An immunohistochemical study of the pancreatic endocrine cells of the nude mouse, Balb/c-nu/nu

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The regional distribution and frequency of the pancreatic endocrine cells in the nude mouse, Balb/c-nu/nu were studied by immunohistochemical (peroxidase anti-peroxidase; PAP) methods using specific antisera against insulin, glucagon, somatostatin and human pancreatic polypeptide (hPP). The pancreas of the mouse was divided into two lobes, the splenic and duodenal lobes, and each lobe was subdivided into three regions, the pancreatic islets (central and peripheral regions), the exocrine region and the pancreatic duct region (consisting of duct epithelium and surrounding connective tissue – sub-epithelial connective tissue). In the pancreatic islets, most of insulin-immunoreactive (IR) cells were located in the central region, and glucagon-, somatostatin and hPP-IR cells were located in the peripheral region regardless of the lobe. In the splenic part, glucagon-IR cells were also located in the central regions, and more numerous somatostatin-IR cells were detected in the central regions compared to those of the duodenal part. hPP-IR cells were restricted to the peripheral regions in both lobes but more numerous cells were detected in the duodenal portion as compared to those of the splenic portion. In the exocrine parenchyma of the splenic lobe, only insulin-, glucagon- and somatostatin-IR cells were detected.. Here, the insulin- and glucagon-IR cells formed cell clusters, while somatostatin-IR cells were present as solitary cells. In the exocrine region of the duodenal portion, only insulin-, somatostatin- and hPP-IR cells were observed, with the same distributional pattern as that found in the splenic lobe. However, clusters of cells consisting only of hPP-IR cells were distributed in the pancreas parenchyma as small islets. In the pancreatic duct region, only solitary hPP-IR cells were demonstrated in the sub-epithelial connective tissue regions of the splenic portion. In conclusion, some strain-dependent characteristic distributional patterns of pancreatic endocrine cells, especially of the hPP-IR cells, were found in the nude mouse. In addition, somewhat different distributional patterns were found between the two pancreatic lobes.

Key words: Balb/c-nu/nu mouse, Endocrine cells, Pancreas, Immunohistochemistry.

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Balb/c-nu/nu is an outbred Balb/c background mouse. The original was first reported in 1966 as a hairless mouse occurring as a spontaneous mutation. This strain is a T cell-deficient immunodifficient mouse used extensively in cancer research (Gao *et al.,* 2004; Han *et al.,* 2005). This mouse strain is essential for studying xenograft models in cancer research, especially in tumor cell lines of human origin (Singh *et al.,* 2004). In addition, it is also used in human hepatitis C virus work (Shan *et al.,* 2005). It is generally known that the pancreas of vertebrates is subdivided into two regions, one is the exocrine region where digestive enzymes are released and the other is the endocrine region where regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are released into the blood. Anatomically, the mouse pancreas has two lobes – the splenic and duodenal lobes (Hsu and Crump, 1989). The pancreas is a valuable organ for endocrine studies, with the endocrine pancreas being extensively studied in diabetes (Jansson and Saudler, 1988).

The appearance, regional distribution and relative frequency of the regulatory hormones, namely insulin, glucagon, somatostatin and pancreatic polypeptide (PP), in the endocrine pancreas have been well characterized by methods in 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), secretin- (Lee *et al.,* 2003) and chromogranin family- (Rindi *et al.,* 1986; Ito *et al.,* 1987) immunoreactive (IR) cells have been demonstrated in the vertebrate pancreas. Furthermore, investigations of gastroenteropancraetic (GEP) endocrine cells have been considered as an important part of a phylogenet-

ic 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 gained 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 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 distributions of these IR cells were also detected with the increasing production of genetically mutated laboratory animals and breeding of specific laboratory animals having a specific disease or a unique property, 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; Ku and Lee, 2005). Although, the distribution of pancreatic endocrine cells in the hyperglycemic nude mouse has already been reported focussing on the hyperglycemic status (Zeidler *et al.,* 1989), reports dealing with the distribution and frequency of pancreatic endocrine cells in the splenic and duodenal lobes of the normal nude mouse have not yet been forthcoming.

The object of this study was to clarify the regional distribution and frequency of the endocrine cells in the duodenal and splenic parts of the pancreas of the nude mouse, Balb/c-nu/nu by immunohistochemistry, using specific antisera against insulin, glucagon, somatostatin and human PP (hPP).

Materials and Methods

Ten SPF Balb/c-nu/nu female mice (6-weeks old upon receipt) were acquired from Charles River (Yokohama, Japan) and used in this study after acclimatization for 4 weeks. After food restriction for about 24 hours, the animals were phlebotomized under ethyl ether anaesthesia. Samples from the splenic and duodenal lobes of pancreas were fixed in Bouin's solution. After paraffin embedding, 3-4? 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). Blocking of nonspecific peroxidase reactions was performed with methanol containing 0.1% H_2O_2, and to avoid non-specific reactions with the background, the sections were incubated with normal goat serum prior to incubation with the specific antibodies (Table 1). The staining procedure reported by Ku and Lee (2004): after rinsing in phosphate buffered saline (PBS; 0.01 M, 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 finally 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 analysed with the use of a light microscope. After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin, and the IR cells were observed under light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979), including the replacement of

Table 1. Antisera used in this study.

*Antisera**	*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
Human pancreatic polypeptide	A619	DAKO corp., Carpenteria, CA, USA	1 : 600

**All antisera were raised in rabbits except for insulin, which was raised in a guinea pig.*

specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen – insulin, glucagon, somatostatin and hPP. In addition, to test the cross reactivity of specific antisera with other antisera, immunohistochemical staining was conducted using specific antiserum, which had been preincubated with all other antigens except for its corresponding antigen and the results compared to that of immunohistochemical staining using specific antiserum. No cross reactivities are detected in this study. The frequency of IR cells was calculated as mean ± standard deviation (S.D.) of 10 parts (n=10) of islets, exocrine and/or duct regions according to that performed in the SKH-1 hairless mouse (Ku *et al.,* 2002a). Among parenchymal cells showing a nucleus, numbers of cells showing immunoreactivities against each antiserum were counted using automated image analysis (Soft Image System, Germany) combined with light microscopy. In the pancreatic islets and duct regions (consisting of duct epithelium and surrounding connective tissues – sub-epithelial connective tissues), numbers of IR cells were counted among 100 cells located in the each region. In addition, the number of each IR cell type was also counted among 1000 parenchymal cells that were located in the exocrine regions, and the percentage of each endocrine cell was also calculated in both the splenic and duodenal lobes.

Results

In this study, all four types of the IR endocrine cells were detected with the antisera against insulin, glucagon, somatostatin and hPP in both lobes of the pancreas of the nude mice. The pancreatic islets of this study were distinguished as two distinct regions, central and peripheral regions, with their composition of IR cells. According to the lobe of the pancreas, different regional distributions and frequencies of these IR cells were observed as shown in Tables 2 and 3. Spherical to spindle or occasionally oval to round-shaped IR cells were located in the pancreas.

The pancreatic islets region

In the splenic lobe, most of insulin-IR cells were located in the central region and indeed were the most predominant cell type in this region of the pancreatic islets with a frequency of 65.20±12.43/100 cells. In addition, these cells were also detected in the peripheral regions, but

Table 2. Regional distributions and frequencies of the endocrine cells in the splenic lobe of the pancreas of the nude mouse, Balb/c-nu/nu.

IR cell type	*IR cells in the islets**		*IR cells in the extra-islet regions*		*Percentage of each IR cell*
	Central	*Peripheral*	*Exocrine***	*Pancreatic duct**	
Insulin	65.20 ± 12.43	5.20 ± 2.74	3.70 ± 2.31	ND	59.27 ± 5.56
Glucagon	7.20 ± 3.33	17.20 ± 4.71	3.00 ± 2.71	ND	21.76 ± 3.93
Somatostatin	2.10 ± 1.66	15.60 ± 2.95	2.30 ± 0.82	ND	16.03 ± 3.01
hPP [1)]	ND[2)]	2.90 ± 1.45	ND	0.80 ± 0.63	2.94 ± 1.31

*Quantitative frequencies were calculated using automated image analysis (Soft Image System, Germany) combined with light microscopy; *Cell number/100 parenchymal cells; **Cell number/1000 parenchymal cells; 1) hPP: human pancreatic polypeptide; 2) ND: not detected.*

Table 3. Regional distributions and frequencies of the endocrine cells in the duodenal lobe of the pancreas of the nude mouse, Balb/c-nu/nu.

IR cell type	*IR cells in the islets**		*IR cells in the extra-islet regions*		*Percentage of each IR cell*
	Central	*Peripheral*	*Exocrine***	*Pancreatic duct**	
Insulin	69.00 ± 8.26	10.10 ± 3.68	6.00 ± 5.52	ND	61.55 ± 5.54
Glucagon	ND 2)	20.40 ± 1.51	ND	ND	15.00 ± 3.17
Somatostatin	0.60 ± 0.70	8.90 ± 7.65	1.30 ± 0.82	ND	7.32 ± 4.38
hPP 1)	ND	11.30 ± 2.36	11.50 ± 8.42	ND	16.13 ± 3.31

*Quantitative frequencies were calculated using automated image analysis (Soft Image System, Germany) combined with light microscopy; *Cell number/100 parenchymal cells; **Cell number/1000 parenchymal cells; 1) hPP: human pancreatic polypeptide; 2) ND: not detected.*

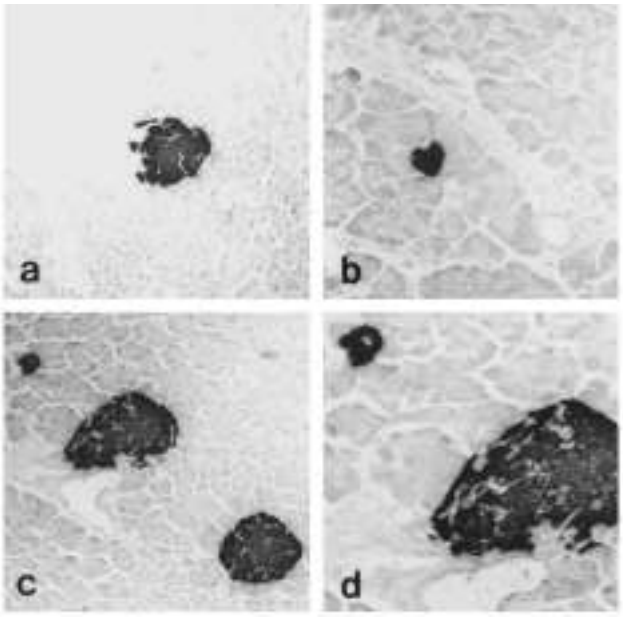

Figure 1. Insulin-IR cells in the splenic (a, b) and duodenal (c, d) lobes of the pancreas of Balb/c-nu/nu mice. Most of cells were situated in the central regions of the pancreatic islets (a, c, d) and they were also located in the exocrine region as cell clusters in both the splenic and duodenal lobes (b-d). a, c. z75; b, d. x300; PAP method.

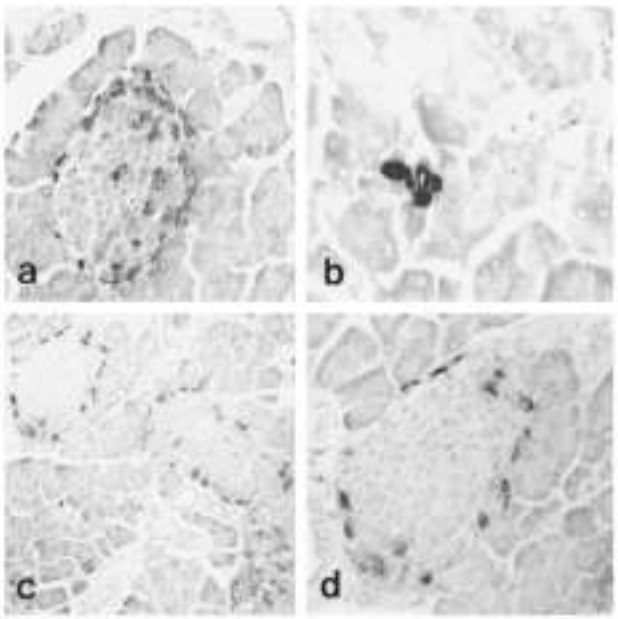

Figure 2. Glucagon-IR cells in the splenic (a, b) and duodenal (c, d) lobes of the pancreas of Balb/c-nu/nu mice. Most of the cells were found in the peripheral regions of the pancreatic islets (a, c, d). In addition, some cells were also observed in the central regions of the pancreatic islets in the splenic lobe (a). In the exocrine region of the splenic lobe, they were detected as cell clusters (b). Solitary cells were distributed in the exocrine region (c) and also located in the islet-like cell clusters (d, e). a, d. x150; b. x300; c. x75; PAP method.

with lower frequency 5.20±2.74/100 cells (Figure 1a). Glucagon-IR cells were located in the peripheral region of the pancreatic islets and a somewhat lower frequency of cells was noticed in the central region intermingled with insulin-IR cells (Figure 2a). These showed a frequency of 7.20±3.33 and 17.20±4.71/100 cells in the central and peripheral regions, respectively. Somatostatin-IR cells showed distributional patterns similar to those of glucagon-IR cells with 2.10±1.66 and 15.60±2.95/100 cells frequencies in the central and peripheral regions, respectively (Figure 3a, b). Relatively low numbers of hPP-IR cells were found, and these were restricted to the peripheral regions of pancreatic islets with 2.90±1.45/100 cells frequency (Figure 4a, b).

In the duodenal lobe, similar to that of the splenic lobe, most of the insulin-IR cells were located in the central region with 69.00± 8.26/100 cells frequency; they were also detected in the peripheral regions with 10.70±3.68/100 cells frequency (Figure 1c, d). Glucagon-IR cells were located in the peripheral regions of pancreatic islets, while no such cells were detected in the central regions (Figure 2c, d). These cells showed 20.40±1.51/100 cells frequency. Somatostatin-IR cells showed a quite similar distributional pattern in the splenic portion (Figure 3d, e) with frequencies of 0.69±0.70 and 8.90±7.65/100 cells in the central and peripheral regions, respectively.

Relatively higher numbers of hPP-IR cells compared to that of the splenic lobe were found and again were restricted to the peripheral regions of pancreatic islets (Figure 4d, e) with 11.30± 2.36/100 cells frequency.

The exocrine region

In the splenic lobe, insulin-, glucagon- and somatostatin-IR cells were demonstrated with 3.70±2.31, 3.00±2.71 and 2.30±0.82/1000 cells frequencies, respectively. However, no hPP-IR cells were detected. The insulin-IR cells were detected as cell clusters consisting of 3-7 cells among acinar cells (Figure 1b). Similar distribution patterns were also detected for glucagon- (Figure 2b) and somatostatin-IR cells (Figure 3c).

In the duodenal lobe, insulin-, somatostatin- and hPP-IR cells were demonstrated with 6.00±5.52, 1.30±0.82 and 11.50±8.42/1000 cells frequencies, respectively. However, no glucagon-IR cells were detected. Insulin-IR cells were detected as

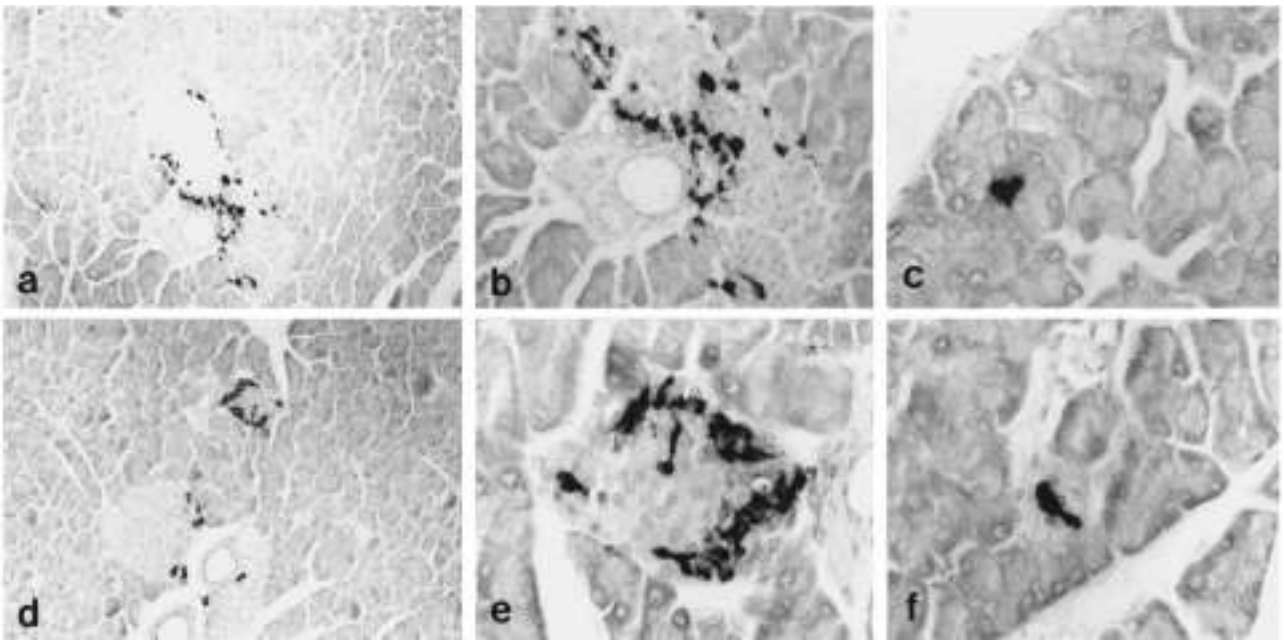

Figure 3. Somatostatin-IR cells in the splenic (a-c) and duodenal (d-f) lobes of the pancreas of Balb/c-nu/nu mice. These cells were located in the regions similar to those for glucagon-IR cells in the pancreatic islets in both splenic (a, b) and duodenal lobes (d, e). They were also located in the exocrine as cell clusters in the splenic lobe (c) and as solitary cells in the duodenal lobe (f). a, d. x75; b, c, f. x300; e. x150; PAP method.

cell clusters consisting of 3-7 cells among acinar cells (Figure 1c, d). In addition, somatostatin-IR cells were detected as solitary cells (Figure 3f). Unique distribution patterns of hPP-IR cells were detected in this region. They were demonstrated as islet-like cell clusters (Figure 4d, f).

The pancreatic duct region

Only hPP-IR cells were detected in this region of the splenic lobe with 0.80±0.63/100 cells frequency. They were situated in the sub-epithelial connective tissues of some pancreatic ducts (Figure 4c).

Percentages of the IR cells

In the splenic lobe, insulin-, glucagon-, somatostatin- and hPP-IR cells occupied approximately 59.27±5.56, 21.76±3.93, 16.03±3.01 and 2.94±1.31% of the total IR cell population, respectively (Table 2). The insulin-IR cell is clearly the most abundant cell type in the splenic lobe of the pancreas of the nude mouse.

In the duodenal lobe, insulin-, glucagon-, somatostatin- and hPP-IR cells occupied approximately 61.55±5.54, 15.00±3.17, 7.32±4.38 and 16.13±3.31% of total IR cell population, respectively (Table 3). Again, the insulin-IR cell is the most abundant cell type, as in the splenic lobe, but differently from that of the splenic lobe, hPP-IR cells ranked second in order, followed by glucagon- and somatostatin-IR cells in the duodenal portion of the pancreas.

Discussion

Because the function of hormones released from pancreatic endocrine cells is directly related to the regulation of pancreatic digestive enzymes 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 the endocrine cells in the gastro-entero-pancreatic system originate from the ectoderm. Therefore, it cannot be excluded that the 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 and duodenal lobes of nude mouse, Balb/c-nu/nu.

The regional distribution and relative frequency of the pancreatic endocrine cells have been reported in various species of mammals including some mouse strains (Krause *et al.,* 1989; Sasaki

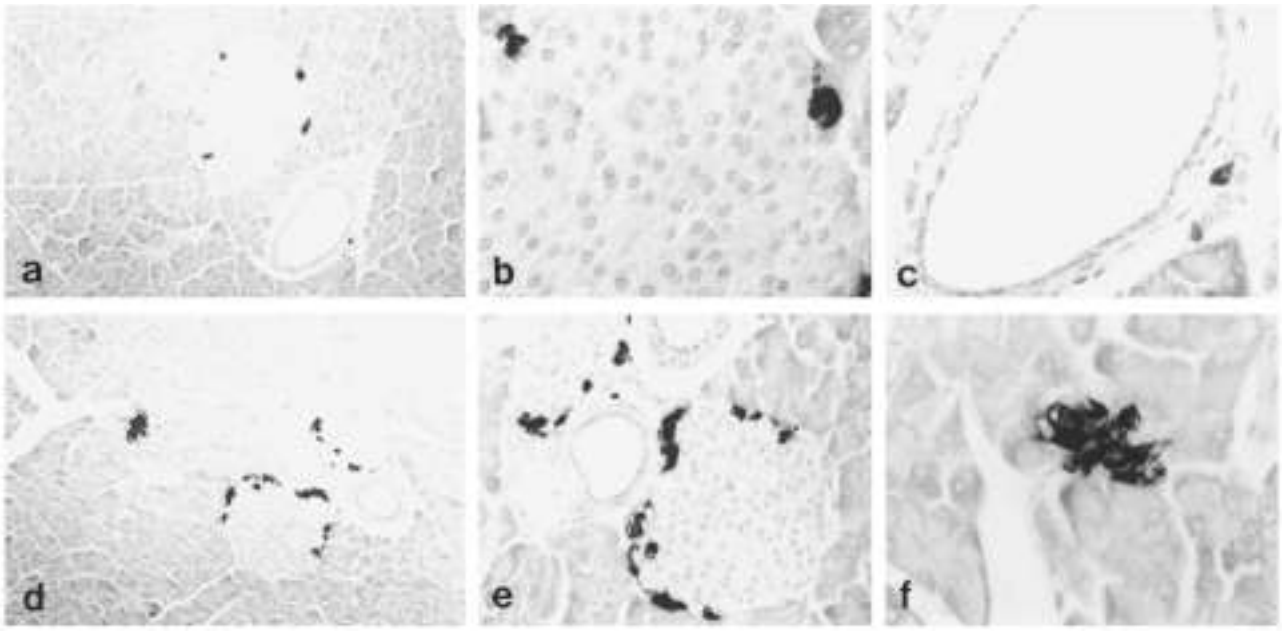

Figure 4. hPP-IR cells in the splenic (a-c) and duodenal (d- f) lobes of the pancreas of Balb/c-nu/nu mice. They were restricted to the peripheral regions of the pancreatic islets in both splenic (a, b) and duodenal lobes (d, e). They were also located in the sub epithelial connective tissues of pancreatic duct regions of the splenic lobe as solitary cells (c) and as cell clusters like small-islets in the duodenal lobe (f). a, d. x75; b. x150; c, e, f. x300; PAP method.

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 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. They have also been demonstrated to be associated with acinar cells and pancreatic duct. However, unlike other researchers, Reddy *et al.,* (1986) observed insulin-IR cells in most islets where they occurred as groups of cells peripherally and within the pancreatic islets of several marsupial species. In addition, in the naked mole-rat pancreas, insulin-IR cells formed the mantle while alpha cells formed the core of the islets; quite differently from other mammals (Kramer and Buffenstein, 2004) but similar to that of equine pancreas in which glucagon-IR cells were found in the center of pancreatic islets (Helmstaedter *et al.,* 1976). In the present study, similar to that found in 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), we found that most of the insulin-IR cells were situated in the central regions of the pancreatic islets, and similar distributional patterns of insulin-IR cells were observed in the splenic and duodenal lobes of the nude mouse. The characteristic distribution of pancreatic endocrine cells detected in the Balb/c mouse (whose cell clusters consisted of insulin-IR cells only in the pancreatic duct, Ku *et al.,* 2002b) was not noticed in the present study.

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 in the splenic lobe. While these results differ from those observed in other mammals, such distributional patterns were also demonstrated in some mouse strains, such as the SKH-1 hairless mouse (Ku *et al.,* 2002a) and the Balb/c mouse (Ku *et al.,* 2002b). In addition, in the equine and the naked mole-rat pancreas, glucagon-IR cells were found in the center of pancreatic islets (Helmstaedter *et al.,* 1976; Kramer and Buffenstein, 2004); it was also reported that under specific disease conditions, such as obese and diabetic 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). It is considered to be a strain-dependent characteristic of the nude mouse that there exists a difference in the

distributions of glucagon-IR cells in the central region of the pancreatic islets between the splenic and duodenal lobe.

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 PP is not clear; however, inhibition of food intake has been postulated (Hsu and Crump, 1989). The general distribution and frequency of somatostatin- and hPP-IR cells in the pancreas of the nude mouse was found to be similar to that 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). However, cell clusters consisting of hPP-IR cells only were detected in the exocrine region and restricted to the duodenal portion. These results are also considered as strain-dependent characteristics of the nude mouse. In the present study, somewhat different regional distribution and frequency of pancreatic endocrine cells were detected between the splenic and duodenal lobes. In the splenic portion, somatostatin-IR cells were more numerous than hPP-IR cells in all regions; but in the duodenal portion, hPP-IR cells outnumbered the somatostatin-IR cells in all regions. The hPP-IR cells outnumbered glucagon-IR cells by an even greater amount. These differences were considered as strain-dependent characteristics of the nude mouse and it was also considered that these findings are directly related to the digestive function of hPP (Hsu and Crump, 1989).

In conclusion, some strain-dependent characteristic distributional patterns of pancreatic endocrine cells, particularly the hPP-IR cells, were found in the nude mouse, Balb/c-nu/nu. In addition, somewhat different distributional patterns were demonstrated in the two pancreatic lobes.

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