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Regional Distribution and Relative Frequency of Gastrointestinal Endocrine Cells in Large Intestines of C57BL/6 Mice

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Abstract

The regional distributions and relative frequencies of some gastrointestinal endocrine cells in the three portions (cecum, colon and rectum) of the large intestinal tract of C57BL/6 mice were examined with immunohistochemical method using 7 types of specific antisera against chromogranin A (CGA), serotonin, somatostatin, human pancreatic polypeptide (HPP), glucagon, gastrin and cholecyctokinin (CCK)-8. In this study, all 3 types of immunoreactive (IR) cells were identified. Most of these IR cells in the large intestinal portion were generally spherical or spindle in shape (open-typed cell) while cells with a round shape (close-typed cell) were found in the intestinal gland. Their relative frequencies varied according to each portion of the large intestinal tract. CGA-IR cells were found throughout the whole large intestinal tract but were most predominant in the colon. Serotonin-IR cells were detected throughout the whole large intestinal tract and showed highest frequency in the colon. Peculiarly, glucagon-IR cells were restricted to the colon with a low frequency. However, no somatostatin-, HPP-, gastrin- and CCK-8-IR cells were found in the large intestinal tract. In conclusion, some peculiar distributional patterns of large intestinal endocrine cells were identified in C57BL/6 mice.

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

Introduction

C57BL/6 mice are inbred black mice and are probably the most widely used of all the inbred strains. Although in many ways, they appear to be atypical of inbred strains of laboratory mice. These mice generally have a good breeding performance, depending on the substrain, and have been used as the genetic background for a large number of congenic strains covering both polymorphic and mutant loci. This strain of mouse is resistant to chloroform toxicity[1], to the induction of a cleft palate by cortisone[2], to the lethal effects of ozone[3] and to colon carcinogenesis from 1,2-dimethylhydrazine[4]. In addition, it is also the recommended host for the following transplantable tumors: mammary adenocarcinoma BW 10232 melanoma B16, myeloid leukaemia C 1498 and preputial gland carcinoma ESR586. The histological and immunohistochemical profiles of the pancreas from C57BL mice have been extensively studied because it has been used as an animal model for non-obese diabetes[5].

Gastrointestinal endocrine cells dispersed in the epithelia and gastric glands of the digestive tract synthesize various types of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract[6]. Thus far, investigations of gastrointestinal endocrine cells have been considered an important part of a phylogenic study[7]. In addition, the regional distributions and relative frequencies of these endocrine cells vary according to the animal species and feeding habits[8]. Many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the gastrointestinal tract (GIT) of various vertebrates including various rodent species. Moreover, there is much data on gastrointestinal endocrine cells in other mouse strains. In Rodentia, the location of endocrine cells in the GIT of the Manchurian chipmunk[9, 10] and gerbil[11] was demonstrated, and the distribution of endocrine cells in the GIT was also detected in the Korean tree squirrel[12, 13]. In addition, Spangeus *et al*[14, 15] investigated the endocrine cells in the GIT of homozygous obese mice, and Pinto *et al*[16] reported that the gastrointestinal endocrine cells in genetically diabetic (db/db) mice had quite different distributional patterns compared to that of nondiabetic control (db/+) mice and abnormalities of the small intestinal[17] and antral[18] endocrine cells in non-obese diabetic mice were also compared to that of normal BALB/cJ mouse were also reported. In addition, changes in the regional distribution and relative frequency of some gastrointestinal endocrine cells in aging mice have also been reported[19-21].

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Although many studies have explained the regional distribution and relative frequency of the different endocrine cells in the GIT of the various vertebrates including various species and strains of rodents, there is a dearth of reports dealing with the endocrine cells in the large intestine of the C57BL/6 mice. This is despite its biological, physiological and anatomical differences from other rodents and its utility in many research fields. The objective of this study was to clarify the regional distribution and relative frequency of endocrine cells in the large intestine of C57BL/6 mice by specific immunohistochemistry using 7 types of antisera against chromogranin A (CGA), serotonin, somatostatin, human pancreatic polypeptide (HPP), glucagon, gastrin and cholecyctokinin (CCK)-8.

Materials and Methods

Five adult male and female C57BL/6 mice (6-wk old, 21-26 body weight upon receipt) were acquired from the Charles River Laboratories (Yokohama, Japan) after acclimatization for one week. The animals were placed at 5 per polycarbonate cage in a temperature (20-25℃) and humidity (30-35%) controlled room during the acclimatization periods. The light : dark cycle was 12hr : 12hr, and food (Samyang, Korea) and water was supplied *ad libitum*. After anesthetizing with ethyl ether, the large intestinal tract of the mice was divided into 3 portions according to the general classification of the mammalian GIT[22]. In order to induce gastric and/or intestinal emptying, the animals were fasted for approximately 24 hours. After phlebotomization, samples from the cecum, colon and rectum 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 an optical microscopic examination of the normal gastrointestinal architecture.

The each representative section was deparaffinized, rehydrated and immunostained using the peroxidase-anti peroxidase (PAP) method[23]. The nonspecific reactions were blocked with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS; 0.01M, pH 7.4), the sections were incubated in a secondary antiserum. They were subsequently washed in a PBS buffer and the PAP complex was finally prepared. The peroxidase reaction was carried out in a solution of 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive (IR) cells were observed by optical microscopy.

The specificity of each immunohistochemical reaction was determined according to the recommendation by Sternberger[23], including the replacement of specific antiserum by the same antiserum, which was preincubated with its corresponding antigen and the relative frequency of the occurrence of each type of IR cell was placed into one of five categories according to their observed numbers.

Results

In this study, three out of seven types of IR endocrine cells were detected using the antisera against CGA, serotonin, somatostatin, HPP, glucagon, gastrin and CCK-8 in the GIT of the C57BL/6 mice (Table 2). Different regional distributions and relative frequencies of these IR cells were observed according to their location of the large intestinal tract, and these differences are shown in Table 2. The regional distribution and relative frequency of the endocrine cells varied according to where in the large intestinal tract they were found, and some peculiar distributional patterns were observed in the C57BL/6 mice. Most of these IR cells in the large intestinal portions were generally spherical or spindle in shape (open-typed cell), while occasionally, round (close-typed cell) cells were also found in the intestinal gland regions.

Table 1. Antisera used in this study

Antisera raised[1]	Code	Source	Diluton
Cg A[2]	A430	DAKO Corp., Carpinteria	1 : 1,000
Serotonin	BO68082C	Bio Genex Lab., San Ramon	1 : 20
Somatostatin	PUO421295	Bio Genex Lab., San Ramon.	1 : 20
HPP[2]	A610	DAKO Corp., Carpinteria	1 : 600
Glucagon	927604	Dia Sorin, Stillwater	1 : 2,000
Gastrin	PUO190796	Bio Genex Lab., San Ramon	1 : 20
CCK-8[2]	750257	Dia Sorin, Stillwater	1 : 500

[1]All antisera were raised in rabbits.

[2]Cg A: chromogranin A, hPP: human pancreatic polypeptide, CCK-8: cholecystokinin-8

Table 2. Regional distributions and relative frequencies of the endocrine cells in the large intestinal tract of the C57BL/6 mouse

	CgA[1]	Serotonin	Som[1]	HPP[1]	Glucagon	Gastrin	CCK-8[1]
Cecum	++[2]	++	−	−	−	−	−
Colon	+++	+++	−	−	+	−	−
Rectum	+	+	−	−	−	−	−

[1] Cg A: chromogranin A, Som: somatostatin, hPP: human pancreatic polypeptide, CCK-8: cholecystokinin-8
[2] Relative frequencies; +++: numerous, ++: moderate, +: a few, ±: rare, −: not detected.

CGA-IR cells

CGA-IR cells were observed throughout the large intestinal tract and they showed highest frequency in the colon (Table 2). They were located in the intestinal glands in the cecum, which were located the basal portion of the mucosa with a moderate frequency. The open and close typed CGA-IR cells were located mainly in the intestinal gland regions. However, no cells were observed in the inter-epithelial cell regions (Fig. 1a, b). In the colon, open typed cells with long cytoplasmic processes were observed in the inter-epithelial cells regions and close typed cells were restricted to the intestinal gland regions with varying frequencies (Fig. 1c, d). In the rectum, the CGA-IR cells were restricted to the basal portions of the acinar cells of the intestinal glands with a low frequency and they were of the open type (Fig. 1e).

Serotonin-IR cells

Serotonin-IR cells were observed throughout the large intestinal tract in various numbers according to each portion of the large intestinal tract and had the highest frequency in the colon (Table 2). In the cecum, they were found in either the inter-epithelial cells or the intestinal glands, which were located in the basal portion of mucosal layer with moderate frequency. The open typed cells were restricted to the inter-epithelial cell regions while the close typed cells were found in the intestinal gland regions (Fig. 2a). The open and closed typed serotonin-IR cells were widely dispersed in the mucosa of the colon with a high frequency, which was similar to that found in the cecum (Fig. 2b). In the rectum, the serotonin-IR cells were restricted to the inter-epithelial cell regions with a low frequency and were open typed (Fig. 2c).

Somatostatin-IR cells

No somatostatin-IR cells were observed throughout the large intestinal tract (Table 2).

HPP-IR cells

No HPP-IR cells were observed throughout the large intestinal tract (Table 2).

Glucagon-IR cells

Close typed glucagon-IR cells were observed in the intestinal glands of the colon (Fig. 3). However, no glucagon-IR cells were observed in the remaining portions of the large intestinal tract of this strain of mouse.

Gastrin-IR cells

No gastrin-IR cells were observed throughout the large intestinal tract (Table 2).

CCK-8-IR cells

No CCK-8-IR cells were observed throughout the large intestinal tract (Table 2).

Discussion

It is generally accepted that endocrine cells in the alimentary tract differ remarkably betweeen animal species in terms of the regional distribution, relative frequency, cell types and each regional part of the GIT. In addition, many studies have investigated the regional distribution and relative frequency of the different endocrine cells in the GIT of various vertebrates including rodents. Moreover, there is a great deal of data regarding the gastrointestinal endocrine cells in mouse strains[15, 16]. Gastrointestinal endocrine cells are generally divided into two types, the round to spherical shaped close-typed cells, which are located in the intestinal gland regions, and the spherical to spindle shaped open typed cells, which are found in the epithelial lining of the intestinal regions. These findings correspond well with the results of this study.CG A belongs to a family of large anionic proteins (CG A, B and secretogranin Ⅱ). Members of this family are found in the secretory granules of a broad spectrum of amine and peptide-producing cells of the adrenal medulla and gastrointestinal endocrine system, and in some neurons of the peptidergic and catecholaminergic nervous system in several mammals[24, 25]. CGs have been found in large variety of endocrine organs and cells outside the adrenal medulla, and they have been reported to be common "markers" for all neuroendocrine cells[26, 27]. Although, reports on the distribution patterns of the CGA-IR cells in the GIT of Rodentia were rare, Hawkins *et*

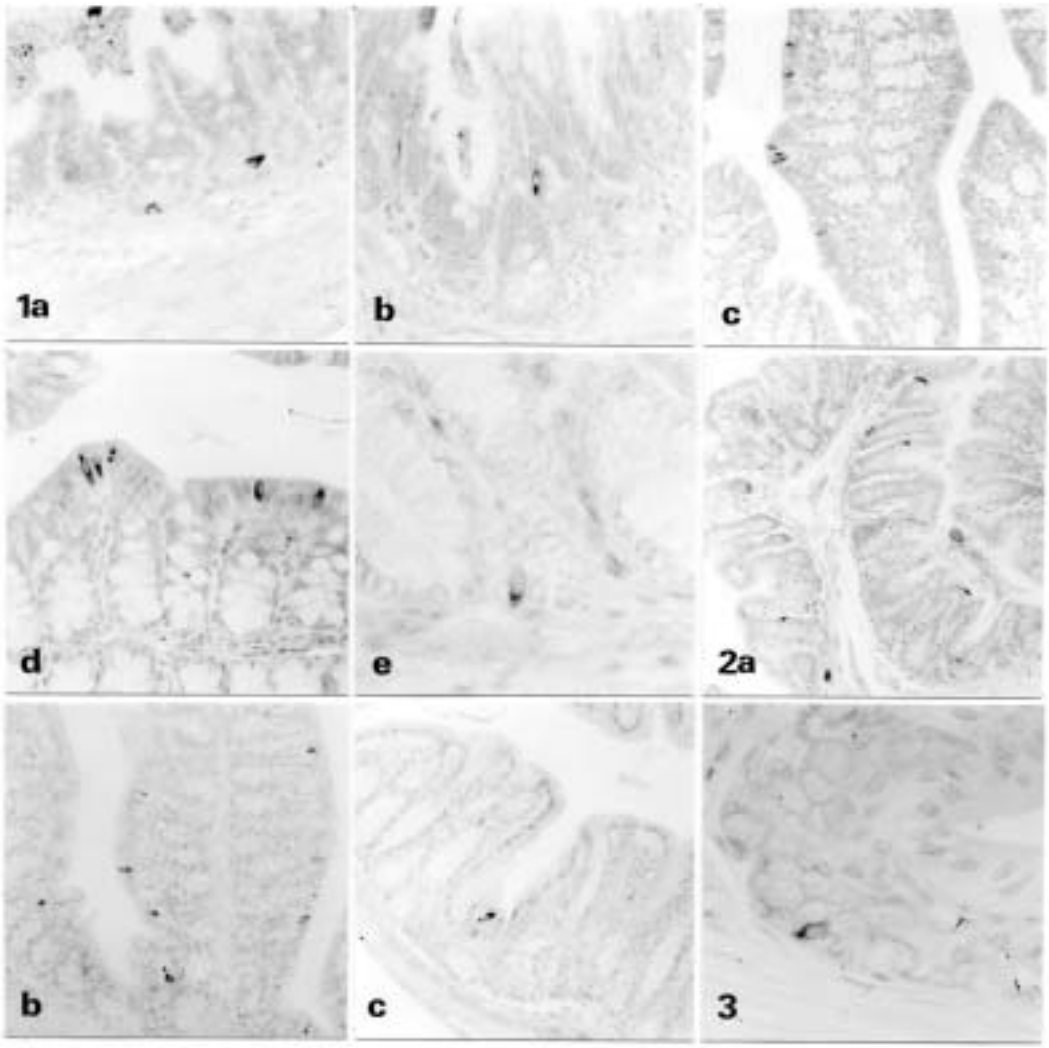

Fig. 1. CGA-IR cells in the large intestinal tract of C57BL/6 mice. Note the various distributions and relative frequencies of these cells throughout whole large intestinal tract. These cells were detected in the cecum (a, b), colon (c, d) and rectum (e). a, b, d: ×240; c: ×120; e: ×480.

Fig. 2. Serotonin-IR cells in the large intestinal tract of C57BL/6 mice. Note the various distributions and relative frequencies of these cells throughout whole large intestinal tract. These cells were detected in the cecum (a), colon (b) and rectum (c). a-c: ×120.

Fig. 3. Glucagon-immunoreactive cells in the large intestinal tract of C57BL/6 mice. Note that they were restricted to the colon. ×480.

al[28] reported CGA-IR cells throughout the whole GIT of 7 species of laboratory animals including mice. In this study, CGA-IR cells were detected throughout the whole large intestinal tract of C57BL/6 mice. These results corresponded well with those of previous studies[24, 25, 28]. However, the single use of CGA as an endocrine cell marker is not recommended, as the relative frequencies of CGA-IR cells are slightly lower than the serotonin- and other IR cells in case of some regions. If mixed or concomitantly immunostained with other types of CGs, then CGs can be considered as a suitable marker of other endocrine cells.

Serotonin consists of monoamines and is widely distributed in the nervous system and gastro-entero-pancreatic endocrine cells[29]. The main functions of serotonin are the inhibition of gastric acid secretion and smooth muscle contraction in the GIT[30]. El-Salhy *et al*[29] reported that serotonin-IR cells were found throughout the GIT of all species and were established in the GIT in the early stage of vertebrate evolution. In addition, these IR cells were detected in the whole alimentary tract including the esophagus of lower vertebrates[31]. Serotonin-IR cells were detected throughout the GIT of the gerbil[11], common tree shrew[32],

Philippine carabao[33], Manchurian chipmunk[10], rat[34] and mouse[21]. In this study, serotonin-IR cells were found throughout the whole large intestinal tract and exhibited the highest frequencies in the colon. These results are similar to those reported for most other mammals[10, 11, 21, 29, 31-34].

Somatostatin consisting of 14 amino acids was isolated from the hypothalamus of sheep for the first time, and can be divided into the straight form and cyclic form[35]. This substance inhibits the secretion of the other neuroendocrine hormones[36]. It is known that somatostatin-IR cells show have the widest distribution in the whole GIT except for the large intestine of all vertebrate species investigated, including primitive agnathans with serotonin-IR cells[37]. However, species-dependent variations on the distributional pattern of these IR cells have been reported. In the GIT of the Manchurian chipmunk, they were detected throughout the whole GIT and showed the highest frequencies in the pylorus[10]. However, they were restricted to the pylorus of the gerbil[11]. In mouse strains, a decrease in the number of somatostatin-IR cells in the duodenum of aging NMRI mice[21] and the antral of diabetic mouse regardless of whether they were obese, has been reported[15, 18]. In this study, somatostatin-IR cells were not detected in the large intestinal tract of C57BL/6 mice.

Since PP was isolated from an insulin extraction of the pancreas in 1961, the regional distribution of PP-IR cells in mammalian species was relatively well known. However, species-depended differences exist among mammals[10-12, 32, 33]. These IR cells have been found in areas from the fundus to the jejunum of the Manchurian chipmunk[10] but no cells were detected in the GIT of gerbils[11]. In this study, no HPP-IR cells were found in the large intestinal tract. Glucagon is synthesized in the A cells of the pancreas and regulates the serum glucose levels. These IR cells are found in various mammals, and they have been demonstrated in the GIT of the common tree shrew[32] and musk shrew[38]. However, Baltazar *et al*[33] suggested that these IR cells could only be detected in the intestinal tract of the *Philippine carabao* and Lee *et al*[12] reported that they were restricted to the cardia and fundus of the Korean tree squirrel. In addition, glucagon-IR cells were identified in the stomach and small intestine of the Manchurian chipmunk[9]. Overall, the distributional patterns of glucagon-IR cells in the GIT of mammals show species-dependent variations. In particular, appearances of these IR cells in the large intestine have also been reported in mice[14, 19]. However, no glucagon-IR cells were found in the GIT of the gerbil[11]. In this study, the glucagon-IR cells were restricted to the colon with a low frequency. These findings are quite different from those of previous studies[11-14, 19, 32, 38], and these differences are considered to be species and/or strain-dependent variations.

It is generally accepted that gastrin and CCK-8 originated from same ancestor. In the human duodenum, a large fraction of these cells, besides reacting with the non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also show immunoreactivity with the C-terminal gastrin-34 antibodies, co-localized with CCK in varying portions of secretory granules[39]. Gastrin secreted by intestinal G cells, promote gastric acid secretion, and the CCK secreted by intestinal I cells stimulates pancreatic enzyme secretion. In this study, gastrin- and CCK-8-IR cells were not found in the large intestinal tract.

In conclusion, some characteristic differences compared to previous reports were observed in the present study. These differences were attributed to differences in the antisera tested or the methods and/or species differences used in each study[40-42].

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