Cluster Formation of Basal-Granulated Cells in the Intestinal Villi of the Fetal Japanese Monkey (*Macaca fuscata fuscata*)

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Abstract Segi’s cap, an aggregation of the basal-granulated cells in the intestinal villi of the fetal Japanese monkey was investigated by means of light microscopy and immunocytochemical techniques. Characteristic Segi’s caps were observed at the tips of the villi in the duodenum and upper jejunum in fetal Japanese monkeys, aged 120 and 140 days. The Segi’s cap of Japanese monkeys is characterized by the following morphological features: 1) There are relatively fewer caps per section in comparison with humans. 2) The number of basal-granulated cells of the cap is also less than in humans. 3) The characteristic indentation at the center of the Segi’s cap, reported in humans, cattle and pigs is only rarely present. 4) Argentaffin cells, which are now known as serotonin-containing cells, are present in the monkey Segi’s cap. 5) Somatostatin-, motilin- and CCK-immunoreactive cells are found in the monkey Segi’s cap.

Key Words: Segi’s cap, basal-granulated cells, immunocytochemistry, intestinal villi, Japanese monkey fetus

INTRODUCTION

Gut hormones, such as secretin, gastrin, and motilin are secreted by basal-granulated cells at particular levels of the gut. These cells are dispersed within the epithelium of the gastroenteric mucosa, and it is a peculiarity of these cells that they are not assembled into a mass (Fujita and Kobayashi, 1977; Grube and Forssmann, 1979). An obvious exception was noted by Segi (1935, 1936), who described large aggregations of basal-granulated cells at the tips of the villi in the duodenum and upper jejunum of human fetuses aged five or more months. In recent years, Kobayashi *et al.* (1980) confirmed the Segi’s findings and named the structure “Segi’s cap.” Subsequently, Iwanaga *et al.* (1980) have shown that gastrin-, somatostatin- and motilin-immunoreactive cells are present in the Segi’s cap of the human fetus. Similar clusters of basal-granulated cells also have been reported in the cattle and pig fetuses in the latter half of pregnancy (Yamada *et al.*, 1981). At present, information concerning Segi’s cap is completely lacking in species other than those listed above. Thus, from the comparative point of view it is pertinent to ask whether this particular structure occurs in nonhuman primates. Furthermore, fetal development of this structure has been studied so far only in
humans (Segi, 1935, 1936).

In the present study, we have examined whether the Segi’s cap is present in the Japanese monkey fetus, and further, we have examined the ontogenetic development of this structure by means of light microscopy and by use of immunocytochemical methods.

MATERIALS AND METHODS

Animals

Animals used in this study were fetal and newborn Japanese monkeys (Macaca fuscata fuscata). The gestational period of Japanese monkeys has been reported to be 167±7 days in our indoor colony (Shimizu, 1988). Accordingly, the following four developmental stages were chosen for study: (1) 60±5 days of gestation (n=2), (2) 120±5 days of gestation (n=2), (3) 140±5 days of gestation (n=2), and (4) newborn within 24 hrs after delivery (n=2). The studied newborns were normally delivered at 162±5 and 169±5 days of gestation.

To determine the gestational ages of the fetuses, we employed the timed mating method, and monitored the length of the biparietal diameter of the fetus periodically, using ultrasonography (Shimizu, 1988). The fetuses were obtained by Cesarean section under Ketamin hydrochloride and halothan anesthesia according to “The Guide for the Care and Use of Laboratory Primates,” a protocol prepared by the Primate Research Institute, Kyoto University, Japan (1986).

Tissue preparation

Fetal and newborn monkeys were deeply anesthetized by use of sodium pentobarbital (Nembutal, 35 mg/kg i.p.) and were perfused through the heart with 0.01 M phosphate buffered saline (pH 7.4), followed by perfusion of sublimated Bouin-Holland solution. The intestinal tissues were dissected out, and dehydrated through a series of increasing concentrations of ethanol. To remove deposits of mercuric chloride, after 90% ethanol, the tissues were immersed in a solution containing iodine-potassium iodide in 90% ethanol for 24 hrs. After embedding in Paraplast, serial sections 6 μm in thickness were cut and mounted on glass slides pretreated with egg albumin.

Silver impregnation and classical tinctorial stain

Argyrophil reactivity of the basal-granulated cells was studied by use of the method described by Grimelius (1968). Enterochromaffin cells were visualized by using Masson-Hamperl’s method (Singh, 1962) or a Fontana-Masson method (Masson, 1928). Azan stain was performed for general histological observations.
**Immunocytochemistry**

Immunocytochemical staining was performed by use of a Vectastain avidin-biotin peroxides complex (ABC) kit (Vector Labs, Burlingame, CA). In brief, sections were deparaffinized in xylene, hydrated in a graded ethanol series, and washed in phosphate-buffered saline (PBS). They were incubated for 30 min in methanol containing 0.3% hydrogen peroxide to block endogenous peroxidase activity, and washed in PBS. Then they were treated with normal blocking serum for 30 min to reduce nonspecific staining. Primary antisera were applied to the sections for 1 to 2 hrs, and biotinylated secondary antibody solution, and ABC reagents were each applied for 1 hr. The final reaction product was visualized with 3,3'-diaminobenzidine tetrahydrochloride in 0.003% hydrogen peroxide in Tris-HCl buffer, pH 7.4. The sections were then counterstained with hematoxylin, washed in running water, dehydrated through an increased ethanol series, cleared in xylene and coverslipped. All procedures were performed at room temperature, and incubations of sections with antibodies and reagents were performed in closed moist chambers.

The following primary rabbit antibodies were used: (1) anti-mammalian somatostatin-14, obtained from Polysciences, lot 10963 (Nozaki et al., 1988a), working dilution 1:500; (2) anti-motilin was obtained from Cambridge Research Biochemicals, lot 06429, working dilution 1:5000; (3) anti-porcine cholecystokinin (CCK)-27 was kindly supplied by Dr. N. Yanaihara, Shizuoka Prefectural University, Shizuoka, Japan, working dilution 1:1800; (4) anti-human-glucagon was raised by M. Nozaki (Nozaki et al., 1988b), working dilution 1:4000; (5) anti-bovine pancreatic polypeptide (PP) was kindly supplied by Dr. E. M. Plisetskaya, University of Washington, Seattle, U.S.A., working dilution 1:1000; (6) anti-porcine vasoactive intestinal polypeptide (VIP) was kindly supplied by Dr. N. Yanaihara, working dilution 1:450.

The specificities of the immunocytochemical reactions were checked by these methods: (1) incubation of the sections with normal rabbit sera in place of the primary antisera; and (2) incubation of the sections with antigen-inactivated antisera.

**RESULTS**

**Segi’s cap formation**

Characteristically, the so-called Segi’s cap is an aggregation of the basal-granulated cells at the tips of the villi in the duodenum and the upper part of the jejunum. It could be observed in fetal Japanese monkeys, aged 120 and 140 days (Fig. 1). Although the number of Segi’s caps varied among sections, they were relatively most abundant in the duodenum, and least in the jejunum. Segi’s caps were more numerous in the fetuses aged 140 days than in those aged 120 days. Individual caps consisted of approximately 10 to 20 basal-granulated cells and a few goblet cells...
Fig. 1. Two successive sections (a and b) and an adjacent section (c) of the 140–days fetal duodenum. Segi’s caps are shown at the tip of a villus after Azan stain (a and c) and Grimelius’ silver impregnation (b). In (b), note two argyrophil cells in the Segi’s cap (arrowhead). The individual cap consisted of approximately ten to twenty basal-granulated cells. A typical indent is shown at the center of the aggregated basal-granulated cells (arrow in c). ×390
Segi’s Cap in the Japanese Monkey Fetus

(Fig. 1). The basal-granulated cells were generally grouped in the deeper part of the epithelium and most of them extended cytoplasmic processes to the intestinal lumen between other epithelial cells (Fig. 1). The aggregations of these cells were, as a whole, fingertip-shaped, but sometimes they were shallow dish-shaped (Fig. 1). Such aggregations of basal-granulated cells were not observed on the villi of the ileum, appendix, colon or rectum at any of the ages of fetuses listed above. Segi’s caps were not observed anywhere in the intestines of 60-days fetus or in newborns.

Argyrophil cells

Following Grimelius’ silver impregnation, many basal-granulated cells in the intestine were stained. These Grimelius-positive, argyrophil cells were dispersed in the duodenal intestinal mucosa of fetuses from 60 to 140 days of gestation. In fetuses aged 120 and 140 days, some of the argyrophil cells were aggregated at the tips of the duodenal villi, forming a so-called Segi’s cap cluster (Fig. 2). Thus, the majority of the basal-granulated cells were silver-stained positively. Clusters of argyrophil cells were rarely observed in the upper part of the jejunum, and were not found in the ileum or the large intestine of any of the developmental stages examined.

Fig. 2. Argyrophil cells in a Segi’s cap of the 140-days fetal duodenum stained by Grimelius’ silver impregnation. Aggregations of basal-granulated cells are shown at and near the tip of the villus. ×410
Enterochromaffin cells

Following the application of Masson-Hamperl’s method, as modified by Singh (1962), which stains finely granulated argyrophilic granules of the enterochromaffin cells (or argentaffin cells), positive reaction was observed in the basal-granulated cells of the villi and the intestinal gland in the small intestine and appendix at all stages examined. Cells so stained are now known to be serotonin containing cells (Sano, 1976, pp. 589–591). In particular, numerous enterochromaffin cells were found in the mucosa of the duodenum. They comprised less than 50% of the total populations of argyrophilic cells of the Segi’s cap structures in the duodenum (Fig. 3).

Summary of distribution of other gut peptide-containing cells

1) Somatostatin-immunoreactive cells were demonstrated throughout the intestine at all developmental stages examined. They were generally scattered at the deeper or basal zones of the epithelium of the villi and the intestinal glands but rarely they were found at the apical parts of villi. They were relatively numerous in the duodenum and jejunum, but they were very rare in the large intestine. 2) Motilin-immunoreactive cells were found in the epithelium of the villi throughout

Fig. 3. Adjacent section of Fig. 2. Enterochromaffin cells in a Segi’s cap of the 140-days fetal duodenum. They occupied about 50% of the total populations of argyrophilic cells of the Segi’s cap formation. Masson-Hamperl’s method modified by Singh. ×410
the intestine at all stages examined. They were more numerous in the small intestine than in the large intestine. 3) CCK-immunoreactive cells were observed in the epithelium of the villi and the intestinal glands, but only in the small intestine at all stages examined. 4) Glucagon-immunoreactive cells were scattered in the epithelium of the small intestine at all stages examined, but they were not observed in the large intestine. 5) PP-immunoreactive cells were found throughout the intestine at all stages examined. They were dispersed in the basal zone of the villar epithelium and the intestinal glands. They were numerous in the duodenum and jejunum, but were very rare in the large intestine. 6) VIP-immunoreactive cells could not be found in the intestinal mucosa at any of stages examined.

The numbers of cells immunoreactive to above-mentioned peptides listed above increased in correlation with developmental ages, but were relatively few in fetuses aged 60-days. The shapes of the individual immunoreactive cells were not significantly different among stages.

*Occurrence of gut peptide-containing cells in the Segi's cap*

Of the 6 peptides examined, only somatostatin-, motilin-, and CCK-immunoreactive cells were found in Segi's caps (Figs. 4a–c). However, they accounted for less than 10% of the total population of basal-granulated cells of the Segi's caps.

**DISCUSSION**

The present study clearly shows that Segi’s caps are limited to the duodenum and upper jejunum of the fetal Japanese monkeys aged 120 and 140 days. Since the gestational period of Japanese monkeys has been reported to be 167±7 day in our colony (Shimizu, 1988), our study shows that the Segi’s cap is present in the intestine in fetal Japanese monkeys in late pregnancy. In his earlier studies, Segi (1935, 1936) reported that the characteristic accumulations of basal granulated cells was observed in human fetuses after 5 months of gestation. In cattle and pigs, Segi’s caps were reported in the middle and late periods of pregnancy (Yamada et al., 1981). In sum, prior studies indicate that Segi’s cap is formed in the intestine after the mid-pregnancy.

The Segi’s caps of Japanese monkeys are characterized by the following morphological features: 1) The number of caps per section is relatively few in comparison with those reported in humans (Segi, 1935, 1936). 2) The number of basal-granulated cells of the cap is also less than in humans (Segi, 1935, 1936). 3) The characteristic indentation at the center of the Segi’s cap reported in humans (Segi, 1935, 1936) and cattle and pigs (Yamada et al., 1980) is only rarely observed in monkeys. 4) Argentaffin cells, which are now considered to be serotonin-containing cells, are present in the Segi’s caps of Japanese monkeys, but the numbers of
Fig. 4. Three closely adjacent, but not successive sections (a, b, c) of a duodenal villus of the 140 days fetus. Somatostatin- (a), motilin- (b) and CCK-immunoreactive cells (c) are shown in a Segi’s cap. ×340
argentaffin cells in the cap are much lower than in humans, in which about a half of the basal-granulated cells in the Segi’s cap is reportedly argentaffin cells (Iwanaga et al., 1980). 5) In agreement with previous studies in humans (Iwanaga et al., 1980), cattle and pigs (Yamada et al., 1981), somatostatin-, motilin- and CCK-immunoreactive cells are observed in the Segi’s cap of Japanese monkeys.

In this study, Segi’s caps were not seen in the intestines of newborn monkeys obtained within 24 hrs after delivery. This result is in accordance with the reports of Segi (1935, 1936) in the human. Although the timing of disappearance of Segi’s caps from the intestine is not clear, it may be soon after delivery, when the intestine has begun to digest and absorb the milk. It is most likely that the newborn monkeys used in this study had already suckled at the mother’s breast before sampling.

The functional significance of Segi’s cap in the fetus is not known. However, the fact that the basal-granulated cells of the Segi’s cap are located at the tips of the villi, and are in contact with the intestinal lumen, invites some speculations. It is possible that cells of the cap contact amniotic fluid in the digestive tract and they may receive certain chemical and/or osmotic information from the amniotic fluid. Recent studies have shown that the human fetus drinks amniotic fluid through the mouth, and that amniotic fluid contains high concentrations of prolactin (Riddick and Daly, 1982). Segi’s cap may also be involved in the production of meconium from the substances carried by amniotic fluid. Furthermore, since we have shown that there are some endocrine cells in Segi’s cap, this structure may be the intestinal hormone-producing organ functioning particularly in the later period of gestation.

In conclusion, Segi’s cap, an aggregation of basal-granulated cells was observed at the tips of the villi of the duodenum and the upper jejunum of the fetal Japanese monkey at 120 and 140 days of gestation. Moreover, somatostatin-, motilin-, and CCK-immunoreactive cells, and presumable serotonin containing cells, were consistent constituents of the Segi’s caps of the Japanese monkey fetus. In this respect they resemble other mammalian species so far examined.

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REFERENCES


