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Immunohistochemical Study of the Pancreatic Endocrine Cells in the BALB/c mice: An Unique Distributional Pattern of Glucagon

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Abstract

The regional distribution and relative frequency of insulin-, glucagon-, somatostatin- and pancreatic polypeptide (PP)-producing endocrine cells in the pancreas of BALB/c mouse were investigated by immunohistochemical method. The pancreas of mice was divided into two portions; pancreatic islets and exocrine portions, and pancreatic islets were further subdivided into two regions (central and peripheral regions) and the relative frequency and regional distribution of immunoreactive cells against insulin, glucagon, somatostatin and PP antisera were monitored. In the pancreatic islet portions, insulin-immunoreactive cells were located in the central regions and they were randomly dispersed in the whole pancreatic islets in some case of the small islets. Quite different from those of other mammals, glucagon-immunoreactive cells were dispersed throughout central to peripheral regions in case of large islets and in the smaller ones, most of these cells were situated in the peripheral regions. Somatostatin-immunoreactive cells were detected in the peripheral regions with various frequencies. Although some cells were demonstrated in the central regions of pancreatic islets, most of PP-immunoreactive cells were located in the peripheral regions. In the exocrine portions, all four types of immunoreactive cells were demonstrated in the BALB/c mouse. Some peculiar distributional patterns of pancreatic endocrine cells were found in BALB/c mouse, especially in case of glucagon-immunoreactive cells.

Key words : BALB/c mouse, pancreatic endocrine cell, immunohistochemistry

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Introduction

BALB/c mouse is an inbred albino (*A,b,c.*) mouse. Now it is widely distributed and one of the most widely used inbred mouse strains. This strain is particularly well known for the production of plasmacytomas on injection with mineral oil. These tumours form the basis for the production of monoclonal antibodies. So it is used as a general-purpose strain in many different disciplines. Besides, it has good breeding performance and long reproductive life span. Normally this strain has low mammary tumor incidence but can be infected with the mammary tumor virus by fostering C3H (which carries the virus), and it then gets a high incidence of mammary tumors[1, 2]. In addition, this strain shows high sensitivity to X-irradiation[3, 4] and low LD_{50} to X-irradiation[5] and recommended host for transplantable tumours: melanoma HP and pleomorphic sarcoma 5180.

It is generally known that pancreas of vertebrates is subdivided into two portions, one is exocrine portions where digestive enzymes are released and the other is endocrine portions where regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are released into blood circulation. The appearance, regional distribution and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas were well recognized by histochemistry[6], immunofluorescence method[7] and immunohistochemistry[8]. Except above regulatory hormones, peptide YY-, neuropeptide YY-[9], motilin-[10] and chromogranin family-[11, 12] immunoreactive cells were also demonstrated in the vertebrate pancreas. The pancreas has been treated as a valuable organ for endocrine studies and endocrine pancreas has been extensively studied, associated with diabetes[13]. In addition, the investigations of gastroenteropancreatic endocrine cells have been considered as an important part of a phylogenetic studies[14].

Until now, the regional distribution and relative frequency of major four types of endocrine cells, insulin, glucagon, somatostatin and PP, were reported in the pancreas of the hamster[15], wood mouse[16], C57BL/6 mouse[17], preobese and obese yellow Avy/- mouse[18], vole[19], obese ob+/ob+ mouse[20],

sand rat[21], Japanese field vole[22], guinea pig[23] and ICR mouse[24]. In addition, angiotensin Ⅱ-immunoreactive cells were found in the pancreas of mouse[25] and appearances of calcitonin gene-related peptide- and cholecystokinin- immunoreactive cells in the rat pancreas were also reported[26, 27]. With the increasing demands of diabetic animal models and usefulness of irradiation in many fields, the regional distribution and relative frequency of pancreatic endocrine cells, especially insulin- and glucagon-producing cells in the laboratory animals have been concerned in recent years[17, 18, 28]. Many researchers suggested that species-dependent characteristic distribution of cells producing different hormones in the pancreas of each species of animals might be due to feeding habits and now it is are generally accepted[29]. In addition, it was also reported that different regional distribution and relative frequency of endocrine cells in the pancreatic islets were demonstrated in different portions of the pancreas even if they were same pancreas of same animal[16]. And strain-dependent characteristic distribution of these immunoreactive cells was also detected with the increase of producing genetically mutated laboratory animals and breeding of specific laboratory animals having specific disease or unique nature, especially in rat and mouse[16-18, 20, 24]. Although many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the pancreas of the various vertebrates including various species and strains of rodents, the reports were seldom dealing with the endocrine cells in the pancreatic islets of BALB/c mouse in spite of its biological, physiological and anatomical differences from the other rodents.

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

Materials and Methods

Five adult BALB/c mice (7-wk old, 26-38 body weight upon receipt) were acquired from the Charles River Laboratories (Yokohama, Japan) and they were used in this study without sexual distinction. After phlebotomized under anesthetizing with ethyl ether, samples from the pancreas were fixed in Bouin's solution. After paraffin embedding, 3-4 ㎛ serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal pancreatic architecture.

Each representative section was deparaffinized, rehydrated and immunostained with the peroxidase anti-peroxidase (PAP) method[30]. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsed in phosphate buffered saline (PBS; 0.01M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3'- diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05M, pH 7.6). After immunostained, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger[30], including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen and the relative frequency of occurrence of each type of immunoreactive cells was placed into one of five categories according to their observed numbers as seen using light microscopy. The relative frequency of occurrence of each type of IR cell was placed into one of five categories, not detected (−), rare (; mean values were below 2/one filed), a few (+; mean values were below 5/one filed), moderate (++; mean values were below 10/one filed) and numerous (+++; mean values were up to 20/one filed), according to their observed mean numbers as seen under one filed of light microscope (×200).

The local animal research committee approved the experimental protocol. The animals used were cared for in accordance with the principles of the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences (NIH publication 86-23 revised 1985).

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Diluton
Insulin	842613	Diasorin, Stillwater, Minnesota	1 : 2000
Glucagon	927604	Diasorin, Stillwater, Minnesota	1 : 2000
Somatostatin	917600	Diasorin, Stillwater, Minnesota	1 : 1000
PP[1)]	A619	DAKO Corp., Carpinteria, California	1 : 600

*All antisera were raised in rabbits, [1)] PP: human pancreatic polypeptide

Table 2. Regional distributions and relative frequencies of the endocrine cells in the pancreas of BALB/c mouse

Immunoreactive cells	Pancreatic islets portion		Exocrine portion	Pancreatic duct portion
	Central	Peripheral		
Insulin	+++	+	±	−
Glucagon	++	++	+	−
Somatostatin	−	++	++	−
PP[1)]	±	±	++	−

* Relative frequencies; +++: numerous, ++: moderate, +: a few, ±: rare, −: not detected
1) PP: human pancreatic polypeptide.

Results

In this study, all four kinds of the immunoreactive endocrine cells were detected with the antisera against insulin, glucagon, somatostatin and PP in the pancreas of BALB/c mice. The pancreatic islets of this study were distinguished into two distinct layers, central and peripheral regions with their composition of immunoreactive cells. According to the regions of the pancreas, different regional distribution and relative frequency of these immunoreactive cells were observed and these differences are shown in Table 2. Spherical to spindle or occasionally oval to round-shaped immunoreactive cells were located in the pancreas.

Insulin-immunoreactive cells

These cells were located in the central regions with numerous frequency. In addition, cells showing a few frequency were also demonstrated in the peripheral regions (Fig. 1a). However, some insulin-immunoreactive cells were randomly distributed throughout the whole pancreatic islets in a case of relatively small islets, which were mainly located between pancreatic duct and exocrine portions (Fig. 1b, c). In the exocrine portion, they were randomly scattered between pancreatic acinar cells or interlobular connective tissues with rare frequency (Fig. 1d). No insulin-immunoreactive cells were detected in the pancreatic duct portions.

Glucagon-immunoreactive cells

In case of relatively large pancreatic islets, they were dispersed throughout central to peripheral regions intermingled with other 3 types of immunoreactive cells and showed regular relative frequencies between central and peripheral regions, with moderate frequency (Fig. 2a-c). In case of relatively small pancreatic islets, most of glucagon-immunoreactive cells were situated in the peripheral regions but no cells were found in the central regions (Fig. 2d). In the exocrine portion, they were randomly scattered between pancreatic acinar cells or interlobular connective tissues with a few frequency (Fig. 2e). No glucagon-immunoreactive cells were detected in the pancreatic duct portions.

Somatostatin-immunoreactive cells

These immunoreactive cells were located in the peripheral regions with moderate frequency. However, no somatostatin-immunoreactive cells were demonstrated in the central regions where numerous insulin-immunoreactive cells were found (Fig. 3a). In the exocrine portion, they were randomly scattered between pancreatic acinar cells or interlobular connective tissues with moderate frequency (Fig. 3b). No somatostatin-immunoreactive cells were detected in the pancreatic duct portions in this study.

PP-immunoreactive cells

Although some cells were demonstrated in the central regions of pancreatic islets with rare frequency, most of these cells were located in the peripheral regions with a few frequency (Fig. 3c, d). In the exocrine portion, they were randomly scattered between pancreatic acinar cells or interlobular connective tissues with moderate frequency (Fig. 3e). However, no cells were detected in the pancreatic duct portions in this study.

DISCUSSION

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose levels[31]. In the mammals, the regional distribution and relative frequency of insulin-immunoreactive cells in the pancreas were reported in the wood mouse[16], hamster[15], C57BL/6 mouse[17], voles[19], ICR mouse[24], three-toed sloth[32], Australian brush-tailed possum[33], opossum[34] and laboratory animals[29]. From these previous reports, it is well recognized that insulin cells are situated in the central regions of pancreatic islets and other cells, such as glucagon-, somatostatin- and PP-immunoreactive cells, surround them. And they were also demonstrated frequently associated with acinar cells and pancreatic duct. However, somewhat different from other researchers, Reddy

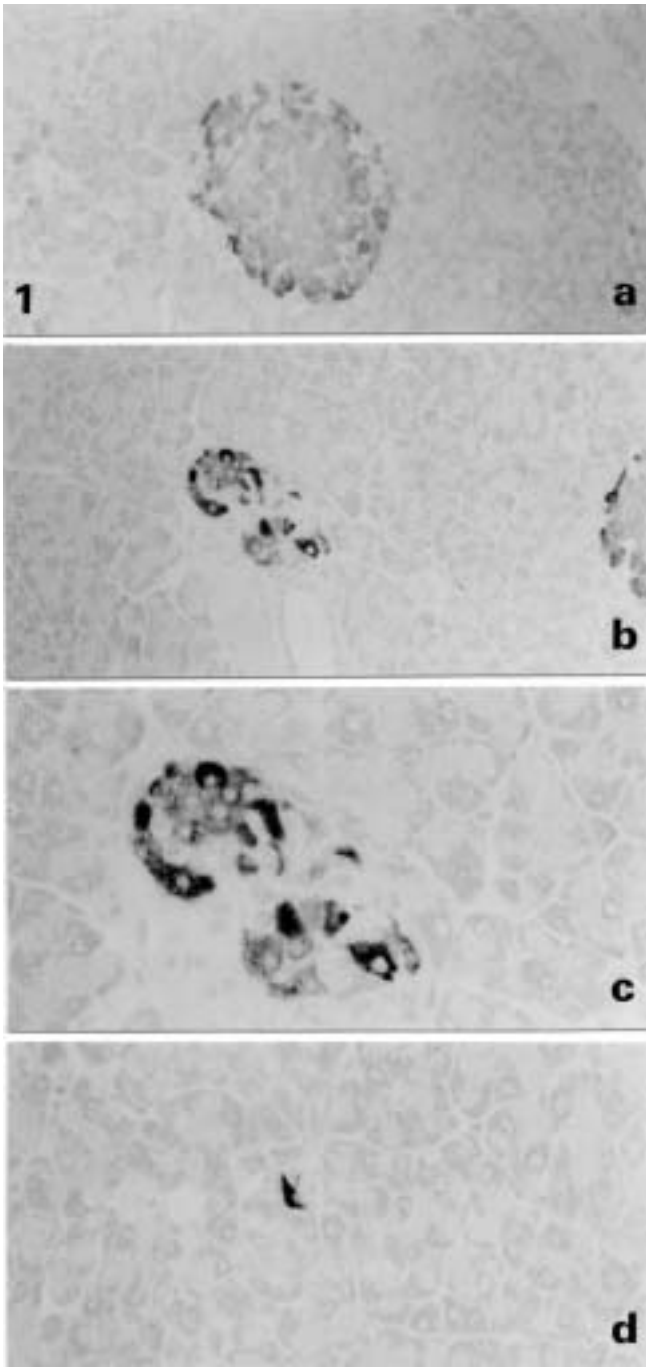

Fig. 1. Insulin-immunoreactive cells in the pancreas of the BALB/c mice; Most of immunoreactive cells were situated in the central regions of pancreatic islets (a) and they were randomly dispersed in the whole pancreatic islets in some case of the small islets (b; c is high magnification of b). In addition, insulin-immunoreactive cells were also detected in the exocrine portions (d). **a**, **b**: ×150; **c**, **d**: ×300, **PAP method.**

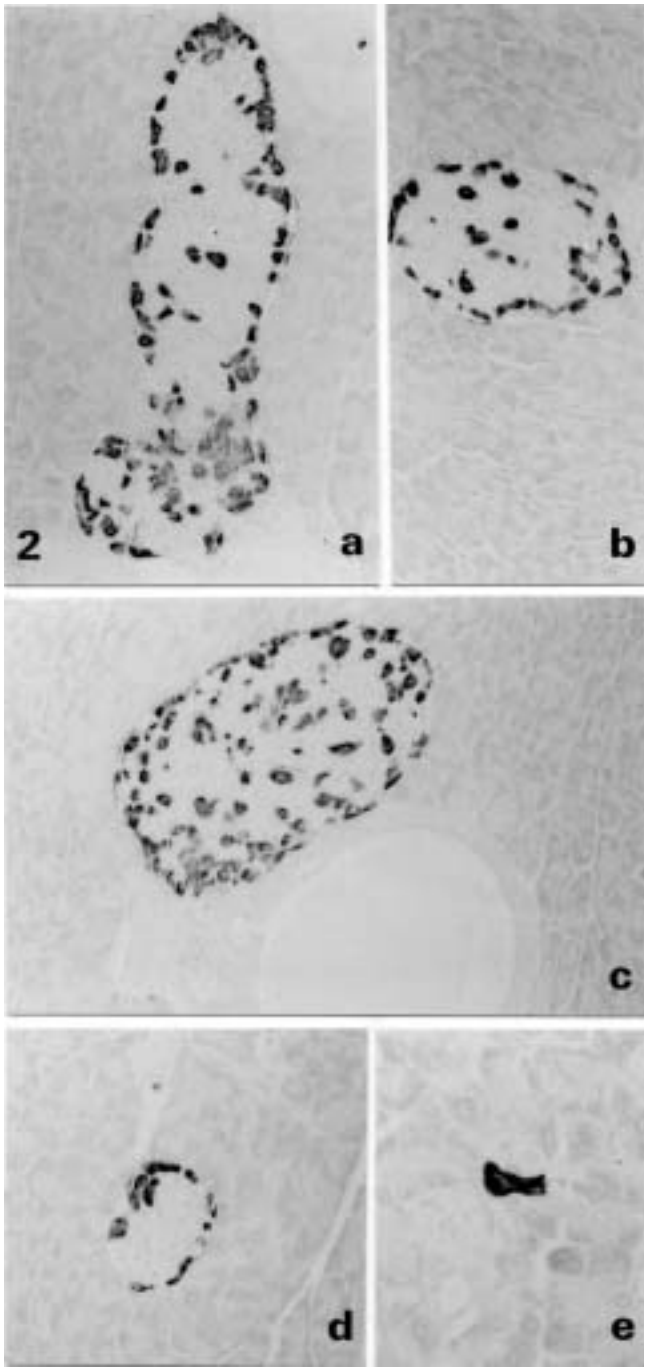

Fig. 2. Glucagon-immunoreactive cells in the pancreas of the BALB/c mice; They were dispersed throughout central to peripheral regions in a case of large islets (a ~ c) and in the smaller ones, most of immunoreactive cells were situated in the peripheral regions (d). In addition, some cells were demonstrated in the exocrine portions (e). **a** ~ **d**: ×150; **e**: ×300, **PAP method.**

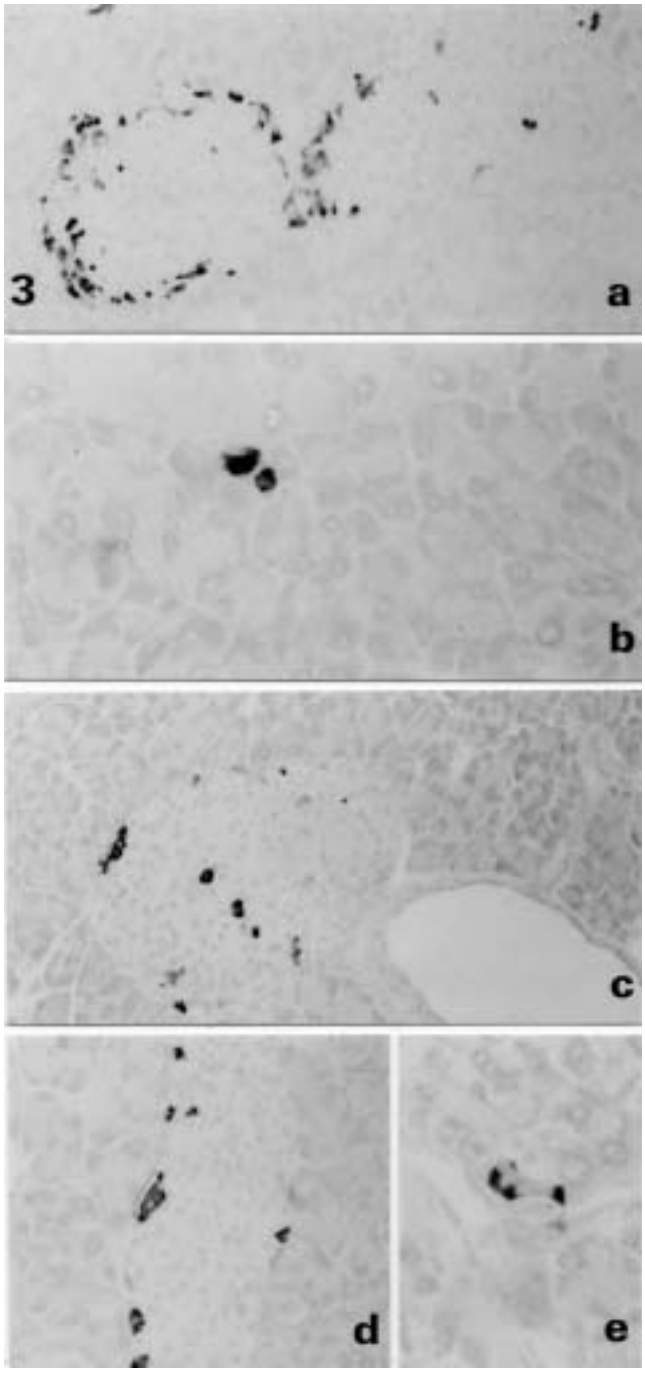

Fig. 3. Somatostatin- and PP-immunoreactive cells in the pancreas of the BALB/c mice; Somatostatin- immunoreactive cells were located in the peripheral regions of the pancreatic islets regardless of their size (a) and some cells were also demonstrated in the exocrine portions (b). Although some cells were demonstrated in the central regions of pancreatic islets (c), most of PP-immunoreactive cells were located in the peripheral regions (d). In addition, some PP-immunoreactive cells were also detected in the exocrine portions (e) **a**, **c**, **d**: ×150; **b**, **e**: ×300, **PAP method.**

et al[35] reported that they 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, most of insulin-immunoreactive cells were restricted to the central regions of islets similar to that of previous rodents[15-19, 24, 28]. Different from other rodents, where these cells were found in the lining epithelium of pancreatic duct, no insulin-immunoreactive cells were situated in the pancreatic duct portions. In addition, some cells were randomly distributed in case of relatively small islets of BALB/c mouse. According to the location of these smaller islets, which were correlated with pancreatic duct in this study and the previous reports that showed possibility of insulin cells originated from pancreatic duct stem cells[36, 37], these smaller ones were regarded as a juvenile or infant typed cell clusters composed of insulin-immunoreactive cells. Anyway, these differences compared to that of other rodents were considered as peculiar distributional patterns of BALB/c mouse.

Glucagon is synthesized in the A cells of the pancreas and regulates glucose levels in blood[31]. Morphologically similar cells are also observed in the digestive tract of the dog. Although glucagon-immunoreactive cells were located in the mantle and peripheral regions of mammalian pancreatic islets, exocrine portions and pancreatic duct[15-19, 24, 29, 32-34], species-dependent variations were also reported. In the equine pancreas, A-cells demonstrated by anti-glucagon were found in the center of pancreatic islets where in most vertebrates, insulin-immunoreactive cells were numerously found[38]. In addition, it was also reported that under specific disease conditions such as obese (diabetic condition) mouse, glucagon-immunoreactive cells were intermingled with insulin-immunoreactive cells in the central regions of pancreatic islets, in contrast, normal non-obese littermates showed a peripheral localization of these immunoreactive cells[20]. Although glucagon-immunoreactive cells were situated in the peripheral regions in case of relatively small islets, they were randomly distributed in the central to peripheral regions in case of relatively large ones where numerous insulin-immunoreactive cells were located and these results were different from those of other mammals. In addition, they were not demonstrated in the pancreatic duct portions. These distributional patterns were considered as peculiar patterns of BALB/c mouse.

Somatostatin, which consisted of 14 amino acids, was isolated from hypothalamus of sheep for the first time. It could be divided into straight form and cyclic form[39]. This substance inhibited the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid[40] and the absorption of amino acid, glucose and fatty acid in the gastrointestinal tract[41]. So far as investigated, somatostatin-immunoreactive cells are located in the peripheral regions of mammalian pancreatic islets and exocrine portions[15-19, 24, 29, 32-34]. Well corresponding to these previous studies, most of somatostatin cells were found in the peripheral regions

where intermingled with glucagon- and PP-immunoreactive cells and they occupied outmost regions of pancreatic islets.

PP is a peptide hormone containing 36 amino acids, which is synthesized by F cells in the pancreatic islets[31]. The specific function of this peptide is not clear, however, inhibition of food intake has been postulated as a possible function of this peptide[31]. And Polak ***et al***[41] reported that it promoted the secretion of gastric acid and stimulated the glycolysis of liver in avian species. It has been revealed that PP-immunoreactive cells were conspicuously distributed in the peripheral regions of pancreatic islets and exocrine portions in mammalian species, if they occurred[15-19, 24, 29, 33, 34]. In addition, colocalization with serotonin in the pancreatic islets of the opossum[34] and cattle[42] was also demonstrated. Anyway, da Mota ***et al***[32] reported that PP-immunoreactive cells were not found in the pancreas of the three-toed sloth. In the present study, well corresponding to previous studies[15-19, 24, 29, 33, 34], PP-immunoreactive cells were detected in the outmost regions of pancreatic islets, although rare frequencied cells were intermingled with other immunoreactive cells in the central regions where insulin-immunoreactive cells were most predominant.

In conclusion, some peculiar distributional patterns of pancreatic endocrine cells, especially, glucagon-immunoreactive cells, were demonstrated in the BALB/c mice.

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