatation of pancreatic ducts by proliferation of their epithelial cells (34) was not observed in line-1 Reg-Tg mice. Therefore, proliferating ductal epithelial cells were presumed to differentiate into islet cells, especially $\beta$ cells. Indeed, insulin-producing epithelial cells were detected in line-1 Reg-Tg mice, while glucagon-producing epithelial cells were not (data not shown).

Reg I may stimulate the proliferation of ductal epithelial cells rather than islet cells, leading to islet neogenesis. Reg I is primarily produced by acinar cells and secreted into pancreatic ducts (6, 35), and the ductal epithelial cells are exposed to a much higher concentration of Reg I than islet cells. Moreover, ordinarily Reg I is not expressed in the islet cells. This may be a mechanism for preventing $\beta$-cell injury due to high concentration of Reg I protein. Reg II protein was reported to promote the regeneration of motor neurons (36). Like Reg I in pancreatic islets, ordinarily Reg II protein is not expressed in mature motor neurons, but is produced during regeneration of motor neurons and also during embryogenesis (36). In gastric enterochromaffin-like cells, Reg I is usually expressed in association with continuous destruction and regeneration of

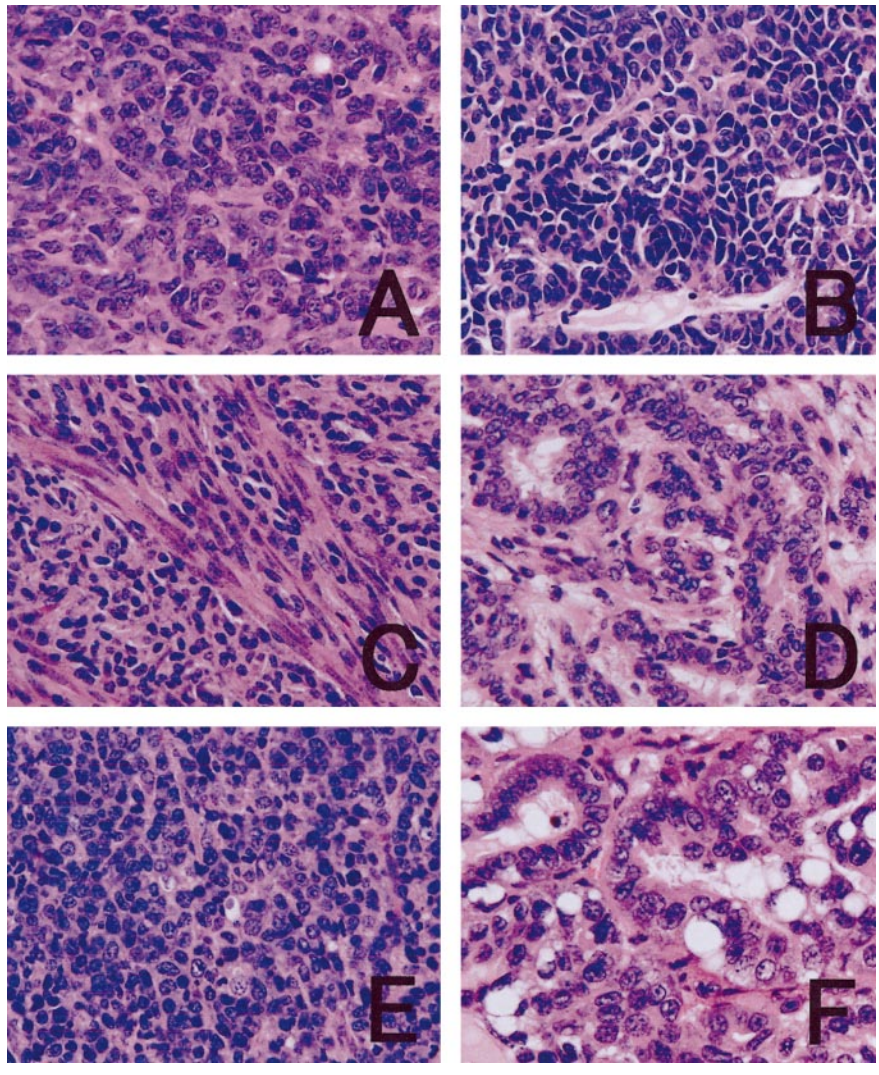


**FIG. 7.** Tumor formation in line-1 Reg-Tg mice. (A) Pancreatic cancer. (B) Hepatocellular carcinoma. (C) Uterine leiomyosarcoma. (D) Ovarian adenocarcinoma. (E) Cervical lymphoma. (F) Lachrymal gland adenocarcinoma. Magnification, ×400.

gastric epithelial cells (37). These reports suggest that Reg I and II are necessary only during regeneration, and that the absence of Reg I and II is favorable under normal conditions.

In F1 Reg-Tg mice of line 1, 6 out of 10 nondiabetic mice developed various malignant neoplasms, which were probably caused by the increase in the blood concentration of Reg I protein. Although the blood concentration of Reg I protein could not be determined in this study, a considerable increase in Reg I protein in blood is speculated for the following reasons: Reg I protein is primarily secreted into pancreatic ducts by exocrine cells, and not into blood, whereas in Reg-Tg mice, Reg I protein is secreted from $\alpha$ cells directly into the blood under the control of the glucagon promoter. The glucagon promoter has a relatively high activity, and in transgenic mice expressing IFN-$\gamma$ driven by this promoter, the average concentration of serum IFN-$\gamma$ was 50 times higher than in wild-type mice (22). Although tumor formation in transgenic mice expressing growth factors is relatively rare, HGF-transgenic mice developed a remarkably broad array of histologically distinct tumors of both mesenchymal and epithelial origin (38). In HGF-transgenic mice, the forced misdirection of HGF expression induced aberrant morphogenesis and subsequent malignant transformation of cells of diverse origins. Because the glucagon promoter starts to function from embryonic day 9.0 in mice, overexpressed Reg I in Reg-Tg mice may also affect normal cell differentiation and lead to tumorigenesis. Indeed, most tissues express Reg receptor and receive Reg I signal (33). Overexpressed Reg I may promote the growth of previously-developed microscopic malignant tumors, or protect these tumor cells from apopto-

sis. Although the mechanism for tumor formation by overexpressed Reg I remains unknown, the tumor-promoting activity of Reg I protein should be considered for its possible clinical applications.

In conclusion, overexpression of Reg I protein from transgenic $\alpha$ cells induced a decrease in the number of $\beta$ cells by apoptosis and promoted the formation of diverse malignant tumors.

## ACKNOWLEDGMENTS

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