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ARTICLE

In Vivo Gene Transfer to Healthy and Diabetic Canine Pancreas

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Gene therapy may provide new treatments for severe pancreatic disorders. However, gene transfer to the pancreas is difficult because of its anatomic location and structure, and pancreatitis is a serious concern. Like the human pancreas, the canine pancreas is compact, with similar vascularization and lobular structure. It is therefore a suitable model in which to assess gene transfer strategies. Here we examined the ability of adenoviral vectors to transfer genes into the pancreas of dogs in which pancreatic circulation had been clamped. Adenoviruses carrying the β-galactosidase (β-gal) gene were injected into the pancreatic–duodenal vein and the clamp was released 10 min later. These dogs showed β-gal-positive cells throughout the pancreas, with no evidence of pancreatic damage. β-Gal was expressed mainly in acinar cells, but also in ducts and islets. Moreover, transduction was prominent in connective tissue of the lobe septa. β-Gal expression in the exocrine pancreas of a diabetic dog was also found to be similar to that observed in healthy dogs. Thus, efficient gene transfer to canine pancreas *in vivo* may be achieved by adenovirus injection after clamping pancreatic circulation. This technique may be used to assay new gene therapy approaches for diabetes mellitus and other pancreatic disorders.

Key Words: pancreas, dog, adenoviral vectors, gene transfer, diabetes

INTRODUCTION

Beagle dogs have been used as large animal models of many human diseases, and several gene therapy approaches, such as strategies for hemophilia and retinal degeneration, have been assayed [1–5]. However, gene transfer to canine pancreas has not yet been reported. Genetic manipulation of this organ requires thorough knowledge of its anatomy, especially the vascularization, since there are major differences compared to the rodent pancreas. It is located in the dorsal part of the abdominal cavity close to the proximal part of the duodenum. In the dog, it has the classic V shape, consisting of two lobes (right and left) that emerge from the pancreatic body, surrounded by a delicate capsule of connective tissue. Septa from the capsule divide the pancreas into lobules, delimited by connective tissue, which produce a nodular surface with irregular crenate margins. Between the lobules, connective tissue surrounds the larger ducts, blood vessels, and nerve fibers. Branches from the celiac and the cranial mesenteric arteries supply blood to the pancreas. The pancreatic branches of the splenic artery irrigate the left lobe, whereas the right lobe is irrigated by the cranial and caudal pancreaticoduodenal arteries (branches from the gastroduodenal artery (cranial) and cranial mesenteric artery (caudal), respectively) (Fig. 1A). Anastomoses are common in pancreatic circulation. Veins are parallel to the arteries and eventually drain into the portal vein (Fig. 1A).

In type 1 and type 2 diabetes, hyperglycemia develops when pancreatic insulin secretion fails, as a result of β-cell loss. Gene transfer to the pancreas to induce β-cell regeneration from islet cell precursors *in vivo* may revert these diseases. Successful genetic engineering of the pancreas *in vivo* will depend on the appropriate choice of both the route of adminis-

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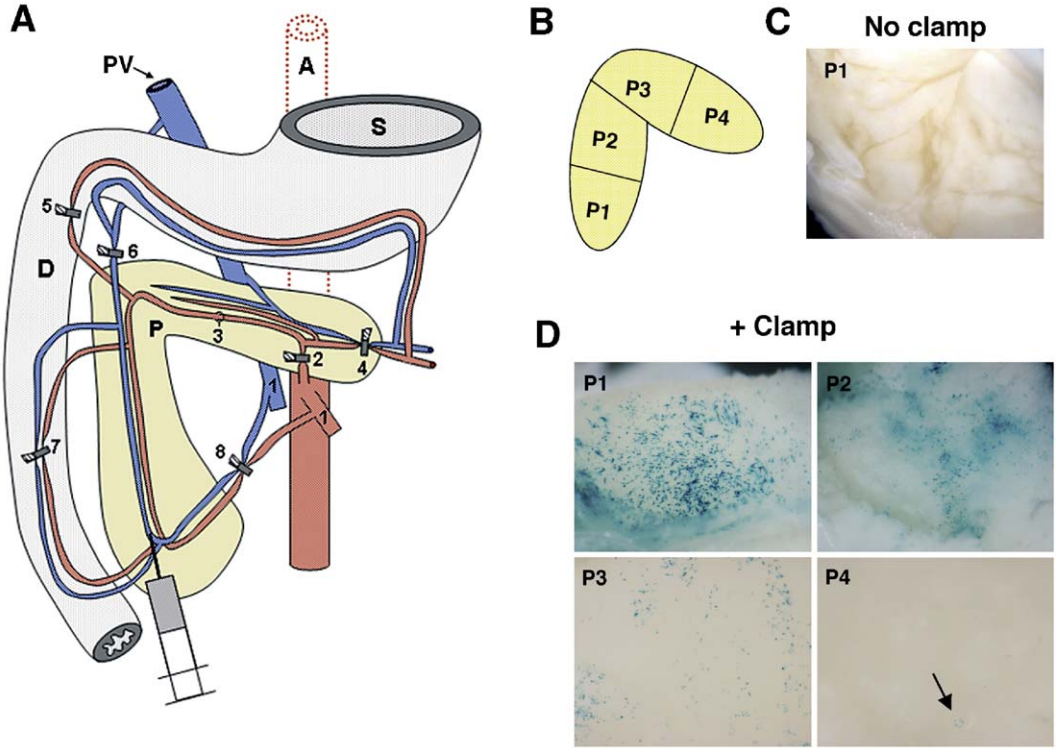

FIG. 1. (A) Scheme of canine pancreatic circulation showing the clamping sites. Stomach (S), duodenum (D), pancreas (P), aorta (A), and portal vein (PV) are indicated. The arterial system is colored in red and venous system in blue. 1, Cranial mesenteric artery and vein. 2, Celiac artery. 3, Hepatic artery. 4, Splenic artery and vein. 5, Right gastroepiploic artery. 6, Gastroduodenal vein. 7, Cranial pancreaticoduodenal artery and vein. 8, Caudal pancreaticoduodenal artery and vein. The syringe shows the site of virus injection. (B–D) Pancreas gene transfer of dogs that underwent pancreatic circulation clamp. (B) Pancreas from clamped dogs were removed 5 days after adenoviral vector administration and divided into four parts, P1, P2, P3, and P4, as indicated. (C) No X-gal staining was detected in pancreas of nonclamped dogs after *in toto* analysis. (D) X-gal staining of the pancreas revealed that adenoviral transduction was extended mainly from P1 to P3, although a few transduced cells in P4 (arrow) were also observed. Original magnification 25×.

tration and the vector. In rodents, systemic delivery of adenovirus does not infect the pancreas *in vivo,* because the liver rapidly clears the virus from circulation [6]. However, we have shown that systemic injection of adenovirus after a temporary closure of the portal vein, hepatic artery, and bile duct (portal clamp) results in increased concentration of circulating virus during the clamp and in transduction of both the exocrine and endocrine pancreas [7]. An approach of this kind is difficult to apply in large animals, since liver ischemia could result, and adenoviral vectors could be toxic when injected systemically in large amounts [8,9]. However, taking into account the anatomy and vascularization of the dog pancreas, a clamp of pancreatic circulation may be applied *in situ* and vectors can be injected directly into the pancreatic vessels. This may increase pancreas transduction and avoid liver damage. Several vectors have been used to transfer foreign genes to pancreatic islets and pancreatic β-cell lines *in vitro* [10–21]. Among them, adenovirus shows β-cell tropism and high transduction efficiency both *in vitro* [13,15,17,18] and *in vivo* [7].

Since gene transfer to canine pancreas *in vivo* is difficult because of its anatomic location and structure, we performed a local clamp of pancreatic circulation, followed by *in situ* injection of adenoviral vectors. This strategy led to the successful transfer of the β-galactosidase (β-gal) gene to the exocrine and endocrine pancreas. Surgery and vector administration were also performed in a diabetic dog, and exocrine pancreas β-gal expression was similar to that of healthy dogs. Thus, this methodology may be used to assay new gene therapy approaches for diabetes mellitus.

RESULTS

Adenovirus-Mediated Gene Transfer to Pancreas was Achieved in Dogs with Clamped Pancreatic Circulation

Since blood supply to the pancreas comes from multiple vessels, we established clamps at several sites to achieve blood stasis. We clamped the following vessels before vector administration: celiac, splenic, gastroduodenal, gastroepiploic, and cranial and caudal pancreaticoduode-

nal arteries. As the veins parallel the arteries, clamps occluded both types of vessel (Fig. 1A). Once the clamp was established, we infused a solution containing adenoviral vectors (2×10^{10} IU (infection units)/dog) carrying the β-galactosidase marker gene under the control of the CMV promoter (AdCMV/β-gal) into the pancreaticoduodenal vein (Fig. 1A).

We euthanized the dogs 5 days later. To study the distribution of the β-gal-expressing cells we sectioned the pancreas into four pieces, P1, P2, P3, and P4 (Fig. 1B). Five days after vector injection into the pancreatic–duodenal vein, β-gal was expressed throughout the pancreas (Fig 1D). However, β-gal expression was higher in P1 and P2, which were closer to the site of vector injection. Transduction was lower in P3 and much lower in P4, in which only a few cells were β-gal positive (Fig 1D). Thus, while efficient transduction was achieved when viruses were injected through the pancreatic–duodenal vein in clamped dog pancreas, in nonclamped pancreas transduction was not observed (Figs. 1C, 2A, and 2D). We have quantified the percentage of positive cells in histological sections stained for β-gal. In the whole pancreas 1.7 ± 0.7% of cells were transduced (P1, 3.3 ± 1.7%; P2, 1.7 ± 1; P3, 0.3 ± 0.2%; and P4, 0.01 ± 0.008%; $n = 3$).

β-Gal expression after injection into the pancreaticoduodenal artery was similar to that observed following injection into the vein (Fig. 2). These results suggest that both routes of administration, vein and artery, may be used when blood stasis is established within the pancreas.

Cell Types Transduced by Adenovirus in Canine Pancreas

β-Gal-positive cells were located near the lobe septum (Fig. 3A), especially in the connective tissue forming the septa (Figs. 3B and 3C). Furthermore, acinar cells showed prominent β-gal expression (Fig. 3E). In addition, we detected positive nuclei for β-gal staining in ducts of clamped animals (Fig. 3G). After both venous and arterial delivery of the vectors we observed that acinar cells were preferentially transduced. Neither acinar nor ductal cells expressed β-gal in dogs that were not clamped (Fig. 3D and 3F, respectively).

We also analyzed the distribution and shape of dog islets by insulin and glucagon immunostaining of pancreatic sections (Figs. 4A–4F). Canine pancreas contained large number of small islets homogeneously distributed throughout the section (Fig. 4D). The mouse pancreas, in contrast, contained significantly fewer islets (dogs, 11.7 ± 1.1 islets/mm^2 of pancreas, vs mice, 1.47 ± 0.1 islets/mm^2 of pancreas, $P < 0.05$), although they were larger (mouse islet area, 3878 ± 190 μm^2, vs dog, 1075 ± 96 μm^2, $P < 0.05$) (Fig. 4A). In mouse islets, glucagon-producing α cells were distributed mainly at the periphery, whereas insulin-producing β cells were seen in the core (Figs. 4B and 4C). In contrast, dog islets showed irregular α-and β-cell distribution (Figs. 4E and 4F). Furthermore, we observed scattered endocrine cells, either α or β cells, throughout the dog pancreas (Figs. 4D–4F). Double immunostaining revealed cells in the islets that were positive for insulin and β-gal (Fig. 4G). Furthermore, we also detected many β-gal-positive cells around the islets, suggesting that adenovirus transduction of dog pancreas was not selective for a specific cell type.

When we released the clamp adenovirus escaped from the pancreatic circulation, into the general circulation, and was taken up by the liver. However, β-gal expression in the liver was similarly low in clamped and nonclamped animals (Fig. 4H).

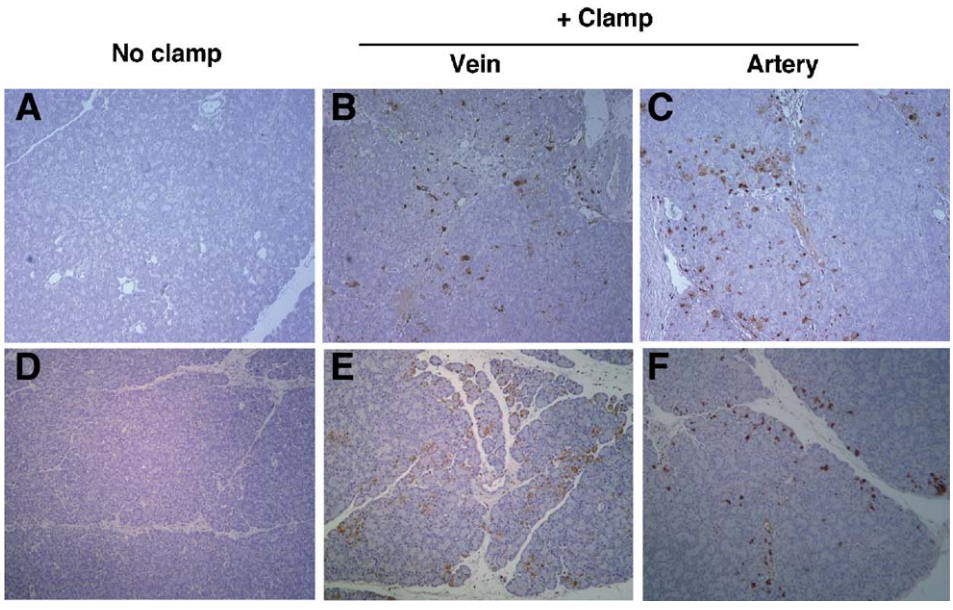

FIG. 2. Immunohistochemical analysis of β-gal expression in the pancreas. (A, D) Nonclamped and (B, C, E, F) clamped dog pancreas were analyzed 5 days after vector administration. (A, D) No β-gal expression was observed in nonclamped animals. (B, C, E, F) Pancreas gene transfer was achieved in clamped dogs when the injection of the adenovirus was performed either by the vein (B, E) or by the artery (C, F). Original magnification 100×.

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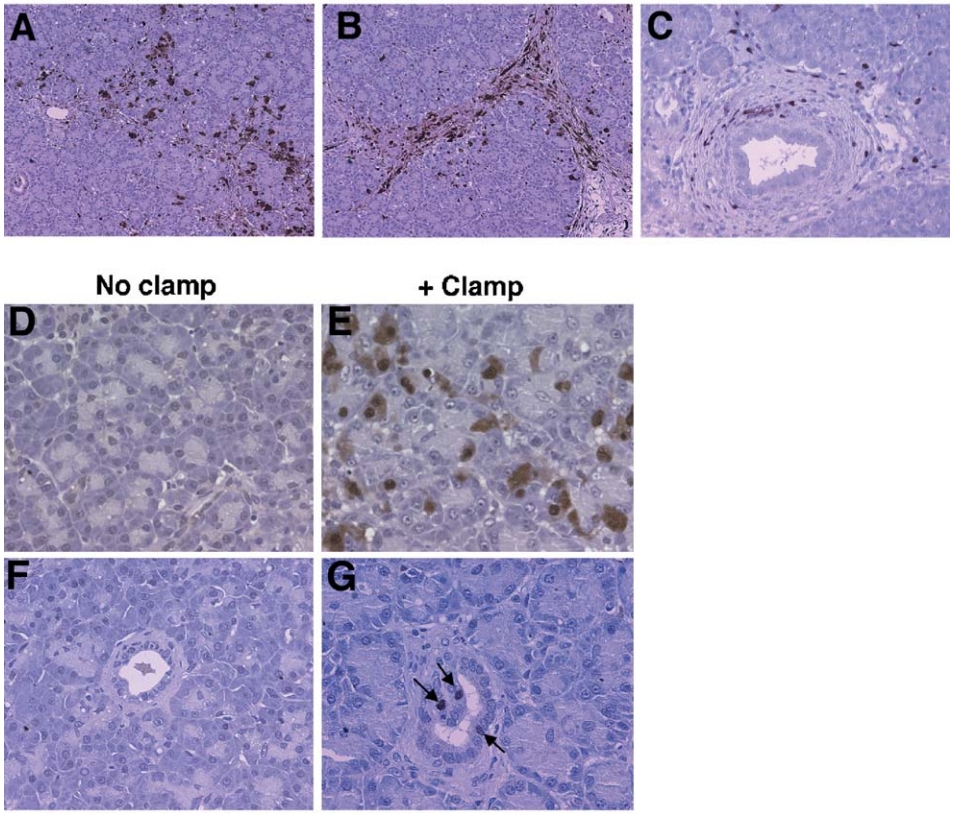

FIG. 3. (A–C) Connective tissue in the septa was efficiently transduced. Septa divide the pancreas into lobules, bounded by connective tissue. Between the lobules, connective tissue surrounds the larger ducts, blood vessels, and nerve fibers. (A) β-Gal-positive cells located around the septa can be observed (original magnification 100×). (B) Adenovirus transduction of the connective tissue in a longitudinal section (original magnification 100×). (C) β-Gal-positive cells in the connective tissue surrounding pancreatic duct in a cross section (original magnification 200×). (D–G) Gene transfer to acinar and ductal cells of dog pancreas. β-Gal immunostaining was performed in pancreatic sections 5 days after adenovirus injection. (E) Acinar cells were highly transduced by the adenovirus in clamped dog. (D) In contrast, exocrine pancreas was not transduced in dogs without clamp. (G) Ductal cells were also transduced by the adenovirus in animals that underwent pancreatic clamp. Original magnification 400×.

To evaluate pancreas and liver damage caused by the clamp and/or the adenoviral vectors, serum samples were taken the day before surgery (control value) and 1, 2, 5, and 15 days thereafter. Amylase and lipase are common markers of pancreatic damage, and alanine-amino transferase (ALT) is a marker of liver damage. ALT, amylase, and lipase levels were within the normal range 5 days after surgery (Table 1). Only one of the 11 dogs that were clamped showed amylase and lipase levels above normal values 5 days after the surgery. However, we observed no symptoms of severe pancreatitis, such as vomiting or diarrhea.

Gene Transfer to the Pancreas of a Diabetic Dog

We treated one dog with a single intravenous injection of a streptozotocin (STZ) and alloxan mixture to induce experimental diabetes [22]. Since destruction of β cells led to massive insulin release a few hours after the STZ/alloxan injection, the dog developed hypoglycemia. To maintain normoglycemia during this period, we monitored the dog and controlled glycemia by glucose infusion. Two days after STZ/alloxan injection, we detected hyperglycemia (>250 mg/dl) and maintained blood glucose levels below 300 mg/dl by subcutaneous injections of 8 IU of soluble insulin (Fig. 5A). Afterward, we carried out surgery on the diabetic dog, clamped the pancreatic circulation, and injected 2×10^{10} IU of AdCMV/β-gal into the pancreaticoduodenal vein. Insulin immunostaining confirmed the loss of β cells in the diabetic pancreas (Figs. 5B and 5C). This was consistent with the decrease in serum insulin levels (data not shown). β-Gal was expressed throughout the pancreas in the diabetic dog (Fig. 5E), mainly in the acinar cells and similar to healthy dogs (Fig. 5D). Thus, these results indicate that surgery and vector administration were feasible under diabetic conditions and that the pancreas was efficiently transduced.

DISCUSSION

Successful genetic engineering of the pancreas *in vivo* will depend on the appropriate choice of both the route of administration and the vector. Large animals are good models of human diseases, especially the dog, but canine pancreas and murine pancreas are different. Adenoviruses show high efficiency in infecting islets *in vitro* [13,15, 17,18] as well as mouse exocrine and endocrine pancreas *in vivo* [7,23–30]. In mice, direct pancreatic injection of adenoviruses leads to high transduction of exocrine pancreas around the site of injection, but only a few endocrine cells in the periphery of islets are infected [28]. In contrast, in mice with clamped hepatic circulation, systemic injection of adenoviral vectors leads to transduction of most islets, and scattered acinar cells express-

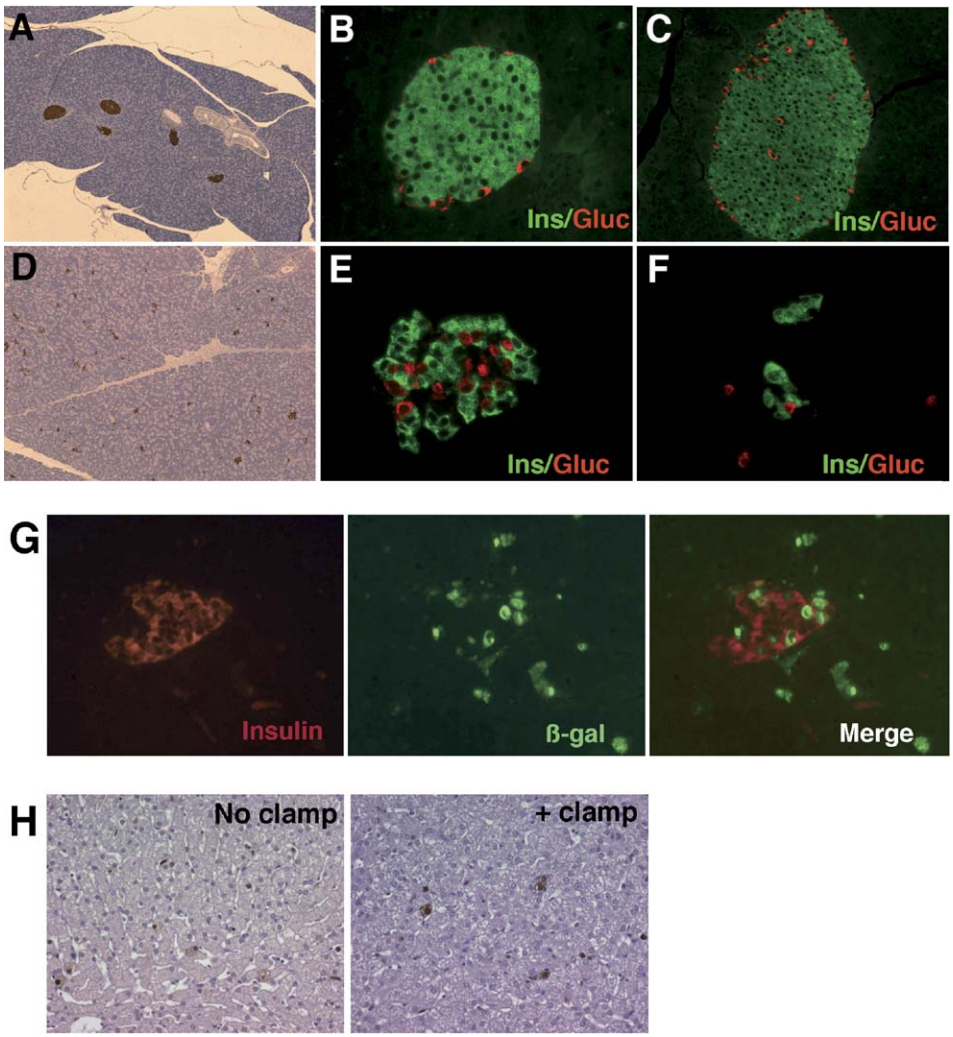

FIG. 4. Analysis of dog endocrine pancreas. (A–F) The distribution of insulin-producing cells in canine islets was determined in paraffin sections and compared with that of mouse islets. Mouse pancreas (A) showed a small number of larger islets. Islet architecture was examined by double immunostaining of insulin (green) and glucagon (red). In mouse islets, β cells reside in the core, whereas the α cells are located in the periphery (B, C). Altered distribution of β and α cells was observed in canine islets (E, F). Furthermore, small groups of α and β cells distributed throughout the pancreas can also be observed (F). Original magnifications 40× (A, D), 200× (C), and 400× (B, E, F). (G) Endocrine pancreas transduction. Insulin and β-gal double immunostaining was carried out in transduced pancreas. β cells expressing β-gal can be observed (original magnification 400×). (H) Liver transduction. Transduction level was similar in clamped and nonclamped animals. Equal amounts of viral vectors were used. β-Gal immunostaining of liver sections is shown. Original magnification 200×.

ing β-gal are also observed throughout the pancreas [7]. Thus, intravascular delivery of adenoviral vectors allows transduction of larger areas of the pancreas than direct injection, which can also induce severe inflammation and toxicity [26–28]. In the present study in dogs, injection of adenoviral vectors into the pancreatic circulation led to efficient *in vivo* gene transfer throughout the pancreas. Most of the β-gal immunostaining was seen in acinar cells and in connective tissue surrounding the main blood vessels and in the islets and ducts. However, after intravascular injection, the pattern of transduction differed from that seen in mice, in which β cells are preferentially transduced rather than acinar cells, because of the high vascularization of islets and the fenestrated structure of islet capillaries [31]. The presence of endocrine cells scattered throughout the pancreas and morphological differences in canine islets might explain these discrepancies. Furthermore, the local clamp induced blood stasis in the dog pancreas, so distribution of the adenoviruses was limited. In addition, dogs and rodents also differ in their lobe structure and blood supply. In dogs, connective tissue surrounds the blood vessels, ducts, and nerve fibers, as in the human pancreas. Since vectors were injected intravascularly, transduction was high in connective tissue and acinar cells and pancreatic islets near the septa showed higher β-gal expression than those located in the center of the lobe.

Dogs were euthanized 5 days after adenoviral vector administration, when maximal expression of the delivered gene was expected. Long-term expression cannot be expected using the β-gal reporter gene and first-generation adenoviruses. These vectors induce an immune response against transduced cells, since viral proteins are expressed at low levels [32]. Furthermore, β-gal can also induce an immune response, since it is a foreign protein, in which case transduced cells are eliminated [33,34]. To avoid undesirable effects of first-generation adenoviral vectors that lead to transient

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TABLE 1: Serum enzyme activity after surgery

	ALT (U/L)				Amylase (U/L)				Lipase (U/L)			
	D 0	D 1	D 5	D 15	D 0	D 1	D 5	D 15	D 0	D 1	D 5	D 15
Dog 1	26	29	96	—	1162	1248	1173	—	111	134	72	—
Dog 2	41	36	75	—	1129	825	1166	—	87	21	76	—
Dog 3	96	65	61	—	1173	1471	1948	—	72	72	83	—
Dog 4	63	—	31	—	832	—	1170	—	196	—	126	—
Dog 5	39	41	101	—	690	839	501	—	87	277	265	—
Dog 6	87	92	78	—	1063	849	850	—	257	93	76	—
Dog 7	25	—	29	—	1096	—	1299	—	225	—	216	—
Dog 8	44	—	1395[a]	—	1041	—	4000[a]	—	111	—	6628[a]	—
Dog 9	34	—	25	27	938	—	1057	911	81	—	53	52
Dog 10	34	—	36	29	704	—	554	600	57	—	48	41
Dog 11 (nonclamped)	24	—	23	—	637	—	713	—	107	—	62	—
Dog 12 (diabetic)	—	—	—	—	1385	1299[b]	—	—	263	211[b]	—	—

Serum samples were taken the day before surgery (control value) and 1, 2, 5, and 15 days thereafter. Normal values: ALT, 21–102 U/L; amylase, 185–2000 U/L; lipase, 13–200 U/L. Dogs 1 to 10 were healthy dogs that underwent pancreatic clamp. Dog 11 was unclamped. Dogs 1–8 were injected with 2×10^{10} IU of adenoviral vectors. Dogs 9 and 10 were injected with saline. Dog 12 was a diabetic dog that underwent pancreatic clamp. —, not determined.

[a] Parameter out of the upper range.

[b] Serum value of day 2 instead of day 1 after the surgery.

expression of the gene of interest, helper-dependent adenoviral vectors may be used [35–37]. These vectors minimize the host adaptive response and improve the efficacy and duration of gene transfer *in vivo* [1,38–43]. In addition to adenoviruses, adenoassociated viruses (AAV) can be used for pancreas gene transfer *in vivo* [23]. It has been shown that AAV8 is more efficient than AAV2 in transducing mouse pancreas after direct injection, whereas AAV5 injection does not lead to any detectable transgene expression [23]. Nevertheless, the study of Wang *et al.* also shows that adenoviral vectors are more efficient than AAV8 for engineering pancreas *in vivo* [23]. It has been recently reported that AAV8 vectors injected into mouse pancreatic duct led to efficient transduction of acinar cells with less than 5% of ductal cells being transduced and a minimal amount of β cells transduced [44]. However, AAV1 serotype was more efficient than AAV8 in rat pancreas [44].

In addition to pancreas transduction we also detected liver transduction. To prevent expression of the gene of interest in undesired tissues, cell-type-specific promoters could be used to direct the expression of the genes of interest to β cells or acinar cells. In addition, the use of tissue-specific promoters reduces the immune response against the transgene when adenoviral vectors are used [45].

Several studies have shown that β-cell precursors reside both in pancreatic ducts and inside mouse islets [46–49]. Since we observed transduced cells in ducts and inside the islets, genes involved in pancreas regeneration, β-cell differentiation, β-cell preservation, and function may be expressed in canine pancreas *in vivo* using this approach. Furthermore, acinar cells were highly transduced and they might be used to express and secrete proteins that may act in the surrounding islets in a paracrine manner. Thus, progress in diabetes therapy by transferring key genes directly to the pancreas in large animals should be possible and may lead to the development of treatment for humans. In addition, this methodology may be used to study new therapeutic strategies for other pancreatic disorders.

MATERIALS AND METHODS

Animals. Male beagle dogs, 6–12-months of age, were used (Isoquimen, Barcelona, Spain). They were fed once a day with a standard diet and kept under a natural light cycle. Male C57BL/6SJL mice, 2 months of age, were used (CBATEG, Barcelona, Spain). Animal care and experimental procedures were approved by the Ethics Committee on Animal and Human Experimentation of the Universitat Autònoma de Barcelona.

Recombinant adenoviral vector. Human E1-deleted recombinant serotype 2 adenoviruses carrying the cytomegalovirus promoter/β-galactosidase chimeric gene (AdCMV/β-gal) were generated as described previously [50]. Infectious units were determined by infecting 293 cells with serial dilutions of the virus and then counting β-gal-expressing cells after 48 h. The β-galactosidase contains a nuclear localization signal. The particle/IU ratio of the virus stock used in the experiments was between 10 and 40.

Local pancreatic clamp and administration of adenovirus. All dogs received an im injection of a neuroleptoanalgesic combination of acepromazine (0.05 mg/kg) and buprenorfine (0.01 mg/kg). Thirty minutes after the preanesthetic medication, anesthesia was induced by intravenous injection of 4 mg/kg propofol and maintained with 1–2% isoflurane in oxygen. Following laparotomy, pancreatic circulation was clamped (including celiac, splenic, gastroduodenal, gastroepiploic, and cranial and caudal pancreaticoduodenal arteries). Blood vessels were occluded with hemostatic clamps. Since pancreas and duodenum share a common blood supply we also clamped the duodenum by plastic-protected intestinal clamps. Afterward, 2 ml of virus suspension (in 150 mM NaCl) was injected into the pancreaticoduodenal vein (or artery). A total viral dose of 2×10^{10} IU per animal was used. Ten minutes later, the clamp was removed and the abdominal wall was sutured. Surgery and

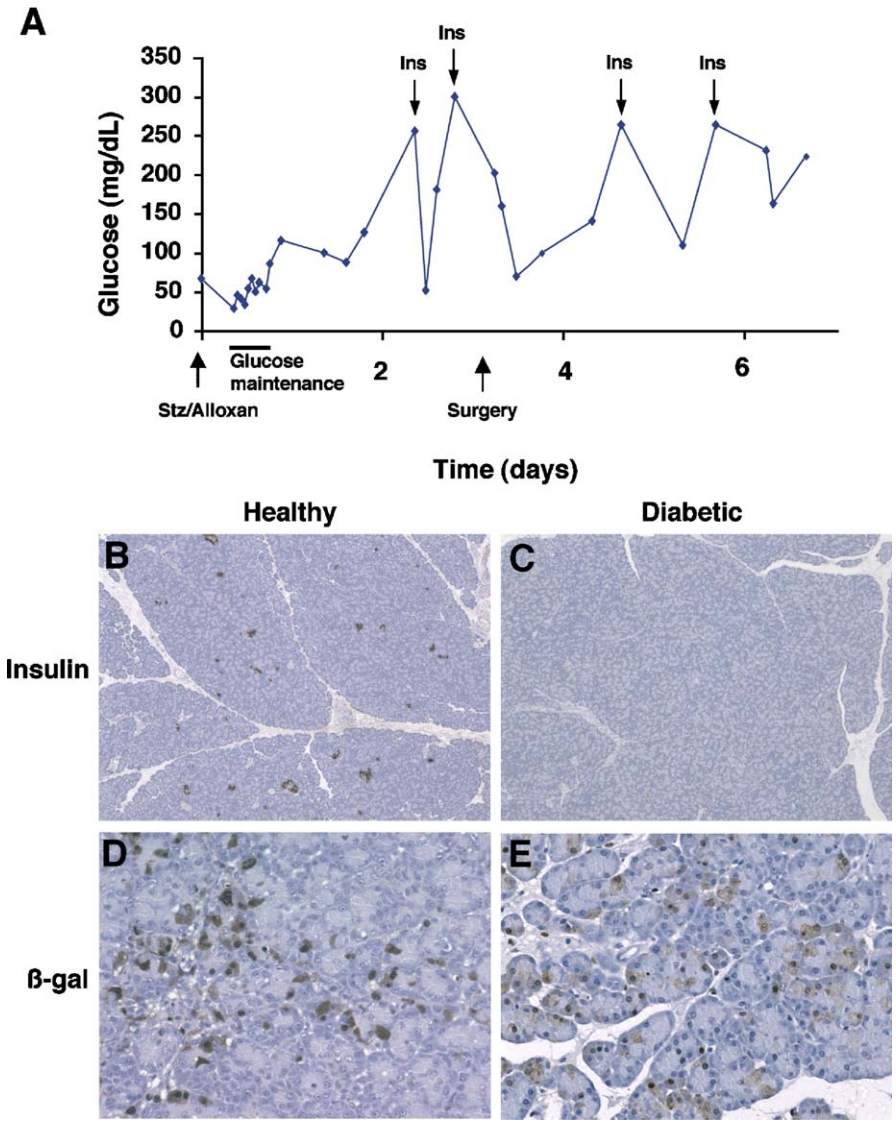

FIG. 5. Gene transfer to diabetic canine pancreas. (A) Experimental diabetes was induced by streptozotocin/alloxan injection as described under Materials and Methods. A few hours afterward, the animal developed hypoglycemia due to massive destruction of β cells and insulin release. Glycemia was controlled by glucose infusion. When hyperglycemia was observed, the dog was treated with subcutaneous injection of 8 IU of soluble insulin (Ins). Surgery and vector administration were performed 3 days after diabetes induction. (B, C) Reduction of β-cell mass was observed in the diabetic dog after insulin immunostaining of pancreatic sections. (D, E) Gene transfer was also achieved in diabetic pancreas. Pancreatic sections of a healthy (D) and a diabetic (E) dog immunostained with β-gal antibody (brown) are shown. Original magnifications 40× (A, B) and 200× (D, E).

vector dose (injected into the pancreaticoduodenal vein) used in unclamped dog were the same as in clamped; however, blood vessels were not occluded. Five days after vector administration, animals were euthanized with intravenous injection of pentobarbital overdose, and the pancreas and liver were removed. Experimental diabetes was induced in one dog by a single intravenous injection (by cephalic vein) of an STZ (35 mg/kg body wt) and alloxan (40 mg/kg body wt) mixture [22].

Analysis of β-galactosidase expression in tissue samples. To detect the presence of β-galactosidase in pancreas and liver *in toto,* samples were fixed for 1 h in 4% paraformaldehyde, washed twice in phosphate-buffered saline (PBS), and then incubated in X-gal (5-bromo-4-chloro-3-β-D-galactopyranoside) in 5 mM $K_3Fe(CN)_5$, 5 mM $K_4Fe(CN)_6$, and 1 mM $MgCl_2$ in PBS for 6–8 h in the dark at 37°C.

Immunohistochemical and morphometrical analysis. For immunohistochemical detection of β-galactosidase, insulin, and glucagon, dog and mouse pancreas were fixed for 12 to 24 h in formalin, embedded in paraffin, and sectioned. Sections were then incubated overnight at 4°C with rabbit anti-β-galactosidase antibody ab616 (Abcam, Cambridge, UK) diluted at 1:900, with a guinea pig anti-porcine insulin antibody (DAKO Corp., Carpinteria, CA, USA) at 1:100 dilution, or with a rabbit anti-human glucagon antibody (ICN Biomedicals, Inc., Cleveland, OH, USA) at 1:4000 dilution. As secondary antibody rabbit anti-guinea pig immunoglobulin G, coupled to peroxidase (Roche Molecular Biochemicals), or biotinylated goat anti-rabbit antibody and ABC complex (Vector, Burlingame, CA, USA) was used. 3′,3′-Diaminobenzidine was used as the substrate chromogen. Sections were counterstained in Mayer's hematoxylin. For immunofluorescence, FITC-labeled goat anti-rabbit (Southern Biotechnology Associates, Inc., Birmingham, AL, USA) or TRITC-conjugated rabbit anti-guinea pig (Sigma Chemical Co.) was used as secondary antibody. For morphometrical analysis of the islets, pancreas was obtained and immunohistochemical detection of insulin was performed on four (2 to 3 μm) sections per animal (n = 3 dogs and n = 6 mice). The total number of islets/mm^2 was calculated by dividing the total number of islets in one section by the total area of this section. The area (mm^2) of each section was determined using a Nikon Eclipse E800 microscope (Nikon Corp., Tokyo, Japan) connected to a video camera with a color monitor and to an image analyzer (analySIS 3.0; Soft Imaging System Corp., Lakewood, CO, USA). The area of the islets (mm^2) was measured using the same software, and more than 500

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islets were counted both in dogs and in mice. Quantification of pancreatic cell transduction was performed in histological sections immunostained for β-gal. The percentage of transduction was obtained counting all positive cells in 20 microscopy fields in P1–P4 (40× magnification).

Determination of serum parameters. Before and after the surgery blood samples were taken for biochemical analysis. Blood glucose levels were measured with a Glucometer Elite analyzer (Bayer AG, Leverkusen, Germany). Determination of serum ALT, amylase, and lipase levels were determined spectrophotometrically using specific kits from ABX Diagnostics (Montpellier, France).

Statistical analysis. All values are expressed as the means ± SEM. Differences between groups were compared by the Student *t* test. *P* values less than 0.05 were considered statistically significant.

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