Undernutrition does not alter the activation of β-cell neogenesis and replication in adult rats after partial pancreatectomy

E. Fernández,1,*, M. A. Martín,1,*, S. Fajardo,1 D. Bailbé,2 M. N. Gangnerau,2 B. Portha,2 F. Escrivá,1 P. Serradas,2 and C. Álvarez1

1Departamento de Bioquímica y Biología Molecular II, Facultad de Farmacia, Universidad Complutense, Madrid, Spain; and 2Laboratory of Physiopathology of Nutrition, CNRS UMR 7059, Université Paris 7/D, Diderot, France

Submitted 19 December 2005; accepted in final form 28 May 2006

Fernández, E., M. A. Martín, S. Fajardo, D. Bailbé, M. N. Gangnerau, B. Portha, F. Escrivá, P. Serradas, and C. Álvarez. Undernutrition does not alter the activation of β-cell neogenesis and replication in adult rats after partial pancreatectomy. Am J Physiol Endocrinol Metab 291: E913–E921, 2006. First published June 6, 2006; doi:10.1152/ajpendo.00638.2005.—In previous work, we demonstrated that a 65% protein calorie food restriction started during the third trimester of gestation in rats caused a reduced β-cell mass at 4 days of life that persisted until adult age. In this study with adult offspring neonates at day 1 and day 4 of postnatal life, we investigated whether undernutrition affects the β-cell growth potential and both β-cell proliferation and differentiation and 2) the implication of the IGFs, highly responsive to nutritional status, in these processes. To this end, we used the 90% pancreatectomy (Px) procedure in U and control (C) adult rats. The results show that, on day 2 after Px, β-cell replication was significantly higher in C rats, whereas the β-cell neogenesis was markedly increased in U/Px rats. Both the serum levels of IGF-I and the liver IGF-I mRNA expression were reduced in adult U rats before and after Px compared with C rats. Pancreatic IGF-I mRNA expression was reduced in U animals on day 0. However, on day 2 after Px, the increase of pancreatic IGF-I mRNA expression was significantly higher in U rats than in C rats. These data suggest that β-cells still have the capacity to regenerate in the adult U rats, with a higher efficiency than C rats on day 2, and that both β-cell neogenesis and β-cell replication are stimulated. The increased pancreatic IGF-I mRNA may be instrumental in these processes.

β-cell regeneration; proliferation; insulin-like growth factors

DIETARY INFLUENCES DURING EARLY STAGES of development present a risk factor for the onset of both perinatal and later life diseases. As indicated by the “thrifty phenotype hypothesis” (23), the endocrine pancreas may be particularly susceptible to the effects of poor maternal nutrition, since fetal and postnatal periods are critical for β-cell development and maturation of pancreatic function. In concert, several studies in experimental models with rats subjected to different patterns of malnutrition have reported that maternal food restriction significantly affects the β-cell mass in the fetuses (2, 4, 49) and in the offspring neonates at day 1 (19) and day 4 of postnatal life (37). This effect persists until adulthood (37) and can provoke long-lasting consequences related to the plasticity of the endocrine pancreas under situations of increased insulin demand, such as aging (19) and pregnancy (5). However, to our knowledge, the effect of prolonged global malnutrition starting in the fetal period on the potential for regeneration of the pancreatic islets in adulthood has not been investigated.

The adult pancreas has the capacity to respond to changing physiological needs such as the requirement for increased β-cell mass/function during pregnancy, obesity, or insulin resistance and an ability to regenerate cells (both replication and neogenesis), including β-cells, which has been convincingly demonstrated in animal models of pancreatic injury and diabetes (46, 47). One of these models is the partial pancreatectomy in rats (7, 11). In this model, the pancreatic regeneration involves, first, replication of preexisting differentiated endocrine and exocrine cells, and second, proliferation of ducts and subsequent differentiation into new acini and islets (8), and these processes of pancreas regeneration are similar to those described for endocrine pancreas development (24). After pancreatectomy, several growth factors, including insulin-like growth factor-I (IGF-I), transforming growth factor-β (TGF-β), and hepatocyte growth factor, showed enhanced expression at different times during the regenerative process (8).

The IGF system is a family of peptide growth factors that includes two ligands (IGF-I and -II), two cell surface receptors, the IGF-binding proteins (IGFBPs), and IGFB proteases (1). IGF-I and -II stimulate cell proliferation and differentiation (31, 35) and are expressed in developing tissues (28) and in response to injury (48). During mid- to late gestation, IGF-II gene expression is widespread in fetal tissues and is more abundant than IGF-I in most species studied (27). In rodents, IGF-II expression disappears from most tissues except the brain by weaning (27), and plasma IGF-I levels increase rapidly after birth, mainly as a result of the onset of growth hormone (GH)-stimulated IGF-I production by the liver (17). Therefore, in adults, IGFs are synthesized primarily by the liver, but they are also locally produced by many tissues, including the pancreas, where they act in an autocrine/paracrine manner. Several lines of evidence suggest that IGF-I may be involved in both endocrine and exocrine pancreatic growth. Specific receptors for IGF-I have been described in rat pancreatic α- and β-cells and murine acinar tissue (52), and IGF-I has been localized to islet β-cells by immunohistochemistry (26). The IGF axis is highly responsive to nutritional status (51), and experimentally induced growth retardation in the rat has been associated with a reduction in circulating IGF levels and altered presence of IGFBPs (38, 50). Furthermore, it has been described that IGF-I is expressed in areas of regeneration after partial pancreatectomy (20), suggesting that IGF-I may play a
main role in the growth and differentiation of normal pancreatic tissues.

We previously showed (37) that a 65% protein-caloric food restriction started during the third week of gestation and continued until adult age led to reduced β-cell mass. The aim of the present study was to determine whether undernutrition affects β-cell growth and proliferation potential in adult undernourished rats and the possible implication of IGF-I and -II in these processes. To that end, we performed a 90% partial pancreatectomy (Px) in 8- to 10-wk-old undernourished (U) and control (C) male rats (8). Spontaneous β-cell regeneration was evaluated by β-cell mass quantitation using immunohistochemistry and morphometry. Involvement of β-cell replication and β-cell neodifferentiation from ductal precursors was evaluated as well. IGF-I and -II serum concentrations were measured as well as IGF RNA expression in liver and pancreas. All determinations were performed before Px (day 0, D0) and day 2 (D2) and 14 days (D14) after Px.

MATERIALS AND METHODS

Animals and diets. Wistar rats bred in our laboratory under a controlled temperature and artificial dark-light cycle (from 0700 to 1900) were used throughout the study. Females were caged with males, and mating was confirmed by the presence of spermatozoa in a vaginal smear. Animals were fed a standard laboratory diet (19 g protein, 56 g carbohydrate, 3.5 g lipid, 4.5 g cellulose/100 g, plus salt and vitamin mixtures). Rats were 65% food restricted during the last week of gestation, and the food restriction was extended during the suckling and adult period until 70 days of life. C rats were given access to food ad libitum. Water was available ad libitum to all groups. Food intake of C and U rats has been reported previously (15).

Male adult rats (8–10 wk old) were anesthetized with pentobarbital sodium (5.0 mg/kg body wt ip), and partial (90%) Px was performed as described by Bonner-Weir et al. (8). Briefly, a midline abdominal incision was performed, and the complete portion of the tail and most of the head of the pancreas were removed by gentle abrasion with cotton applicators, the major blood vessels being left intact so as not to compromise other organs. The remnants (residual pancreases) were anatomically well defined, comprising the tissue within 1–2 mm of the common pancreatic duct and extending from the duct to the first part of the duodenum. The corresponding portion of the pancreas in non-Px rats is referred to as the remnant equivalent. For 2 wk postoperatively, on D2 and D14, body weight, plasma glucose, and plasma insulin concentrations were studied. Blood was collected by snipping the tail of nonfasting rats, and plasma or serum was separated by centrifugation and stored frozen at −20°C until analyzed.

Animals were divided into six groups as follows: non-Px C and U rats (D0; n = 8–10) and C and U rats (n = 8–10) killed on D2 or D14 after Px. Remnant pancreases and a piece of liver were rapidly excised at each time of study, frozen in liquid nitrogen, and then stored at −70°C until RNA preparation. For immunohistochemical study, pancreases from each group were removed, weighed, fixed in aqueous Bouin’s solution overnight, and embedded in Paraplast Plus (Labonord, Templemars, France). All studies were conducted according to the principles and procedures outlined in the National Institutes of Health Guidelines for Care and Use of Experimental Animals.

Determination of plasma insulin and glucose levels. Plasma insulin was determined with a rat insulin RIA (Linco Research, St. Louis, MO), with rat insulin used for the standard curve. Sensitivity of 0.1 ng/ml was achieved with overnight equilibrium using a 100-μl serum sample. The coefficients of variation within and between assays were 10%. Aliquots of 10 μl obtained from 30 μl of Ba(OH)₂-ZnSO₄ deproteinized blood were used to determine glucose by a glucose oxidase method (Boehringer Mannheim, Mannheim, Germany).

Determination of serum IGF-I. IGF-I in serum was measured by enzyme immunoassay (EIA) using a rat IGF-I EIA kit from Diagnostic Systems Laboratories (Webster, TX). The method incorporates a sample pretreatment to avoid interference from IGFBPs. The intra- and interassay coefficients of variation were 6.5 and 9.4%, respectively.

Immunohistochemistry and morphometry. Each pancreatic block was serially sectioned (7 μm) throughout its length to avoid any bias due to regional changes in islet distribution and islet cell composition and was then mounted on slides. Sections at fixed intervals throughout the block (every 72nd section) were immunostained for insulin by use of a technique adapted from peroxidase indirect labeling, as previously described (41). Anti-insulin serum purchased from ICN (reference 65-104; ICN, Orsay, France) was raised in guinea pig against porcine insulin. Labeling was performed using a peroxidase-conjugated rabbit anti-guinea pig IgG (reference P0141; Dako, Trappes, France). The activity of peroxidase complex was revealed with 3,3′-diaminobenzidine tetrahydrochloride (DAB) using a peroxidase substrate kit DAB (Biosys, Compiegne, France). After staining, sections were mounted in Eukitt (Labonord). Quantitative evaluation was performed using computer-assisted image analysis performed by means of an Olympus BX40 microscope connected via a video camera to a PC computer and using the Visiolab 1000 software (Bioscom, Les Ulis, France). The area of insulin-positive cells and that of total pancreatic sections were evaluated in each stained section.

β-Cell relative volume was determined according to stereological methods by calculating the ratio between the area occupied by insulin immunoreactive cells and that occupied by total pancreatic cells. Total β-cell mass per pancreas was derived by multiplying the total pancreatic weight by the β-cell relative volume.

β-Cell replication. Other sections from the pancreases used for morphometric studies were used to measure β-cell replication rate (8–10 animals per group). In each group, animals were injected with bromodeoxyuridine (BrDU, 50 mg/kg body wt ip; Sigma, St. Louis, MO) 1 h before being killed. Sections were double stained for BrDU with a cell proliferation kit (reference RPN 20 Cell Proliferation kit; Amersham, Les Ulis, France) and for insulin. Briefly, sections were incubated with a mixture of nuclease and mouse monoclonal anti-BrdU antibody for 1 h at room temperature, washed with Tris·HCl (pH 7.6), incubated with a peroxidase anti-mouse IgG (IgG2) for 30 min, and stained with DAB using a peroxidase substrate kit DAB. After BrDU labeling, tissue sections were washed in Tris·HCl and stained for insulin. Insulin staining was performed using a guinea pig polyclonal anti-insulin antibody (ICN). This antibody was recognized with a sheep anti-guinea pig alkaline phosphatase-conjugated antibody (reference BI 2505/8323, Biosys). This last antibody was revealed with alkaline phosphatase substrate kit I (reference SK-5100, Biosys). In these double-stained sections, β-cells exhibited red cytosol, and BrDU-positive cells exhibited brown nuclei. To estimate the β-cell replication rate, β-cells and BrDU-positive β-cells were counted using an Olympus BX40 microscope (×40 objective). Results were expressed as the percentage of BrDU-positive β-cells. At least 500 β-cells were counted per remnant or remnant equivalent pancreas.

β-Cell neoformation from ductal precursors. To obtain an estimation of β-cell neogenesis activation, we quantified on every section immunostained for insulin and used for β-cell mass quantitation the number of single β-cells incorporated into the duct epithelium on D0, D2, and D14 after Px. Results were expressed as a percentage of values in non-Px groups (8–10 animals per group).

Preparation of total RNA. Total RNA was isolated from pancreases and livers with TRIzol reagent according to the manufacturer’s instructions (Invitrogen Life Technologies, Carlsbad, CA). RNA concentration was determined by absorbance at 260 nm. Samples were electrophoresed through 1.1% agarose and 2.2 mol/l formaldehyde gels and then stained with ethidium bromide to render the 28S and 18S ribosomal RNA bands.
18S ribosomal RNA visible and thereby confirm the integrity of the RNA and normalize the quantity of RNA in the different lanes. A p7T RNA 18S antisense (Ambion, Austin, TX) was used for lane loading control.

Riboprobes. Rat IGF-I and -II cDNAs were kindly provided by Drs. C. T. Roberts Jr. and D. LeRoith (National Institutes of Health, Bethesda, MD). Rat IGF-I cDNA ligated into a pGEM-3 plasmid (Promega Biotech, Madison, WI) was linearized with HindIII and incubated with T7 RNA polymerase to generate a riboprobe that recognized a fragment of 386 bp. Rat IGF-II cDNA ligated into a pGEM-3 plasmid was linearized with HindIII and incubated with T7 RNA polymerase to generate a riboprobe that recognized a fragment of 700 bp. S18 cDNA was incubated with T7 RNA polymerase to produce a 109 nucleotide runoff transcript, 80 nucleotides of which are complementary to human 18S ribosomal RNA. The above riboprobes were synthesized with [32P]UTP, purchased from ICN (Nuclear Iberica, Madrid). The Riboprobe Gemini II Core System (Promega) was used for the generation of RNA probes.

Solution hybridization/RNase protection assay. Solution hybridization/RNase protection assays were performed as previously described (50). Autoradiography was performed at –70°C against a Hyperfilm MP film between intensifying screens. Bands representing protected probe fragments were quantified using a Molecular Dynamics scanning densitometer and accompanying software. RNase A and T1 were purchased from Boehringer Mannheim.

Statistical analysis. All data are presented as means ± SE. The difference between two mean values was assessed using Student’s t-test. For multiple comparisons, significance was evaluated by ANOVA followed by the protected least significant difference test. A P value of < 0.05 was considered statistically significant.

RESULTS

Biological characteristics of animals. Non-Px adult U rats displayed lower body and residual pancreatic weight, plasma insulin, and serum IGF-I levels compared with C rats (P < 0.05; Table 1). Circulating IGF-II levels were undetectable by radioreceptor assay (RRA). After Px, residual pancreatic weight increased in both groups of animals. In C/Px rats, a moderate hyperglycemia (P < 0.05) developed on D14. However, U rats remained normoglycemic during the entire study. Two days after Px, in C rats basal plasma insulin levels dropped to 50% of non-Px values (P < 0.05), but on D14 these levels returned to initial values; in U rats, plasma insulin was independent of Px. No change in serum IGF-I was observed in C animals after Px. In contrast, on D2, the U group showed increased IGF-I levels compared with D0 values (P < 0.05), although such a tendency was only transient.

Pancreatic β-cell mass and proliferation. Figure 1A shows the total pancreatic β-cell mass values (μg) in the Px remnants and the remnant equivalent pancreases. The β-cell mass was significantly (P < 0.05) higher in the remnant equivalents of non-Px C rats compared with the corresponding U group. Although in both groups β-cell mass reached a value significantly higher on D2 and D14 (P < 0.05) compared with that in the remnant equivalent of non-Px rats, total β-cell mass values were significantly lower in the U population during all the study (P < 0.05). Representative images are shown in Fig. 3, A–F.

Relative increase of β-cell mass (Fig. 1B), expressed as the percentage of the corresponding value on D0, demonstrated that β-cell regeneration became significantly higher in U/Px rats compared with the C group on D2 and D14 (P < 0.05). The regeneration of endocrine cells started immediately after surgery in both populations.

The β-cell replication rate was measured by double immunohistochemical staining for insulin and BrdU (Fig. 1C). The BrdU labeling index (BrdU LI) was significantly higher (P < 0.05) in non-Px C rats than in the corresponding U group. After Px, on D2 and D14, the BrdU LI of β-cells increased in both C/Px and U/Px rats compared with corresponding non-Px animals. However, values of the same parameter in the U/Px population became significantly lower compared with the age-related C/Px groups during all of the study (P < 0.05).

β-Cell neof ormation from ductal precursors. β-Cell neof ormation from ducts, single β-cells incorporated into the ductal epithelium, and β-cell clusters in close contact to ducts are shown in Fig. 2A. Representative images are shown in Fig. 3, G–J. The relative increase of β-cell neof ormation is expressed as the percentage of the corresponding value on D0 (Fig. 2B). Although in both groups β-cell neof ormation from ducts was significantly enhanced on D2 compared with D0 (P < 0.05), in U/Px rats this increase was significantly higher than in C/Px animals (3.8- vs. 1.6-fold). On D14, the values of this parameter almost returned to initial levels, although β-cell neof ormation continued being significantly higher in U/Px rats compared with age-related C/Px group (P < 0.05).

Pancreas IGFs mRNA expression in C and U adult rats. RNase protection assay of pancreas was performed to evaluate IGF mRNA expression in C and U rats. Densitometric measurements of protected probe fragments are expressed as the percentage of the corresponding C/non-Px group. IGF-II mRNA could not be detected in pancreas of either group of rats. Figure 4 shows that, before Px, IGF-I mRNA expression in U rats was significantly lower compared with the corresponding C/non-Px group (P < 0.05). Although Px caused a marked increase in IGF-I mRNA levels in both populations, on D2 this was higher in the U than in the C group (4- vs. 3-fold). On D14 after Px, IGF-I mRNA levels returned to basal values.

Table 1. Effects of 90% Px on body and residual pancreatic weight, plasma glucose, plasma insulin, and serum IGF-I levels in control and undernourished rats

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<th>Control</th>
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<td></td>
<td>D0</td>
<td>D2</td>
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<tr>
<td>Body weight, g</td>
<td>318±7</td>
<td>299±7</td>
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<tr>
<td>Residual pancreatic weight, mg</td>
<td>118±3</td>
<td>173±9†‡</td>
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<tr>
<td>Plasma glucose, mg/dl</td>
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<td>87±3</td>
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<tr>
<td>Plasma insulin, μU/ml</td>
<td>27±6</td>
<td>12±2*</td>
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<tr>
<td>Serum IGF-I levels, ng/ml</td>
<td>1,432±65</td>
<td>1,372±61</td>
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Data are means ± SE; n = 8–10 animals per conditions. D, day; Px, pancreatectomy. *P < 0.05 vs. age-related control group; †P < 0.05 vs. respective non-Px groups on D0; ‡P < 0.05 vs. respective non-Px groups on D2.
in C rats, but in U rats this parameter remained significantly higher compared with U/non-Px rats ($P < 0.05$).

Liver IGF-I and -II mRNA expression in C and U adult rats. Liver IGF-I mRNA expression was independent of Px in both groups of animals, although the value of this parameter was significantly lower ($P < 0.05$) in U rats compared with C rats during the entire study. IGF-II expression was similar in the two groups of animals and was independent of Px (data not shown).

**DISCUSSION**

The results presented in this study show that β-cell regeneration after 90% Px is not inhibited by undernutrition and that pancreatic IGF-I overexpression may be instrumental in this process.

A 90% Px has been well documented to induce chronic hyperglycemia and the loss of glucose-induced insulin release in well-nourished rats. In our hands, a moderate hyperglycemia was observed in C rats following Px, whereas U rats remained normoglycemic, suggesting an appropriate and functional β-cell-regeneration in both C and U rats. In both C/Px and U/Px rats, the increase of β-cell mass reflected hyperplasia, as the individual β-cell size did not change significantly over the period of study (data not shown). β-Cell regeneration was significantly higher on D2 in U/Px rats compared with related C/Px rats. These results showed that, after Px, β-cells still have the capacity to regenerate in adult U rats and again that the precursor/stem cell population is not susceptible to damage by undernutrition. Although the β-cell mass increases after Px in U rats, no changes are detected on D2 and D14 after Px in serum insulin levels. This result agrees with the increased insulin sensitivity of peripheral tissues reported in our model of chronic undernutrition (15).

According to the results of Bonner-Weir and colleagues (7, 11), the regeneration of endocrine cells after a 90% Px takes place by neogenesis from proliferating ductules and by proliferation of preexisting differentiated cells. Similarly, in our conditions, the rate of β-cell replication and neogenesis increased after Px in both C and U adults rats but with a different timing and intensity. In C rats, we found that β-cell mass increased mainly through β-cell replication, although β-cell neogenesis may also have contributed to the increased β-cell

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**Fig. 1.** A: total β-cell mass in control (C) and undernourished (U) rats on days (D)0, D2, and D14 after 90% pancreatectomy (Px). B: relative increase of β-cell mass (% corresponding values on D0) in C and U rats on D0, D2, and D14 after Px. C: bromodeoxyuridine (BrdU) labeling index of β-cells in C and U groups on D0, D2, and D14 after Px. Values are means ± SE for 8–10 observations in each experimental group. ● $P < 0.05$ vs. age-related control group, ▲ $P < 0.05$ vs. respective non-Px groups on D0, *$P < 0.05$ vs. respective groups on D2.

**Fig. 2.** A: β-cell neof ormation from ducts in C and U groups was evaluated through quantitation of single β-cells located in duct epithelium and β-cell clusters close to ducts per unit of total tissue area on D0, D2, and D14 after Px. Values are means ± SE for 8–10 observations in each experimental group. β-data are expressed as percentage (±SE) of corresponding values in non-Px groups. ● $P < 0.05$ vs. age-related control group, ▲ $P < 0.05$ vs. respective non-Px groups on D0, *$P < 0.05$ vs. respective groups on D2.
Fig. 3. Immunostaining for pancreatic insulin (brown) in adult non-Px C and U rats on D0 (A and B), D2 (C and D), and D14 (E and F) was used to measure β-cell mass. β-Cell neoformation from ducts was illustrated by single β-cells incorporated into ductal epithelium and β-cell clusters in close contact with duct on D2 (G and H) and D14 (I and J).
mass. These data are in accord with previous findings (11, 25). Our present observations indicate that, after surgery, regeneration by replication is quantitatively more important on D2 in C/Px than in U/Px adult rats but not on D14, probably as a result of decreased β-cell mass in our model of chronic undernutrition before Px (37).

Neogenesis of β-cells in the adult pancreas has been observed in normal postnatal development (9) and has been documented in experimental models of pancreas damage (reviewed in Ref. 29). However, β-cell neogenesis had not been studied in the undernourished adult rats after Px. In the present study, the observation of single insulin-positive cells or clusters in close contact with ducts in U/Px rats suggests an active process of differentiation from precursor cells on D2 compared with C/Px rats, indicating the predominance of neogenesis vs. replication in U/Px rats at that time. On D14 in both C/Px and U/Px rats, β-cell neogenesis returned to basal values. These results suggest that, in U/Px rats, regeneration of β-cells on D2 occurs mostly through β-cell neogenesis, whereas in C/Px rats it occurs by β-cell replication.

The result in C/Px rats is consistent with the finding that, after partial Px in adult rats, mature duct cells can transiently regain a less differentiated state after replication, and second, this less restricted phenotype can serve as a multipotent progenitor capable of differentiating and generating new islets (10, 22). Transdifferentiation of pancreatic ductal cells into β-cells is a physiological process generally more efficiently activated by increased metabolic demand an tissue injuries (10). A variation of this hypothesis, the transdifferentiation of acinar cells to islets, has been supported by Bouwens et al. (3). Thus, in U/Px rats, β-cell neogenesis may increase immediately after surgery because the duct cells may be less differentiated as a consequence of undernutrition and respond to injury by differentiating into islet cells. These processes of pancreas regeneration are similar to those of endocrine pancreas development (24), and it is possible that the same fetal pattern of factors is involved in the regeneration in adult age (7).

This finding is in contrast with other theories suggesting that β-cell replication is the only source for new β-cells in adult life during physiological regeneration of β-cell mass (6, 13). Dor et al. (13) also investigated the origin of new β-cells after partial Px in mice and found no evidence for β-cell neogenesis. The discrepancies between these findings and ours may be a matter of difference between species and/or may be due to technical issues.

In our conditions, the β-cell regeneration in C/Px rats occurred mostly through the replication of β-cells and secondarily by neogenesis. Waguri et al. (53) found in a diabetic mouse model induced by selective perfusion of alloxan after clamping of the mesenteric artery that the regeneration of islet endocrine cells occurs mostly through the proliferation of preexisting intraislet β-cells in the non-damaged pancreatic region and by neogenesis in the damaged region. Thus it is also possible that undernutrition alters pancreatic islets and favors neogenesis from ductal cells but not replication of β-cells immediately after surgery.

In contrast with our results, other authors have reported that, in malnourished animals previously treated with streptozotocin (STZ) (20) or in the Goto-Kakizaki rat, a model of genetic diabetes, treated with STZ (43), the regeneration by neogenesis is impaired despite normal β-cell proliferation.

Glucose is a well-known stimulus to induce β-cell proliferation in vitro and in vivo (9). Marked β-cell regeneration occurs in the presence of moderate hyperglycemia after partial Px, but the relative influence of this hyperglycemia on new β-cells is uncertain (16). Several authors have reported that chronic hyperglycemia in vivo leads to progressive loss of β-cell differentiation that can be reversed by normalization of circulating blood glucose levels by insulin administration (22, 30, 42). The question remains as to whether insulin directly promotes β-cell regeneration or acts indirectly through normalization of serum glucose levels. In contrast, using alloxan-induced diabetic mice treated with epidermal growth factor (EGF) and gastrin, Roozen et al. (45) concluded that the observed normalization of blood glucose and increased islet
mass resulted from increased neogenesis from ducts. In the present study, the U/Px rats on D2 and D14 after surgery were normoglycemic, and this situation may have contributed to increased neogenesis in the U rats, or normoglycemia might have resulted from increased neogenesis on D2. On the other hand, in partially duct-ligated mice, Li et al. (34) suggest that islet cell neogenesis is not necessarily glucose dependent and could be related to local production of growth factors.

Our second purpose was to investigate whether IGF-I and -II would affect the regenerative response after Px in U adult rats. IGFs are highly responsive to nutritional status (51), and IGF-I, which is expressed in adult pancreas (12), is independent of GH (33, 39). Most studies on nutritional regulation of IGF-I have shown that undernutrition decreased circulating IGF-I and/or hepatic IGF-I mRNA expression and protein abundance in the neonatal and adult periods (17, 40, 44, 54) as well as in the fetal period (14, 38, 50). The decreased serum IGF-I levels observed in U adult rats before Px (Table 1), probably due to decreased liver IGF-I mRNA expression, are in accord with the above-mentioned studies. Interestingly, we found in the present study that pancreatic IGF-I mRNA expression was decreased in U adult rats prior to Px, suggesting that pancreatic IGF-I is also affected by nutrition. In contrast, we observed that liver IGF-II mRNA expression was unaffected by general food restriction in U adult rats before and after Px. Thus our results indicate that, in the adult period as well as in the fetal period (17, 38), IGF-I is more affected by nutritional restriction than IGF-II.

IGF-I can stimulate both cell proliferation and differentiation, (48) but the role of IGF-I on pancreatic growth is not clear, since the results obtained under culture conditions may not exactly reflect the in vivo state. In C adult rats after 90% Px, pancreatic IGF-I mRNA levels increase rapidly. It is localized to focal areas of regeneration and may play an important role in pancreatic regeneration by autocrine or paracrine mechanisms to stimulate DNA synthesis or may act as a differentiation factor in the proliferating ductules (48). In agreement with George et al. (21), overexpression of IGF-I in both C/Px and U/Px animals did not lead to hyperinsulinemia or hypoglycemia.

The peak of pancreatic IGF-I in C/Px rats on D2 coincided predominantly with a large rise in the mitotic index of β-cell following partial Px and with little β-cell neogenesis. These results are in accord with the finding of Smith et al. (48). In contrast, the peak of pancreatic IGF-I in U/Px rats coincided with a great rise of β-cell neogenesis and with little β-cell replication. Thus, at that time, the effect of local pancreatic IGF-I in both C/Px and U/Px adult rats was different. These results indicate that, after Px, the local production of pancreatic IGF-I affects primarily the replication process in C/Px adult rats and neogenesis in U/Px rats. It is possible that pancreatic IGF-I expression in U/Px rats stimulates first β-cell neogenesis, because in U adult rats the β-cell mass is decreased (37) and/or the duct cells may be less differentiated as a consequence of undernutrition and respond to local stimuli to differentiate into islets cells. 

In accord with Smith et al. (48), pancreatic IGF-I mRNA levels returned to basal levels on D14 in C/Px rats but in U/Px rats continued being significantly higher compared with U rats (U/non-Px). This result suggests that enhancement of pancreatic IGF-I mRNA expression is longer in conditions of chronic malnutrition, which favors replication of new β-cells produced after Px in an autocrine/paracrine manner. Thus interaction of IGF-I with IGF-I receptors (IGF-IRs) in islets and activation of the IGF-I signaling pathway would stimulate β-cell proliferation in both C/Px and U/Px adult rats. In line with this, we have shown that maternal undernutrition increased β-cell proliferation in U fetuses due to locally increased IGF-I in the pancreas and IGF-IR protein content in islets (38) and that these effects protect the fetal endocrine pancreas from the impact of maternal undernutrition. This idea is also consistent with findings that refer to the protective effect of IGF-I against the oxidative and apoptotic effects of STZ (21), diabetes (32), and brain injury (36).

In this study, we did not measure IGF-IRs, but it cannot be ruled out that Px may increase islet IGF-IRs in adulthood, suggesting that adult pancreas responds to pancreatic injury in a similar manner to what fetal pancreas does to the impact of maternal malnutrition (38). This observation agrees with the increased splenic IGF-IR expression during protein malnutrition (40).

In summary, by using the 90% Px procedure, we have tested, for the first time, the β-cell growth potential in the adult undernourished rat, a model of chronic undernutrition with reduced β-cell mass. Our results clearly show that 1) β-cells have the capacity to regenerate in U adult rats; 2) the contribution of β-cell neof ormation from foci of regeneration is very much increased in U/Px rats immediately after surgery and plays an important role during the early phase of pancreatic regeneration; and in contrast, 3) β-cell proliferation occurred mainly at later periods. Our finding that IGF-I is locally overexpressed in the pancreas suggests that this factor could promote regeneration of β-cells in U rats following Px by stimulating β-cell neogenesis in the short term and β-cell replication in the longer term. Therefore, understanding differences in the relationship between the IGF system and the process of β-cell regeneration in malnutrition may lead to therapeutic and preventative interventions in diabetic patients with previous episodes of undernutrition.

ACKNOWLEDGMENTS

We thank Drs. Luis Goya and Jean Pierre Chanoine for critical review of this manuscript.

GRANTS

This work was supported by Grants BFI2002-00253 and BFU2005-02849 from Ministerio de Ciencia y Tecnologia, Spain, and by the France/Spain Program for Science (Cooperation Franco-Espagnole Centre National de la Recherche Scientifique/Consejo Superior Investigaciones Cientı´ficas, Project 7963). C. Alvarez thanks the Naturalia et Biologia (NEB) Association for grant travel allowing a 4-weekstay training period in the French laboratory.

Parts of this work were presented at the 40th European Association for the Study of Diabetes (EASD), Munich, Germany (Abstract in Diabetologia 47, Suppl 1: A159, 2004).

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