Rapid Communication

Appearance of a Regenerating (reg) Gene Protein in Pancreatic Islets of Remission BB/Wor//Tky Rats

CHIKARA ISHII, SHOJI KAWAZU, SHOICHI TOMONO,
TOMIO OHNO, MITSUO SHIMIZU, NORIHIRO KATO,
MAYUMI FUKUDA, YOSHITO ITO, SHUICHI KURIHARA,
KAZUHIKO MURATA, AND KAJURO KOMEDA*

The 2nd Department of Internal Medicine, Gunma University
School of Medicine, Gunma 371, and *Animal Research Center,
Tokyo Medical College, Tokyo 160, Japan

Abstract. Remission of diabetes, i.e. significant amelioration from absolute insulin-dependency, has been sometimes observed in diabetic BB/Wor//Tky rats which were treated with insulin. In remission BB/Wor//Tky rats, plasma glucose levels improved to near normal level and insulin content was also preserved as much as that between diabetic and non-diabetic rats. In this process, we hypothesized that autoimmune insulinitis was suppressed and remaining islet B-cells was restored from severe destruction by recovering in number and/or function. While, recently, a novel regenerating (reg) gene, identified in the regenerating pancreatic islets of surgical models, is reported to be related to the replication of pancreatic B-cells *in vitro*. Based on these findings, we histologically investigated whether the reg protein could be actually expressed or not in the islets from remission BB/Wor//Tky rats. As expected, reg protein was observed in the islets from remission BB/Wor//Tky rats mainly in accordance with pancreatic B-cells. Thus, the present findings suggested that the regeneration of pancreatic B-cells represented by the expression of reg protein might be, at least in part, relevant to remission induced by insulin therapy in spontaneously occurring Type 1 diabetes in BB/Wor//Tky rats.

Key words: BB/Wor//Tky rats, Pancreatic islets, Insulinitis, Reg protein, Remission of diabetes.

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IN BB/WOR//TKY rat, an animal model of Type 1 diabetes, autoimmune insulinitis is believed to result in absolute insulin dependency by the complete destruction of pancreatic B-cells [1, 2]. During the maintenance of this animals in our laboratory, remission of diabetic BB/Wor//Tky rats has been sometimes experienced soon after the insulin treatment, like the case of remission of human type 1 diabetes [3–5]. Really, animals in remission were free from insulin requirement to survive without glycosuria. On the other hand, Okamoto H. and his colleagues have found a novel “reg”

gene in the regenerating pancreatic islets of surgical models, and then identified the reg protein in B-cells [6, 7].

Recently, Francis *et al.* have reported an clear association between reg gene expression and pancreatic B-cell replication *in vitro* [8].

In this context, we histologically investigated the pancreas of BB/Wor//Tky rats in remission.

Materials and Methods

(1) Animals

The animals used in this study were BB/Wor//Tky rats before onset (1 month of age; n=4), those in remission (4–5 months of age; n=5),

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Correspondence to: Dr. Shoji KAWAZU, The 2nd department of internal medicine, Gunma University school of medicine, 3–39–22 Showa, Maebashi, Gunma 371, Japan

diabetic ones (3–4 months of age; $n=18$) and young normal Wistar rats as controls (1 month of age; $n=7$).

BB/Wor//Tky rats have been maintained in our laboratory by brother-sister mating after the kind donation from Prof. A. A. Like, Massachusetts Medical School, Massachusetts, USA. These rats developed diabetes about 80% by 120 days after birth (F0-33) [2]. During these experiences, as already reported, we sometimes found diabetic rats which were induced to remission by insulin treatment (Ultralente, Novo Nordisk Pharm. Co., Ltd., Denmark; 2–5 U/day s.c.), usually soon after the onset of diabetes. We defined remission when urinary glucose turned to be negative by Testape (Elli Lilly, Co., Ltd., USA) continuously more than a week entirely without insulin injection. The incidence of remission of diabetic BB/Wor//Tky rats was 10 to 20%, approximately, up to 120 days of age. Normal Wistar rats were purchased from Imai Animal Laboratory Co., Ltd., Japan.

(2) Methods

Biochemical studies

After anesthesia with ethyl ether, blood was drawn by heart puncture *ad libitum* and pancreas was taken for biochemical and histological examinations. Plasma glucose level was measured by glucose-oxidase method (Glucose B-test, Wako Pure Chemical Industry Co., Ltd., Osaka, Japan) and insulin content of pancreas was measured after overnight acid-ethanol extraction by radioimmunoassay kit commercially available (Phadeces Insulin RIA kit, Pharmacia Diagnostics Co., Ltd., Sweden).

Histological observations

For light microscopy, pancreatic tissues were immediately fixed in 10% formalin solution and then embedded in paraffin. The sections were immunohistologically stained either with mouse anti-rat reg protein monoclonal antibody (1:500) or guinea pig anti-swine insulin antibody (1:200; DAKO Co., Ltd., Calif., USA) applying LSAB Kit (DAKO Co., Ltd., Calif., USA). Counterstaining was done with Mayer's hematoxylin as usual method.

Anti-rat reg protein antibody was kindly donated from Prof. Okamoto, H., the 1st Department of Biochemistry, Tohoku University School of Medicine, Miyagi and Shionogi Research Laboratories, Osaka, Japan.

(3) Statistical analysis

Unpaired Student's *t*-test was used for statistical analysis with 95% confidence of significance.

Results

Biochemical studies

As shown in Table 1, plasma glucose levels in remission BB/Wor//Tky rats were almost the same as those in young preonset BB/Wor//Tky rats and normal Wistar rats. Insulin content of pancreas from these animals was apparently higher than those of diabetic ones, and ranged between diabetic and non-diabetic rats.

Table 1. Biochemical data of rats in each category

Category	N	Plasma glucose (mg/100 ml)	Insulin content (μ g/g-panc)
Normal Wistar rat (1 month)	7	115.0 $\pm$ 7.0	149.0 $\pm$ 11.0
BB/Wor//Tky rat			
preonset (1 month)	4	90.8 $\pm$ 9.8*	84.4 $\pm$ 40.7*
diabetic (2~3 month)	18	329.5 $\pm$ 207.8	1.6 $\pm$ 4.7***
remission (2~3 month)	5	143.9 $\pm$ 36.7	31.5 $\pm$ 8.4

The values are the mean $\pm$ SD (standard deviation) and were analysed statistically by unpaired Student's *t*-test, expressing the significance as follows: *, $P<0.05$; ***, $P<0.001$ vs. remission BB/Wor//Tky rats.

Fig. 1. Representative pictures of histological observations (I). The pictures show the islet from normal Wistar rat (A and B), from BB/Wor//Tky rat before onset of diabetes (preonset, C and D), from diabetic BB/Wor//Tky rat (E and F) and from remission BB/Wor//Tky rat (G and H), respectively. Each islet was stained by anti-insulin antibody (A, C, E and G) and anti-rat reg protein antibody (B, D, F and H) applying LSAB Kit.

Fig. 2. Representative pictures of histological observations (II). The pictures show the islet from remission BB/Wor//Tky rats which were stained by anti-insulin antibody (A), anti-reg protein antibody (B) and anti-glucagon antibody (C), respectively.

Histological observations

Representative pictures of islets from each category of rats are shown in Figs. 1 and 2. Islets from preonset BB/Wor//Tky and normal Wistar rats were remained almost intact ($n=4$ and 7), and were positively stained only with anti-insulin antibody (Fig. 1-A and -C), but not with anti-reg protein antibody (Fig. 1-B and -D). Islets from diabetic BB/Wor//Tky rats ($n=18$), which were often infiltrated by mononuclear cells and destructed in those structure, were small in general and a few in number, and were negative for reg protein (Fig. 1-E and -F). While, almost all the islets of remission BB/Wor//Tky rats ($n=5$), ameliorated to near normal level in glucose metabolism, were clearly stained positive with both antibodies, irrespective of the presence of mononuclear cell infiltration (Fig. 1-G and -H). Furthermore, the reg protein seemed to be also expressed in accordance not only with insulin positive cells but also sometimes partially with glucagon positive cells (Fig. 2-A, -B and -C). The distribution of reg protein positive cells were observed mainly in the islets of remission BB/Wor//Tky rats, but not in the exocrine cells or pancreatic ducts, including the other categories of BB/Wor//Tky rats.

Discussion

In islets of remission BB/Wor//Tky rat, reg protein was strongly expressed in almost all the

islets observed and was found mainly in accordance with pancreatic B-cells under light microscope. In this study, we could not always identify the origin of regenerating pancreatic B-cells whether they were coming from themselves or from pancreatic ducts or from other cells. Anyway, however, it is possible to assume that quantitative recovery of pancreatic B-cells could contribute enough to ameliorate a diabetic state in this animals, since the pancreatic insulin contents in diabetic BB/Wor//Tky rats were always extremely low. These results suggest that reg protein expression in pancreatic B-cells could be one of the indispensable factors to induce remission of diabetes. In our experience, the long-term survival of BB/Wor//Tky rats in remission more than a year was achieved completely without glycosuria in the absence of insulin treatment. In this study, we also found the possible presence of reg protein in pancreatic A-cells, which are reported to be increased in diabetic state. The data are not shown, however, cyclosporin and FK506 also induce remission, more or less, and these phenomena do not seem to be specific for insulin treatment [5]. Including both the suppression of insulinitis and the promotion of reg gene expression in pancreatic islet cells, the precise mechanisms of insulin induced remission remain to be clarified. In conclusion, the expression of reg gene product i.e., a reg protein was identified in pancreatic B-cells of BB/Wor//Tky rats in remission and it was suggested that the regeneration of pancreatic B-cells could, at least in part, be relevant to

“remission”, i.e., the recovery from diabetes.

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