Distribution and frequency of endocrine cells in the pancreas of the ddY mouse: an immunohistochemical study

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The regional distribution and frequency of pancreatic endocrine cells in ddY mice were studied by an immunohistochemical (peroxidase anti-peroxidase; PAP) method using four types of specific antisera against insulin, glucagon, somatostatin and human pancreatic polypeptide (hPP). In the pancreatic islets, most of insulin-immunoreactive (IR) cells were located in the central portion. Most of glucagon- and somatostatin-IR cells were observed in peripheral regions although a somewhat smaller number of cells were also located in the central regions. HPP-IR cells were randomly distributed throughout the entire islets. In the exocrine pancreas, insulin-, glucagon-, somatostatin- and hPP-IR cells were detected; they occurred mainly among the exocrine parenchyma as solitary cells. Cell clusters consisted of only insulin- or only glucagon-IR cells and were distributed in the pancreas parenchyma as small islets. In addition, insulin- and glucagon-IR cells were also demonstrated in the pancreatic duct regions. Insulin-IR cells were located in the epithelium and sub-epithelial connective tissue regions as solitary cells and/or clusters (3-4 cells), and glucagon-IR cells were mainly located in the epithelium as solitary cells. Overall, there were 63.89±5.39% insulin-, 26.52±3.55% glucagon-, 7.25±2.83% somatostatin- and 1.90±0.58% hPP-IR cells. In conclusion, some strain-dependent characteristic distributional patterns of pancreatic endocrine cells were found in the ddY mouse.

Key words: ddY mouse; pancreas; Endocrine cells; Immunohistochemistry;

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Paper accepted on November 11, 2004

European Journal of Histochemistry
2005; vol. 49 issue 2 (Apr-Jun): 19-24

The ddY mouse is a closed colony albino mouse. From non-inbred dd mice of the Institute of Infectious Diseases, University of Tokyo, strain 1953 was selected (Tajima, 1968) as the most suitable among 6 different strains, and was tested for the culture of *Clonorchis sinensis* (Kim *et al.*, 1992). Strain 1953 is now widely distributed and is one of the most widely used inbred mouse strains in Japan and other countries. This strain is generally used in the induction of osteoporosis by ovariectomy (Yamaguchi *et al.*, 1999) and/or sciatic neurectomy (Sakai *et al.*, 1996). The regional distribution and relative frequency of endocrine cells in the gastrointestinal tract has been relatively well-documented in recent years, and some characteristic, strain-dependent distributional patterns of gastrointestinal endocrine cells were found in this strain of mouse as compared to those of other strains (Ku *et al.*, 2004).

The occurrence, regional distribution and relative frequency of the regulatory hormones insulin, glucagon, somatostatin and pancreatic polypeptide (PP), in the vertebrate endocrine pancreas are well known from their histochemistry (Kobayashi and Ali, 1981), immunofluorescence (Orci, 1982) and immunohistochemistry (Sternberger *et al.*, 1970). In addition to the above regulatory hormones, peptide YY-, neuropeptide YY- (Alli-Rachedi *et al.*, 1984), motilin- (Yamada *et al.*, 1986) and chromogranin family- (Rindi *et al.*, 1986; Ito *et al.*, 1987) immunoreactive (IR) cells have also been demonstrated in the vertebrate pancreas. The pancreas has been treated as a valuable organ for endocrine studies, and the endocrine pancreas has been extensively studied in association with diabetes (Jansson and Sandler, 1988). In addition, the investigation of gastroenteropancreatic (GEP) endocrine cells has been considered as an important part of a phylogenetic study (D'Este, *et al.*, 1994). With the increasing demand for diabetic animal models in many fields, the regional distribution and relative frequency of

pancreatic endocrine cells, especially insulin- and glucagon-producing cells in laboratory animals have been of great interest in recent years (Warbritton *et al.*, 1994; Gomez-Dumm *et al.*, 1995; Fu *et al.*, 1996). In addition, it has been reported that the regional distribution and relative frequency of immunoreactive (IR) endocrine cells in the pancreatic islets are different in different portions of the pancreas even within the same pancreas of the same animal (Yukawa *et al.*, 1999), and a species-dependent characteristic distribution of pancreatic endocrine cells originating from feeding habits has also been suggested (Wieczorek *et al.*, 1998). The strain-dependent characteristic distribution of these IR-cells were also detected with the increased availability of genetically mutated laboratory animals and breeding of specific laboratory animals having specific or unique diseases, especially in rats and mice (Starich *et al.*, 1991; Warbritton *et al.*, 1994; Gomez-Dumm *et al.*, 1995; Fu *et al.*, 1996; Yukawa *et al.*, 1999; Ku *et al.*, 2002a-c).

The object of this study was to clarify the regional distribution and frequency of the endocrine cells in the pancreas of the ddY mouse by immunohistochemistry using four types of specific antisera against insulin, glucagon, somatostatin and hPP.

Materials and Methods

Ten adult female ddY mice (7-weeks old, 25-27g body weight upon receipt), acquired from SLC (Shizuoka, Japan) were used in this study. After food restriction for about 24 hours, the animals were phlebotomized under diethylether anaesthesia. Samples from the splenic lobes of the pancreas were fixed in Bouin's solution. After paraffin embedding, 3-4μm serial sections were prepared and stained with hematoxylin and eosin for light microscopic examination of the normal pancreatic architecture. Other sections were used for immunostaining using the peroxidase anti-peroxidase (PAP) method (Sternberger, 1979). Nonspecific peroxidase reactions were blocked with methanol containing 0.1% H_2O_2. To avoid non-specific background reactions, the sections were incubated with normal goat serum prior to incubation with the specific antibodies (Table 1). After rinsing in phosphate-buffered saline (PBS; 0.01M, pH 7.4), sections were incubated with secondary antibodies (goat anti-rabbit IgG or goat anti-guinea pig IgG, dilution, 1:200; Sigma, St. Louis MO, USA). Sections were then washed in PBS buffer and incubated with PAP complex (dilution, 1:200; Sigma). The peroxidase reaction was carried out using a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6). After immunostaining, sections were

Table 1. Antisera used in this study.

Antisera raised*	Code	Source	Diluton
Insulin	842613	DiaSorin, Stillwater, MN, USA	1 : 2,000
Glucagon	927604	DiaSorin, Stillwater, MN, USA	1 : 2,000
Somatostatin	PU0421295	BioGenex Lab., San Ramon, CA, USA	1 : 20
hPP[1)]	A619	DAKO corp., Carpenteria, CA, USA	1 : 600

**All antisera were raised in rabbits except for insulin, which were raised in a guinea pig; [1)]hPP: human pancreatic polypeptide*

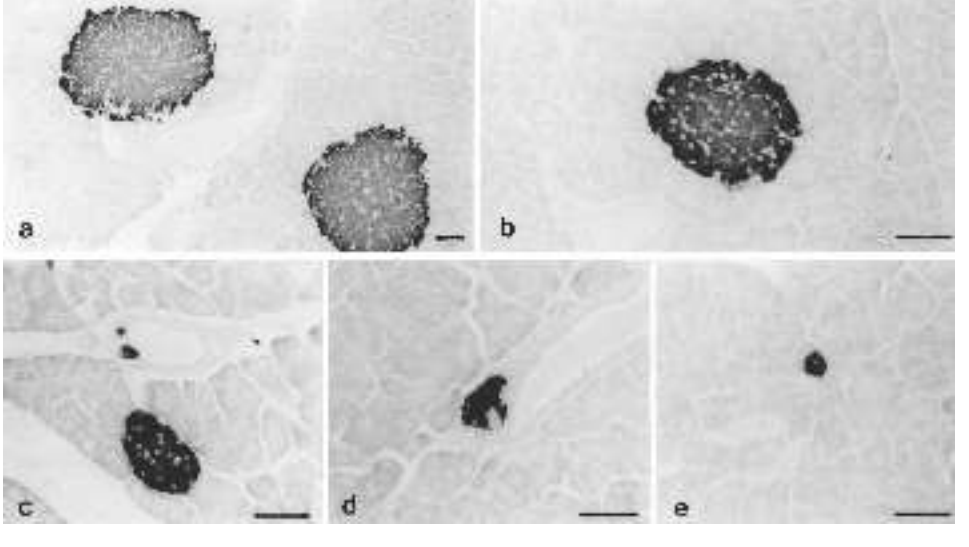

Figure 1. Insulin-IR cells in the pancreas of the ddY mouse. Most of cells were situated in the central regions of the pancreatic islets (a, b). They were also located in the islet-like cell clusters situated in the exocrine (c) and subepithelial connective tissues of the pancreatic duct (d). Solitary cells were located in the epithlia of the pancreatic duct (c) and 3-4 cells were observed between acinar cells of the exocrine (e). Scale bar, 42?.

Table 2. Regional distributions and frequencies of the endocrine cells in the pancreas of the ddY mouse.

IR cells	IR cells in the islets*	Immunoreactive cells in the exocrine pancreas			Percentage of total IR cells
		Pancreatic duct*	Cell cluster [1)]	Acinar regions**	
Insulin	65.00±6.13	10.20±4.42	21.20±4.94	4.20±2.15	63.89±5.39
Glucagon	18.20±6.14	0.30±0.48	19.30±7.56	3.90±1.73	26.35±3.55
Somatostatin	10.10±5.40	0.00±0.00	0.00±0.00	1.50±0.71	7.25±2.83
hPP 2)	1.70±0.82	0.00±0.00	0.00±0.00	1.30±0.48	1.90±0.58

*Quantitative frequencies were calculated using an automated image analysis process (Soft Image System, Germany) attached to light microscopy; *Cell number/100 parenchymal cells; **Cell number/1000 parenchymal cells; 1) Cell cluster: cell clusters consisted of only insulin- and/or glucagon-IR cells; 2) hPP: human pancreatic polypeptide.*

analysed with the use of a light microscope. After being immunostained, the sections were lightly counterstained with Mayer's hematoxylin, and the IR cells were observed under the light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979), including the replacement of the specific antiserum by the same antiserum which had been preincubated with its corresponding antigen, i.e. insulin, glucagon, somatostatin or hPP. The frequencies of IR cells were calculated as the mean ± standard deviation (S.D.) of 10 parts (n=10) of islets, exocrine and/or duct regions according to that described by Ku *et al.,* (2002a). for the SKH-1 hairless mouse. For every 100 cells, the number of cells showing immunoreactivities against each antiserum were counted using an automated image analysis process (Soft Image System, Germany) attached to light microscopy. In the pancreatic islets and duct regions, the number of IR cells was counted per 100 cells located in each region. In addition, the number of each IR cells was also counted per 1000 parenchymal cells that were located in the exocrine regions.

Results

In this study, all four types of IR endocrine cells were detected with the antisera against insulin, glucagon, somatostatin and hPP in the pancreas of the ddY mice. The pancreatic islets were distinguished as two distinct layers and central and peripheral regions from the composition of their IR cells. Depending on the region of the pancreas, different regional distributions and frequencies of the IR cells were observed, as shown in Table 2. Spherical to spindle, or occasionally oval to round-shaped IR cells were located in the pancreas.

In the pancreatic islet portion

Most of insulin-IR cells were located in the central regions (Figure 1a, b) and were the most predominant cell type in the pancreatic islets, with a frequency of 65.00±6.13 cells/100 cells. In addition, the peripheral regions of the pancreatic islets showed stronger reactivity than that of central regions. Glucagon-IR cells were located in the peripheral regions of pancreatic islets; a somewhat smaller number of these cells were also observed in the central regions intermingled with insulin-IR cells (Figure 2a, b). They showed a fre-

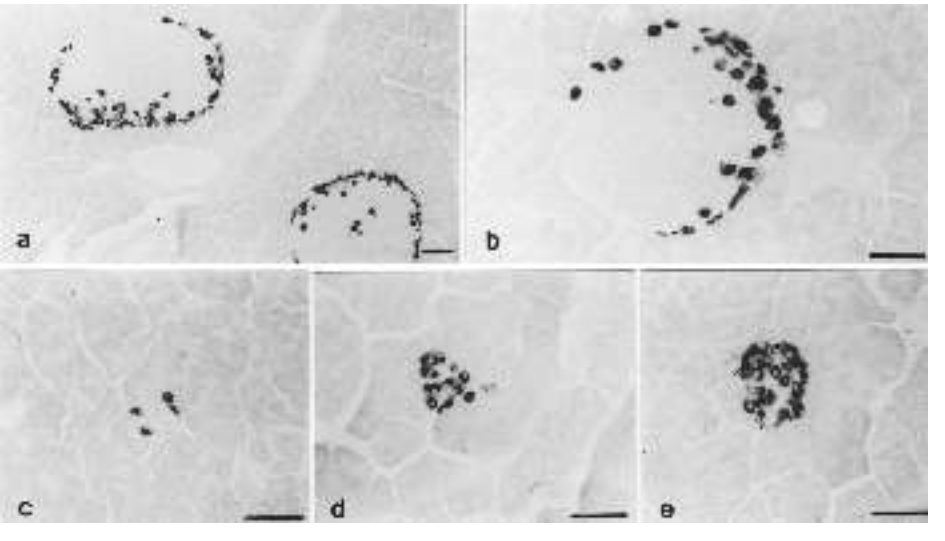

Figure 2. Glucagon-IR cells in the pancreas of the ddY mouse. They were found in the peripheral regions of pancreatic islets (a, b). Solitary cells were distributed in the exocrine (c) and also located in the islet-like cell clusters (d, e). Scale bar, 42?.

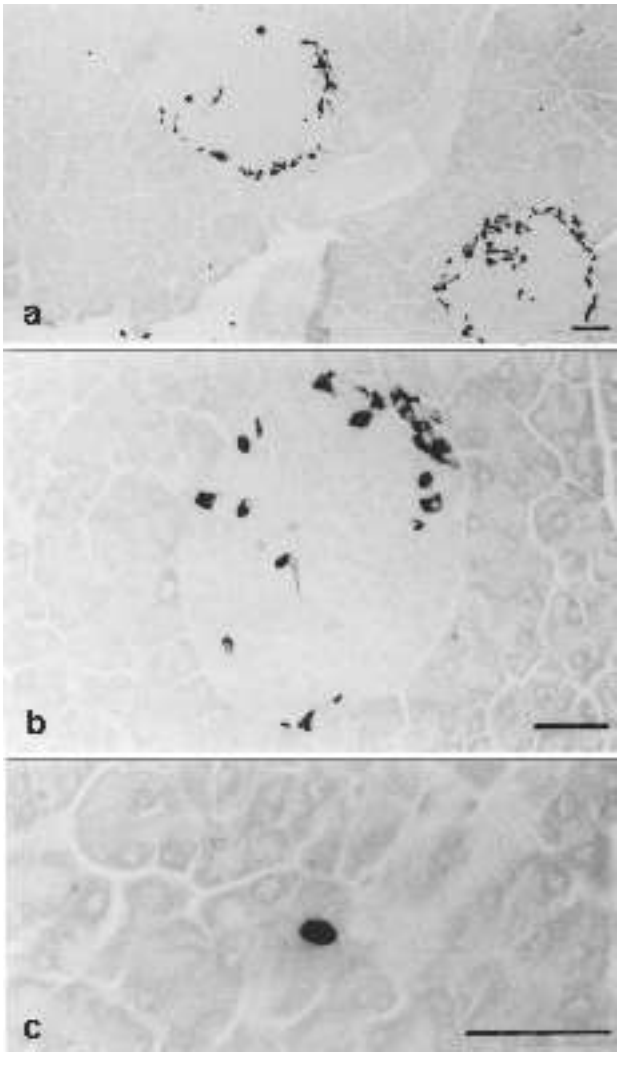

Figure 3. Somatostatin-IR cells in the pancreas of the ddY mouse. These cells were located in regions similar to those of glucagon-IR cells in the pancreatic islets (a, b) and the exocrine pancreas (c). Scale bar, 42?.

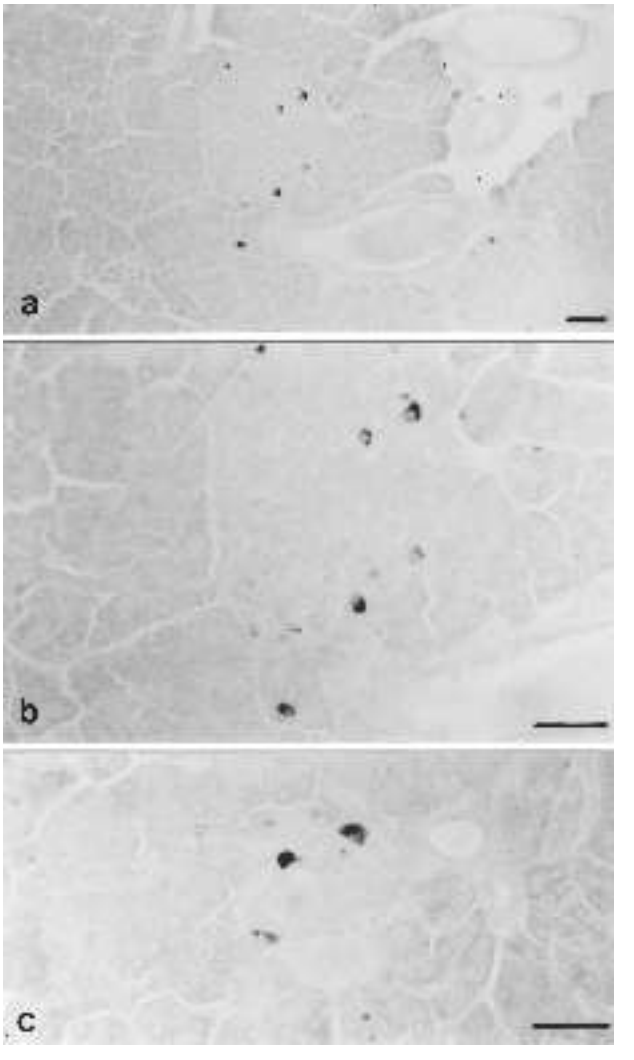

Figure 4. hPP-IR cells in the pancreas of the ddY mouse. hPP-IR cells were randomly distributed in the pancreatic islets and solitarily distributed in the exocrine pancreas (a ~ c). Scale bar, 42?.

quency of 18.20±6.14 cells/100 cells. Somatostatin-IR cells showed a distributional pattern similar to that of the glucagon-IR cells (Figure 3 a, b) with a frequency of 10.10±5.40 cells/100 cells. Relatively few hPP-IR cells were randomly located in the pancreatic islets (1.70±0.82 cells/100 cells) (Figure 4a-c).

In the exocrine portion

Insulin- (Figure 1e), glucagon- (Figure 2c), somatostatin- (Figure 3c) and hPP- (Figure 4a, b) IR cells were demonstrated in this portion with 4.20±2.15, 3.90±1.73, 1.50±0.71 and 1.30±0.48/1000 cells frequencies, respectively. They were randomly scattered in the exocrine acinar regions as clusters (consisting of 2 - 4 cells) or as solitary cells. Small islet-like cell clusters consisting of only one type of IR cell, i.e. insulin (Figure 1c) and/or glucagon (Figure 2d and e) were also observed in this region. Clusters consisted of 21.20± 4.94 cells of tightly distributed insulin-IR cells or 19.30±7.56 cells of loosely distributed glucagon-IR cells (Table 2).

In the pancreatic duct portion

Only insulin- (Figure 1c, d) and glucagon-IR cells were detected in this region with 10.20±4.42 and 0.30±0.48/100 cells frequencies, respectively.

Insulin-IR cells were situated in the epithelia and the subepithelial connective tissues; in some pancreatic ducts they formed islet-like cell clusters (Figure 1d).

Occupied percentages of each IR cell

Insulin-, glucagon-, somatostatin- and hPP-IR cells constituted approximately 63.89±5.39, 26.35 ±3.55, 7.25±2.83 and 1.90±0.58% of the total IR cell population, respectively (Table 2). Insulin-IR cells are the most abundant cell type followed in order by glucagon-, somatostatin- and hPP-IR cells in the pancreas of the ddY mouse.

Discussion

Because the function of hormones released from pancreatic endocrine cells is directly related to the regulation of pancreatic digestive enzyme and serum glucose levels (Hsu and Crump, 1989), the different distribution patterns and frequency of these pancreatic endocrine cells are considered to be a result of differences in feeding habits, especially for glucose and proteins. In addition, most of endocrine cells in the GEP system originate from ectoderm. Therefore, it cannot be excluded that differences have genetic or phylogenetic backgrounds (D'Este *et al.*, 1994). In this study, the regional distribution and relative frequency of the pancreatic endocrine cells were determined in the splenic lobes of the ddY mouse.

The regional distribution and relative frequency of the pancreatic endocrine cells have been reported in various species of mammals including some strains of mice (Krause *et al.*, 1989; Sasaki *et al.*, 1991; da Mota *et al.*, 1992; Leigh and Edwin, 1992; Gomez-Dumm *et al.*, 1995; Wieczorek *et al.*, 1998; Yukawa *et al.*, 1999; Camihort *et al.*, 2000; Ku *et al.*, 2002a-c). From these previous reports, it is well recognized that insulin-IR cells are situated in the central regions of pancreatic islets and other cells, such as glucagon-, somatostatin- and PP-IR cells, surround them; and they were also demonstrated to be associated with acinar cells and the pancreatic duct. However, unlike other researchers, Reddy *et al.,* (1986) reported that insulin-IR cells were observed in most islets where they occurred as groups of cells peripherally and within the pancreatic islets of several marsupial species. In the present study, differently from other rodent and mouse strains (Sasaki *et al.*, 1991; Warbritton *et al.*, 1994; Gomez-Dumm *et al.*, 1995; Wieczorek *et al.*, 1998; Yukawa *et al.*, 1999; Camihort *et al.*, 2000; Ku *et al.*, 2002a-c), insulin-IR cells are situated in the islet-like cell clusters located in the subepithelial connective tissues of the pancreatic duct. In addition, cell clusters consisting of insulin- or glucagon-IR cells only were demonstrated in the exocrine pancreas as small islets. Because insulin and glucagon regulate serum glucose levels, it is suggested that the ddY mouse may have peculiarly regulated pathways of glucose metabolism. However, it cannot be excluded that these characteristic distributions originated from some disease states and stress. In addition, the different reactivity of insulin in islet regions was also considered as a species-dependent characteristic although it also could not be excluded that this originated from some technical problems such as sectioning and immunostaining.

Although most of the glucagon-IR cells were situated in the peripheral regions of the pancreatic islets, they were also demonstrated in the central regions where numerous insulin-IR cells were located. While these distributional patterns are different from those of other mammals, they have been demonstrated in some strains of mice (Ku *et al.*, 2002a, b). In addition, in the equine pancreas, glucagon-IR cells were found in the center of pancreatic islets (Helmstaedter *et al.*, 1976). It was also reported that under specific disease conditions, such as the diabetic condition of obese mice, in contrast to normal non-obese littermates, glucagon-IR cells were intermingled with insulin-IR cells in the central regions of pancreatic islets (Starich *et al.*, 1991). Generally, somatostatin inhibits the secretion of other hormones and gastric acid (Kitamura *et al.*, 1984), and the absorption of amino acids, glucose and fatty acids (Brazeau *et al.*, 1973).

The specific function of hPP is not clear; however, inhibition of food intake has been postulated (Hsu and Crump, 1989). The distribution and frequency of somatostatin- and hPP-IR cells in the pancreas of the ddY mouse were quite similar to those of other rodents (Sasaki *et al.*, 1991; Warbritton *et al.*, 1994; Gomez-Dumm *et al.*, 1995; Wieczorek *et al.*, 1998; Yukawa *et al.*, 1999; Camihort *et al.*, 2000; Ku *et al.*, 2002a-c).

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