Regional Differences in the Cellular Proliferation Activity of the Regenerating Rat Pancreas after Partial Pancreatectomy*

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Summary. The proliferation activity of component cells and its regional differences in the regenerating rat pancreas after 90% pancreatectomy were examined by bromodeoxyuridine (BrdU) immunohistochemistry. Cells of the ductal system and the centroacinar cells showed a rapid increase in labeling indices at day 2 after pancreatectomy, followed by a second peak of a mild increase at days 5 to 7. No regional difference in the labeling index was recognized in the ductal elements. In contrast, the labeling index of acinar cells started to increase at day 3, reaching a definite peak at day 5. Furthermore, acinar cells in the region close to the duodenum had labeling indices more than 2 times higher than those in the portions further away from the duodenum. Acinar cells increased in number as early as from day 3 after surgery. These result suggested that the parental cells of regeneration were located in the ductal epithelium. It is highly probable that the proliferation of acinar cells is controlled by some unknown trophic factor(s) which is released locally from the duodenum, but does not involve a neural or a circulatory route. The phenomenon may be closely linked to the known fact that the incidence of pancreatic cancer is highest in the head region.

The process of organ regeneration provides a useful model to investigate cellular proliferation and differentiation. Regeneration of the pancreas has been studied in experimental animals after various treatments including drug-induced pancreatitis (Fitzgerald and Alvizouri, 1952; Herman and Fitzgerald, 1962a, b; Fitzgerald et al., 1966; Lampel and Kern, 1977; Adler et al., 1979; Elsasser et al., 1986, 1992), excretory duct ligation (Boquist and Edström, 1970; Hultquist et al., 1979; Pound and Walker, 1981; Walker, 1981; Walker et al., 1992; Abe and Watanabe, 1995; Watanabe et al., 1995), and partial pancreatectomy (Fitzgerald et al., 1968; Lehv and Fitzgerald, 1968; Pearson et al., 1977; Brockenbrough et al., 1988; Ohashi et al., 1991; Bonner-Weir et al., 1993; Hayakawa et al., 1996). These studies clearly indicate that the pancreatic tissue can regenerate to a limited extent after partial loss of the pancreatic cell mass, although the degree of regeneration is lower than that in the liver (Lehv and Fitzgerald, 1968).

Much controversy can be found in the literature regarding the original cells and the rate of proliferation in the regenerating pancreatic tissue (Elsasser et al., 1993). These different results appear to be caused by differences not only among experimental animals and the methods of pancreatectomy but also the regions of the remnant tissue studied.

This situation led us to reexamine the proliferation activity of the component pancreatic cells during regeneration after partial pancreatectomy, paying special attention to regional differences in the remnant tissue. For this purpose, we examined remnant pancreatic tissue removed en bloc with surrounding tissues including the duodenum and the hepatic portal tissues, and positioned the tissue block precisely on a microtome so as to obtain sections covering the whole area of the remnant pancreas from the intestinal portion to the resected margin, including the common bile duct. Results showed that the highest proliferative activity in acinar cells was in the portion closest to the duodenum. This will be discussed.

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from the viewpoint of the higher incidence of pancreatic head cancer over other regions.

MATERIALS AND METHODS

Experimental animals and pancraectomy
Male Wistar rats (6-week-old, weight ranged from 130 to 145 g) were used. They were housed in an air conditioned room at 23°C on a diurnal light and dark schedule, and were allowed free access to water and standard food before and after the operation. A group of rats received partial pancreatectomy by SCOW’s method (1957) after being anesthetized with an intraperitoneal injection (1 ml/100 g body weight) of a mixture containing 35 mg Na-pentobarbital (Dainihon Pharmaceutical Co., Ltd., Osaka, Japan) and 15 mg ketamin hydrochloride (Sankyo Pharmaceutical Co., Ltd., Tokyo, Japan) in 1 ml physiological saline. The gastric, splenic, and duodenal segments (about 90% of the total weight) of the pancreas were resected, leaving only parabiliary segment which was the smallest portion closely attached to the duodenum together with the common pancreatic duct (Fig. 1) (RICHARDS et al., 1964). Pancreatic resection was performed using a battery-operated small vessel cauterizer equipped with a straight tip (Fine Science Tools, Heidelberg, Germany), which allows easy recognition of the resected margin and prevents the post-operative adhesion of the remnant tissue to the surrounding peritoneum. A group of rats were sham operated under the same anesthesia by the same procedures as in the experimental groups, except that pancreatic tissue separated from the mesentery was rubbed gently by fingertip several times instead of being resected. After surgery, all the animals were kept for a maximum of 4 weeks in the above conditions. All surgical procedures in this study were started at 10 a.m. and were completed within approximately 20 min.

Tissue preparation
Rats were sacrificed daily under ether anesthesia from days 1 to 5, and at weeks 1, 2 and 4 after surgery. The pancreatic remnants were removed en bloc with the surrounding gastric pylorus, duodenum, common bile duct, and hepatic portal area (Fig. 1A). Sampling was performed at 2 p.m. Each sample was fixed as one block overnight in Carnoy’s fixatives, and then embedded in paraffin. Each paraffin block was positioned precisely on a microtome in order to obtain sections that covered the entire cross section of the remnant pancreas from the duodenal portion to the resected margin. Sections cut at 3 μm in thickness were stained with hematoxylin and eosin and examined by light microscopy.

The proliferation activity of pancreatic cells was examined by the incorporation of bromodeoxyuridine (BrdU) (DEFAZIO et al., 1987) using a cell proliferation kit (RPN201, Amersham Pharmacia Biotech Inc., Piscataway, USA). A rat was injected with BrdU (30 mg/kg body weight) intraperitoneally at 10 a.m. on the indicated day after pancreatectomy, and the

![Fig. 1](image-url)  
**Fig. 1.** Schematic illustration of the normal rat pancreas and the method of partial resection. **A.** Among 4 segments of the pancreas, the gastric, splenic and duodenal segments were resected surgically at the dotted line leaving only the parabiliary segment with the common bile duct. **B.** Examination was made by dividing the remnant pancreas into three regions: ① duodenal, ② medial and ③ stump regions. L liver, S stomach, D duodenum, SP spleen.
pancreas was removed 4 h later and processed for light microscopy in the same manner as stated above. Sections were stained for 1 h with anti-BrdU monoclonal antibody as the first antibody after denaturing the intrinsic DNA with 1N HCl for 5 min, and treated with peroxidase-conjugated mouse IgG2a for 1 h. After extensive rinsing with PBS, peroxidase activity was developed using a mixture of 0.05% 3,3'-diaminobenzidine-4HCl and 0.01% H2O2. Endogenous peroxidase was inactivated in advance by treatment with 2% H2O2 in methanol for 20 min. Sections were observed after hematoxylin staining.

The labeling index was calculated by the number of labeled cells in each of more than 500 centroacinar, intercalated, and inter- and intra-lobular duct cells and 10,000 acinar cells. Calculations were conducted by dividing the remnant pancreas into three microscopic regions: the portion nearest to the duodenum (duodenal region), the middle portion (medial region), and the portion from the common bile duct to the resected margin (stump region) (Fig. 1B). In each region, the acinar, centroacinar, intercalated ductal, and intra- and interlobular duct cells were counted separately. The labeling index for each component cell was compared among the three different regions of the remnant pancreas.

Statistical analysis

The data obtained from at least 5 rats were expressed as mean ± SD, and differences between groups were analyzed using student’s t-test. A p value of less than 0.01 was regarded as significant.

RESULTS

General status and macroscopic alterations in remnant pancreas after pancreatectomy

Rats showed a decrease in body weight by an average of 22 g at days 1–2 after partial pancreatectomy, and recovered to the original body weight by day 7. At two weeks following the operation and thereafter, the rate of body weight gain was the same as for non-operated and sham operated control rats. Less than 5% of the pancreatectomized rats died from obstructive jaundice or hemorrhagic diathesis; these rats were excluded from the study.

Macroscopic examination indicated an increase in the volume of the remnant pancreas from day 3 after operation, followed by gradual growth until day 28. When the remnant pancreas at day 5 was compared with the pancreas of a non-operated 6-week-old rat (Fig. 2), growth of the pancreatic tissue was evident with a tendency to surround the duodenum from the left with many lobule-like expansions, resulting in an increase in thickness toward the antero-posterior axis. However, tissue growth was not evident in the region near the resected margin throughout the period studied.

Fig. 2. Macroscopic regeneration of the pancreatic remnant at day 5, compared with that immediately after the operation. Over 5 days, the pancreatic remnants have obviously grown toward the direction of the duodenum, forming many small nodules. A and B: 5 days after partial pancreatectomy, C and D: immediately after operation. A and C: anterior view corresponding to Figure 1B, B and D: posterior view. The scale is in millimeters.
Fig. 3  Legend on the opposite page.
Fig. 4. Light micrograph showing the incorporation of BrdU at day 2 after surgery. A large number of ductal cell show a positive reaction; in contrast, only few acinar cells are labeled. Scale bar indicates 100 μm.

Fig. 5. Incorporation of BrdU in three regions 7 days after pancreatectomy. Labeled cells have decreased in number rapidly in all three regions. Compare with Figure 3. A: duodenal, B: medial and C: stump regions. Scale bar indicates 50 μm.

Fig. 3. Incorporation of BrdU in three regions at day after 5 after operation, compared with non-operated control. Sporadic distribution of BrdU-positive cells is recognized in all regions in the sham-operated control pancreas (A–C). Increase in number of BrdU-positive cells is detected in the remnant pancreas at day 5, and BrdU-positive acinar cells in the portion of the duodenal region outnumber those in other regions (compare D with E and F). A portion of the duodenum, in which the incorporation of BrdU is high into crypt epithelial cells, is included in the uppermost of A and D, and common bile duct epithelial cells can be seen in the upper portions of C and F. A–C: sham-operated control pancreas, D–F: 5 days after the pancreatectomy. A and D: duodenal, B and E: medial, and C and F: stump regions. Counter stained by hematoxylin. Scale bar indicates 50 μm.
Histological changes in remnant tissue after pancreatectomy

Light microscopic examination of the standard hematoxylin-eosin stained sections of the remaining pancreas immediately after pancreatectomy indicated slight edema, the infiltration of inflammatory cells in the interstitium, and the death of acinar cells. Generally, the histological picture gave less consistent impression. The inflammatory changes regressed and complete recovery was recognized by day 7. Meanwhile, a gradual increase in the number of acinar cells was recognized at day 3 after surgery and continued through day 14. At around day 14 after operation, the density of the parenchymal cells in the remnant pancreas recovered to a level comparable to that of 6-week-old control rats. From days 14 to 28 after pancreatectomy, no evidence of further proliferation was recognized in the exocrine elements. An increase in the number of cells at the mitotic stage was recognized after the pancreatectomy. In the non- or sham-operated pancreas, one mitotic cell appeared in about 10 or more visual fields at ×400 magnification. However, a more than 5 times greater increment of mitotic cells could be detected 2 days after the pancreatectomy, and the frequency of mitotic cells gradually decreased to the control level through day 7. Observations of hematoxylin-eosin stained sections under a light microscope showed no remarkable histological difference among the three regions during the course of examination. Nevertheless, in the section at 5 days after the pancreatectomy, an almost five-fold increase in mitotic cells as compared to the control was realized in the region close to the duodenum, and the frequency was obviously higher than that in the medial and stump regions.

Incorporation of BrdU and change in labeling index after pancreatectomy

BrdU incorporation in cells was demonstrated by light microscopy as dark deposits in the nuclei. In the pancreas of non-operated normal 6-week-old rats, only a small number of BrdU-positive nuclei were found distributed uniformly in all the cell types.
Fig. 7. Regional differences in labeling indices of acinar cells. Labeling indices of acinar cells were compared among the duodenal, medial and stump regions. Acinar cells in the duodenal region showed a significantly high proliferative activity as compared to those in medial and stump regions (p<0.01).

throughout the tissue (Fig. 3A–C). In contrast, the remnant pancreas showed an increase in the number of BrdU-positive cells as early as from day 2 after pancreatectomy, and the frequency of labeled cells changed depending on the cell type as well as the time after pancreatectomy. The ductal elements including the intercalated ducts and the centroacinar cells were predominant positive cells for BrdU immunoreaction at day 2 after the operation (Fig. 4). The frequency of labeled acinar cells was found to increase at days 4–5 (Fig. 3D–F). At day 7 after the operation, only a small number of labeled all cells were detected in all cell types (Fig. 5). Labeling in cell types of exocrine elements gradually returned to the normal control level at 2–4 weeks after operation.

When the frequencies of BrdU-positive cell were compared among the three regions at day 5 after pancreatectomy, a very high proliferative activity in acinar cells was recognized in the region closest to the duodenum, and the BrdU-positive acinar cells seemed to decrease gradually with distance from the duodenum (Fig. 3D–F).

These results were analyzed quantitatively by determining the frequencies of BrdU-positive nuclei in four groups of cells: inter- and intra-lobular ductal cells, intercalated ductal cells, centroacinar cells, and acinar cells. The proliferation activity was expressed as a labeling index which was calculated from the proportion of BrdU-positive cells in each group. The average labeling index of each component cell type was calculated from randomly sampled light micrographs, covering the total cross-sectional area of the remnant pancreas including the duodenal, medial and stump regions, and compared according to the period after pancreatectomy. The results are illustrated in Figure 6A–D. The labeling index in the inter- and intra-lobular intercalated ductal cells, and in the centroacinar cells showed an immediate sharp increase at day 2, followed by a second mild increase at days 5–7 (Fig. 6B–D). A 3 to 5-fold increase in labeling index was observed at day 2. In contrast, the labeling index in acinar cells showed a peak at day 5 after pancreatectomy (Fig. 6A). The labeling index in both elements gradually recovered to the control level toward day 28.

These data were further analyzed comparing the labeling indices in the three regions. As a result, a marked regional difference in labeling indices was noted in the acinar cells (Fig. 7). The labeling index of acinar cells in the region closest to the duodenum showed a gradual increase starting from day 2 to reach a peak at day 5. At the maximum point, a labeling index of 12% was noted in this particular region, which was significantly higher than in the medial and stump regions (p<0.01). In contrast, there were no statistically significant regional differences in the labeling index in the lobular ductal cells, intercalated ductal cells, and centroacinar cells (data not shown).
DISCUSSION

Technical evaluation of pancreatectomy

There are numerous studies available on pancreatic regeneration after partial pancreatectomy. However, these have varied in the extent of resection, the region of the remnant organ examined as well as the animal species used, making a conclusive comparison of the published data rather difficult. According to PEARSON et al. (1977), the larger the portion resected, the higher the rate of proliferation was recognized in the remnant pancreas. Since a growing body of experimental evidence has accumulated on the events after 90% pancreatectomy, we applied this method of resection in this study. The resection was precisely performed using a battery-operated cautery, which enabled us to prepare sections including the total cross-sectional area of the remnant tissue from the duodenal to the stump on a single slide, and allowed accurate examinations of regional differences.

Through preliminary studies, we realized that the histology of regeneration changed according to the method of pancreatic cell mass reduction. The appearance of a large number of duct-like tubules was a characteristic feature in the regenerating pancreas after ductal ligation (WATANABE et al., 1995). Pancreatectomy may cause ductal obstruction in a certain segment due to the anatomical route of the ductal system; therefore special attention was paid in this study to exclude any mingling of a small remnant of the duodenal segment.

Cellular proliferation

The average proliferation activity of the component cells in the total region of the remnant pancreas after partial pancreatectomy essentially confirmed previous studies in rats by FITZGERALD et al. (1968), BROCKENBROUGH et al. (1988) and BONNER-WEIR et al. (1993). Cells of all ductal elements and the centroacinar cells showed the first, sharp increase in the labeling index at day 2, followed by a second less obvious peak at around days 5 to 7. The quantitative results were confirmed by the frequent occurrence of mitotic cells at the particular stage after the pancreatectomy. Among the first peaks, proliferation activity in the cells of thicker ducts tended to have higher labeling indices. Contrary to the proliferation pattern of ductal cells, the labeling index increase in acinar cells was slightly delayed, starting at day 3 to reach a peak around day 5. This result was similar to that of ELSÄSSER et al. (1986) who investigated the rate of thymidine incorporation in different component cells in the regenerating pancreas after experimental pancreatitis. In spite of the slightly delayed labeling index increase in acinar cells, an increase in cell number was recognized as early as 3 days after operation. BONNER-WEIR et al. (1993) pointed out that the proliferation of ductal cells produced focal small duct-like cells with the potential to transform into exocrine as well as endocrine cells. It is very probable that the first wave of ductal cell proliferation produced exocrine acinar cells which in turn divided to increase the number of acinar cells. If the speculation is correct, the exocrine element of the pancreas may be regenerated from the ductal cells. In the process of normal development, the common progenitor for exocrine and endocrine cells are believed to be located in the embryonic endoderm (PICTECT et al., 1972; SLACK, 1995; PERCIVAL et al., 1999). The regeneration of both exocrine and endocrine cells after partial pancreatectomy has been indicated to recapitulate embryonic development in that the proliferation of duct-like tubules is followed by differentiation (BONNER-WEIR et al., 1993).

Regional difference

A noteworthy finding in the present study was that the acinar cells in the region closest to the duodenum showed a proliferation rate more than twice that of other regions. This finding coincides well with the macroscopic observation that the pancreatic tissue proliferated toward the duodenum forming small nodules, and the histological finding that acinar cells proliferated as early as 3 day after operation. The results indicate that the proliferative activity of acinar cells is enhanced in response to cellular destruction, and that the proliferative response of these cells depends on the distance of the cells from the duodenal end. Furthermore, our quantitative examination indicated the highest occurrence of mitotic cells in the duodenal region at 5 day after the pancreatectomy. To our knowledge, this is the first report describing regional variation in proliferation activity in the pancreas. LEHY et al. (1968) reported a uniform distribution of cells that incorporated thymidine by radioautography.

Although further studies will be required to elucidate the detailed mechanisms of the higher rate of acinar cell proliferation in the region close to the duodenum, it is hard to relate the phenomenon to neural regulation as well as stimulatory effect via circulation. Several agents have been reported to have the capability to control pancreatic cell proliferation, including hormones and growth factors (for review, see GITHENS, 1993; LEE and LEBENTHAL,
Among these factors, the cholecystokinin and secretin that are present in the small intestinal mucosa have been shown to stimulate pancreatic cell development (Leung and Leventhal, 1989). It is very likely that some unidentified trophic factor(s) that diffuses from the duodenum to the pancreatic tissue has a significant effect on its regeneration.

The present observation might be correlated with the preferential occurrence of pancreatic cancer in the head portion (Pour et al., 1994). It is possible that local induction by some unknown factors released from the duodenum give rise to a higher incidence of malignant transformation through repeated acinar cell proliferation after chronic inflammatory cell death. Examination of this possibility and the actual situation is ongoing in our laboratory.

Partial pancreatectomy has been known to induce regeneration of not only exocrine but also endocrine cells (Brockenbrough et al., 1988). The present study deals with the proliferation activity only of the exocrine elements. The regeneration of the cells of Langerhans after partial pancreatectomy will be described in a separate paper.

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REFERENCES


