Endocrine Cell Populations in the Pancreas of Diabetic WBN/Kob Rats

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(Received 17 September 1991/ Accepted 13 January 1992)

ABSTRACT. Numerical changes of insulin-, glucagon- and somatostatin-positive cells in the pancreas of WBN/Kob male rats with spontaneously occurring diabetes were examined. The rats examined were divided into three different age groups: Groups I (12 weeks old) and II (33 weeks old) were clinically prediabetic and group III (60-90 weeks old) was diabetic. Serum glucose value was in the normal range in groups I and II, while it was much higher in group III. B and A cells were markedly decreased in number in groups II and III. In group II, the ratio of B to A cells was normally preserved, though the total endocrine cell number was markedly decreased as compared with that in group I. In group III, the percentage of B cells was decreased significantly. The normal ratio in group II seemed to keep serum glucose within the normal level. In addition to the total endocrine cell reduction, an altered ratio of B and A cells was considered to cause the diabetic condition.

KEY WORDS: diabetes, glucagon, insulin, somatostatin, WBN/Kob rat.


The WBN/Kob rat is a newly established diabetic strain [22]. The diabetic condition is characterized by long-lasting hyperglycemia, glycosuria, polyuria and polydipsia, and develops in aged males showing late onset (approximately 9 months of age), with primary histological lesions in the pancreas [12, 22]. In this animal strain, pancreatic lesions occur after showing the impaired glucose tolerance in the younger period and are progressive with age [6, 22]. The advanced stages of the condition are associated with diabetic complications such as nephropathy, cataract, and peripheral motor neuropathy [6, 10, 12, 15, 21, 23]. Therefore, this rat strain is considered to be a useful animal model for human type II, non-insulin dependent diabetes mellitus (NIDDM).

Histologically, the pancreas of diabetic aged male WBN/Kob rats revealed atrophy of both exocrine and endocrine tissues with diffuse fibrosis and fatty infiltration [22]. These end-stage lesions are a sequel to focal to multi focal widespread inflammatory changes in the prediabetic young males. However, the pathogenesis of the initial inflammatory changes and hyperglycemia in this rat strain is still obscure.

Quantitative investigations of pancreatic endocrine cells in nondiabetic and diabetic humans and animals have been performed by immunofluorescence or immunohistochemical methods [7, 9, 19, 20].

This paper describes numerical changes of endocrine cells in WBN/Kob rats of different ages and refers to the histopathogenesis of diabetes mellitus in the rat.

MATERIALS AND METHODS

Animals: Thirty male WBN/Kob rats from an inbred colony (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) was used in this study. They were divided into three different age groups with 10 animals each; 12 weeks (group I), 33 weeks (group II), and 60 to 90 weeks (group III). Groups I and II were normoglycemic, though the glucose tolerance was impaired in the latter group, and group III was diabetic [22]. The rats were fed a standard rat chow (Funabashi Farm, Funabashi, Japan) and water ad libitum, and housed in a barrier-sustained animal room which was air-conditioned at 24±1°C and 50±10% of relative humidity with 12 hrs artificial lighting from 8:00 to 20:00.

Serum chemistry: Blood samples were collected from the abdominal aorta of all rats after 16 hrs fasting under light ether anesthesia before necropsy. Serum glucose level was measured with an automatic analyzer (Hitachi Automatic Analyzer 706 D: Hitachi Ltd., Tokyo, Japan).

Immunohistochemistry: Pancreatic tissues for immunohistochemistry were sampled from three different regions, which were the head (duodenal), body
(biliary) and tail (splenic) parts, and fixed in 10% formalin. Tissue blocks were embedded in paraffin wax after a routine processing and sectioned at 5 μm. Immunohistochemical stainings were carried out by the indirect peroxidase-antiperoxidase (PAP) method using commercial kits (Bio Genex Laboratories, Dublin, CA, U.S.A.). The antisera used were rabbit anti-porcine insulin, glucagon and somatostatin, and anti-synthetic human pancreatic polypeptide (PP) (MILAB A Medscand Company, Malmö, Sweden). The staining specificity of the antibodies was confirmed in the islets of normal pancreas from F344 male rats aged 24 months. After immunohistochemical stainings, sections were counterstained with Mayer's hematoxylin.

Quantitation of the endocrine cell population: On immunostained sections, positive endocrine cells for each hormone were counted and their proportion to total endocrine cells was compared among the three groups. In addition, the total pancreatic parenchymal tissue area from each animal was measured on sections by using a Histopathological Image Analyzing system (PC 9801, Ikegami Frame Memory, Tokyo, Japan). Both exocrine and endocrine tissues including the fibroser tissue were measured as parenchymal tissue. Mesenchymal tissues such as adipose tissue and large caliber ducts or vessels were not included in the area. Data were assessed using Wilcoxon’s rank sum test. Statistical differences were given by p values which were regarded significant when P<0.05.

RESULTS

On immunostained sections, endocrine cells showed positive reaction with either insulin, glucagon or somatostatin, but did not react with PP. In the intact islets, not involved in inflammatory changes, of groups I and II, B cells were the most numerous and located in the center of islets while A cells distributed peripherally (Fig. 1). A few D cells were randomly present. Those were similar to that of normal rats. Fibrotic lesions resulting from inflammation appeared in rats 33 and more than 60 weeks of age. In these lesions, small aggregations of endocrine cells separated by fibrous tissue were observed, suggesting a regenerative proliferation. Endocrine cells in the fibrotic tissue also reacted with either insulin, glucagon or somatostatin. In the small aggregations of A and B cells, A cells tended to be in the periphery as seen in the normal islets (Fig. 2).

Fasting glucose values, pancreatic parenchymal areas and total endocrine cell numbers of the different age rats are shown in Fig. 3. The mean serum glucose value in group II (143.8±20.2 mg/dl) was approximately the same as that in group I (142.3±18.0 mg/dl), and the individual values in

Fig. 1. Immunostained sections of the non-affected pancreas of a prediabetic rat (12 weeks old). B cells are located centrally (left), and A cells (arrows) are present peripherally (right) in the islet. Left: anti-insulin, Right: anti-glucagon. × 100.
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Fig. 2. Immunostained sections of the pancreas of a prediabetic rat (33 weeks old). Fibrosis is prominent. B cells are located centrally in each endocrine cell cluster (left) and A cells are at the periphery (right). Left: anti-insulin, Right: anti-glucagon. × 100.

Fig. 3. Glucose value, pancreatic parenchymal area, and total endocrine cell number in individuals of different age groups. Lines represent the mean value. I: 12 weeks old; II: 33 weeks old; III: 60–90 weeks old. *P<0.05, **P<0.01.
both groups were within the normal range. The mean glucose value in group III (391.5±107.4 mg/dl) was much higher than that in group I or II, showing a significant difference by Wilcoxon’s rank sum test (P<0.01). On the other hand, the pancreatic parenchymal area and total endocrine cell numbers were markedly decreased with age. The mean size of the areas in groups I, II and III were 55.52±8.88 mm², 25.56±5.60 mm² and 17.87±6.81 mm², respectively. The mean number of total endocrine cells in groups I, II and III were 3071±577, 1674±615 and 785±432, respectively. There were significant differences between these groups as shown in Fig. 3. Individual numbers of each endocrine cell type in different age groups are shown in Fig. 4. Although their numbers varied widely in individuals, B and A cells were markedly decreased in number with age, showing significant difference between groups I and II or III (P<0.01). A remarkable decrease in number of B cells was seen, particularly in group III. D cells were not markedly changed in number with age, although those in group II were fewer than in groups I (P<0.05) and III (P<0.01). In individual percentages of endocrine cell types shown in Fig. 5, significant changes were observed mainly in group III. The percentage of B cells was lower in group III as compared with that in groups I and II (P<0.01). The percentage of D cells was higher in group III as compared with that in groups I and II (P<0.01), and that of A cells was also higher in 5 cases of group III.

DISCUSSION

Human type I diabetes (IDDM) results from an absolute lack of insulin caused by a reduction in the B cell mass [2, 18]. Most patients with type II diabetes (NIDDM) also show a relative or absolute deficiency of insulin, though it is much milder than in type I [2]. Therefore, numerical studies of
pancreatic endocrine cells seem to give useful information to understand the pathogenesis of diabetes [2, 18].

In the present study, both A and B cells were decreased in number in the rats more than 33 weeks of age. D cells, on the other hand, were temporarily decreased in number at 33 weeks old and increased thereafter. These findings suggest that all the endocrine cell types have initially been injured in this strain of rats. Thus, the mechanism of B cell reduction in WBN/Kob rats seem to differ from that in other diabetic animal models [1, 8, 13, 14] and probably from that in human diabetes [18].

The numerical changes in the endocrine cell types of the present rats reflects the degree of derangements in carbohydrate metabolism during the disease process. At 33 weeks old, the ratio of B to A cells was normally preserved, though the total endocrine cell number was markedly decreased. This normal ratio of B to A cells may keep the serum glucose within the normal level. As reported in our previous paper [22], the glucose tolerance was impaired in the prediabetic animals of 33 weeks old. This prediabetic condition is considered to be caused by a decrease in the absolute number of B cells. On the other hand, the diabetes at 60 to 90 weeks of age is thought to result from an imbalance of B to A cells with a decrease in the total endocrine cell number.

Somatostatin-containing D cells were increased in number at 60 to 90 weeks of age in this study. It has been known that the pancreatic somatostatin content and D cell number increase in chronic insulin-deficient conditions [4, 9, 16], and that somatostatin inhibits the insulin secretion [3]. A close numerical relationship between B and D cells was noted in the present diabetic rats. On the other hand, high level of serum glucose and glucagon results in D cell hyperfunction in the diabetic state [5, 17].

The present study showed a decrease in the total endocrine cell number in the pancreas with the progression of inflammation. The decrease in number of endocrine cells might be provoked by two mechanisms; 1) the destruction due to the pancreatic inflammation and 2) the atrophy and loss independent of the inflammation. Further study is needed to elucidate the details of pathogenesis of the pancreatic endocrine cell reduction in this rat strain.

Acknowledgements. The authors wish to thank Mr. Takayoshi Itoh and Miss Yuko Okuda for their skillful technical assistance.
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