Regeneration of the Pancreas in Adult Zebrafish

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OBJECTIVE—Regenerating organs in diverse biological systems have provided clues to processes that can be harnessed to repair damaged tissue. Adult mammalian β-cells have a limited capacity to regenerate, resulting in diabetes and lifelong reliance on insulin. Zebrafish have been used as a model for the regeneration of many organs. We demonstrate the regeneration of adult zebrafish pancreatic β-cells. This nonmammalian model can be used to define pathways for islet-cell regeneration in humans.

RESEARCH DESIGN AND METHODS—Adult transgenic zebrafish were injected with a single high dose of streptozotocin or metronidazole and anesthetized at 3, 7, or 14 days or pancreatectomized. Blood glucose measurements were determined and gut sections were analyzed using specific endocrine, exocrine, and duct cell markers as well as markers for dividing cells.

RESULTS—Zebrafish recovered rapidly without the need for insulin injections, and normoglycemia was attained within 2 weeks. Although few proliferating cells were present in vehicles, ablation caused islet destruction and a striking increase of proliferating cells, some of which were Pdx1 positive. Dividing cells were primarily associated with affected islets and ducts but, with the exception of surgical partial pancreatectomy, were not extensively β-cells.

CONCLUSIONS—The ability of the zebrafish to regenerate a functional pancreas using chemical, genetic, and surgical approaches enabled us to identify patterns of cell proliferation in islets and ducts. Further study of the origin and contribution of proliferating cells in reestablishing islet function could provide strategies for treating human diseases. ***Diabetes*** **58:1844–1851, 2009**

The adult endocrine pancreas functions as a regulator of blood glucose levels by virtue of insulin-secreting β-cells contained within the pancreatic islets of Langerhans. Destruction or malfunction of β-cells results in hyperglycemia and diabetes, often requiring lifelong insulin therapy. Damage to differentiated tissues, such as the human liver, can elicit regeneration through the activation and division of reserve quiescent adult cells. However, the pancreas has little capacity to be replaced when damaged and typically responds to insults through inflammatory repair mechanisms (1). Unlike repair, regeneration is a process of controlled cell proliferation and patterning that restores the entire tissue. Regeneration is associated with neogenesis in the adult and the replacement of diverse cell types. Adult zebrafish are capable of regrowing an entire fin, including cartilage, muscles, and nerves, by a process known as epimorphic regeneration (2). In contrast, a severed mammalian appendage fails to regenerate, although newborn mice temporarily retain the regenerative memory that zebrafish can utilize for wound repair throughout their life span (3). Limited regrowth of adult mammalian islets can occur after injury; however, if hyperglycemia persists, insulin therapy is required and inflammatory responses severely limit the restitution of lost tissue.

The main zebrafish pancreas is both anatomically (4) and developmentally (5) similar to the mammalian pancreas, whereas major differences are apparent in other fish species. In adult zebrafish, the main pancreas contains several principal islets surrounded by exocrine tissue. A tail of single islets embedded in exocrine tissue and fat extends caudally along the intestine. In contrast, the β-cells of tilapia (another glucose-sensitive teleost fish) reside in Brockman bodies or isolated islets located along the mesentery that are not surrounded by exocrine tissue (6,7). In embryonic zebrafish, recent investigation has revealed the capacity to recover ablated β-cell mass with restoration of the islet without a requirement for exocrine cells (8,9). However, adult zebrafish were not evaluated in these studies and it is not clear that β-cell recovery after removal of the drug was a reinitiation of developmental pathways. In adult mammals, cell division from preexisting, differentiated β-cells appears to be the primary means of tissue replacement (10), although severe forms of impairment such as partial duct ligation induce replication of insulin-negative β-cell progenitors within ducts that later become insulin-positive β-cells (11). In mammals, wounding the pancreas by chemical (12) or physical (13) means does not result in a significant replacement of lost tissue, while subsequent insulin administration increases the number of β-cells per islet, especially in young animals. Chemical-induced pancreatitis causes a hyperplasia of tubular structures (14), and islet morphogenesis from ducts has been observed in several developmental as well as disease models (15), supporting the role of pancreatic ducts in islet neogenesis. In contrast to these mammalian models, the current study demonstrates a functional regeneration of the adult zebrafish islet after ablation using two different models of drug-induced damage and surgical removal. We visualized islets directly in the adult because transgenic zebrafish expressed bright fluorescence only in β-cells. Islet regeneration resulted in a return to normal size and function of the islets with no requirement for insulin therapy. Analysis of the expression of hormone (glucagon, insulin), Pdx1 (transcription factor), and a marker of zebrafish ductal and vascular eptithelium [cyto-

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Received 9 May 2008 and accepted 14 May 2009.

Published ahead of print at http://diabetes.diabetesjournals.org on 9 June 2009. DOI: 10.2337/db08-0628.

keratin 18 (CK18)] revealed a contribution of $Pdx1^+$ dividing cells to the regenerate. The unusual ability of the adult zebrafish to regenerate appendages and internal organs provides new opportunities for probing changes in β-cell mass in the adult, with the ultimate goal of adapting knowledge gained from this unique system to the development of strategies for inducing human β-cell regeneration.

RESEARCH DESIGN AND METHODS

Zebrafish strains and maintenance. Adult InsGFP (16) and wild-type (Tübingen AB) zebrafish maintained in our recirculating system (Aquatics Habitat) were 1 year old before manipulation. InsGFP zebrafish (Tg(−1.0ins:eGFP)sc1) are now available from ZIRC (http://zfin.org). Tg(T2Kins:nfsB-mCherry)^{ih4} fish (abbreviated to InsNTR) express a nitroreductase (NTR) mCherry fusion protein in the β-cells (8) and were used at 8 months old for ablation studies. After treatment, experimental fish were kept in isolation tanks with daily water changes. Institutional animal facility Institutional Animal Care and Use Committee protocols were followed for all experiments.

Streptozotocin or metronidazole treatment and surgical resection. The dose of streptozotocin (STZ) (no. S0130, Sigma) required to extinguish β-cell GFP fluorescence was first determined by culturing pancreatic explants in supplemented RPMI media (17) at 28°C without CO_2. A similar dose of alloxan (no. A6316, Sigma) also caused β-cell necrosis after 3 days in culture (data not shown). A dose curve using 200, 400, 800, and 1,000 mg/kg STZ ($n = 6$ for each dose) indicated that a single-high intraperitoneal dose of 1 g/kg STZ caused loss of green fluorescent protein (GFP) fluorescence. Metronidazole (MET) (no. M3761, Sigma) was injected at 0.25 g/kg. Intraperitoneal injections of alloxan (≤0.2 g/kg) caused greater lethality and less reproducibility, primarily because of liver failure. STZ or MET dissolved in 5 mmol/l citrate, pH 5, was injected with pulled capillary pipets inserted into the holder of a microinjector (Eppendorf). The effectiveness of the injection was monitored by adding 0.25% phenol red, resulting in a diffuse pink color throughout the fish within 15 min. Zebrafish were anesthetized with tricaine (18) and revived within 30 s after gentle swirling of system water through the gills. Surgeries were aided using a dissection microscope equipped with fluorescence (Leica) to detect endogenous islet InsGFP expression. Anesthetized adults were placed on a sponge exposing their right side. Number 55 forceps were used to penetrate the body wall and remove the GFP-positive main pancreas. Experimental subjects recovered within 1 min and were maintained individually for 3, 7, or 14 days in 28°C system water while monitored for rapid gill movement or hemorrhage.

Blood glucose determination. Fasted adults were weighed, anesthetized, and placed on a wet sponge, and a pulled capillary pipet was inserted into the atrium of the beating heart. Approximately 50 nl of blood was removed and rapidly transferred to a Freestyle glucose monitor strip. We did not use blood from the tail vein because this technique caused more lasting damage than heart puncture. Blood glucose measurements from InsNTR fish were taken at sacrifice and anesthetized in 4°C fish water for 2 min because of required changes in our animal protocol. Recordings were evaluated statistically using Student's t test. At least six vehicle-injected and 10 drug-injected animals were used at each regeneration time point. At the termination of each experiment, zebrafish were weighed and final blood glucose determinations were made. Zebrafish were killed according to established protocols (18).

Histology immunohistochemistry and microscopy. Excised gut sections were placed in buffered 2% paraformaldehyde overnight at 4°C. Paraffin embedding was achieved using minimal times in solutions (≤30 min), after which 5 micron serial sections were cut. We were able to select GFP- or mCherry-positive sections while still in paraffin before, as well as after, antibody treatment (19). Fluorescence microscopy images were recorded with a Hamamatsu CCD camera using a Leica MZFlIII dissecting microscope or a Zeiss Axioskop. Confocal images were made on a Zeiss LSM 410 microscope. Primary antibodies were used according to the manufacturer's recommendations: proliferating cell nuclear antigen (PCNA), NeoMarkers; insulin, Linco Research; glucagon, Dako; (cyto) keratin 18, Progen (Germany). The Pdx1 guinea pig anti-zebrafish peptide polyclonal antibody was a generous gift from Chris Wright (Vanderbilt). The secondary antibodies for 488, 546, and 647 nm were acquired from Molecular Probes (Alexa Fluor). We reproduced PCNA results with BrdU (no. B9285, Sigma) injections (data not shown). Antigen retrieval was preformed using Dako products. Images were acquired and processed using Openlab software (Improvision) and Adobe Photoshop CS2.

RESULTS

Zebrafish islet tissue is destroyed by exposure to STZ. We initially tested whether zebrafish islets were susceptible to the toxic effects of STZ, a nitrosourea causing DNA damage after entering β-cells through the Glut2 receptor. Adult zebrafish containing a stable transgene expressing GFP from the zebrafish insulin promoter only in β-cells (InsGFP [16]) were anesthetized, and the main GFP-positive pancreatic tissue was excised and incubated for 3 days in media used for culturing rat primary islets (17). At 0 or 20 mmol/l STZ, no gross changes in GFP fluorescence or morphology were detectable. However, at 100 and 200 mmol/l STZ, significant loss of GFP-positive cells was observed in culture (Fig. 1*A*). These data provided the basis for examining the physiological effects of β-cell destruction in living InsGFP zebrafish.

The zebrafish main pancreas is located on the right side of the adult fish, attached to the lateral aspect of the duodenum by the pancreatic duct (20). Typically, one large islet and 3–6 smaller islets occupy the main pancreas of the adult zebrafish. The tail of the pancreas is embedded with single β-cells or clusters of small GFP-positive islets and extends caudally along the right side of the intestine (Fig. 1*B*). We evaluated a range of STZ doses from 200 mg/kg to 1,000 mg/kg and determined that a single high dose of 1 g/kg reduced GFP fluorescence in vivo (Fig. 1*B*). This dose suggested a relative resistance of zebrafish islets to β-cell toxins (7) compared to the mouse. Lethality at doses greater than 1 g/kg was correlated with damage to other organs and/or death. Injections were performed on day 0 before the morning shrimp meal. Unlike controls, normal feeding behavior was delayed in STZ-injected zebrafish until 12–24 h postinjection. After 3 days, significant loss of fluorescence in the islets could be observed in injected animals when compared with controls (Fig. 1*B*). To determine if the loss of GFP expression was associated with β-cell necrosis, propidium iodide was applied in situ immediately after death to the pancreas of STZ-treated zebrafish or controls. STZ-injected animals showed a dramatic loss of GFP-positive cells and an increase of necrotic, propidium iodide–positive cells within the islet when compared with vehicle (Fig. 1*C* and *D*).

Functional assessment of β-cell loss in STZ- and MET-injected zebrafish. To evaluate if the loss of fluorescent β-cells produced a physiological change, blood glucose levels in zebrafish at 3, 7 or 14 days after drug injection were measured (Fig. 2). The collected blood was rapidly discharged onto a strip containing adsorbed glucose oxidase, routinely used by human diabetic patients to monitor blood glucose (Freestyle). To acquire a baseline, fasted blood glucose readings were compared between male and female as well as wild-type versus transgenic animals. An average fasted adult zebrafish blood glucose reading was 57.4 mg/dl ± 4.1 mg/dl ($n = 96$). STZ-injected blood glucose levels increased at least two-fold after 3 days and returned to normal after 2 weeks without intervention (Fig. 2, black bars). Reports of STZ-induced rodent hyperglycemia have indicated a much larger increase of blood glucose readings after treatment. This may be due either to greater hydration secondary to hyperglycemia in zebrafish or to liver toxicity from drug administration (data not shown). In consideration of potential detrimental effects of STZ on tissues other than the pancreatic β-cells, we also evaluated a β-cell intrinsic ablation system

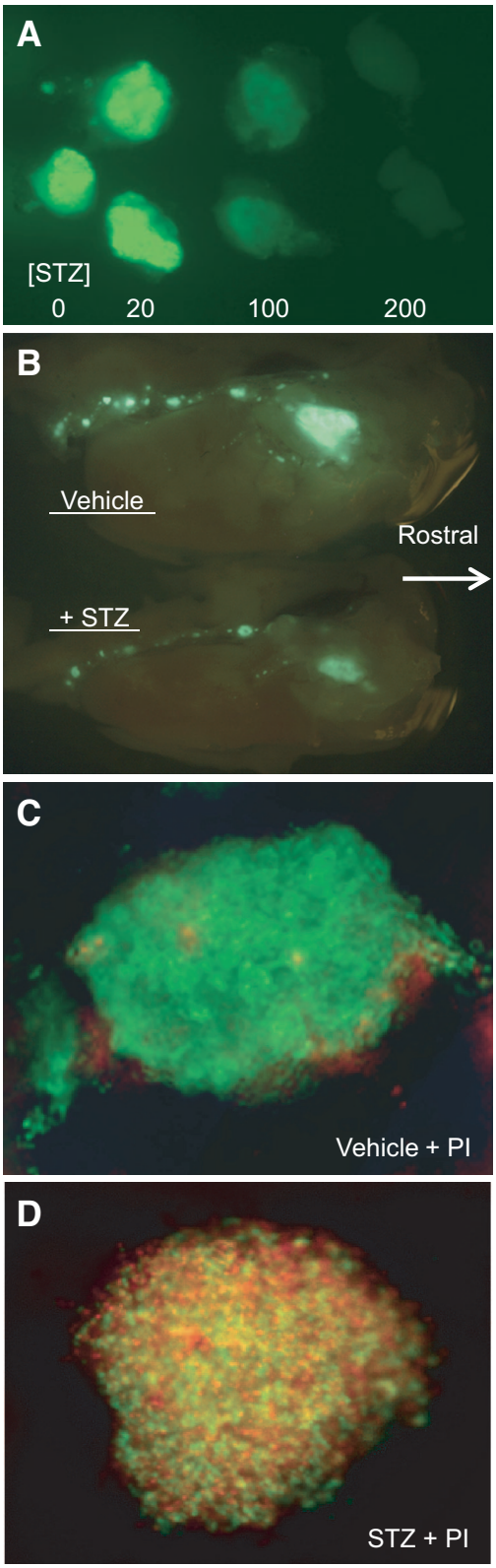

FIG. 1. β-cell destruction by STZ. *A*: GFP fluorescence of zebrafish pancreatic explants after 3 days in culture (100×). *B*: Intact zebrafish: lateral views of right side without skin (50×). *Top panel*: GFP fluorescence in the main pancreas (rostral) and auxiliary islets (green) of vehicle-injected zebrafish. *Bottom panel*: STZ-injected zebrafish killed after 3 days. *C*: Vehicle-injected, killed InsGFP zebrafish. Main pancreas was imaged after in situ treatment with propidium iodide (propidium iodide: red fluorescence). *D*: STZ-injected zebrafish pancreas + propidium iodide after 3 days. (A high-quality digital representation of this figure is available in the online issue.)

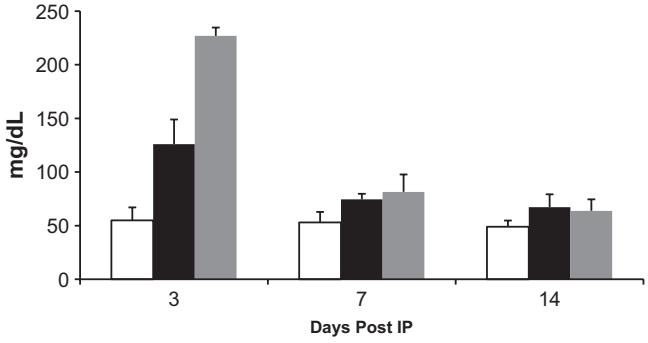

FIG. 2. Blood glucose levels return to normal after 2 weeks. Fasted blood glucose readings were recorded from cardiac blood in STZ-treated (black bars) versus vehicle-treated (white bars) zebrafish at 3, 7, and 14 days from 10 (5 male/5 female) STZ- and 6 (3 male/3 female) vehicle-injected InsGFP 1-year-old siblings. A two-fold reduction in blood glucose was observed 1 week after destruction of the β-cells. Gray bars: blood glucose readings from fasted InsNTR 1 year olds 3, 7, or 14 days after MET treatment. IP, intraperitoneal.

in adult zebrafish (8). NTR converts the prodrug MET to a toxic compound that damages DNA in zebrafish embryos, causing β-cell apoptosis. Blood glucose readings for InsNTR-mCherry transgenics, recorded at time of death, also increased 3 days after injection and then fell to normal levels after 2 weeks. The observation that the 3 day MET blood glucose readings were higher than the STZ-treated reading at this time point suggests that β-cell ablation may have been more complete in the InsNTR transgenics.

Time course of STZ-mediated cell death and regeneration. A histological study of adult zebrafish islets was used to evaluate the surprising return to normoglycemia without the need for insulin administration. Excised gut fragments including the proximal intestine and attached fluorescent main pancreas were fixed and embedded in paraffin. Figure 3 compares stained and immunofluorescence-hybridized sections of vehicles with STZ-injected zebrafish at 3, 7, and 14 days. Hematoxylin and eosin staining showed the main islet was frequently absent and smaller islets were invested with lymphocytic infiltrates and/or necrotic cells after 3 days (Fig. 3*A*). The islet perimeter was often discontinuous or aberrant (Fig. 3*B* and *C*). Weak insulin (Fig. 3*B*) or GFP (Fig. 3*C*) staining after 3 days confirmed a loss of β-cells while dividing PCNA-positive cells appeared at the islet perimeter, whereas vehicle-injected zebrafish contained dividing PCNA-positive cells primarily in the intestine (Fig. 3*B*). Insulin-positive cells were not dividing. Glucagon staining indicated a disruption of islet geometry after 3 days (Fig. 3*C*). After 7 days, small insulin-positive islets were rarely found. We occasionally observed GFP-positive β-cells within ductal epithelium (Fig. 3*B*, *inset*), though dividing cells were not insulin positive. Glucagon marked the perimeter of GFP-positive islets (Fig. 3*C*, *inset*) and outlined adjacent structures that appeared to emanate from ducts, visible after 7 days. After 2 weeks, numerous islets were present, and unlike controls, glucagon-positive cells were prominent in ductal epithelium (Fig. 3*A* and *C*). Larger islets contained scattered PCNA-positive cells comparable to vehicle-injected zebrafish after 14 days (Fig. 3*B*).

Regeneration after MET injection of β-cell–specific NTR conditional transgenics. Because InsNTR zebrafish treated with MET also had elevated blood glucose levels that returned to normal after 2 weeks (Fig. 2), we

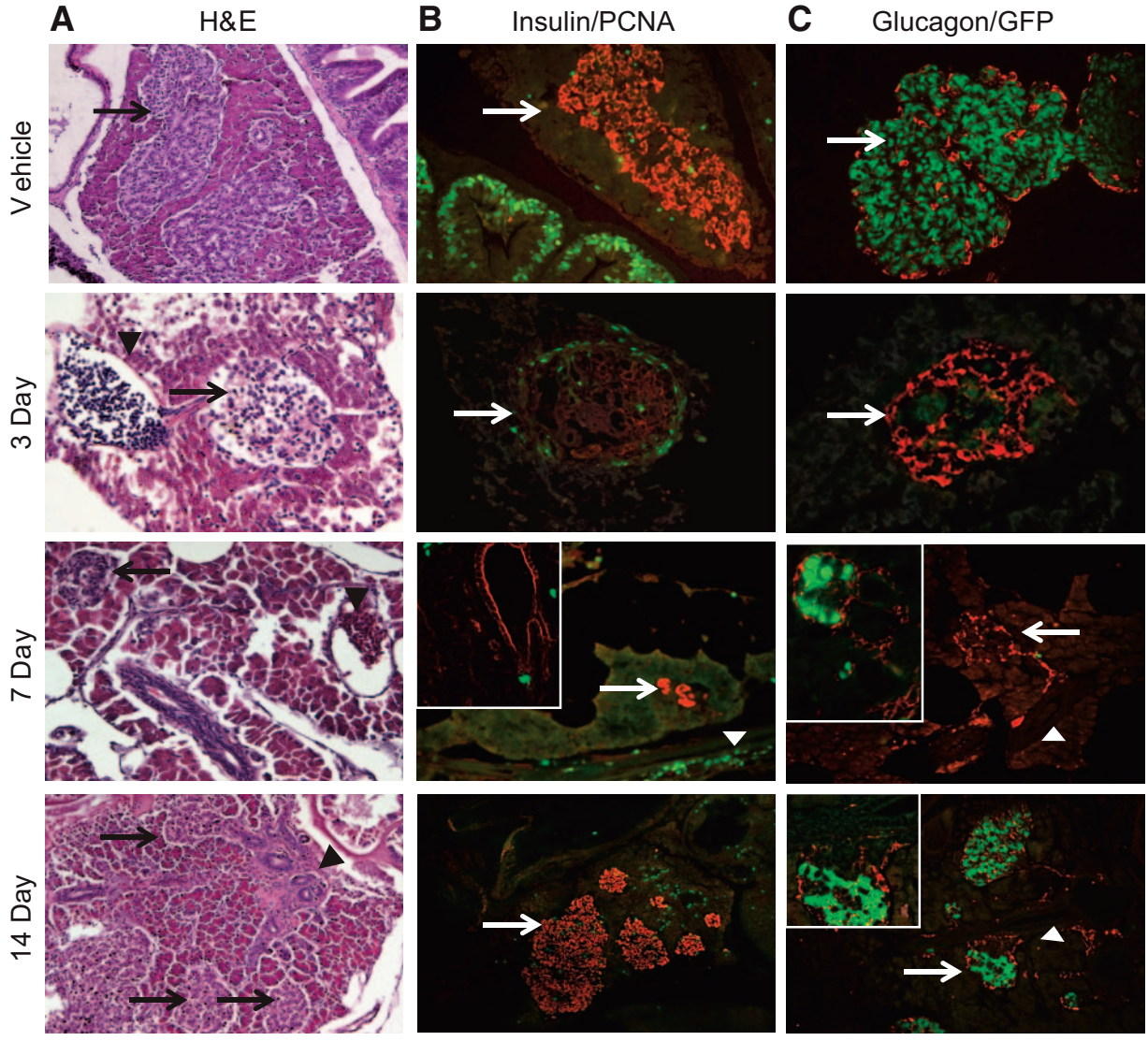

FIG. 3. Regeneration of zebrafish islets after STZ treatment. *A*: Hematoxylin and eosin (H&E) staining of paraffin sections at 3, 7, and 14 days. Vehicle and 14 day STZ: 200× magnification; 3 day and 7 day: 400× magnification. Arrows: islets. Arrowheads: blood vessels. *B*: STZ Ins/PCNA: insulin antibodies (visualized with red fluorescent secondary antibodies) mark β-cells. PCNA$^+$ dividing cells are green. Arrows identify islets. Arrowheads: ducts; vehicle: numerous dividing PCNA$^+$ cells are located at the base of intestinal villi. A few non–insulin expressing dividing cells are scattered throughout the islet and surrounding exocrine pancreas (400× magnification); 3 day STZ: a mantle of PCNA-positive cells surrounds affected islets (400×); 7 day STZ: dividing cells are located in and around ducts (400× magnification); inset (200× magnification): CK18 (red) labeling of ducts. Insulin$^+$ cells are green; 14 day STZ: 200× magnification. Large islets with scattered dividing cells have appearance similar to vehicle-injected zebrafish. Dividing cells surround insulin-negative areas, similar to the PCNA expression observed after 3 days. *C*: STZ glucagon/GFP; vehicle (400× magnification): β-cells (green) and α-cells (red). Arrow: islet; 3 day STZ: glucagon$^+$ cells within islet remnant (400×); 7 day STZ: glucagon labels GFP-negative islet attached to duct (200×). Inset (400×): glucagon (red) outlines islets ± β-cells (green); 14 day STZ: ductal hyperplasia (200×) with glucagon staining (red). Inset (600×): the ductal epithelium is continuous with the islet (confocal image). (A high-quality digital representation of this figure is available in the online issue.)

used these transgenics to further examine islet regeneration in dividing cells and ducts (Fig. 4). A CK18 antibody (20) was used to label ducts. We employed a zebrafish-specific Pdx1 antibody (gift of Chris Wright) to identify prospective β-cells. Pdx1 is a homeodomain protein required for the organogenesis of the pancreas as well as maintenance of the β-cell phenotype (21). Pdx1 expression in wild-type adult zebrafish islets has previously been demonstrated using antisense probes (22). Compared with vehicle-injected InsNTR adults (Fig. 4*A*), PCNA$^+$ cells were found in and around ablated islets after 3 days (Fig. 4*B*), similar to the STZ-ablation phenotype. Insulin$^+$ cells were absent. By 14 days, β-cells had reappeared and divided at levels similar to the vehicle-injected controls. However, more PCNA$^+$ cells were present outside the islet after 2 weeks (Fig. 4*C*). Although islet architecture had been disrupted, α-cells were not actively dividing after 3 or 14 days (Fig. 4*B* and *C*, Glu/PCNA). As for vehicle-injected zebrafish (Fig. 4*A*), the pancreatic ductal epithelial cells were labeled with the Pdx1 antibody at 3 days (Fig. 4*B*). As seen after STZ treatment, more ducts were present 14 days after injection of MET and were labeled by the Pdx1 antibody (Fig. 4*C*). Small numbers of dividing, Pdx1$^+$ cells appeared in control zebrafish (Fig. 4*A*). After 3 days, large numbers of Pdx1$^+$ cells were dividing (Fig. 4*B*) in islets and ducts. Cells labeled with both Pdx1 and PCNA were numerous in both islets and within ductal epithelia after treatment (Fig. 4*C*), although significant numbers of PCNA$^+$/Pdx1$^-$ cells were also observed.

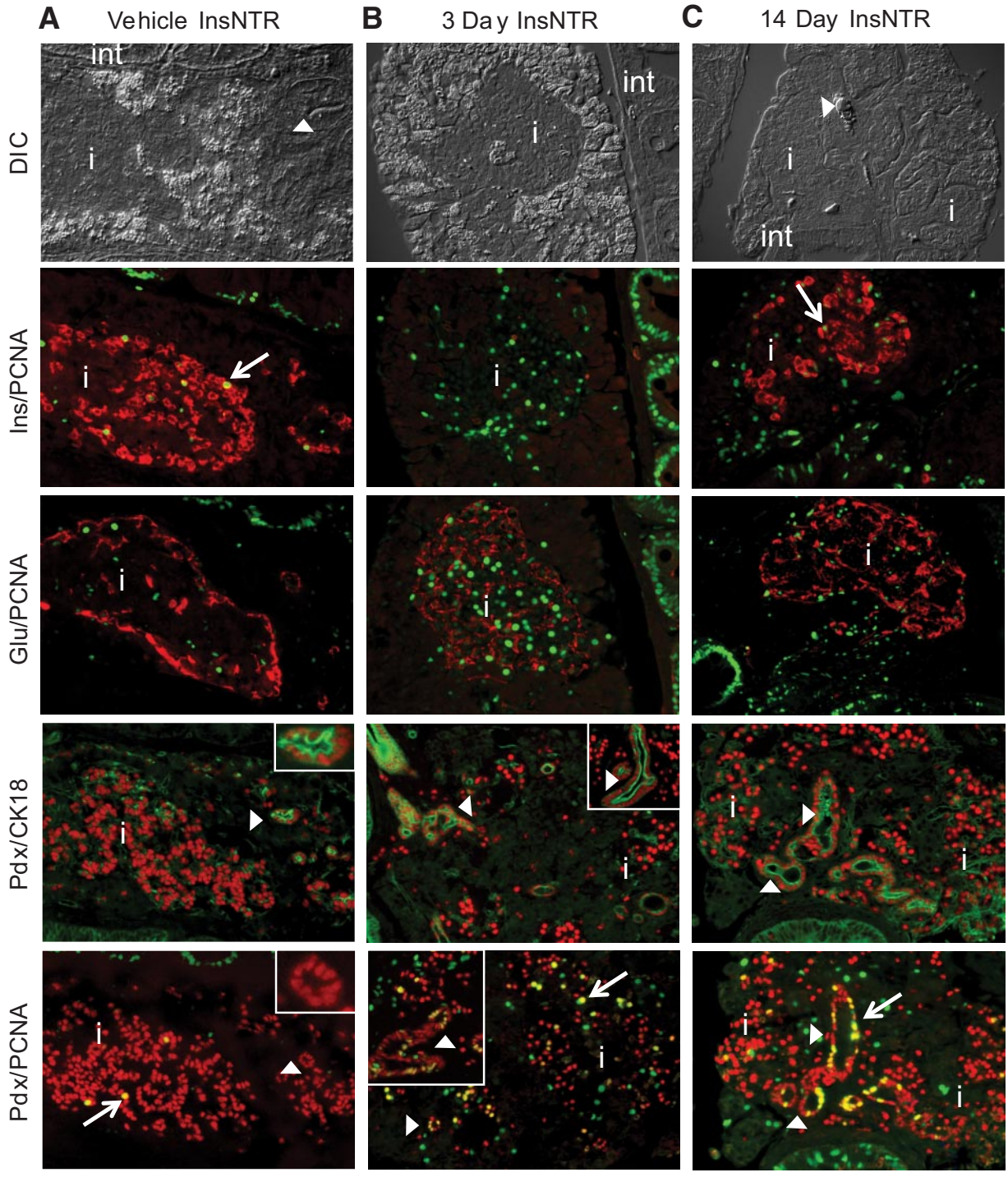

FIG. 4. Regeneration of islets after metronidazole treatment. 200× magnification. i = islets, int = intestine. Each column contains serial sections except one: 14 day InsNTR, Glu/PCNA. *A*: Vehicle InsNTR. DIC: differential interference contrast image indicating location of pancreatic islets and ducts. Ins/PCNA: sparse, dividing PCNA$^+$ cells (green) in endocrine islets (red, insulin). Arrow: dividing β-cell. Glu/PCNA: glucagon$^+$ cells (red) primarily at the perimeter of the islet. Pdx1/CK18: Pdx1$^+$ cells located in CK18$^+$ (green) ductal epithelium (arrowhead and inset). Pdx1/PCNA: nondividing Pdx1$^+$ cells throughout the islet and ducts (arrowhead and inset). Arrow: dividing cell. *B*: Three day InsNTR. Ins/PCNA: β-cells are absent. Dividing cells (green) occupy the islet. Extra-islet cells are also dividing. Glu/PCNA: glucagon$^+$ cells (red) are not dividing. Centrally located α-cells. Pdx1/CK18: Pdx1$^+$ (red) cells depicted (inset) in islets and numerous ducts (arrowheads). Pdx1/PCNA: many Pdx1$^+$ cells (red) are also dividing (arrows and insert). Many dividing Pdx1$^+$ cells (inset) are located in ducts (arrowhead). *C*: Fourteen day InsNTR. Ins/PCNA: regenerated β-cells in islet (red) occasionally divide (arrow). Glu/PCNA: dividing cells (green) located in and around regenerated islet (α-cells are red). Pdx1/CK18: ducts and vascular epithelium (CK18: green) and Pdx1$^+$ cells (red). Pdx1/PCNA: Pdx1 (red) labels islets, ducts (arrowhead), and occasionally intestine (green). Arrows: dividing Pdx1$^+$ cells. (A high-quality digital representation of this figure is available in the online issue.)

Regeneration after pancreatectomy. Anesthetized zebrafish were pancreatectomized (Ptx) or manipulated with forceps without removing GFP-positive tissue (sham-operated control). We removed as much of the GFP-positive tissue as possible without severely damaging the liver, gall bladder, or spleen. After 7 days, the wound had healed and GFP-positive cells were photographed in situ (Fig. 5*A* vs. *B*). Blood glucose readings in Ptx fish killed after 14 days were

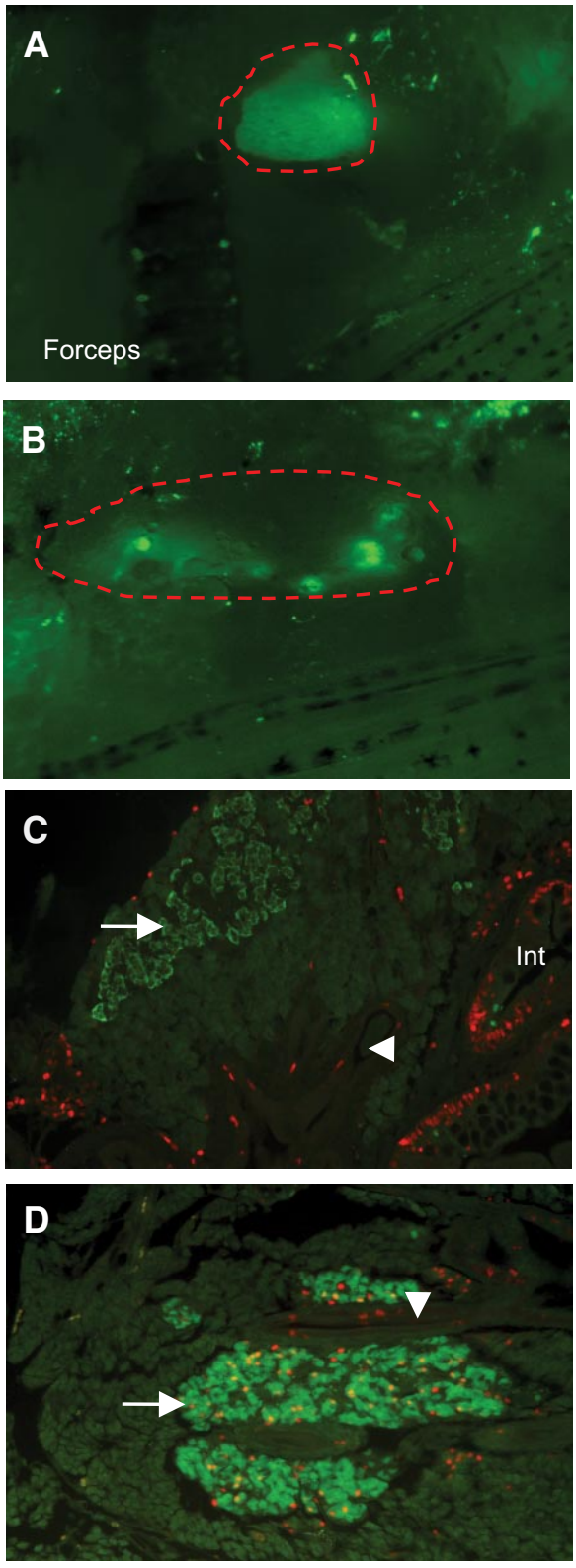

FIG. 5. Regeneration after pancreatectomy. *A* and *B*: Right side of intact, living zebrafish before (sham, *A*) and 14 days after (Ptx, *B*) surgical removal of the GFP$^+$ pancreas (red outline). The tip of the forceps used to remove the pancreas is visible (100× magnification). *C*: Paraffin section of sham-operated pancreas with few PCNA$^+$ dividing cells (PCNA, red) except in the intestine (Int). β-Cells are green (arrow; 200× magnification). *D*: Many red PCNA$^+$ dividing cells in ducts (arrowhead) and in nuclei of regenerating β-cells (yellow; 200× magnification). (A high-quality digital representation of this figure is available on the online issue.)

on average 78.9 mg/dl (n = 6). Sham-operated animals had normal blood sugars (52.4 mg/dl, n = 4). Fibrotic tissue was observed along with a proliferation of small islets. A histological assessment indicated that, like the STZ-treated fish, ductal elements were associated with regenerating tissue. Dividing cells were not prominent in the pancreas of sham-operated animals, whereas numerous PCNA-positive cells were found in pancreatic ducts (Fig. 5*C*). Surprisingly, unlike the chemical-ablation models, PCNA co-stained many insulin-positive cells (Fig. 5*D*). The partial physical ablation caused by pancreatectomy resulted in an increased division of existing β-cells that was not observed after chemical ablation. In contrast, ductal hyperplasia and a prominent association of regenerating islets with ducts were required for both wound repair and the regeneration we observed after STZ or MET treatment.

DISCUSSION

Animal models provide insights as well as controversies regarding β-cell regeneration. In human type 1 diabetes or rodent models of extensive β-cell loss, insulin replacement is required for survival in the face of severe hyperglycemia and other metabolic derangements. Although there is growing evidence that some degree of β-cell regeneration does occur in these settings, it is inadequate to eliminate the need for insulin therapy. In contrast, we observed 8–12 month old zebrafish recovering spontaneously from hyperglycemia after chemical treatment or pancreatectomy without the need for insulin therapy (the average life span for a zebrafish in captivity is 42 months [23]). This physiological improvement correlated with a return of insulin-positive β-cells observed in histological sections. We ascertained whether the population of dividing cells could be characterized using an early pancreatic/β-cell precursor marker such as Pdx1. Our results define some of the dividing cells in both islets and adjacent pancreatic ducts as Pdx1 positive. Although the cells within the islet could have originated locally or migrated from a distance, the presence of dividing Pdx1 cells in the islets as well as within adjacent pancreatic ducts suggests that these cells may be a possible source for β-cell progenitors. Subsequent studies using lineage-tracing strategies will be exploited to more rigorously define the origins of β-cell precursors in the adult zebrafish and delineate the role of pancreatic ducts in the regeneration process.

During epimorphic regeneration, common in urodele amphibians, proliferating undifferentiated mesenchymal cells accumulate at wound sites, promoting regeneration in a process that is distinct from the fibrosis and inflammation associated with mammalian wound repair (24). Although sources of mesenchymal cells are not well understood, proliferation of resident cardiomyocytes occurs after resection of the zebrafish heart to rebuild muscle without scarring (25). These proliferating cells apparently use a genetic program distinct from embryonic heart development because unique genes were upregulated during regeneration (26). Ablative treatment of insulin nitroreductase zebrafish embryos causes existing insulin-positive cells as well as non–insulin expressing cells at the periphery of the islet to divide (8,9). However, during embryogenesis, the recovery of the β-cell population may be a continuation of developmentally programmed organogenesis and different from regeneration in adults (27). In adult zebrafish, it is not clear if islet regeneration recapitulates development.

It appears that the distribution and location of proliferating cells during regeneration depends on the nature and degree of the insult. A number of indications from human and rodent β-cell proliferation studies both support and reject this conclusion. For example, STZ treatment resulted in permanent β-cell loss and hyperglycemia in 6-month-old mice unless hyperglycemia was ameliorated by insulin administration (12). In addition, human autopsy specimens from normal or type 1 diabetic patients provided evidence for limited β-cell replication though no differences were found in pancreatic ducts (28). However, 90% pancreatectomy in rats elicited β-cell proliferation along with apparent differentiation of cells within pancreatic ducts to β-cells (29). Recent work suggests that new β-cells may arise from cells expressing carbonic anhydrase II, presumably pancreatic ductal epithelium (30). In 1-week-old mice, a 70% pancreatectomy caused β-cell expansion only by replication of preexisting β-cells (31), which could be an example of compensatory growth when an adequate number of β-cells remain. In contrast, a robust regeneration of β-cells from undifferentiated progenitors was seen after pancreatic duct ligation in 1-month-old mice (11). Although we identified ductal hyperplasia and elevated numbers of dividing cells in the islets and pancreatic ducts of surgical as well as chemical ablation models, insulin-positive β-cells divided only after pancreatectomy. Surgical removal, which affects many more cell types than β-cells, may have triggered a distinct set of signals that in aggregate leads to β-cell proliferation. Alternatively, surviving β-cells may be induced to undergo compensatory growth in response to unmet systemic demands. Therefore, observations regarding β-cell regeneration are highly dependent on the experimental model employed and islet regeneration processes may be operative in young mammals that are either absent or compromised in older adults. These new adult animal models of diabetes will be useful in defining the roles of dividing cells as well as pancreatic ducts during β-cell regeneration.

We suggest that the capacity of the adult zebrafish to regenerate islet tissue to an extent mammals cannot makes the zebrafish a powerful system with which to address fundamental questions of β-cell regeneration. The ability to specifically test regenerating β-cells in an in vivo compartment where all the environmental influences of cytokines, wound repair, inflammation, etc., are in place provides a platform for discovering the signals required for the reestablishment of β-cell function and glycemic homeostasis. Unique factors may be identified from the zebrafish model that could be applied to diabetes treatment either by inducing endogenous β-cell regeneration or by expanding the β-cell mass of transplanted islets.

ACKNOWLEDGMENTS

This work was generously supported by Juvenile Diabetes Research Foundation grants 1-2005-1177 to L.G.M. and 1-2007-145 to M.J.P. and by Duke University Medical Center Stead Scholarship Grant to M.G.; I.W. was financed by a fellowship of the Max Kade Foundation, New York, awarded by the Austrian Academy of Science.

No potential conflicts of interest relevant to this article were disclosed.

We thank Chris Newgard for thoughtful revisions and Rebecca Schneider for technical help.

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