Korean J Vet Res(1999) 39(4) : 673~678

Immunohistochemical study on the insulin-immunoreactive cells in the developing pancreas of the Korean native goat (*Capra hircus*)

Sae-kwang Ku, Hyeung-sik Lee*, Jae-hyun Lee

Laboratory of Histology, College of Veterinary Medicine, Kyungpook National University
*Department of Biology, Faculty of Basic Science, Kyungsan University**
(Received Feb 5, 1999)

Abstract : The distribution and relative frequency of insulin-immunoreactive cells in the pancreas was studied during developmental stages (fetus, neonate, 1-month-old, 6-month-old and adult) of the Korean native goat by immunohistochemical methods.

The different distribution and relative frequency of glucagon-immunoreactive cells in the pancreas of the Korean native goat was observed during development. Insulin-immunoreactive cells were detected in the exocrine and endocrine portions (pancreatic islets) of the all ages, and in the duct of the 6-month-old. The relative frequencies of these cells were increased in the pancreatic islets with ages but decreased in the exocrine portions. Generally, they were distributed in the interacinar spaces or central zone of the pancreatic islets in all ages. However, the distributions and relative frequencies in the pancreatic islets of the neonate Korean native goat were divided into three patterns : 1) located in the inner zone with numerous frequencies, 2) the peripheral zone of the pancreatic islet with moderate frequencies and 3) the peripheral zone of the pancreatic islet with a few frequencies patterns.

Key words : developing pancreas, immunohistochemistry, Korean native goat, insulin cell.

Introduction

The pancreas of vertebrates were consisted of two seprated parts : the exocrine and endocrine portions (pancreatic islet or islet of Langerhans). The morphological feature and position of the pancreas in the vertebrates were varied among the animals[1]. In the Korean native goat, this organ was divided into 3 parts : body, left and right lobes[2] as the other ruminants[3].

Insulin is synthesized and secreted by the B cells in the islets of Langerhans and consists of two peptide chains, A

Address reprint requests to Dr. Sae-kwang Ku, Laboratory of Histology, College of Veterinary Medicine, Kyungpook National University, Taegu 702-701, Republic of Korea.

and B, which are linked by two disulfide bridges. The major functions of insulin are to control the movement of glucose into cells and intracellular metabolism of glucose[4].

Many studies have been elucidated the distribution and relative frequency of the insulin-immunoreactive cells in the pancreas of various vertebrates[2,5,6], and a few informations that dealt with the changes of the distribution and relative frequency of these cells with developing stages are available [7-10]. In addition to, there are no reports on the change of the distribution and relative frequency of insulin-immunoreactive cells in the developing pancreas of the Korean native goat except for the adult stages[2,5].

In the present study, the distribution and relative frequency of insulin-immunoreactive cells in the pancreas of the Korean native goat were investigated in the 5 stages of development: fetus, neonate, 1-month-old, 6-month-old and adult.

Materials and Methods

Each of 5 healthy fetus (180 days of gestation), neonate, 1-month-old, 6-month-old and adult Korean native goats (*Capra hircus*) were used without sexual distinction. The animals were anesthetized with Rompun® (Bayer, Korea), then the canular was inserted into the left common carotid artery to bleed. After phlebotomized, the pancreas was sampled. The sampled pancreas was fixed in Bouin's fluid. After paraffin embedding, 3~4μm serial sections were prepared with routine methods. Each representative sections were deparaffinized, rehydrated and immunostained with the peroxidase antiperoxi-dase (PAP) methods[11]. Background blocking was performed with normal goat serum prior to incubation with the anti-guinea pig insulin antiserum (BioGenex Lab, USA). After rinsing in phosphate buffered saline (PBS, 0.01M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS 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 immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and immunoreactive cells were observed under light microscope.

Results

According to the developmental stages, changes of the regional distribution and relative frequency of insulin-immunoreactive cells were observed, and these changes are showed in Table 1. Insulin-immunoreactive cells were detected in the exocrine and pancreatic islets of the all stages, and in the duct of the 6-month-old (Table 1). These cells were spherical, round or spindle shaped (Fig 1, 2).

Fetus : Insulin-immunoreactive cells were observed in the pancreatic islets and exocrine portions. Numerous cells were dispersed in the interacinus space of the exocrine portion and these cells were singly or clustered distributed (Fig 1a). Also numerous immunoreactive cells were detected in the inner zone of the pancreatic islets (Fig 2a). However, no cells were observed in the duct.

Table 1. The regional distribution and relative frequency of insulin-immunoractive cells in the pancreas of the Korean native goat with development stages

Groups	Exocrine	Endocrine	Duct
Fetus	+++	+++	-
Neonate	+++	+++	-
1-month-old	++	+++	-
6-month-old	++	+++	+
Adult	+	+++	-

Remark: +++; numerous, ++; moderated, +; a few, -; not detected.

Neonate : Insulin-immunoreactive cells were found in the pancreatic islets and exocrine portions. In the exocrine portions, these cells were singly observed in the interacinus space with numerous frequencies (Fig 1b). In the pancreatic islets, numerous immunoreactive cells were detected. In addition to, the distributions of these cells were divided into three patterns : 1) located in the inner zone with numerous frequencies (Fig 2b), 2) the peripheral zone of the pancreatic islet with moderate frequencies (Fig 2c) and 3) the peripheral zone of the pancreatic islet with a few frequencies (Fig 2d) patterns. However, no cells were observed in the duct.

1-month-old : Insulin-immunoreactive cells were observed in the pancreatic islets and exocrine portions. Single or groups of 2~3 cells were detected in the interacinus space of exocrine portions with moderate frequencies (Fig 1c). Clusters of numerous cells were found in the inner zone of the pancreatic islets (Fig 2e). However, no cells were observed in the duct.

6-month-old : Insulin-immunoreactive cells were detected in the pancreatic islets, exocrine portions and duct. Clusters of a few immunoreactive cells were observed in the subepithelial connective tissues of the pancreatic duct (Fig 3) and single or groups of 2~3 cells were detected in the interacinus space of the exocrine portions with moderate frequencies (Fig 1d). Clusters that were consisted of numerous cells were found in the inner zone of the pancreatic islets.

Adult : Insulin-immunoreactive cells were detected in the exocrine portions (Fig 1e) and pancreatic islets (Fig 2f), and the distributions and relative frequencies of these cells were similar to those of the 6-month-old.

Discussion

Insulin is synthesized in the B cells of the pancreatic islets and regulated the serum glucose levels[4]. The distribution and relative frequency of the insulin-immunoreactive cells in the pancreas was reported in the human[12], horse[13], echidna[14], various laboratory animals[6], birds[5], amphibians[15,16], reptile[17] and fish[18]. According to the previous studies[5,6,12~18], insulin-immunoreactive cells were observed in the pancreatic islets, interacinus space of the exocrine portions and duct. But the distributions of these cells are quite different among the species of animals. These immunoreactive cells were dispersed throughout the whole pancreatic islets of the human[12], baboon[19] and guinea pig[20], and located in the central zone of the pancreatic islets of the reptile[17] and fish[18,21]. However, Hiratsuka *et al*[22] reported that according to the sampling portions, the different distribution of insulin-immunoreactive cells were observed in the pancreatic islets of the cattle.

The changes of the distribution and relative frequency of the insulin-immunoreactive cells in the pancreas were reported in the rat[7], guinea pig[8] and opossum[9]. According to the previous studies[7~9], the endocrine pancreas continues to differentiate and develop throughout postnatal life and into adulthood, and the distribution of insulin-immunoreactive cells are quite different from the development stages but there were no reports that demonstrated the different distribution of insulin-immunoreactive in the pancreatic islets.

In the present study, insulin-immunoreactive cells were detected in the exocrine and endocrine portions (pancreatic islets) of the all ages, and in the ducts of the 6-month-old. The relative frequencies of these cells were increased in the pancreatic islets with ages but decreased in the exocrine portions. Generally, they were distributed in the interacinar spaces or inner zone of the pancreatic islets in all ages. These results are similar to the other mammals[6,12,13,19,20]. However, the distributions and relative frequencies in the pancreatic islets of the neonate Korean native goat were divided into three patterns : 1) located in the inner zone with numerous frequencies, 2) the peripheral zone of the pancreatic islet with moderate frequencies and 3) the peripheral zone of the pancreatic islet with a few frequencies patterns. These changes in the pancreatic islets with the developmental stages are somewhat different from the other mammals[7~9].

In conclusion, the different distribution and relative frequency of insulin-immunoreactive cells in the pancreas of the Korean native goat was observed during development. It is suggest that the changing of feeding habits and physiological conditions during different age causing this differences.

Legends for figures

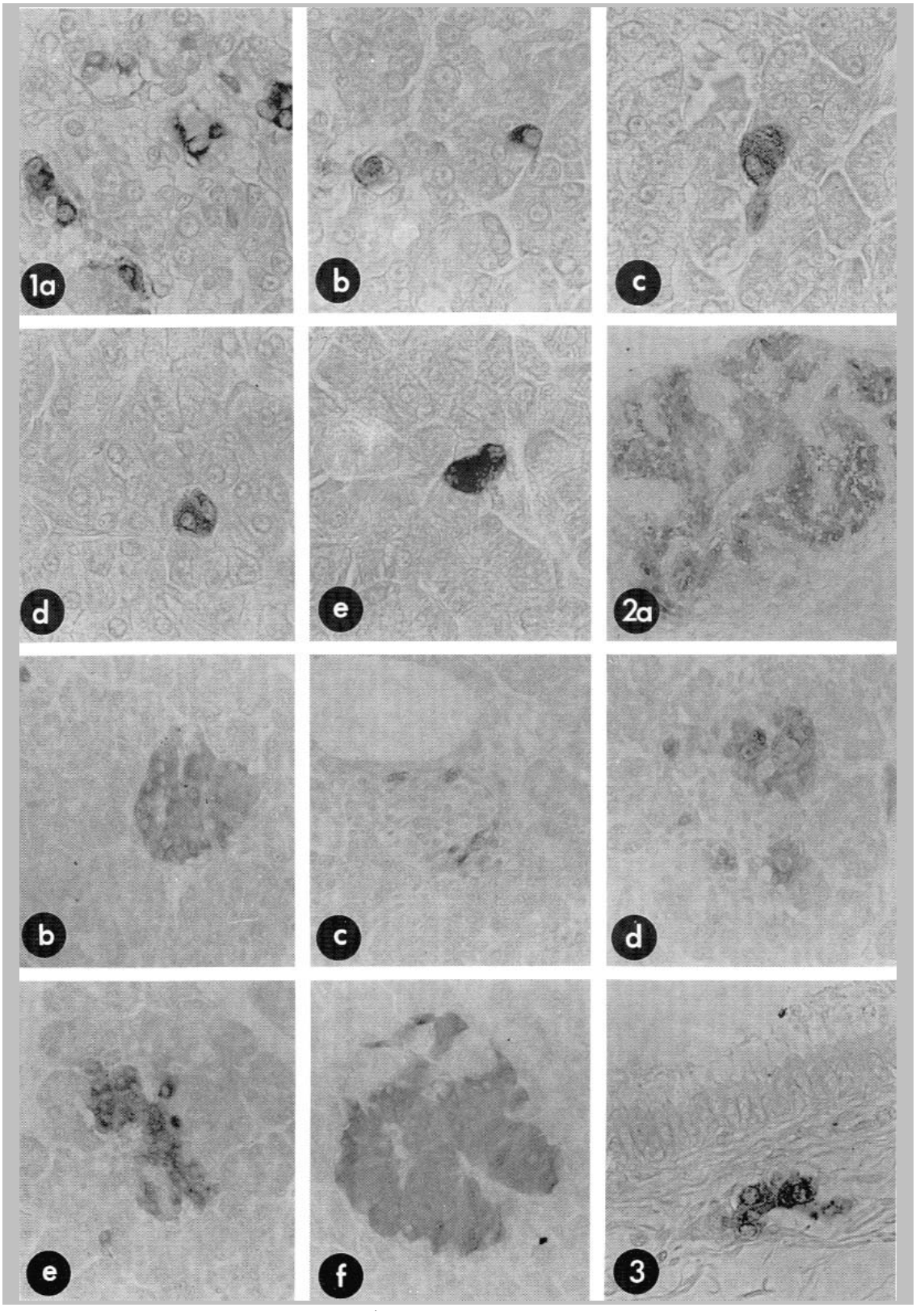
Fig 1. Insulin-immunoreactive cells in the exocrine portions of the pancreas of the Korean native goat.
a. Fetus b. Neonate c. 1-month-old
d. 6-month-old e. adult
a-e : × 480, PAP methods.

Fig 2. Insulin-immunoreactive cells in the pancreatic islets of the pancreas of the Korean native goat.
a. Fetus b-d. Neonate
e. 1-month-old f. adult
a : × 120, b-e : × 240, PAP methods.

Fig 3. Insulin-immunoreactive cells in the duct of the pancreas of the Korean native goat.
× 480, PAP methods.

Reference

1. Dellmann H, Brown EM. Textbook of veterinary histology. 3rd ed, Saunders Company, Philadelphia, 1987.
2. Lee HS, Lee IS, Kang TC, *et al*. Immunohistochemical study on the insulin-, glucagon-, somatostatin- and pancreatic polypeptide secreting cells in Korean native goat. *Korean J Vet Res*, 35:45-54, 1995.
3. Nickel R, Schummer A, Seiferle E. The visera of the domestic mammals. 2nd ed, Verlag Paul Parey, Berlin, 1979.
4. Hsu WH, Crump MH. The endocrine pancreas. In McDonald LE, Pineda MH eds, Veterinary endocrinology and reproduction. Lea & Febiger, Philadelphia, pp 186-201, 1989.
5. Lee JH, Lee HS. Immunohistochemical studies of the pancreatic endocrine cells of the various animals. *Korean J Vet Res*, 32:497-510, 1992.
6. Wieczoreck G, Pospischil A, Perentes E. A comparative immunohistochemical study of pancreatic islets in laboratory animals (rats, dogs, minipigs, nonhuman primates). *Exp Toxicol Pathol*, 50:151-172, 1998.
7. Fujii S. Development of pancreatic endocrine cells in the rat fetus. *Arch Histol Jap*, 42:467-479, 1979.
8. Reddy SN, Bibby NJ, Elliott RB. Cellular distribution of insulin, glucagon, pancreatic polypeptide hormone and somatostatin in the fetal and adult pancreas of the guinea pig : a comparative immunohistochemical study. *Eur J Cell Biol*, 38:301-305, 1985.
9. Krause WJ, Cutts JH Ⅲ, Cutts JH, *et al*. Immunohistochemical study of the developing endocrine pancreas of the opossum (*Didelphis virginiana*). *Acta Anat*, 135: 84-96, 1989.
10. Bagnell CA, Baker NK, McMurtry JP. Immunohistochemical localization of insulin in the chicken embryo during development. *Gen Comp Endocrinol*, 73:293-298, 1989.
11. Sternberger LA (1979) Immunocytochemistry. 3rd ed, John Wiley, New York.
12. Erlandson SL, Hegre OD, Parsons JA, *et al*. Pancreatic islet cell hormones distribution of cell types in the islet and evidence for the presence of somatostatin and gastrin within the D cells. *J Histochem Cytochem*,


1a
b
c
d
e
2a
b
c
d
e
f
3

24:883-897, 1976.

13. Helmstaedter V, Feurle GE, Forssmann WG. Insulin-, glucagon-, and somatostatin-immunoreactive endocrine cells in the equine pancreas. *Cell Tiss Res*, 172:447-454, 1976.
14. Yamada J, Krause WJ, Edwin N, *et al*. A survey of endocrine cells in the pancreas of the echidna (*Tachyglossus aculeatus*) with special reference to pancreatic motilin cells. *J Anat*, 171:223-231, 1990.
15. Foty RA, Lai-Fook JE, Liversage RA. Localization of insulin, glucagon and somatostatin in the pancreas of adult newt, *Notophthalmus viridescens*. *Tissue Cell*, 21:1-10, 1989.
16. Kaung HC, Elde RP. Distribution and morphometric quantitative of pancreatic endocrine cell types in the frog, *Rana pipens*. *Anat Rec*, 196:173-181, 1980.
17. Rhoten WB. Immunohistochemical localization of four hormones in the pancreas of the garter snake, *Thamnophis sirtalis*. *Anat Rec*, 208:233-242, 1984.
18. Youson JH, Elliott WM, Beamish RJ, *et al*. A comparison of endocrine pancreas tissue in adults of four species of lamprey in British Columbia: a morphological and immunohistochemical study. *Gen Comp Endocrinol*, 70:247-261, 1988.
19. Wolfe-Coote SA, Du Toit DF. Morphology and endocrine production of cells in the islets of Langerhans of chacma baboon. *Anat Res*, 218:56-65, 1987.
20. Baskin DG, Gorray KC, Fujimoto WY. Immunocytochemical identification of cells containing insulin, glucagon, somatostatin and pancreatic polypeptide in the islets of Langerhans of guinea pig with light and electron microscopy. *Anat Rec*, 208:567-578, 1984.
21. Hansen GN, Hansen BL, Jorgensen PN. Insulin-, glucagon- and somatostatin-like immunoreactivity in the endocrine pancreas of lungfish, *Neoceratodus forsteri*. *Cell Tiss Res*, 248:253-261, 1987.
22. Hirastuka T, Abe M, Takehana K, *et al*. Immuno-histochemical analysis of the endocrine cells in the pancreatic islets of cattle. *Okajima Folia Anat Jpn*, 72: 285-295, 1996.