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Apoptosis Participates in the Remodeling of the Endocrine Pancreas in the Neonatal Rat*

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ABSTRACT

In rodents, shortly after birth a lack of increase in pancreatic weight and in islet mass have been reported during a time of overall body weight increase. To understand this regulation of the neonatal growth of the β cell mass, we studied Sprague Dawley rats at 2, 9, 13, 17, 20, 24, and 31 days of age for β cell replication, β cell mass, and cell size and for the presence of β cell apoptosis. β cell mass was stable from 2–20 days (range: 0.91 ± 0.2 to 1.33 ± 0.23 mg) and increased thereafter. β cell replication progressively decreased. Condensed apoptotic nuclei were identified and counted on paraffin sections using the fluorescent dye propidium iodide. Apoptotic β cell nuclei were found at a basal rate (1.54 ± 0.22%) at 2, 9, and again after 20 days of age. However, at 13 and 17 days, the incidence of apoptosis was significantly increased (3.64 ± 0.45%). The decreased replication and the increased incidence of apoptosis in the β cells strongly suggest a wave of neogenesis of β cells to maintain the constant β cell mass. These data show that the endocrine pancreas undergoes significant modification during neonatal life and that apoptosis is an important mechanism in this remodeling of the β cell mass. Whether a selective deletion of some population of β cells occurs is unclear, but a dysregulation of this remodeling process could have important effects on the pancreatic β cell mass. (*Endocrinology* **138:** 1736–1741, 1997)

THE COMPLEX mechanisms that determine growth and development of any organ are still poorly understood. Remodeling of developing organs in the perinatal period has been suggested by recent reports of concomitant high levels of apoptosis and replication in the nervous system (1, 2), the adrenal gland (3), intrahepatic bile duct (4), and the kidney (5). In each tissue studied, such remodeling occurred before massive growth in the postnatal period, suggesting that this process may generally occur in organ development.

In the pancreas, a surprising discrepancy between the growth in body weight and pancreas weight shortly after birth has been reported in both rats and mice (6, 7). Even though the body weight increased, the pancreatic weight did not increase from 2–20 days. In this same period, β cell mass does not increase (8–11). β cell mass can increase by replication, by hypertrophy of the individual cells, or by new formation (neogenesis) of islets by differentiation and budding from ducts (12). The lack of increase is surprising because β cell replication rate is high (as compared with that of older animals) during this time (11, 13). In a study of perinatal islet development, Kaung found a discordance between the calculated and actual islet cell population growth and suggested that there may be some β and α cell loss during the first 10 days (13). Because no evidence of islet cell death was seen, she hypothesized that the loss was due to transdifferentiation. However, we have reported apoptotic β cells in the neonatal rat (11). This finding is consistent with our mathematical model of the dynamics of the growth of the endocrine pancreas; this model predicts a wave of cell death between 2 and 18 days of age, followed by increased neogenesis shortly before weaning (11).

Our model strongly suggested that the developing endocrine pancreas undergoes substantial remodeling and that apoptosis, replication, and neogenesis play roles in this process. Therefore, in this study we quantified these parameters, that is β cell mass, replication, cell size, and cell death, in the same tissue from developing neonatal rat pancreas to determine if remodeling of the endocrine pancreas occurs and by what mechanisms. These findings provide support for the predictions of the model.

Materials and Methods

Animals

Neonatal Sprague Dawley rats (Taconic Farms, Germantown, NY) of both sexes were kept under conventional conditions in climatized rooms and were nursed by the mother until killing. At 2, 9, 13, 17, 20, 24, and 31 days of age (birth = 0) animals were killed by decapitation or by overdose of anesthesia (sodium amobarbital, amytal sodium, Eli Lilly, Indianapolis, IN, 200 mg/kg BW). In addition, five nonpregnant, female adult Sprague Dawley rats (approximately 3 months of age, mean body weight 230 g) were similarly treated. All the procedures were approved by our institutional animal care committee.

β cell replication

Rats (three to four per age) were injected with 5-bromo-2′deoxyuridine (BrdU, Sigma, St. Louis, 100 mg/kg BW ip) 6 h before killing. BrdU is a thymidine analog that is incorporated in newly synthesized DNA and thus labels replicating cells (14). The G_2 + M phases of the β cell cycle have been estimated to be 6 h (15); therefore, a 6-h BrdU incorporation time was used to maximize the number of labeled cells while still avoiding the detection of labeled daughter cells. After killing, the pan-

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creas was excised, cleared of fat and lymph nodes, weighed, fixed in Bouin's solution, and embedded in paraffin.

Sections (5 μm) were double stained with immunoperoxidase for BrdU and for the endocrine non-B cells of the islets. Immunostaining for BrdU used a Cell Proliferation Kit (Amersham International, Amersham, UK). Sections were incubated 30 min at room temperature with a mouse monoclonal antibody anti-BrdU, washed with PBS (pH 7.4), incubated with peroxidase-linked sheep antimouse Ig and stained with 3,3′-diamino-benzidine tetrahydrochloride (DAB) plus a substrate/intensifier containing hydrogen peroxide and nickel chloride/cobalt chloride. The sections were then washed with 0.01 mM HCl, then rinsed with distilled water, soaked in PBS plus 1% lamb serum (Life Technologies), and stained for the endocrine non-β cells using a cocktail of antibodies: rabbit antibovine glucagon (final dilution 1:3000, gift of Dr. M. Appel), rabbit antisynthetic somatostatin (final dilution 1:300, made in our own laboratory), and rabbit antibovine pancreatic polypeptide (final dilution 1:3000, gift of Dr. R. Chance, Eli Lilly Co., Indianapolis, IN). The sections were incubated with this cocktail of antibodies overnight at 4 C, washed with Tris buffer, pH 7.4, sequentially incubated with goat antirabbit Ig and rabbit peroxidase antiperoxidase (PAP) (Cappel Laboratories, Cochranville, PA), stained with DAB and counterstained with hematoxylin. On stained sections the islets were identified by a mantle of endocrine non-β cells with orange-brown cytosol, a core of β cells with unstained cytosol, and BrdU positive cells with blue-black nuclei.

BrdU positive and negative β cells were counted using an Olympus BH-2 microscope connected by a video camera to a black and white monitor at a final magnification of 680×. All the β cells in each section were counted (range 507-1288 β cells/animal, 844 mean), and the results expressed as percentage of BrdU positive β cells, giving an estimate of the number of β cells in replication.

β cell mass

β cell mass was measured by point counting morphometry on the same stained section as above. Each section was covered systematically at a magnification of 340 × using a 48-point grid to obtain the number of intercepts over β cell, endocrine non-β cell, exocrine pancreatic tissue, and nonpancreatic tissue. The β cell relative volume was calculated by dividing the intercepts over β cells by intercepts over total pancreatic tissue; the β cell mass was then estimated by multiplying the β cell relative volume by the corrected pancreatic weight. The non-β cell mass was similarly calculated. Pancreatic weight was corrected by subtracting from the pancreas weight a correction factor obtained by multiplying pancreatic weight by the ratio of intercepts over non pancreatic tissue to intercepts over total tissue. A nomogram relating number of points counted to volume density and expected relative standard error in percentage of mean (<10%) had been used to determine the number of intercepts needed for a representative sampling (16).

β cell size

β cell size (cross-sectional area) was measured on electron micrographs. To this end, the pancreas from three rats at each age were removed, minced in 2.5% glutaraldehyde, 0.1 M phosphate buffer pH 7.4, and routinely processed with osmication and subsequently embedded in Araldite. Ultrathin sections of islets were stained with lead citrate and uranyl acetate and photographed on a Phillips 301 electron microscope. For each time point, at least three random islets were analyzed.

To minimize the variations in cross-sectional area due to different planes of section of the cells, only β cells that had a visible capillary face and a nuclear cross-section were measured for cross-sectional area using an electronic planimetry program (Sigma Scan, Jandel Scientific). For each age cross-sectional areas of 60–100 cells were measured. The magnification of each set of micrographs was calibrated using a photograph of a calibration grid. In addition, apoptotic cells were photographed from these samples.

β cell death

For quantification of apoptotic β cell death, sections were both immunostained for non-β endocrine cells and stained with propidium iodide. Propidium iodide is a fluorescent dye that binds to nucleic acids and therefore allows the detection of condensed or fragmented nuclei characteristic of apoptotic cells. Sections (3 μm) from the same paraffin blocks used for β cell replication and mass were deparaffinized with xylene and rehydrated, stained for endocrine non-β cells as described above, soaked with PBS pH 7.4 and incubated for 10 min with propidium iodide 10 μg/ml and RNAse A 100 μg/ml at 37 C. Sections were then washed extensively with PBS, rinsed with distilled water and mounted with an aqueous media (AFT, Behring Diagnostics, Somerville, NJ).

On a fluorescent microscope, islet tissue was identified on bright field and then using a rhodamine filter set, normal, and apoptotic nuclei were counted. Sections were covered systematically, and all the β cells in each section were counted (range 203-1315, mean 737 for neonates; at least 1100 for each of 5 adult animals). The results expressed as percentage of fragmented and condensed nuclei (apoptotic cells) estimate the occurrence of cell death.

Statistical analysis

All data are expressed as mean ± [sem]se. Comparison between groups was performed by one-way ANOVA with multiple comparisons by Fisher's PLSD. Differences between groups were evaluated using the unpaired two tailed Student's t test. A P value ≤ 0.05 was considered significant. For analysis of the distribution of cell population, a multivariate analysis was performed and the Chi Square with a P value ≤ 0.05 was considered significant.

Results

Body and pancreatic weights

The body weight of the neonatal rats increased in a linear manner, being significantly increased at each sampled time point compared with the preceding time point (Fig. 1A). In contrast to the literature, the pancreatic weight (Fig. 1B) also significantly increased between each time point, with the exception at day 31 that did not differ from day 24. The rate of increase was highest in the preweaning period (17–24 days), with a doubling of pancreatic weight between 17 and 20 days. Between 2 and 17 days, linear regressions of the values of body weight ($y = 7.448x + 4.407$) and pancreatic weight ($y = 2.263x + 2.557$) show marked differences in slopes.

Endocrine cell mass

β cell mass increased over the neonatal period from 0.91 ± 0.20 mg at 2 days and 3.28 ± 0.97 mg at 31 days (Fig. 2). However, β cell growth was not linear. In fact, β cell mass remained stable from 2–20 days of age; only at 24 and 31 days had the β cell mass significantly increased. The non-β cell mass also remained stable from 2–20 days (2 day: 0.28 ± 0.08 mg; 20 day: 0.58 ± 0.12 mg) with significant increase only at 24 and 31 days (24 day: 0.82 ± 0.06 mg; 31 day: 0.83 ± 0.07, $P < 0.005$ as compared with 2 day).

β cell replication

β cell replication progressively decreased with time, starting at 2 days of age at 4.7 ± 0.3%/6 h and reaching 1.8 ± 0.2%/6 h at 31 days (Fig. 3). During the period of flat growth of β cell mass (between 2 and 20 days), the β cell replication rate are substantial compared with our previously reported 0.22 ± 0.06%/6 h in adult female (17) and 0.38 ± 0.05/6 h in adult male rats (18). Moreover, neogenesis of islets is occurring: BrdU positive cells were found in the small ducts and budding of islets from these ducts was seen at 13 days and later (data not shown). The frequency of non-β cell replication was too low for quantification.

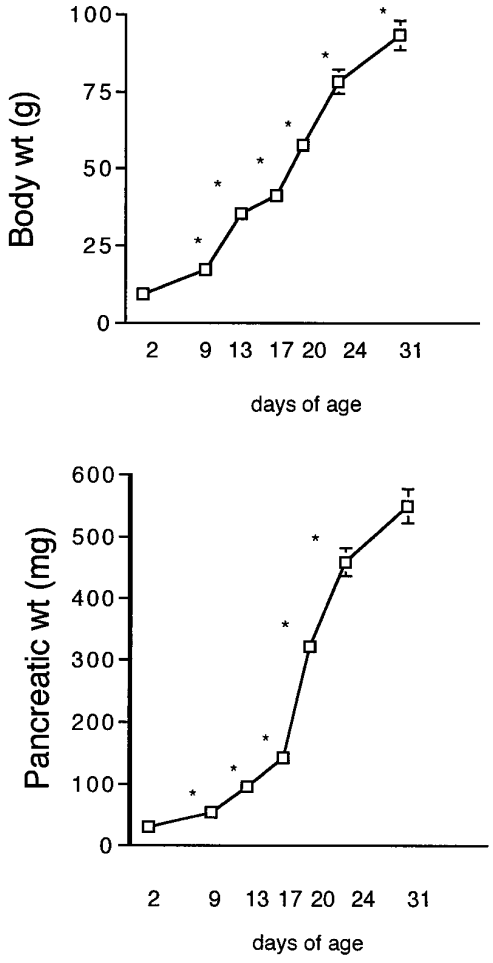

FIG. 1. Body and pancreatic weight. During the neonatal period, the body weight increases linearly, being significantly different at each time sampled (*, $P < 0.05$ as compared with preceding sample). The pancreatic weight also increases during this time period but is not linear (*, $P < 0.02$ at each time point compared with preceding time)

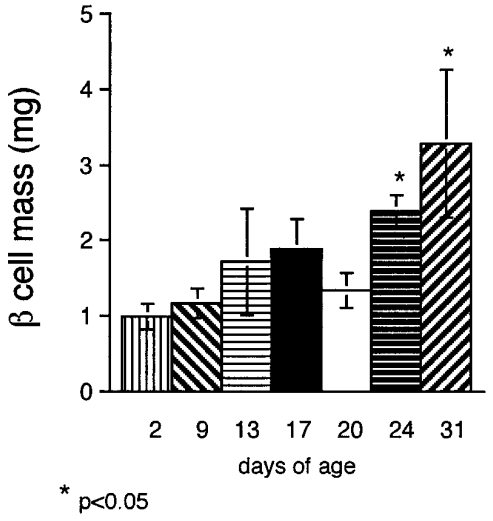

FIG. 2. β cell mass in the neonatal rat. Over the neonatal period, the β cell mass does not increase between 2 and 20 days but then increases thereafter. Immunostained sections from three to four rats per age were quantified by point counting morphometrics. Data given as mean ± SEM, n= number of animals. *, $P < 0.05$ as compared with 2-day value.

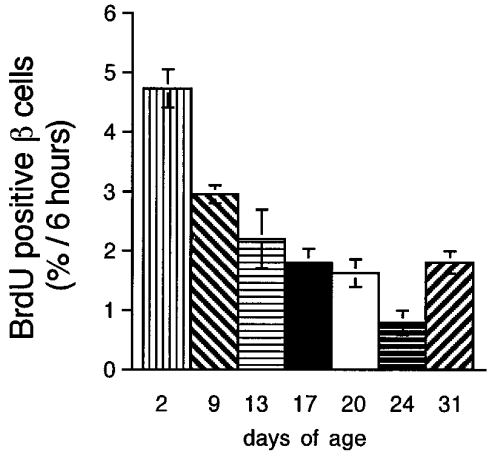

FIG. 3. β cell replication in the neonatal rat. The replication of preexisting β cells declines after birth but during the neonatal period is still increased over adult values (our previously reported of 0.22 ± 0.06%/6 h in adult female (17) and 0.38 ± 0.05/6 h in adult male rats (18). Quantification of BrdU positive nuclei on immunostained sections of the same animals as in Fig. 1. Data given as mean ± SEM.

β cell size

The mean β cell size (cross-section area) was stable through this neonatal period, except for a significant decrease at 17 days (59.2 ± 2.3 $\mu m^{2)}$ as compared with 2 and 9 days (69.9 ± 2.3 and 70.5 ± 2, respectively) (Fig. 4). A multivariate analysis of the distribution of cells by size (Fig. 5) shows a loss of the larger cells (area > 80.2 μm^2) at 17 days as compared with all the other ages (chi square test $P < 0.02$). Such a change in distribution was only evident at day 17.

These size data can be used with the formula for a sphere to calculate the mean β cell volume, and then an estimate of the number of β cells can be made from the β cell mass. With such calculations, the number of β cells between 2 days and 20 days of age does not significantly change ($2.1 \pm 0.5 \times 10^6$ cells at 2 days to $3.2 \pm 0.6 \times 10^6$ cells at 20 days), but almost triples by 24 days ($5.9 \pm 0.5 \times 10^6$ cells).

β cell death

With propidium iodide and islet cell immunostaining, apoptotic β cells could be identified (Fig. 6); their condensed or fragmented nuclei are readily discriminated from mitotic β cells. At all the neonatal ages examined the percentage of apoptotic β cells (Fig. 7) was high (1.51 ± 0.21% at day 2) as compared with levels found in 3-month-old adult rats (0.37 ± 0.06%). At both 13 (3.5 ± 0.42%) and 17 days of age (3.08 ± 0.12%) the percentage of apoptotic β cells was significantly increased compared with that of 2-day-old rats. Throughout the neonatal period both exocrine and duct cells with fragmented or condensed nuclei were also observed but these were not quantified.

Estimation of contributions of apoptosis and replication to stable β cell mass

Using our mathematical model (11) with the present data for β cell mass, volume and replication rates, estimates of the

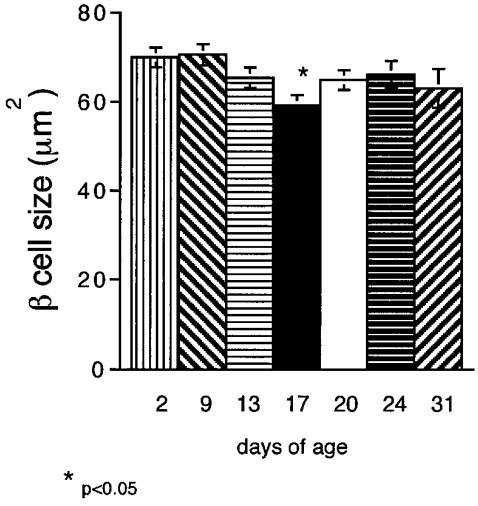

FIG. 4. β cell size in the neonatal rat. The mean cross-sectional area (size) of the β cells did not change in the neonatal period except at 17 days. Planimetry of electron micrographs of 60–100 β cells from at least 3 islets from a total of three rats per age were measured. In comparison, adult β cells had a mean cross-sectional area of 95.0 ± 3.4 μm^2 (Scaglia, unpublished data). Data given as mean ± SEM; n= number of cells. *, $P < 0.05$ as compared with 2-day value.

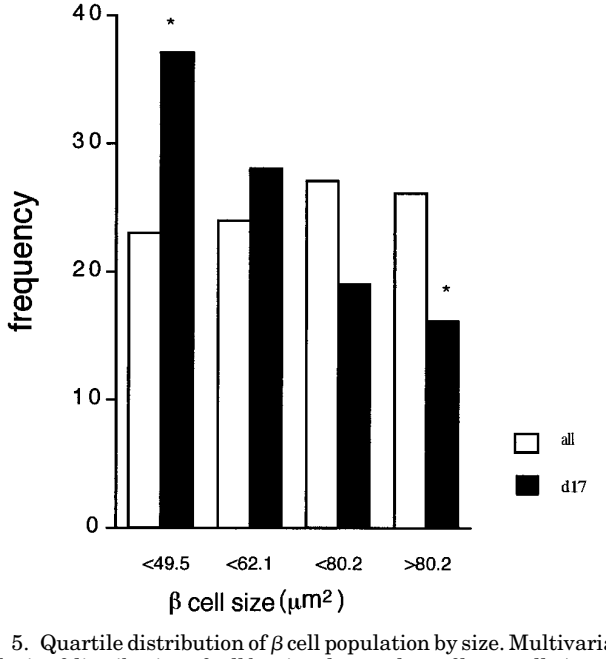

FIG. 5. Quartile distribution of β cell population by size. Multivariate analysis of distribution of cell by size shows a loss of large cells (area > 80.2 μm^2) (and concomitant increase in smallest cells) at 17 days as compared with all the other ages. *, Chi square $P < 0.02$.

number of cells added by replication, and lost by apoptosis can be made (Fig. 8). The calculated net cell death is the difference between the rate of change of the number of β cells and the rate of replication. Because neogenesis cannot be measured in cell number, net cell death actually equals total cell death minus the new cells formed through neogenesis or other processes that are not accounted for by BrdU incorporation.

Discussion

The growth and development of the pancreas has been studied extensively (9, 10, 13, 19). Nonetheless, a previously reported discrepancy between the growth in body weight, pancreas weight, and β cell mass had no clear explanation. Here we have shown that from 2–17 days after birth the pancreatic growth is slower than that of the whole animal. In addition, the β cell mass and the non-β cell mass did not increase until 20 days. The flatness of the curve could not be due to an absence of replication because it was found to be substantial at all the ages examined, being 8–10 fold that of adult rats. In parallel to this enhanced replication was increased frequency of apoptotic β cells. Throughout the neonatal period, this frequency was 3-fold higher than in the normal adult rat, and at 13 and 17 days of age, was 10-fold increased. The occurrence of both β cell apoptosis and high cell birth in the neonatal endocrine pancreas suggests a remodeling as has been found in other developing organs, such as the kidney (5), the adrenal gland (3), and the central nervous system (1, 2).

Kuang also predicted an increased occurrence of cell loss over this time, but in the absence of morphological evidence of cell death, she suggested that transdifferentiation occurred (13). In this latter process, β and α cells would differentiate into other islet cells and thus leave their respective mass compartments. Such an occurrence would be consistent with the concept of islet endocrine cells passing through a stage of expressing more than one islet hormone before final differentiation into a specific islet cell type (20–22). While we have no data addressing a loss of coexpression of islet hormones, Bouwens *et al.* (8) could not find neonatal rat islet cells differentiating from non-β to β cells. Our findings of the presence of apoptotic β cells by both light (immunostaining) and electron microscopy (specific granule morphology) support the actual cell death of β cells.

The mass of β cells is dependent on the number of cells and their mean cell size. A significant decrease in β cell size was found at 17 days of age, but this decrease in cell size at only one time point hardly explains the flat curve of β cell mass. The multivariate analysis of the distribution of β cell population by size showed a significant difference when β cells from 17 day old pancreas were compared with the other groups. There was a specific loss of the larger cells (>80.2 μm^2) and an increase in the smaller ones (<49.5 μm^2). The reason of this change remains unclear. One of the features of apoptosis is condensation of the cell and thus a reduction in its size. However, only those β cells with normal nuclei were measured on electron micrographs, and therefore the reduction of cell size cannot be due to the larger number of apoptotic β cells. Another possibility for this shift in the distribution curve is that replicating cells and/or newly differentiated cells are smaller. β cell replication was, however, similarly high at all the ages examined with a trend to decrease with time, so it seems impossible that replicating cells could contribute to changes in cell size population at only one time point. It is not known whether β cells newly differentiated from duct epithelium are smaller since no markers have been identified yet that could follow these cells. Unfortunately. the potential markers cytokeratin 19

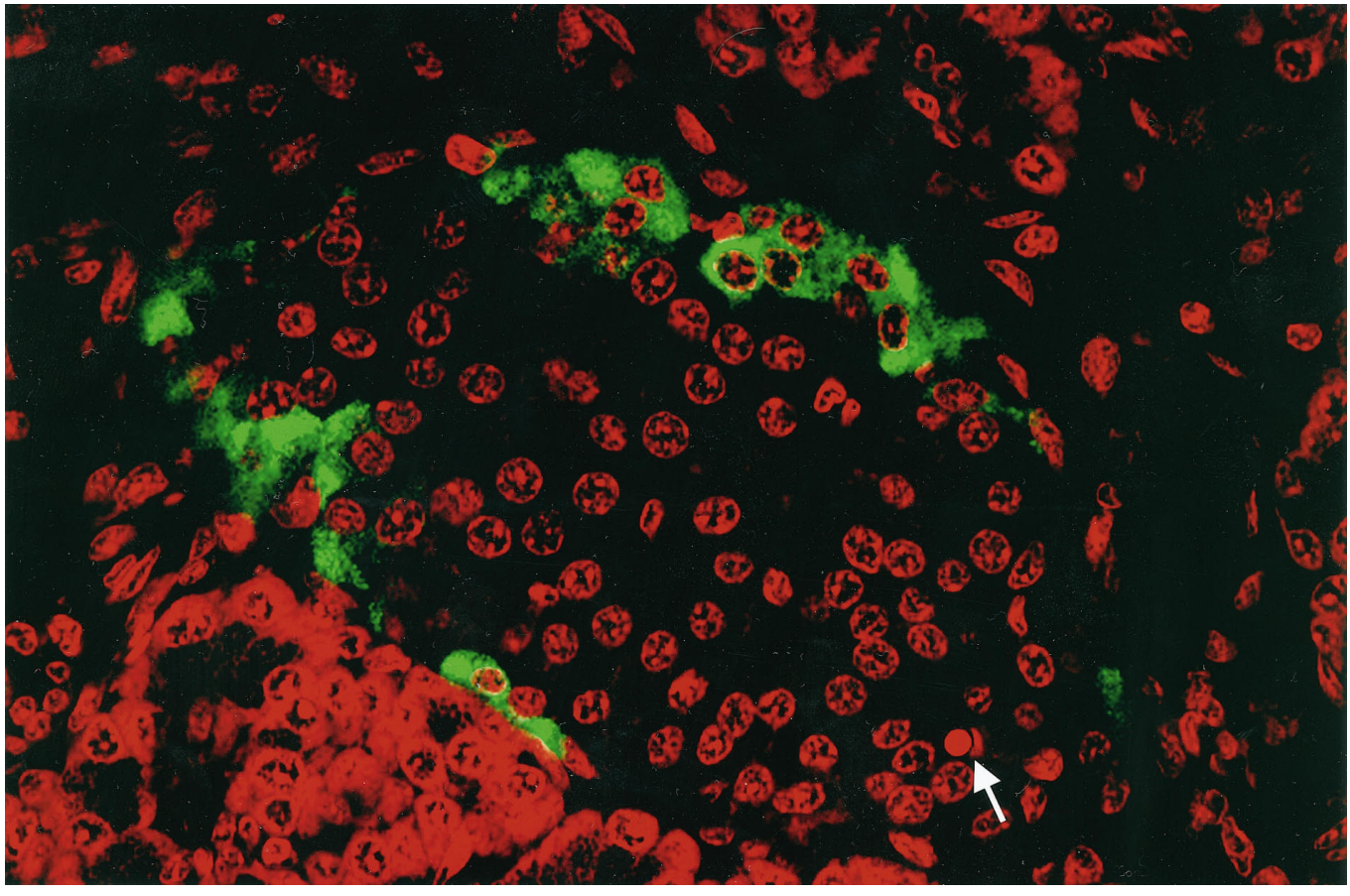

FIG. 6. Immunofluorescent micrograph of apoptotic β cell. Sections from animals used for morphometric analysis were immunostained with cocktail of antibodies against the non-β cell islet hormones (fluorescein isothiocyanate, *green*) to show the boundaries of the islet and then reacted with propidium iodide (*red*). The propidium iodide binds to the nuclei in all cells because this is fixed tissue; however, the condensed nuclei of an apoptotic β cells is clearly evident (*arrow*) in contrast to the nuclei with just rim of heterochromatin. Image was photographed on a Zeiss LSM confocal microscope using filters appropriate for fluorescein isothiocyanate and rhodamine.

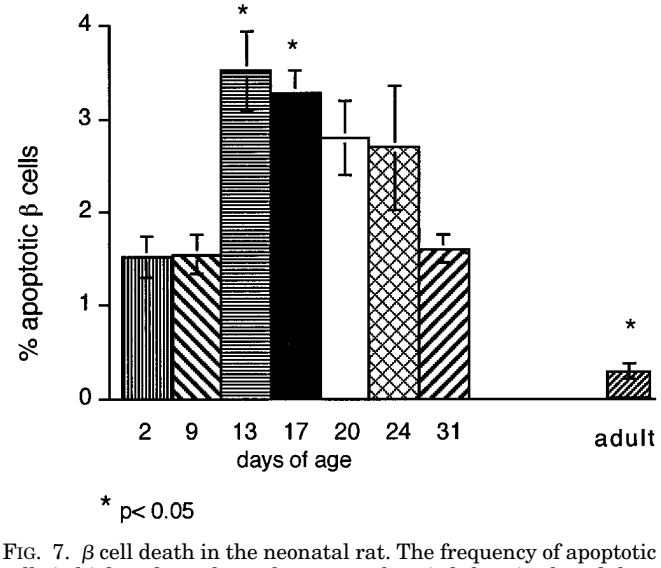

FIG. 7. β cell death in the neonatal rat. The frequency of apoptotic β cells is higher throughout the neonatal period than in the adult rat, but it is further increased at 13 and 17 days after birth. Apoptotic β cells were quantitated on sections from the same animals as used for mass and replication but immunostained as illustrated in Fig. 5. *, $P < 0.05$ as compared with 2 day.

and 20 are quickly lost in newly formed β cells (8). Further investigations are needed to elucidate the relevance and relationship of this change in β cell size to the development and metabolic function of the endocrine pancreas.

The contribution of replication and apoptosis to the overall stability of the β cell mass can be estimated with our mathematical model (11). During the early phase (2–9 days), the number of cells dying is equal to about half of the number formed by replication. So only a slight increase in cell number will occur without the mass being significantly increased. During the middle phase (13–17 days), equal numbers of cells are dying as result from replication, so the number/mass does not increase at all. One should remember that we did observe neogenesis from 13 days onwards. The growth of mass begins to be significant by 24 days with a tripling of the number. Initially, this growth is due to increased neogenesis (so net cell death is now negative), but by 31 days is due to an increase in replication, very low cell death and possibly continued neogenesis.

The lack of increase in β cell number in face of both replication and differentiation potentially allows a selective deletion of some β cells. Even though there is considerable evidence of functional heterogeneity of adult β cells (23), nothing is known about the relation of this heterogeneity and the age of the β cells.

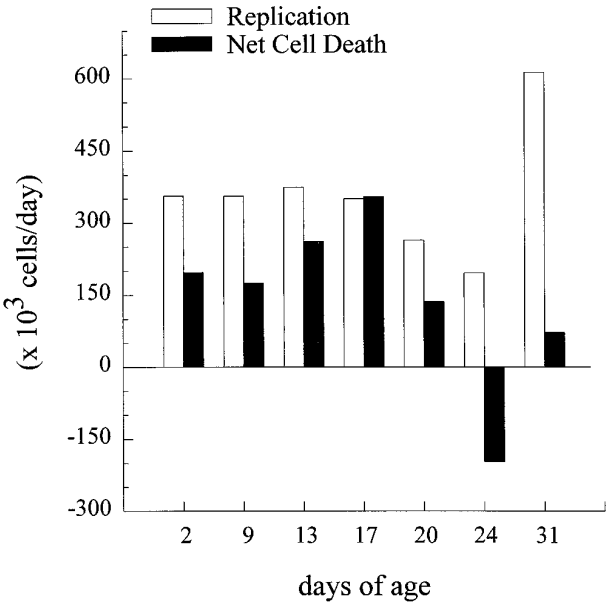

FIG. 8. Estimation of the contributions of apoptosis and replication to the stable β cell mass. Using our mathematical model (11) with the present data for β cell mass, volume, and replication rates, estimates of the number of cells added by replication and lost by apoptosis can be made. The calculated net cell death is the difference between the rate of replication and the rate of change of the number of β cells. Net cell death equals total cell death minus the rate of new cells formed through neogenesis or other processes that are not accounted for by the technique of BrdU incorporation. Replication as cells per day is estimated from the replication rate in percentage/day $\times$ the mass of β-cells divided by the average cell volume.

We do know that the maturation of the insulin response to glucose is seen in the postnatal period, but this occurs earlier, being between days 5 and 8 after birth (24). Recent data on apoptotic regulatory proteins have shown that bcl-2 is found in fetal islets but is no longer detected after birth (25), even though bcl-xL, bax, and bag-1 are expressed in adult islets (26). Perhaps there is a heterogeneity of expression of these proteins within β cells that allows a differential response to apoptotic stimuli. At present the basis of the selective deletion in the endocrine pancreas is unknown.

In addition, the process of cell death can be influenced by the cell's environment; for example, changes in hormones or growth factors can stimulate apoptosis. The large scale cell death in neonatal kidney was reduced by injections of epidermal growth factor (5), suggesting that limited amounts of survival factors trigger the loss of some cells. In the neonatal rat pancreas, there are marked changes in the message RNA levels of both IGF-I and IGF-II as well as transient appearances of IGFBP 1 and 2 between 14 and 21 days (27). Further studies are needed to examine if the IGFs serve as survival factors for the β cells.

There may be important implications of cell deletion within the endocrine pancreas. One can speculate that a defective clearing of the apoptotic bodies in face of the substantial deletion of β cells could lead to a presentation of β cell antigens that could result in autoimmunity.

The present data show that the endocrine pancreas during the neonatal period is subject to substantial remodeling of the β cell population and that apoptosis plays an important role in the final β cell mass. A dysregulation of this process could be of great importance during this period of time when a fine balance between cell replication and cell death determines the development of the pancreatic β cell mass.

Acknowledgments

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References

1. **Oppenheim RW** 1991 Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501
2. **Blaschke AJ, Staley K, Chun J** 1996 Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. Development 122:1165–1174
3. **Spencer SJ, Mesiano S, Jaffe RB,** Programmed cell death in the human fetal adrenal cortex: actions of activin follistatin and TFG-β. Program of the 77th Annual Meeting of The Endocrine Society, Washington DC, 1995, p 611 (Abstract)
4. **Terada T, Nakanuma Y** 1995 Detection of apoptosis and expression of apoptosis-related proteins during human intrahepatic bile duct development. Am J Pathol 146:67–74
5. **Coles HSR, Burne JF, Raff MC** 1993 Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. Development 118:777–784
6. **Sesso PA, Cruz AR, Leite JBA** 1965 La croissance du pancreas chez le rat pendant la vie post-natale. Acta Anat (Basel) 62:283–295
7. **Dore BA, Grogan WM, Madge GE, Webb SR** 1981 Biphasic development of the postnatal mouse pancreas. Biol Neonate 40:209–217
8. **Bouwens L, Wang R-N, De Blay E, Pipeleers DG, Kloppel G** 1994 Cytokeratins as markers of ductal cell differentiation and islet neogenesis in the neonatal rat pancreas. Diabetes 43:1279–1283
9. **McEvoy RC** 1981 Changes in the volumes of the A-, B- and D-cell populations in the pancreatic islets during the postnatal development of the rat. Diabetes 30:813–817
10. **McEvoy RC, Madson KL** 1980 Pancreatic insulin-, glucagon- and somatostatin-positive islet cell populations during the perinatal development of the rat. Biol Neonate 38:248–259
11. **Finegood DT, Scaglia L, Bonner-Weir S** 1995 (Perspective) Dynamics of B-cell mass in the growing rat pancreas: estimation with a simple mathematical model. Diabetes 44:249–256
12. **Bonner-Weir S** 1994 Regulation of pancreatic β-cell mass *in vivo*. Recent Prog Horm Res 49:91–104
13. **Kaung H-LC** 1994 Growth dynamics of pancreatic islet cell populations during fetal and neonatal development of the rat. Dev Dyn 200:163–175
14. **DeFazio A, Leary JA, Heley DW, Tatersall MHN** 1987 Immunohistochemical detection of proliferating cells *in vivo*. J Histochem Cytochem 5:571–577
15. **Swenne I** 1982 The role of glucose in the *in vitro* regulation of cell cycle kinetics and proliferation of fetal pancreatic B-cells. Diabetes 31:754–760
16. **Weibel ER** 1979 Stereological Methods. Academic Press, London, pp 114
17. **Scaglia L, Smith FE, Bonner-Weir S** 1995 Apoptosis contributes to the involution of β cell mass in the post partum rat pancreas. Endocrinology 136:5461–5468
18. **Montana E, Bonner-Weir S, Weir GC** 1994 Transplanted beta cell response to increased metabolic demand. J Clin Invest 93:1577–1582
19. **Pictet R, Rutter WJ** 1972 The endocrine pancreas. In: Steiner D, Freinkel N (eds) Handbook of Physiology. Williams & Wilkins Co, Baltimore, pp 25–66
20. **Upchurch BH, Aponte GW, Leiter AB** 1994 Expression of peptide YY in all four islet cell types in the developing mouse pancreas suggests a common peptide YY-producing progenitor. Development 120:245–252
21. **Herrera P-L, Huarte J, Sanvito F, Meda P, Orci L, Vassalli J-D** 1991 Embryogenesis of the murine endocrine pancreas; early expression of pancreatic polypeptide gene. Development 113:1257–1265
22. **Teitelman G, Alpert S, Polak JM, Martinez A, Hanahan D** 1993 Precursor cells of mouse endocrine pancreas coexpress insulin, glucagon and the neuronal proteins tyrosine hydroxylase and neuropeptide Y, but not pancreatic polypeptide. Development 118:1031–1039
23. **Pipeleers DG** 1992 Heterogeneity in pancreatic β-cell population. Diabetes 41:777–781
24. **Freinkel N, Lewis NJ, Johnson R, Swenne I, Bone A, Hellerstrom C** 1984 Differential effects of age *vs.* glycemic stimulation on the maturation of insulin stimulus-secretion coupling during culture of fetal rat islets. Diabetes 33:1028–1038
25. **Bouwens L, De Blay E** 1996 Islet morphogenesis and stem cell markers in rat pancreas. J Histochem Cytochem 44:947–951
26. **Naik P, Karrim J, Hanahan D** 1996 The rise and fall of apoptosis during multistage tumorigenesis: down-modulation contributes to tumor progression from angiogenic progenitors. Genes Dev 10:2105–2116
27. **Hogg J, Hill DJ, Han VKM** 1994 The ontogeny of insulin-like growth factor (IGF) and IGF-binding protein gene expression in the rat pancreas. J Mol Endocrinol 13:49–58