Rapid Publications
Spontaneous Diabetes in the Gnotobiotic BB/W Rat

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SUMMARY

To determine the influence of infectious agents on the initiation of diabetes in the spontaneously diabetic Bio-Breeding/Worcester (BB/W) rat, susceptible rats were raised in a germ-free environment. Between 2 and 3 mo of age, 3 of 12 pups became diabetic. Histologic examination of the pancreas revealed insulitis or end-stage islets. Culture and smears from various tissues were negative for bacteria or parasites. Serum vital antibody titers for all known rat viruses were undetectable. These data suggest that the diabetic syndrome of the BB/W rat is not dependent on recognized infectious agents. DIABETES 28:1031–1032, November 1979.

A spontaneous diabetic syndrome occurs between 60 and 120 days of age in about 30% of Bio-Breeding/Worcester (BB/W) rats. Acutely these animals develop glycosuria, hyperglycemia, hyperglucagonemia, hypoinsulinemia, and ketoacidosis. Without insulin, most ketotic animals die in 1 or 2 wk. Diabetes occurs with equal frequency in both sexes. Obesity is absent, and the frequency of diabetes increases with selective inbreeding. The most salient feature of this model is the presence of insulitis (infiltration of the pancreatic islets with lymphocytes and macrophages), which is observed just before and early in the course of the clinical syndrome.[1,2] Recent studies have suggested that a cell-mediated autoimmune mechanism may be important in the pathogenesis of this syndrome.[3] The administration of rabbit anti-rat lymphocyte serum (ALS) normalized plasma glucose in 36% of acutely diabetic rats and prevented overt hyperglycemia in susceptible nondiabetic littermates.

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Insulitis has been reported in two-thirds of juvenile diabetics who died within 6 mo of being diagnosed diabetic.[4] Temporal relationship between clinically apparent viral infections and the onset of diabetes has prompted speculation that a virus may be important in the pathogenesis of some cases of human diabetes.[5–7] The association of a viral infection and immunopathologic changes (islet cell antibodies and insulitis) in a case of acute, fatal juvenile diabetes was recently demonstrated.[8] To evaluate the importance of infectious agents in the initiation of BB/W diabetes, susceptible rats were raised in a germ-free environment. In spite of an effective gnotobiotic environment, BB/W rats nonetheless became diabetic.

MATERIALS AND METHODS

A term BB/W female, who had previously delivered diabetic offspring, was given a cesarean section. After the rats were killed by cervical dislocation, a midline incision was made and sterile clamps applied just medial to both ovaries and proximal to the cervix. The uterus was removed, washed sequentially in solutions of Betadine and Clorox, and brought into a germ-free surgical isolator through a sterile plastic pipe. In the isolator, the clamps were removed and 12 viable pups were delivered. The pups were foster parented by a commercially obtained gnotobiotic nursing mother. The germ-free isolators were maintained in accordance with standard practices developed for this purpose[9] and were monitored weekly for sterility. All anaerobic and aerobic cultures and smears were negative.

RESULTS

Beginning at 60 days of age the animals were tested weekly for glycosuria with Clinistix (a gift from Ames Division, Miles Laboratories, Elkhart, Indiana). Among the 12 gnotobiotically derived BB/W rats, two males and one female developed glycosuria; this occurred between 84 and 91 days of age. These animals were immediately removed from the isolator and killed. Anaerobic and aerobic cultures of stool, peritoneum, and urine were sterile. Stains of cecal contents

were also negative for bacteria and parasites. Serum was sent to Microbiological Associates (Bethesda, Maryland) for murine virus antibody determinations. Hemagglutination inhibition titers were undetectable for pneumonia virus of mice, reovirus type 3, encephalomyelitis virus, Kilham rat virus, and Toolan H-1 virus. Complement fixation titers were negative for Sendai virus, mouse adenovirus, mouse hepatitis virus, lymphocytic choriomeningitis virus, and rat coronavirus. Plasma glucose concentrations were determined by the Beckman glucose oxidase technique.[10] At the time of sacrifice, blood sugar values of the two males were 319 and 393 mg/dl and the urine samples revealed a 1+ ketonuria. The female was severely emaciated, having a plasma glucose of 480 mg/dl and no ketonuria. Histologic examination of the pancreata revealed either insulitis or end-stage islets comprised only of A, D, and PP cells.

DISCUSSION

These data suggest that the diabetic syndrome of the BB/W rat is not dependent on recognized infectious agents. Although the rats reported in these experiments were raised gnotobiotically (*gnos,* known + *bios,* life), there still exists the possibility that some hitherto unknown infectious agent was present to trigger the diabetic syndrome. However, to date, infectious agents have not been detected in properly reared germ-free rats.[11] Unlike the mouse, in which viruses form part of the genome, this vertical transmission phenomenon has not been described in the rat.[12] The above data suggest that a factor or factors other than presently known infectious agents initiate the diabetic syndrome. Our current view is that the islet pathology of the BB/W rat is genetically determined and the pathogenesis of β-cell destruction is mediated through the immune system. Whether the genetic components are immune system genes, target organ factors, or both remains to be determined.

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