J. Vet. Sci. (2002), 3(4), 327-333

JOURNAL OF
Veterinary
Science

An immunohistochemical Study on the Pancreatic Endocrine Cells of the C57BL/6 Mouse

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Received June 19, 2002 / Accepted November 28, 2002

Abstract

The regional distribution and relative frequency of the pancreatic endocrine cells in the C57BL/6 mouse were studied by immunohistochemical method using four types of specific mammalian antisera against insulin, glucagon, somatostatin and human pancreatic polypeptide (PP). The pancreas of mouse could be divided into three portions; pancreatic islets, pancreatic duct and exocrine portions, and pancreatic islets were further subdivided into three regions (central, mantle and peripheral regions) according to their located types of immunoreactive cells and pancreatic duct portions were also subdivided into two regions (epithelial and connective tissue regions). In the pancreatic islet portions, although some cells were also demonstrated in the mantle regions, most of insulin-immunoreactive cells were located in the central regions and they were randomly dispersed in the whole pancreatic islets. Glucagon-immunoreactive cells were detected in the mantle and peripheral regions. Their relative frequencies in the peripheral regions were somewhat numerous than those of the mantle regions. Somatostatin-immunoreactive cells were detected in the mantle and peripheral regions. However, no PP-immunoreactive cells were demonstrated in the pancreatic islets of C57BL/6 mouse. In the pancreatic duct portions, rare glucagon-immunoreactive cells were situated in the epithelial regions. Cell clusters that consisted of glucagon- or somatostatin-immunoreactive cells were found in some case of connective tissue regions of pancreatic ducts. However, insulin- and PP-immunoreactive cells were not detected in the epithelial nor connective tissue regions. In the exocrine portions, all four types of immunoreactive cells except for PP cells were demonstrated in the C57BL/6 mouse. However, no PP-immunoreactive cells were demonstrated. In conclusion, regional distribution of endocrine cells in the pancreas of C57BL/6 mouse was similar to that of mammals, especially other rodents except for topographically different distribution of endocrine cells compared to that of other rodents.

Key words : C57BL/6 mouse, endocrine cell, pancreas, immunohistochemistry

Introduction

C57BL/6 mouse is an inbred black mouse and is probably the most widely used of all inbred strains, though in many ways it seems to be atypical of inbred strains of laboratory mice. It usually has a good breeding performance, depending on substrain, and has been used as the genetic background for a large number of congenic strains covering both polymorphic and mutant loci. This strain of mouse has resistance to chloroform toxicity [5], to induction of cleft palate by cortisone [18], to lethal effects of ozone [12] and to colon carcinogenesis by 1,2-dimethylhydrazine [10]. In addition, it is also a recommended host for the following transplantable tumours: mammary adenocarcinoma BW 10232 melanoma B16, myeloid leukaemia C 1498 and preputial gland carcinoma ESR586. The pancreas of the C57BL mouse has been concerned to their histological profiles because it has been used as animal models of non-obese diabetes [13].

The appearance, regional distribution and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas were well recognized by various methods including immunohistochemistry [20, 29, 38]. Except for insulin, glucagon, somatostatin and pancreatic polypeptide (PP), peptide YY-, neuropeptide YY- [1], motilin- [42] and chromogranin family- [16, 33] 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, 17]. In addition, the investigations of gastroenteropancraetic endocrine cells have been considered as an important part of phylogenetic studies [6]. Until now,

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the regional distribution and relative frequency of major four types of endocrine cells were reported in the pancreas of the rodents such as hamster [3], wood mouse [43], preobese and obese yellow Avy/- mouse [40], vole [34], obese ob+/ob+ mouse [36], sand rat [8], Japanese field vole [28], gerbil [23] and guinea pig [31]. In addition, angiotensin II -immunoreactive cells were found in the pancreas of mouse [26] and appearances of calcitonin gene-related peptide- and cholecystokinin- immunoreactive cells in the rat pancreas were also reported [7, 35].With the increasing demands of diabetic animal models and/or usefulness of anticancer drugs 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 [11, 13, 40]. 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 generally accepted [41]. 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 the same pancreas of same animal [43]. 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 with specific disease or unique nature, especially in rat and mouse [11, 13, 36, 40, 43]. Gomez-Dumm et al. [13] reported the distributional difference of endocrine cells between normal and diabetic C57BL/6 mouse. However, they have only focused on distributional differences and did not showed sufficient and comparative data about normal C57BL/6 mouse.

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 dealing with the endocrine cells in the pancreas of the C57BL/6 mouse were seldom in spite of its biological, physiological and anatomical differences from the other rodents and usefulness in many research fields. The object of this study was to clarify the regional distribution and relative frequency of the endocrine cells in the pancreas of C57BL/6 mouse by specific immunohistochemistry using four types of specific antisera against insulin, glucagon, somatostatin and PP.

Material and Methods

Five adult C57BL/6 mice (7-wk old, 26-38g body weight) 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 [37]. 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 [37], 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.

Results

In this study, three kinds of the immunoreactive endocrine cells were detected with the antisera against insulin, glucagon and somatostatin in the pancreas of the C57BL/6 mouse. However, no PP-immunoreactive cells were demonstrated in this study. The pancreatic islets of this study were distinguished into three distinct layers, central, mantle and peripheral regions with their composition of immunoreactive cells. In addition, the pancreatic ducts were subdivided into two regions, epithelial and connective tissue regions which were extended regions from lamina propria of the pancreatic ducts into interlobular regions. According to the regions of

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Dilution
Insulin	842613	DiaSorin, Stillwater.	1:2,000
Glucagon	927604	DiaSorin, Stillwater.	1:2,000
Somatostatin	917600	BioGenex Lab., San Ramon.	1:1,000
PP1)	A619	DAKO Corp., Carpenteria.	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 C57BL/6 mice

Immunoreactive cells	Pancreatic islets portion			Exocrine Portion	Pancreatic duct portion	
	Central	Mantle	Peripheral		Epithelium	Connective tissue
Insulin	+++	±	−	++	−	−
Glucagon	−	+	++	+	±	++
Somatostatin	−	+	++	+	−	++
PP1)	−	−	−	−	−	−

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

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 of the C57BL/6 mouse.

Insulin-immunoreactive cells

These immunoreactive cells were located in the central regions with numerous frequency. In addition, insulin-immunoreactive cells showing moderate frequency were also demonstrated in the mantle regions intermingled with other immunoreactive cells (Fig 1a). In the exocrine portion, single or three to four cell clustered insulin-immunoreactive cells were randomly scattered between pancreatic acinar cells with moderate frequency (Fig 1b, c). However, no insulin-immunoreactive cells were detected in the pancreatic duct portions.

Glucagon-immunoreactive cells

In the pancreatic islets, most of glucagon-immunoreactive cells were situated in the peripheral regions with moderate frequency and their cytoplasmic processes were intermingled with other immunoreactive cells, especially somatostatin-immunoreactive cells. In addition, rare cells were also demonstrated in the mantle regions intermingled with insulin-immunoreactive cells but no cells were found in the central regions (Fig 2a, b). In the exocrine portion, they were randomly scattered between pancreatic acinar cells or interlobular connective tissues with a few frequency (Fig 2d). In the pancreatic duct portions, glucagon-immunoreactive cells were demonstrated in the epithelial and connective tissue regions with rare and moderate frequencies, respectively (Fig 2a, c).

Somatostatin-immunoreactive cells

These immunoreactive cells were located in the peripheral and mantle regions with moderate and a few frequencies, respectively. 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 a few frequency (Fig 3c). In the pancreatic duct regions, clusters consisted of somatostatin-immunoreactive cells were detected in the connective tissue regions (Fig 3b).

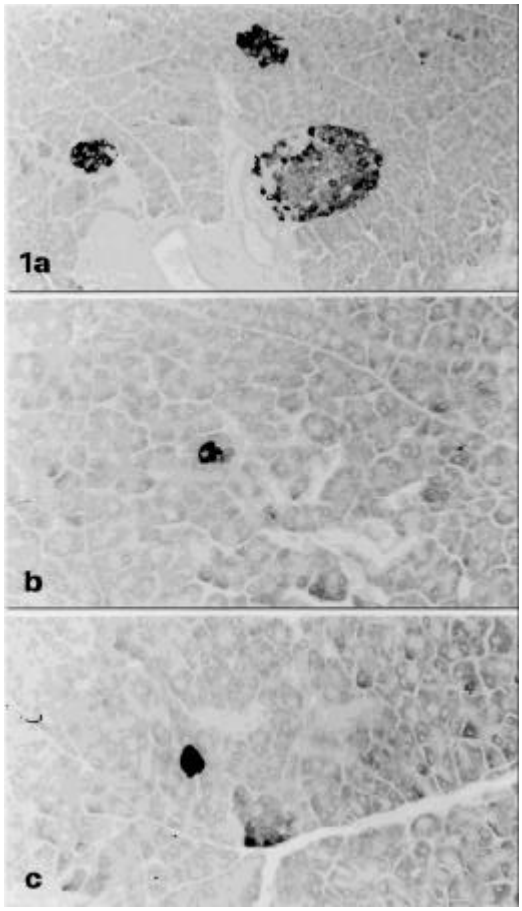

Fig. 1. Insulin-immunoreactive cells in the pancreas of the C57BL/6 mice; Most of immunoreactive cells were situated in the central to mantle regions of pancreatic islets (a). In addition, single or clusters consisted of insulin-immunoreactive cells were also detected in the exocrine portions (b, c). a: ×120; b, c: ×240, PAP method.

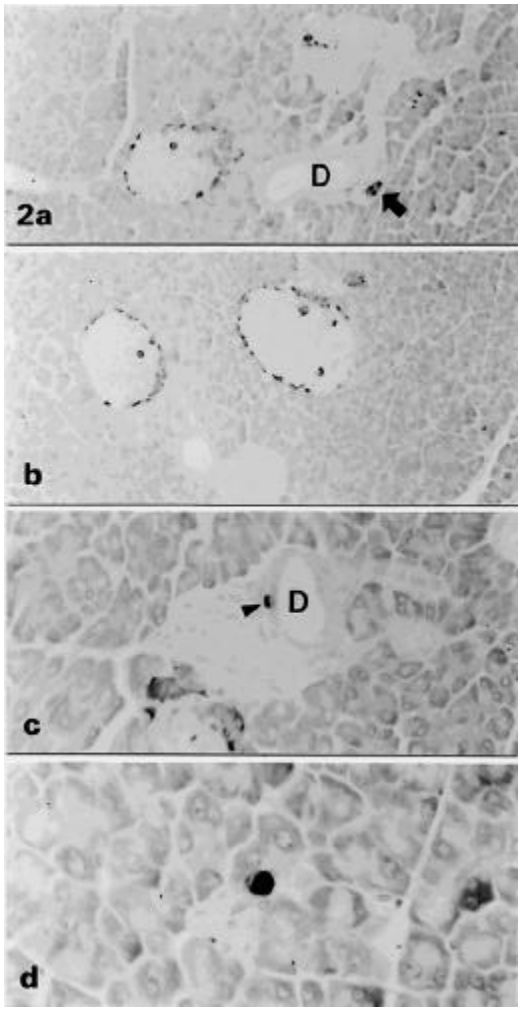

Fig. 2. Glucagon-immunoreactive cells in the pancreas of the C57BL/6 mice; Most of these immunoreactive cells were located to peripheral regions of pancreatic islets and rare cells were also detected in the mantle regions (a, b). Cell clusters consisted of glucagon-immunoreactive cells and single cells were located in the connective tissue (a, arrow) and epithelial (c, arrowhead) regions of the pancreatic duct portions. In addition, some cells were demonstrated in the exocrine portions (d). D: pancreatic duct; a, b: ×120; c: ×240; d: ×480, PAP method.

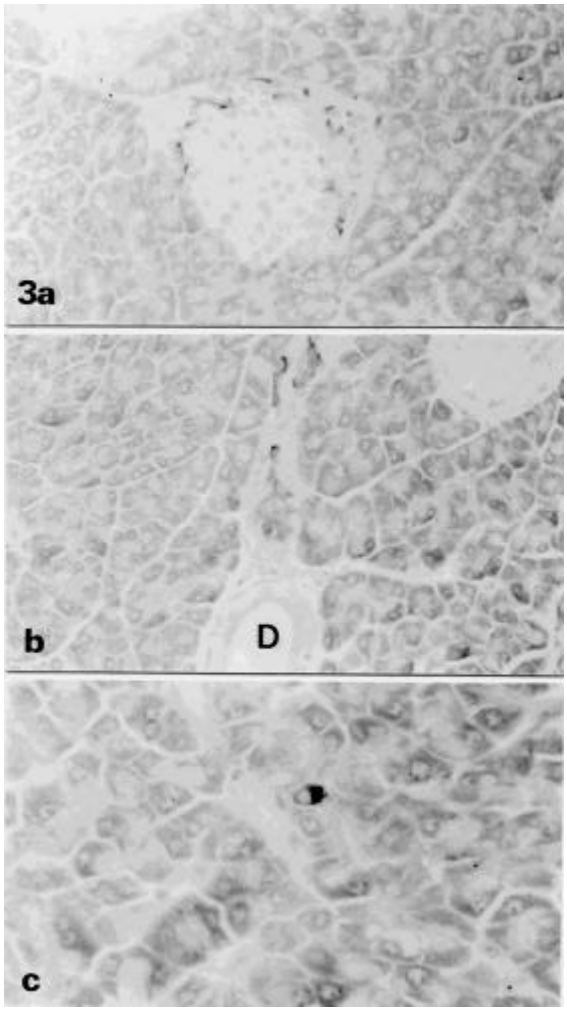

Fig. 3. Somatostatin-immunoreactive cells in the pancreas of the C57BL/6 mice; Somatostatin-immunoreactive cells were located in the mantle and peripheral regions of the pancreatic islets (a) and some cells were also demonstrated in the exocrine portions (c). In addition, cell clusters consisted of these immunoreactive cells were situated in the connective tissues that were extended from lamina propria of the pancreatic ducts to interlobular connective tissues (b). a, b: ×240; c: ×480, PAP method.

Discussion

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose levels [15]. In the mammals, the regional distribution and relative frequency of insulin-immunoreactive cells in the pancreas were reported in the wood mouse [43], hamster [3], gerbil [23], voles [34], three-toed sloth [4], Australian brush-tailed possum [25], opossum [21] and various laboratory animals [41]. From these previous reports [3, 4, 21, 23, 25, 34, 41, 43], 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 et al. [32] 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 [3, 13, 23, 34, 40, 41, 43]. However,

different from other rodents, no insulin-immunoreactive cells were situated in the pancreatic duct portions.

Glucagon is synthesized in the A cells of the pancreas and regulates glucose levels in blood [15]. 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 [3, 4, 13, 21, 23, 25, 34, 40, 41, 43], 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 vertebrate, insulin-immunoreactive cells were numerously found [14]. 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 [36]. In the present study, although cells with relatively low frequency were demonstrated in the mantle regions compared to that of other rodents [3, 4, 13, 21, 23, 25, 34, 40, 41, 43], most of glucagon-immunoreactive cells were situated in the peripheral regions. Although it is seldom in rodents, cell clusters consisted of glucagon-immunoreactive cells located in the connective tissue regions of pancreatic duct portions that are generally detected in higher mammals [22]. The distributional patterns of glucagon-immunoreactive cells in the pancreatic duct portions were considered as strain-dependent characteristics of the C57BL/6 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 [2]. This substance inhibited the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid [19] and the absorption of amino acid, glucose and fatty acid in the gastrointestinal tract [2]. So far as investigated, somatostatin-immunoreactive cells are located in the peripheral regions of mammalian pancreatic islets and exocrine portions [3, 4, 13, 21, 23, 25, 34, 40, 41, 43]. Well corresponding to these previous studies, most of somatostatin cells were found in the peripheral regions where they were intermingled with glucagon-immunoreactive cells and they occupied outmost regions of pancreatic islets. In addition, cell clusters consisted of somatostatin-immunoreactive cells were demonstrated in the connective tissues that were extended from lamina propria of pancreatic duct to interlobular connective tissues of this strain of mice. Although it is seldom in rodents, cell clusters consisted of somatostatin-immunoreactive cells located in the connective tissue regions of pancreatic duct portions are generally detected in higher mammals [24]. The distributional patterns of somatostatin-immunoreactive cells in the pancreatic duct portions were considered as strain-dependent characteristics of the C57BL/6 mouse.

PP is a peptide hormone containing 36 amino acids, which is synthesized by F cells in the pancreatic islets [15]. The specific function of this peptide is not clear, however, inhibition of food intake has been postulated as a possible function of this peptide [15], and Polak et al. [30] 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 [3, 4, 13, 23, 25, 34, 40, 41, 43]. In addition, colocalization with serotonin in the pancreatic islets of the opossum [21] and cattle [27] was also demonstrated. Anyway, da Mota et al. [4] reported that PP-immunoreactive cells were not found in the pancreas of the three-toed sloth. In the present study, well corresponding to that of the three-toed sloth, PP-immunoreactive cells were not detected in the pancreas of the C57BL/6 mouse. However, some researchers [9, 39] suggested that the distribution and appearance of endocrine cells in the pancreas were quite different according to used antiserum. So this different appearance of PP-immunoreactive cells was considered as problems related with used antiserum

In conclusion, some peculiar distributional patterns of pancreatic endocrine cells were demonstrated in the C57BL/6 mouse.

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