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Immunohistochemical Localization of Amylase in Peri- and Tele-Insular Acinar Cells of the Human Exocrine Pancreas

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ABSTRACT

An immunohistochemical localization of amylase was demonstrated in human pancreatic acinar cells using a commercial anti-human pancreatic amylase antibody. The immunofluorescence was mainly localized in the cell apices, and some differences in the intensity of the fluorescence was observed among the acinar cells in respect to their location from the islets of Langerhans. The peri-insular acinar cells showed a brighter fluorescence than the cells of tele-insular acini. This inhomogeneity of pancreatic amylase distribution in the human exocrine pancreas adds a further clue to the concept of insulo-acinar interaction.

Key words: *Amylase, Immunohistochemistry, Peri- and Tele-Insular Acinar Cells, Human Pancreas*

Several reports on the mammalian pancreata have demonstrated morphological differences between the cells of peri- and tele-insular acini[1,2,7]. Immunohistochemical studies on rat pancreatic enzymes have also illustrated a brighter fluorescence in the cells located in the peri-insular region than acinar cells located in the tele-insular tissue[3,4,6]. Studies of human insulin-dependent diabetes have shown a reduced secretion of pancreatic enzymes and bicarbonate in responce to secretin-pancreozymin test[5,10]. All these studies focus on the regulatory role of insulin on the pancreatic exocrine function.

The present work is the first report on the human pancreas to demonstrate an immunohistochemical localization of amylase in the acinar cells by means of a commercial anti-amylase antibody with some inhomogeneity in the intensity of staining of acinar cells in respect to their location from the islets of Langerhans.

MATERIALS AND METHODS

Human pancreatic tissues were obtained from the head, body and tail regions of the pancreas of a 25 years old male with brain tumor during autopsy. The tissue samples fixed in a modified Bouin's fluid (picric acid : formaldehyde = 3 : 1) for 24 hr, dehydrated in graded ethanol series and embedded in paraffin. Serial sections of 4-μm thickness were cut from each region and were mounted on gelatin-coated slides. For routine histological examination, some sections were stained with hematoxylin-eosin. The indirect immunofluorescence method was used to demonstrate amylase immunoreactivity. The deparaffinized sections were pretreated successively with phosphate-buffered saline (PBS) and non-immunized swine serum in PBS (1:15) followed by rinsing with PBS. The sections were incubated with sheep anti-human pancreatic amylase antibody (1 : 25, Serotec Ltd., Oxford, England) in a moist chamber for 2 hr at 37°C. After rinsing with PBS, they were further incubated with FITC-labelled rabbit anti-sheep IgG serum (1:50, Sigma Chemical Co., St. Louis, Mo., U.S.A.) in a moist chamber for 1 hr at 37°C and rinsed with PBS in a dark room. The sections were covered by a cover slip with 10% glycerin in PBS and examined with a Nikon Optiphot XF-EF epifluorescence microscope (Nikon, Tokyo, Japan) with blue excitation.

For controls of immunohistochemical specificity, the sections were incubated with the primary antibody previously adsorbed with an excess of either human pancreatic amylase (Athens Research and Technology, Inc., Athens, Ga., U.S.A.) or human saliva, or only incubated with the FITC-labelled secondary antibody.

RESULTS

By light microscope examination of hematoxylin-eosin stained sections from the head, body and tail regions of the pancreas, both the exocrine and endocrine tissues appeared adequately

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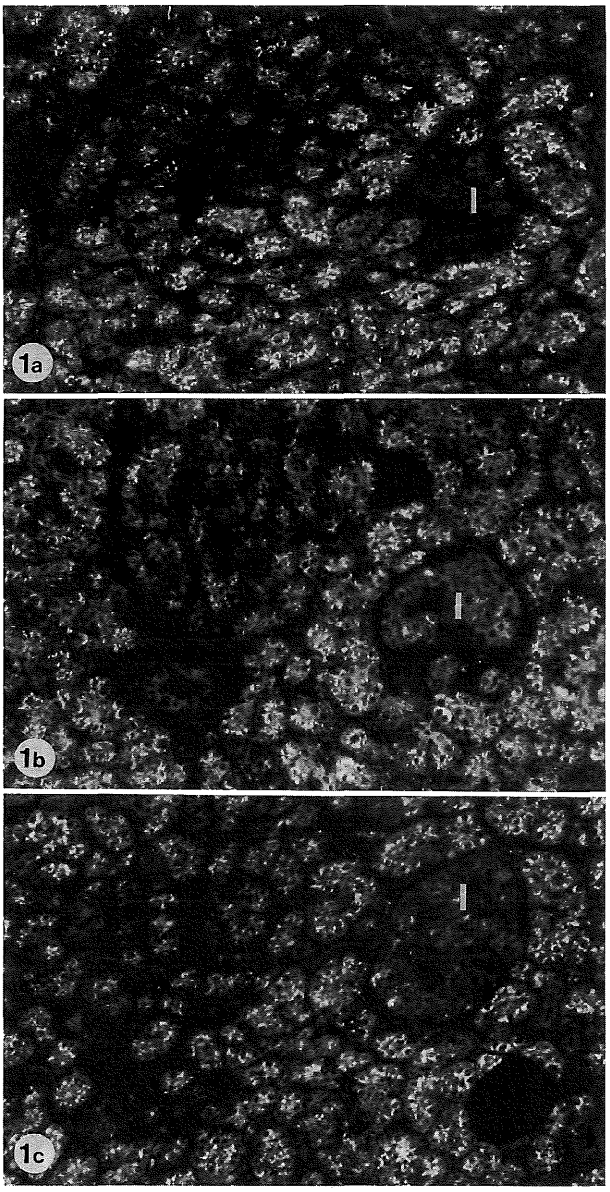

Fig. 1. Fluorescence micrographs of the head (a), body (b) and tail (c) of the pancreas immunostained with the anti-amylase antibody. The acinar cell apices are brightly fluorescent and perinuclear cytoplasm weakly fluorescent, but no stain is observed over the nuclei. In addition, the fluorescence is brighter in the acini around the islets of Langerhans (I) than those far from the islets. (×400).

preserved. No major pathological changes were found in the pancreas except for some fibrous tissue infiltration among the glandular tissue.

In the immunofluorescence study, the acinar cells in all sections examined showed a positive reaction. The most intensive staining was localized over the zymogen granule area, and the perinuclear cytoplasm showed a weak reaction (Fig. 1a, b, and c). Although all acinar cells showed a positive reaction, some inhomogeneity in the intensity of staining was noticed in relation to their location from the islets of Langerhans. Many cells of the peri-insular acini showed a brighter fluorescence than the cells of tele-insular acini (Fig. 1a, b, and c) exhibiting a tendency of halo appearance around the islets. This appearance was consistent in all regions of the pancreas.

Occasionally, the fluorescence was seen in the perinuclear area of a few excretory duct cells and islet cells (Fig. 2a and 2c, respectively).

Complete inhibition of the staining in both acinar cells as well as duct and islet cells was obtained when the primary incubation was omitted or when the primary antibody was preadsorbed with an excess of human pancreatic amylase or with human saliva (Fig. 2b and 2d, respectively).

DISCUSSION

Since all of the previous immunohistochemical studies on pancreatic amylase[2-4,6,14] have been carried out hitherto with non-commercial antibodies, the present study illustrates the usefulness of a commercial sheep anti-human pancreatic amylase antibody in the physio-pathological studies of the human pancreas on autopsied or biopsied samples.

All acinar cells from different regions of the pancreas positively reacted with the anti-amylase antibody: the fluorescence was seen in the acinar cell cytoplasm and most intensely concentrated in granular structures in cell apices, undoubtedly zymogen granules. Amylase is synthesized in the rough endoplasmic reticulum and packaged and concentrated in zymogen granules via the Golgi apparatus[8]. Therefore, we suggest that the present study demonstrates a reasonable immunofluorescence pattern using a commercial antibody in human pancreas obtained during autopsy.

The intensity of immunofluorescence varied among the acinar cells in respect to their location from the islets of Langerhans: peri-insular acinar cells consistently showed a brighter fluorescence than tele-insular acinar cells reflecting the difference in their amylase content. This appearance, so-called peri-insular halo, was noted in every region of the pancreas, although it was not as clear as previously demonstrated in the rat pancreas[4]. The more intense amylase-immunostaining of peri-insular acinar cells shown in the present study can be attributed to the larger number of zymogen granules in the cells, since previous morphometric studies on the rat exocrine pancreas have indicated that the number and volume of zymogen granules in peri-insular acinar cells are twice as much as that of tele-insular acinar cells[1,2]. The other possibility of more intense immunostaining due to high amylase content of a zymogen granule in peri-insular acinar cells cannot be denied.

Most of the human islets had an intralobular location. A scanning electron microscopic study

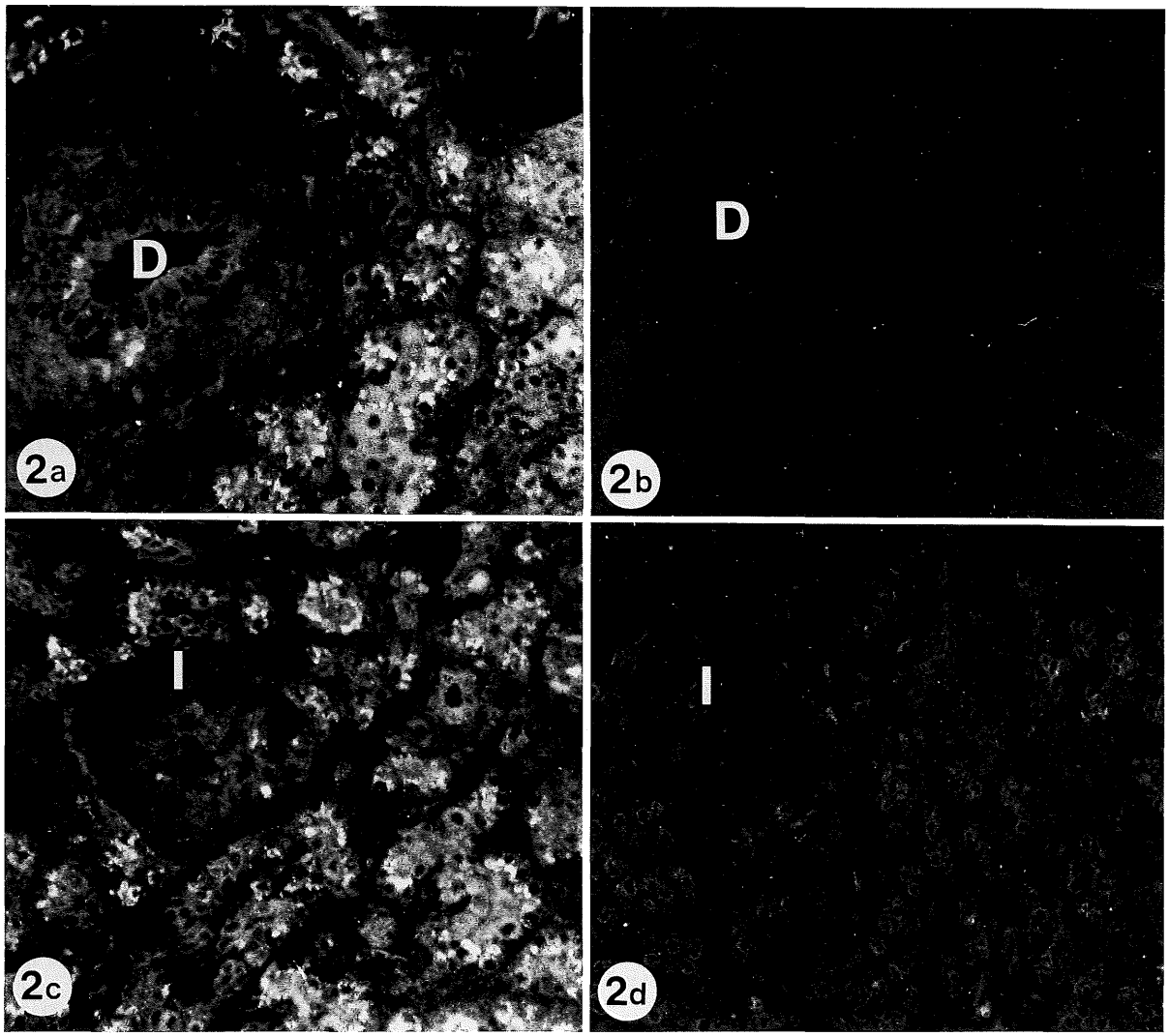

Fig. 2. Fluorescence micrographs of the pancreas showing a few cells of the excretory duct (a) and the islet (c) containing fluorescent spots in the cytoplasm. In consecutive sections reacted with the preadsorbed primary antiserum, no fluorescence was observed in acinar cells (b, d), excretory duct cells (b) and islet cells (d). I, islet; D, excretory duct. (×600).

on the blood vascular bed of the human pancreas showed that these intralobular islets received one or more arterioles and emitted conspicuous insulo-acinar portal vessels which continued into the capillaries of the exocrine tissue, suggesting insular control over the functions of the exocrine pancreas[12]. In fact, it has been well documented that insulin stimulates the synthesis of amylase in pancreatic acinar cells[9,13].

Although this is the first report to demonstrate a tendency of peri-insular halo in human pancreas, some reports on animal pancreata have shown a similar inhomogeneity of the immunostaining pattern of pancreatic enzymes in peri- and tele-insular acinar cells[2,4,14]. Immunohistochemical and cytochemical studies on spontaneous and experimentally-induced diabetes in rats[3,6] have shown a marked reduction in amylase immunolabelling which was restorted to normal after insulin administration.

In the course of the study, we also noticed some fluorescence in a few cells of islets and excretory ducts. The explanation of the nature of this reaction is difficult from the present study, but it seems possible that these cells might represent so-called intermediate acinar-islet cells and amylase containing excretory duct cells. Ultrastructural evidence on the existence of acinar-islet cells has been provided by Marx et al[11] in the regenerating rat pancreas. The other possibility of the fluorescence is that a few cells in islets and excretory ducts contain substance(s) cross reacted with the antibody used in the present study.

In conclusion, our present immunohistochemical results illustrate for the first time an immunohistochemical localization of amylase using a commercial anti-human amylase antibody and a heterogeneity of amylase distribution in the hu-

man pancreatic acinar cells in respect to their location from the islets of Langerhans.

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