

Diabetes Segregates as a Single Locus in Crosses between Inbred BB Rats Prone or Resistant to Diabetes

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Summary

Diabetes-prone (DP) BB rats spontaneously develop insulin-dependent diabetes resembling type 1 diabetes mellitus in man. They also exhibit lifelong T cell deficiency. The segregation of both diabetes and lymphopenia was studied in crosses between this inbred line of rats and the related but nondiabetic and nonlymphopenic inbred diabetes-resistant (DR) BB rat line. Diabetes segregated as a single, autosomal recessive trait and was always accompanied by lymphopenia. Among the limited number of differences in the genomic DNA sequences of the two lines, DP and DR BB, one may account for the development of diabetes and lymphopenia in the DP BB rats. It may be possible to screen the genomic DNA for such differences to detect a marker for the phenotypes.

Diabetes in the spontaneously diabetic and lymphopenic BB rat resembles type 1 insulin-dependent diabetes in man (1). The β cells are selectively destroyed in association with a mononuclear cell infiltration of the islets of Langerhans (reviewed in reference 2). This cell destruction is thought to be of autoimmune origin and the rats die unless exogenous insulin is administered. The lymphopenia affects only T lymphocytes and is thought to result from abnormally low migration of cells out of the thymus (3). Diabetes-resistant (DR) BB rats, bred in parallel with the diabetes-prone (DP) BB rats, were derived from a nondiabetic sibling pair in the fifth generation of inbreeding the BB rats (4). Previous studies of noninbred BB rat lines with varying incidence rates of diabetes have suggested a complex inheritance (4-7).

In this study, we have taken another approach to determine the number of loci necessary for diabetes in the BB rat. Our study is based on a carefully controlled sister-brother breeding of both DP and DR BB rats initiated by Butler et al. (4), and then continued in 1981 at the Hagedorn Research Laboratory (Gentofte, Denmark) (8, 9), and in 1988 at the University of Washington (Seattle, WA). In this colony, the DR BB rats exhibit neither lymphopenia nor diabetes. More importantly, MHC class I and II gene probes failed to detect differences between the DP and DR BB rats that segregate with diabetes (8, 9). Therefore, we speculate that the MHC-associated susceptibility gene is present in the DR BB rats also. The aim of this study was to determine the number of non-MHC-associated diabetogenic loci by crossing the DP and DR BB lines and studying the segregation of diabetes in the F₁, and F₂ generations, and F₂ × F₁ matings.

Materials and Methods

Breeding. The sister-brother breeding of the DP and DR BB rats was carried out in certified SPF facilities. Two crosses were performed, one at the Hagedorn Research Laboratory (HRL), the other at the University of Washington (UW). The temperature, humidity, and light cycle were controlled and the rats had free access to water and food: Altromin®; Chr. Petersen a/s (Ringsted, Denmark) at HRL; and Wayne sterilizable rodent blox®; Continental Grain Co. (Chicago, IL) at the UW. Quarterly quality assurance controls were negative for viruses, mycoplasma, and parasites. Histological examinations failed to detect infectious pathology.

The body weight of each individual rat was recorded daily from 40 d of age. If a rat was not gaining weight the urine was tested for glucose (Testape; Eli Lilly Co, Indianapolis, IN) and if positive, the diagnosis of IDDM was confirmed when the blood glucose concentration exceeded 10 mmol/liter. Diabetic animals were treated with daily injections (0-5 U) of insulin (Lilly U40P; kindly donated by Eli Lilly Co.).

Histology. Rats were anesthetized with 10 mg/kg, i.p., pentobarbital and exsanguinated by cardiac puncture. The pancreas was removed, coded, fixed in Bouin-Holland's solution and paraffin, sections were stained with hematoxylin and eosin. The histologic examination was performed by two investigators independently and with coded slides.

Blood Lymphocytes. Tail vein blood samples were from unanesthetized rats and blood leukocyte numbers were determined in duplicate in a cell counter (model Zbi; Coulter Electronics Inc., Hialeah, FL).

A mouse IgG1 mAb, MRC OX-19 (Serotec, Bio Products for Science, Indianapolis, IN), was used to enumerate CD5⁺ T lymphocytes by indirect immunofluorescence (9). A total of 10³ cells were analyzed, and the percent OX-19 positive lymphocytes was

used to calculate the number of T lymphocytes per microliter of blood. Lymphopenia was defined as <2,500 OX-19-positive cells per microliter.

Results

Parental BB Rats. A total of 468 DP rats were obtained in the 18–29th generations of inbreeding (Table 1). The age at onset has decreased with increasing degree of inbreeding, however, the average frequency of diabetes has remained at or >90% (Fig. 1).

The pancreatic histology of diabetic DP rats showed either marked insulinitis or endstage islets, whereas all DR rats investigated at similar timepoints showed no such islet pathology (Table 2). At all ages, the numbers of OX-19-positive T lymphocytes in the DP rats were significantly less ($p < 0.0005$) than in the DR rats (Fig. 2). The highest number of T lymphocytes observed in a DP rat <60 d was 960 cells per microliter, whereas the lowest number observed in a DR rat was 2,700 cells per microliter.

F₁ Rats. DP BB diabetic males were mated with female DR BB rats to produce F₁ hybrids. None of the 112 F₁ rats followed for an average of 350 d (range, 150–507) became diabetic (Table 3). Tested randomly, including rats older than 350 d, their blood glucose levels were normal (data not shown). Pancreatic histology showed neither islet inflammation nor periductulitis (Table 2). Age-related acinar atrophy, as well as some interstitial inflammation and perivascular infiltrates, were noted in older rats. T lymphocyte numbers were unaffected by age and comparable with those observed in the DR parental line (Figure 2).

F₂ Rats. Sibling F₁ animals were intercrossed to produce F₂ offspring (Table 3). In the first cross, 161 rats from 20 litters were followed until 150 d of age. The observed frequency of diabetes was 24% (40/161). In the second cross, 23% (40/171) of the F₂ rats developed diabetes in a new set of 20 litters, followed for >150 d. The age at onset of diabetes in the F₂ rats was similar to that of the DP parental

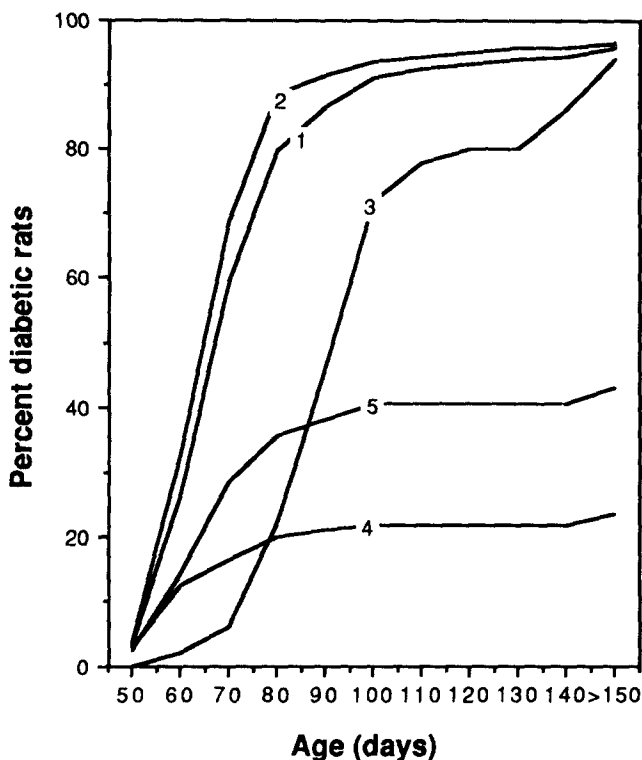


Figure 1. The cumulative percentage of diabetes in DP BB/Hrl rats: generations 18–27 (curve 1); generation 27 (curve 2); generation 18 (curve 3); F₂ rats (curve 4); and F₂ × F₁ matings BB rats (curve 5).

line (Fig. 1). The F₂ diabetic rats had insulinitis as well as end-stage islets (Table 2). The nondiabetic F₂ rats had normal blood glucose concentrations, and their pancreata were free of islet inflammation (Table 2). Leukocyte numbers were similar to those observed in the parent DR line and F₁ hybrids. None of the nondiabetic F₂ rats were found to be consistently lymphopenic. The leukocyte numbers in diabetic F₂ rats were similar to that observed in the parental DP line

Table 1. Incidence of Insulin-dependent Diabetes in DP BB/Hrl Rats

Generation	Age				No diabetes	n	Percent diabetes
	<60 d	60–99 d	100–149 d	>150 d			
18–20	0	67	17	5	3	92	97
21–25*	0	48	8	2	1	59	98
25–29	15	274	12	1	15	317	95
18–19	15	389	37	8	19	468	96
	3.2†	83.1	7.9	1.7	4.1		

* Generation 22 derived by Caesarian section into gnotobiotic environment until 66 d of age, due to severe mycoplasma infection in the preceding generation(s).

† Percent.

Table 2. The Number of Rats with or without Insulinitis or Endstage Islet Morphology in Pancreas Samples from DP, DR, F₁, F₂, and F₂ × F₁ Mating Rats

Generation	Age	No insulinitis	Insulinitis/endstage
	<i>d</i>		
DP	83-467	0	10
DR	107-367	4	0
F ₁	161-507	40	0
F ₂ diabetic	66-317	0	9
F ₂ nondiabetic	66-426*	74	0
F ₂ × F ₁ diabetic	69-107	0	3

* Additional F₂ rats were produced to be killed at timepoints before 150 d of age to study the histology of the pancreas in this age group.

(data not shown). Furthermore, all diabetic rats had T lymphocyte numbers comparable with the DP rats, but reduced numbers compared with the DR, F₁, and nondiabetic F₂ rats at all ages ($p < 0.0005$) (Fig. 2).

F₂ × F₁ Matings. Rats, produced from mating male diabetic F₂ rats with their F₁ mother, showed 43% (18/42) diabetes. The age at onset was similar to that of the DP and diabetic F₂ rats (Fig. 1). All diabetic rats had lymphopenia (Fig. 2) and their pancreata revealed severe insulinitis (Table 2).

Discussion

According to classical Mendelian genetics, an observed frequency of zero diabetic rats among F₁ hybrids is consistent only with a recessive mode of inheritance of this trait. Furthermore, as we did not detect insulinitis, subclinical islet disease as a function of the heterozygous state can be rejected. The observed frequencies of diabetes among the F₂ rats were within the 95% confidence limits for one locus. According to Mendel's law of 1:2:1 segregation in intercrosses between heterozygotes, 25% of the rats would be expected to be

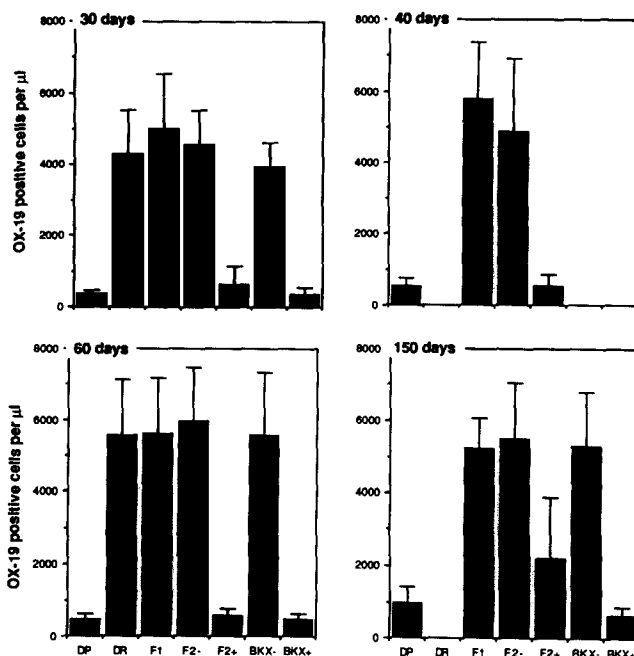


Figure 2. OX-19-positive cells per microliter of peripheral blood in DP, DR, F₁, nondiabetic F₂ (F₂-), diabetic F₂ (F₂+), nondiabetic F₂ × F₁, and diabetic F₂ × F₁ (BKK+) rats compared at different ages. The DR rats were not sampled at 40 and 150 d of age. Shown are mean values ± SD.

homozygotes that express the recessive phenotype. At the gene level, either a single gene or several genes transmitted in close linkage may be involved. If, however, two independently transmitted loci were involved instead of one, then only 1/16 (6.25%) would develop diabetes among the F₂ rats. Clearly, we demonstrate that such a hypothetical segregation cannot account for our findings (Table 3). That the affected F₂ rats were indeed homozygotes was demonstrated by mating them with heterozygous F₁ rats. We observed the expected 1:1 segregation of the diabetic and nondiabetic phenotypes. Again, insulinitis was only seen in the pancreata of such homozygous F₂ animals. All the pancreatic samples from nondiabetic F₂ rats were without subclinical islet inflamma-

Table 3. The Observed Diabetes Frequencies and 95% Confidence Limits of this and of Hypothetical Frequencies of one Pair and Two Independent Pairs of Recessive Alleles for IDDM in the F₁, F₂, and F × F₁ Mating Rats

Cross	Generation	Total	Diabetic	Percent	95% confidence intervals on:		
					Observed	One allele	Two alleles
First	F ₁	<i>n</i> = 30	<i>n</i> = 0	0	0-12	0	0
	F ₂	161	40	24	18-32	18-32	2.5-10
Second	F ₁	82	0	0	0-4	0	0
	F ₂	171	40	23	17-30	19-31	2.6-9.9
	F ₂ × F ₁	42	18	43	28-59	35-65	12-38

tion, which supports the findings in the F₁ rats. We detected some focal inflammation in the acinar tissue randomly among old DP, DR, F₁, and F₂ rats. The presence in DR rats suggested that these changes may be part of an age-related pancreas pathology (10) rather than a diabetes-related process. Therefore, in our rats, diabetes and insulinitis were transmitted together in a recessive fashion. The observed segregation of lymphopenia in our rats is in accordance with previous reports (5, 9). However, the near complete cosegregation of the two phenotypes is somewhat unexpected. Our data suggest that lymphopenia is predictive of future diabetes in the F₂ of the cross between our DP and DR rats. In contrast to our own (9) and others' previous studies, we extended the observation period beyond 150 d of life and detected an additional three F₂ rats with diabetes. The difference in prevalence of diabetes in the F₂ rats in the present study (23%) and those in the literature (2-6%) is not simply explained by a short observation period, but may rather be a consequence of the shared background of the DP and DR lines in this study.

Since only one locus may account for the difference in phenotypic expression of diabetes in our DP and DR BB rats, and since this locus is linked with lymphopenia that maps outside the MHC (5), then the assumption of the presence of the MHC-associated susceptibility gene in the DR rats appears valid. Recent studies have shown similar nucleotide sequences of the coding regions of the class II antigens among DP and DR BB rats, as well as Wistar rats (11, 12).

The cross between our DP and DR rats exhibits a simple mode of inheritance of insulin-dependent (type 1) diabetes. Among the limited number of differences in the genomic DNA sequences of the two lines, one may account for the development of diabetes and lymphopenia in the DP rats. It may be possible to screen the genomic DNA for such differences to detect a marker for the phenotypes. Furthermore, it may eventually be possible to characterize the DNA sequences that confer these phenotypes.

We thank Dr. Thomas Dyrberg, who was instrumental in setting up the colony, and our colleagues for valuable comments on the manuscript. Technical assistance from the Department of Biology, Nordisk Gentofte a/s is also gratefully acknowledged.

This work was supported by National Institutes of Health grants DK-26910 and DK-17047, The Foundation Nordisk Insulinlaboratorium, The Greenwall Foundation, The Juvenile Diabetes Foundation (H. Markholst), and The Danish Media Research Council (H. Markholst).

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Received for publication 21 May 1990 and in revised form 28 August 1990.

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