Genetic Studies in Inbred BB/Wor Rats

Analysis of Progeny Produced by Crossing Lymphopenic Diabetes-Prone Rats With Nonlymphopenic Diabetic Rats

DENNIS L. GUBERSKI, LEONARD BUTLER, WILLIAM KASTERN, AND ARTHUR A. LIKE

BB/Wor diabetes-prone (DP) rats are lymphopenic and frequently develop insulin-dependent diabetes. Diabetes-resistant (DR) BB/Wor rats are not lymphopenic and become diabetic rarely and at a significantly younger age. To examine the genetic basis for diabetes, lymphopenia, and age at onset of diabetes among inbred BB/Wor rats, we crossed nonlymphopenic diabetic rats with lymphopenic DP animals and studied F_1, F_2, and backcross progeny. F_1 rats were neither diabetic nor lymphopenic. Diabetes (both types) and lymphopenia reappeared among F_2 rats, confirming the permissive association of diabetes and lymphopenia and the recessive nature of both. The absence of diabetes in F_1 rats also suggested that the combination of genes responsible for diabetes among lymphopenic and nonlymphopenic rats may be distinct. Nonlymphopenic parental, F_1, and F_2 rats revealed normal lymphocyte subsets, including $CD8^+$ and $RT6^+$ T-lymphocytes. Lymphopenic parental and F_2 rats revealed the absence of $CD8^+$ and $RT6^+$ cells, indicating that these T-lymphocyte abnormalities of lymphopenic DP rats segregate with the lymphopenia gene. The distribution of the ages at onset of diabetes among F_2 lymphopenic and F_2 intercross rats was significantly earlier than among lymphopenic parental and backcross animals, suggesting that the age of diabetes onset is a heritable trait and that the gene(s) or genetic modifier(s) responsible for the earlier onset of F_2 diabetes was acquired from the nonlymphopenic parents. Our genetic studies also confirmed the observations that the 2- and 7-kilobase *Bam*HI fragments of the MHC class I region do not correlate with diabetes or lymphopenia. *Diabetes* 38:887–93, 1989

From the Department of Pathology, University of Massachusetts Medical School, Worcester, Massachusetts; and the Hagedorn Research Institute, Gentofte, Denmark.

Address correspondence and reprint requests to Arthur A. Like, MD, Department of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.

Received for publication 26 September 1988 and accepted in revised form 22 February 1989.

The BB/Wor rat is a useful animal model of human type I (insulin-dependent) diabetes mellitus. Salient features of the syndrome include the abrupt onset of insulin-dependent ketosis-prone diabetes without obesity between 60 and 120 days of age (mean of 92 days) (1). A cell-mediated immunopathogenesis is suggested by genetic predisposition, lymphocytic insulitis, the ability to passively transfer diabetes to naive recipients, and the prevention of diabetes with various immunosuppression and immunomodulating procedures (2–8).

The precise mechanisms responsible for type I diabetes in humans and the BB rat are not fully understood. In both, a gene(s) present in the major histocompatibility complex (MHC) is believed to confer susceptibility for the destruction of pancreatic β-cells (9). In the BB rat, breeding studies suggest that a *u* haplotype in the class II region is required for the development of diabetes (10,11).

A major distinguishing feature of diabetes-prone (DP) BB/Wor rats is the presence of genetically transmitted, lifelong T-lymphocytopenia (12). Diabetes-resistant (DR) animals are not lymphopenic and rarely (<1%) become diabetic (13). The age at onset of diabetes in DR rats is also significantly earlier (mean of 52 days). The factors responsible for the low incidence and early age at onset of diabetes among DR rats are not understood. Because diabetes can be adoptively transferred to DR rats (4) and because X-irradiation (14), cyclophosphamide (4), and the removal of circulating $RT6.1^+$ T-lymphocytes induce diabetes among DR rats (15), it is reasonable to conclude that they possess suitable β-cell target antigens and effector cells.

The University of Massachusetts–Worcester BB/Wor colony began in 1977 with sibling breeding pairs purchased from the BioBreeding Laboratories of Ottawa, Canada. Since 1977, continuous brother-sister matings have been performed, and there are eight family lines that have been through >30 generations of inbreeding. During the development of the BB/Wor colony, two lines of DR animals were

derived from DP stock during the 5th generation of brother-sister matings. DP and DR animals have since been maintained as separate family lines with continued sib matings. DP and DR rats are MHC *RT1*^{u}, but some genetic heterogeneity in MHC has been reported among the various family sublines (16). When analyzed with restriction-fragment–length polymorphism (RFLP) Southern blotting techniques, the DNA of two DP family lines (BA and BB) lack a 2- and 7-kilobase (kb) *Bam*HI fragment. This deletion has been correlated with the absence of serum β_2-microglobulin (17) but does not appear to play a role in the transmission of diabetes (16).

The cumulative incidence of diabetes by 120 days of age in the six DP BB/Wor family lines varies from 50 to 80% (18). The absence of 100% concordance of diabetes among inbred DP animals is not understood. There are, however, no published data concerning the frequency of diabetes in DP rats studied for the full 3 yr of laboratory life expectancy. Because the phenotype diabetes is the culmination of an animal's genetics and environment, it is unclear why inbred animals maintained under identical laboratory conditions do not all become diabetic. However, because ~75% of DP animals that are aglycosuric at 120 days of age have pancreatic insulitis, we anticipate that only a small proportion of these rats would remain normoglycemic if allowed to survive a complete laboratory lifetime. We have reported that the frequency of BB/Wor diabetes among progeny was not influenced by the presence of parental diabetes, providing the breeding pairs were derived from family lines that had been through at least one diabetic (DB) × DB mating. Hence, DB × DB and normal (N) × N mating pairs derived from such family lines yielded an equivalent frequency of diabetic offspring (18,19).

This study was undertaken to investigate the mode of inheritance of diabetes among lymphopenic DP and nonlymphopenic DR BB/Wor rats. In particular, we wanted to test the hypothesis that the genes responsible for diabetes susceptibility in DP and DR rats are the same. We also wanted to determine whether the F_1 progeny produced by mating DP with nonlymphopenic diabetic rats would become diabetic. The study also examined the association of lymphopenia with the absence of cytotoxic/suppressor ($CD8^+$) and $RT6.1^+$ T-lymphocytes and the ages at onset of diabetes in progeny produced during these breeding experiments. Finally, because RFLP studies of nonlymphopenic diabetic DR rats and crosses between diabetic DR and DP rats have not been reported, this study examined the association of the 2- and 7-kb *Bam*HI fragments with both forms of diabetes.

RESEARCH DESIGN AND METHODS

Animals. All BB/Wor rats used in the experiments were raised in the University of Massachusetts Medical School breeding facility. Nonlymphopenic diabetic (NLD) rats were derived from DR breeding stock in the 17th–20th generation of inbreeding, were insulin dependent, and demonstrated normal numbers and phenotypes of peripheral blood lymphocytes (PBLs) (13). Lymphopenic DP rats were in the 20th–21st generation of inbreeding and had decreased numbers of PBLs, low percentages of helper/inducer ($CD4^+$) T-lymphocytes, and a virtual absence of $CD8^+$ and $RT6.1^+$ T-lymphocytes (20,21). Inbred Wistar-Furth (WF) rats were purchased from Harlan-Sprague-Dawley (Indianapolis, IN).

Breeding studies. For the first cross, NLD males were mated with lymphopenic DP females (Fig. 1). All F_1 progeny were nondiabetic, and all tested were nonlymphopenic. For the second cross, F_1 rats were mated, and the F_2 animals produced included diabetic, lymphopenic, and nonlymphopenic progeny. For the third cross, selected F_2 lymphopenic diabetic (LD) rats, with ages at onset of diabetes ≤65 days, were intercrossed. Finally, we performed a backcross. Early-onset (≤65 days old) LD males from the F_2 and intercross animals were mated with female DP parental rats.

Animals were housed under specific pathogen—free conditions with a 12-h light-dark cycle. Purina Chow 5010 and acidified water were provided ad libitum. Animals were kept through 120 days of age and tested for glycosuria 3 times/wk (Tes-Tape). Blood glucose levels were measured (Beckman Glucose Analyzer II, Fullerton, CA) in tail blood of unanesthetized glycosuric animals. Diabetes was defined as 4+ glycosuria with blood glucose >250 mg/dl. Most diabetic blood glucose values were >400 mg/dl.

Peripheral blood. Peripheral blood samples for white blood cell (WBC) counts, differentials, and flow cytometry were collected via orbital venous sinus punctures after light anesthesia (thiamylal sodium, Biotal, Boehringer, St. Joseph, MO). WBC counts were performed with a Coulter Counter, and differential percentages were obtained by microscopic examination of Wright-stained smears.

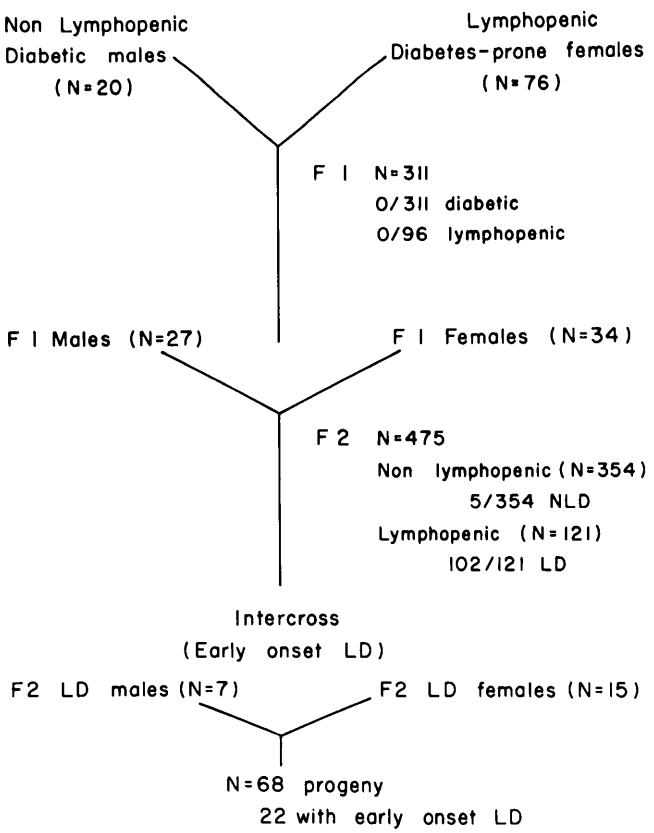

FIG. 1. Schematic of breeding studies. NLD, nonlymphopenic diabetic; LD, lymphopenic diabetic. For data on ages at disease onset, see Table 4.

Flow cytometry. PBLs were obtained from the buffy coats of EDTA-treated whole blood. Splenic lymphocytes were separated in RPMI-1640 tissue culture medium. Two-color flow cytometry was performed as described (6) on 1 × 10^6 PBL aliquots after sequential incubations with OX8-containing tissue culture supernatant (TCS), fluorescein isothiocyanate (FITC)–conjugated F(ab')$_2$ goat anti-mouse IgG, biotinylated OX19, and phycoerythrin-avidin (Molecular Probes, Junction City, OR). Lymph node cells were obtained from cervical lymph nodes and incubated with RT6.1-containing TCS and FITC-conjugated goat anti-rat IgG Fc (Accurate, Westbury, NY). Cells were examined with a Becton Dickinson FACS 440 flow cytometer. For each analysis, ~10,000 viable cells, as described by forward and right-angle scatter, were studied. The data from two-color fluorescence experiments were displayed in contour plots that allowed phenotypic quantification of the relative percentages of natural killer (NK) ($OX8^+/OX19^-$), $CD8^+$ ($OX8^+/OX19^+$), and $CD4^+$ ($OX8^-/OX19^+$) T-lymphocytes (6,20).

Antibodies. OX8 and OX19 monoclonal antibodies (MoAbs) were produced by hybridoma cell lines obtained from A.F. Williams and D.W. Mason (Oxford Univ., Oxford, UK). OX19 reacts with thymocytes and all T-lymphocytes. OX8 reacts with thymocytes, cytotoxic/suppressor (CD8) cells, and NK cells (20,22). RT6.1 MoAbs were produced by a hybridoma cell line (DS4.23) obtained from D. Greiner (Univ. of Connecticut, Storrs, CT). RT6.1 reacts with ~60% of extrathymic T-lymphocytes (23). FITC-conjugated F(ab')$_2$ goat anti-mouse IgG that does not react with rat IgG was purchased from Cappel (Malvern, PA). FITC goat anti-rat IgG Fc was purchased from Accurate.

MHC evaluation. Genomic DNA was prepared from livers frozen in liquid NO_2. Restriction enzyme digestion, agarose gel electrophoresis, Southern transfer to nitrous cellulose membranes, labeling of DNA probes with ^{32}P by nick translation, and DNA hybridization were performed as described (24). Class I MHC 2-kb *Bam*HI fragments were detected with plasmid pKA19, which is a cDNA clone coding for the second extracellular domain of a rat class I antigen (16). Seven-kilobase *Bam*HI fragments were detected with plwo-2, which was a subclone fragment of pKA19.

Morphology. Pancreatic and thyroid tissues of F_1 progeny (n = 38) were fixed in Bouin's solution and prepared for light microscopy as previously reported (13). Hematoxylin-and-eosin–stained sections were studied (A.A.L.) without knowledge of animal category or diabetic status to detect the presence of lymphocytic insulitis and thyroiditis.

Statistical analyses. One-way analyses of variance were performed on a Harris 1000 computer with $SPSS_x$ statistical software to compare the means of experimental groups. Individual pairs of groups were evaluated with the Fisher's least-significant-difference test corrected with a Bonferroni adjustment to compensate for additive type I errors. χ^2-Analyses were performed on contingency tables, and significance was determined from a distribution chart (25).

RESULTS

Diabetes and lymphopenia. The results of the crosses between NLD males (n = 20) and lymphopenic DP females (n = 76) are shown in Fig. 1. None of the F_1 progeny produced by this cross (n = 311) became diabetic, and none of the animals tested (n = 96) were lymphopenic. Microscopic sections of pancreases (n = 38) and thyroids (n = 16) revealed no evidence of lymphocytic insulitis or thyroiditis. Several of the pancreatic sections revealed small scattered foci of lymphocytic infiltration within the exocrine tissue, which in two rats were in close proximity with pancreatic islets (1 islet/animal). However, the islets in question showed no histological evidence of injury.

The 475 F_2 animals were initially divided into nonlymphopenic (n = 354) and lymphopenic (n = 121) groups after examination of WBCs and subsequently subdivided into diabetic and nondiabetic groups on the basis of glycosuria and hyperglycemia. Of the F_2 nonlymphopenic offspring produced, 5 were NLD. Of the lymphopenic progeny produced, 102 were LD. The ratio of nonlymphopenic to lymphopenic F_2 animals was 354:121, which closely matches the 3:1 ratio expected for a homozygous recessive gene (χ^2 = 0.02, df = 1, $P > .1$).

WBC and differential counts of inbred WF, parental NLD, all F_1 rats, and F_2 nonlymphopenic (NLD and nonlymphopenic aglycosuric [NLA]) animals were not significantly different (Table 1). All revealed normal WBCs and differentials. However, WBCs and differentials of lymphopenic and nonlymphopenic progeny were strikingly different from each other ($P < .01$) and essentially mirrored the WBCs and differentials of the respective parental groups or appropriate historic NLD rats (13). F_2 LD and lymphopenic aglycosuric rats revealed slightly but significantly greater percentages

TABLE 1
Peripheral blood leukocytes in parental, F_1, F_2, and Wistar-Furth rats

Rat group	n	White blood cells (×10^3)	Lymphocytes (%)	Polymorphonuclear leukocytes (%)	Total lymphocytes (×10^3)
Wistar-Furth	6	11.9 ± 0.31*	91.5 ± 2.1*	8.5 ± 2.1	1090 ± 24*
NLD	45	11.1 ± 0.41*	86.8 ± 1.4*	12.8 ± 1.4	964 ± 37*
DP	14	5.6 ± 0.61	50.7 ± 3.0	44.6 ± 3.0†	277 ± 28
F_1	96	11.5 ± 0.27*	91.5 ± 0.4*	8.5 ± 0.5	1054 ± 26*
F_2 NLD	5	12.6 ± 1.4*	89.2 ± 1.4*	10.4 ± 1.4	1120 ± 108*
F_2 NLA	349	11.6 ± 0.11*	90.4 ± 0.2*	9.4 ± 0.2	1047 ± 11*
F_2 LD	102	5.6 ± 0.16	66.7 ± 0.9	31.7 ± 0.9†	376 ± 11
F_2 LA	19	6.2 ± 0.46	66.8 ± 2.2	32.3 ± 2.1†	414 ± 37

Values are means ± SE. NLD, nonlymphopenic diabetic; DP, diabetes prone; NLA, nonlymphopenic aglycosuric; LD, lymphopenic diabetic; LA, lymphopenic aglycosuric.
*$P < .01$, significantly greater than DP, F_2 LD, and F_2 LA rats.
†$P < .01$, significantly greater than NLD, F_1, F_2 NLD, F_2 NLA, and Wistar-Furth rats.

of lymphocytes and decreased percentages of polymorphonuclear leukocytes than parental lymphopenic DP rats. Total lymphocytes of parental and F_2 lymphopenic rats were not significantly different.

T-lymphocyte subsets. Flow-cytometry data of PBLs derived from F_1 and nonlymphopenic F_2 animals revealed comparable percentages of $CD4^+$ and $CD8^+$ T-lymphocytes, and these were not significantly different from data derived from NLD and DR rats (Table 2; Fig. 2). PBL T-lymphocyte subsets of parental lymphopenic DP rats revealed the anticipated virtual absence of phenotypic $CD8^+$ T-lymphocytes, the marked reduction of $CD4^+$ cells, and the increased percentages of NK cells. F_2 LD rats also revealed these features of T-lymphocytopenia and increased NK cells. The lower percentages of $CD4^+$ and $CD8^+$ T-lymphocytes given in Table 2 for parental NLD rats reflect the fact that splenic lymphocytes rather than PBLs were studied. Inbred WF T-lymphocyte subsets were significantly greater than those of parental DP and F_2 LD rats ($P < .001$). Although WF, DR, F_1 and F_2 NLD $CD8^+$, and NK cell percentages were comparable, WF $CD4^+$ T-lymphocytes were significantly less than nonlymphopenic BB/Wor $CD4^+$ T-lymphocytes ($P < .001$).

RT6.1 cells. DR, F_1, and nonlymphopenic F_2 animals revealed normal and comparable percentages of $RT6.1^+$ T-lymphocytes. Parental DP and F_2 lymphopenic animals revealed the virtual absence of this subset of PBLs (Table 3; Fig. 3). WF rats possess only $RT6.2^+$ T-lymphocytes (23) and were not phenotyped.

Age at onset of diabetes. Diabetes was almost always detected at an earlier mean ± SD age among nonlymphopenic than lymphopenic rats (51.8 ± 1.8 days [n = 144] vs. 92.7 ± 0.86 days [n = 310]; D.L.G., L.B., and A.A.L., unpublished observations). Virtually all previously studied NLD rats had become diabetic before 70 days of age, with 94% detected between 35 and 62 days of age (Table 4). Diabetes among all F_2 NLD rats occurred before 70 days of age (mean ± SD 48 ± 1.3; n = 5). The age distribution data derived from LD rats in the breeding colony during the experiments are summarized in Table 4. Among these animals, 95% became diabetic between 70 and 150 days of age (18). Parental lymphopenic DP animals used for these breeding studies were not diabetic when mated. The breeders (and their littermates) became diabetic at the expected ages (data not shown).

F_2 LD rats occurred at a significantly earlier age than among historic LD rats (Table 4). Thirty-five percent (36 of 102) of F_2 LD rats were detected before 71 days of age, whereas only 5% (14 of 310) of parental (historic) LD rats occurred during this age range. These F_2 LD animals served as the breeding nucleus for the intercross. Thirty-two percent (22 of 68) of diabetic intercross progeny were detected before 70 days of age.

Early-onset (≤65 days) diabetic males from the F_2 LD and intercross progeny were mated with parental lymphopenic DP females. Although none of the females were diabetic at

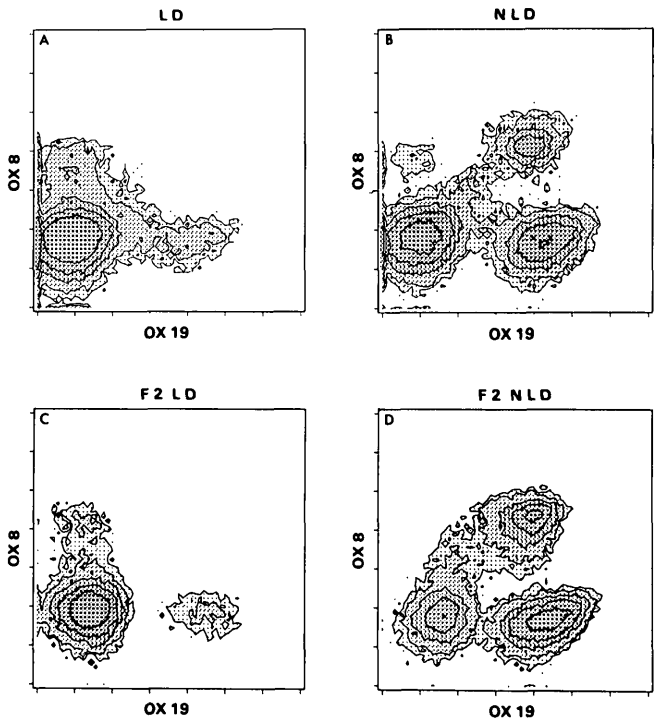

FIG. 2. Two-color fluorescence contour plots of peripheral blood lymphocytes (PBLs) after staining with OX8 ($CD8^+$) tissue culture supernatant and fluorescein isothiocyanate–conjugated goat anti-mouse IgG, followed by biotinylated OX19 ($CD5^+$) and avidin-phycoerythrin. PBL T-lymphocyte subsets of parental lymphopenic diabetic (LD) and F_2 LD reveal virtual absence of $OX8^+/OX19^+$ ($CD8^+$) T-lymphocytes, reduced $OX8^-/OX19^+$ ($CD4^+$) T-lymphocytes, and increased $OX8^+/OX19^-$ natural killer cells. Parental nonlymphopenic diabetic (NLD) and F_2 NLD subsets are normal.

TABLE 2
Flow cytometry results of splenic and peripheral blood lymphocyte subsets

Rat group	n	$OX8^+/OX19^-$ natural killer cells	$OX8^-/OX19^+$ $CD4^+$	$OX8^+/OX19^+$ $CD8^+$
Wistar-Furth	6	3.4 ± 0.4	26.8 ± 0.4*	18.3 ± 0.5
DR	21	3.1 ± 0.3	46.9 ± 1.4	20.1 ± 0.7
NLD†	8	2.7 ± 0.1	23.8 ± 1.5	17.4 ± 0.6
DP	6	6.4 ± 0.6‡	5.9 ± 0.2§	0.85 ± 0.1§
F_1	10	2.8 ± 0.4	45.0 ± 0.9	22.8 ± 0.4
F_2 NLD	5	2.8 ± 1.3	49.0 ± 1.5	25.0 ± 0.8
F_2 LD	15	9.4 ± 1.5‡	8.0 ± 0.8§	0.71 ± 0.1§

Values are means ± SE. DR, diabetes resistant; NLD, nonlymphopenic diabetic; DP, diabetes prone; LD, lymphopenic diabetic.
*$P < .001$, significantly greater than DP and F_2 LD but significantly less than DR, F_1, and F_2 NLD rats.
†Splenic lymphocytes were examined in NLD rats.
‡$P < .05$, significantly greater than DR, NLD, F_1 and F_2 NLD rats.
§$P < .001$, significantly less than Wistar-Furth, DR, NLD, F_1 and F_2 NLD rats.

TABLE 3
Flow cytometry results of cervical lymph node $RT6^+$ cells

Rat group	n	$RT6^+$ cells (%)
DR	10	36.4 ± 2.0*
DP	3	1.5 ± 0.7
F_1	3	47.7 ± 1.3*
F_2 NLA	9	36.1 ± 1.4*
F_2 LA	7	1.1 ± 0.5
F_2 LD	6	0.9 ± 0.4

Values are means ± SE. DR, diabetes resistant; DP, diabetes prone; NLA, nonlymphopenic aglycosuric; LA, lymphopenic aglycosuric; LD, lymphopenic diabetic.
*$P < .01$, significantly greater than DP, F_2 LA, and F_2 LD rats.

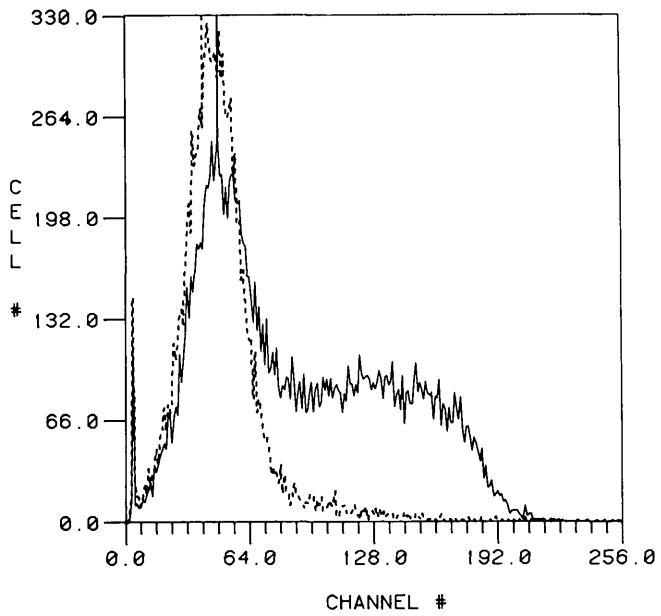

FIG. 3. Superimposed flow cytometry histograms of cervical lymph node cells from F_2 animals. Percentage of RT6.1$^+$ cells is normal in F_2 nonlymphopenic rats (*solid line*) and undetectable in F_2 lymphopenic rats (*dashed line*).

the time of mating (80 days of age), they became diabetic at the expected ages and frequency. The progeny of this mating were classified as backcross. Eleven percent (6 of 56) of the diabetic backcross progeny were detected before 71 days of age, and 82% (46 of 56) were detected between 71 and 99 days of age (Table 4).

MHC studies. Previous studies revealed genetic heterogeneity within the MHC of inbred family lines of the BB/Wor colony (16). Specifically, BA and BB family lines lacked the 2- and 7-kb *Bam*HI DNA fragments within the class I region of the MHC. The 2- and 7-kb *Bam*HI fragments were absent in 15.4% (2 of 13) of the F_2 LA animals and in 15.8% (3 of 19) of the LD animals (Table 5). These differences were not significant (Fisher's exact statistic, $P > .05$). The 2- and 7-kb *Bam*HI fragments were absent in 13.3% (4 of 30) of NLA animals. To determine whether the absence of the 2- and 7-kb *Bam*HI fragments correlated with lymphopenia, a Fisher exact test was performed. When all lymphopenic animals (nondiabetics and diabetics) were considered, 16% (5 of 32) revealed the absence of the *Bam*HI fragments. Among nonlymphopenic rats, 13.3% (4 of 30) lacked the DNA fragments. These differences were not significant.

TABLE 4
Distribution of ages at onset of diabetes

	Age at onset (days)						
	≤70		71–99		100–150		
Rat group	%	*n*	%	*n*	%	*n*	Total
NLD*	94	136	2	3	3	5	144
LD†	5	14	65	202	30	94	310
F_2 NLD	100	5					5
F_2 LD‡	35	36	58	59	7	7	102
Intercross‡	32	22	57	39	11	7	68
Backcross§	11	6	82	46	7	4	56

NLD, nonlymphopenic diabetic; LD, lymphopenic diabetic.
*Unpublished historic data.
†Historic data derived from diabetes-prone breeding stock in mating at time of breeding studies.
‡Ages at onset of diabetes for F_2 LD and intercross rats are significantly less than those of lymphopenic diabetes-prone animals: $\chi^2 = 77.8$ (F_2 LD) and 54.3 (intercross), df = 2, $P < .001$.
§Ages at onset of diabetes in backcross are significantly different than parental LD: $\chi^2 = 14.9$ ($P < .001$), F_2 LD $\chi^2 = 11.4$ ($P < .005$), and intercross $\chi^2 = 9.5$ ($P < .01$).

TABLE 5
Inheritance of 2- and 7-kilobase (kb) *Bam*HI fragments, class I MHC genes, lymphopenia, and diabetes among F_2 progeny

Rat group	2- and 7-kb fragments −	+	Percent negative
Lymphopenic aglycosuric	2	11	15.4
Lymphopenic diabetic	3	16	15.8
Nonlymphopenic aglycosuric	4	26	13.3

DISCUSSION

The absence of diabetes among F_1 animals and its reappearance among nonlymphopenic and lymphopenic F_2 progeny confirm the recessive mode of inheritance of diabetes in lymphopenic BB/Wor rats and suggest that the mode of inheritance of nonlymphopenic diabetes may also be recessive. The lack of diabetes among F_1 animals requires explanation. Because both parental groups were inbred and presumably homozygous for the diabetes gene(s), the progeny from these matings would be expected to have developed diabetes, unless their respective diabetes gene(s) was not identical or unless the *LD* and *NLD* diabetic genes were expressed under different genetically controlled conditions. Support for the hypothesis that *LD* and *NLD* genes are not identical or require a different substratum for expression may be derived from the different ages at onset of diabetes among LD and NLD rats and the evidence that lymphopenic and nonlymphopenic rats use different phenotypic lymphocyte subsets as effector cells responsible for β-cell destruction (26–28). We have reported that removal of NK and CD4$^+$ cells protects against diabetes in lymphopenic DP animals (6). Previous studies also reported that transfusions of DR blood (8) or splenocytes (29) prevent diabetes in lymphopenic rats and that depletion of circulating lymphocytes by cyclophosphamide (4), X-irradiation (14), and injections of anti-RT6.1 MoAbs may induce diabetes in DR rats (15). Hence, we speculated that F_1 animals that were not lymphopenic were protected from NK-mediated β-cell destruction by a restoration of normal percentages of CD8$^+$, CD4$^+$, and RT6.1$^+$ lymphocytes. Depletion of NK cells did not prevent the induction of diabetes in DR rats by RT6.1$^+$ cell depletion (26), and the adoptive transfer of diabetes with concanavalin A–stimulated spleen cells derived from RT6.1$^+$ cell–depleted DR rats requires the presence of CD8$^+$ T-lymphocytes (27,28). It is not clear why F_1 animals endowed with diabetes genes from both NLD and lymphopenic DP parents did not use the available CD8$^+$ T-lymphocytes to mediate β-cell destruction.

Although BB rats were reportedly derived from WF animals (1), we believe that the inbred BB/Wor DR rats are the most

appropriate controls. Although WF, NLD, F_1, and nonlymphopenic F_2 animals have comparable lymphocyte numbers and percentages (Table 1), WF rats had significantly fewer $CD4^+$ T-lymphocytes than DR rats (Table 2) and express the RT6.2 rather than the RT6.1 phenotype.

Note that none of the pancreatic tissues examined microscopically from the F_1 progeny revealed lymphocytic insulitis. This rules out the presence of mild insulitis with incomplete β-cell destruction, a common feature of euglycemic lymphopenic DP animals (6), and suggests that diabetes probably would not have occurred later. Finally, the low incidence of NLD among F_2 progeny (5 of 354) suggests that the mode of inheritance of NLD is multigenic. This hypothesis is supported by the absence of diabetes among progeny (n = 23) obtained from other mating schemes wherein both parents were NLD (data not shown).

The five F_2 NLD rats were detected among 3 of the 52 F_2 litters produced. Furthermore, litters were obtained from the matings of F_1 animals derived from the cross between NLD males and two females from the DP BB family line. Genetic heterogeneity in the MHC of BB/Wor family lines has been reported (16). The BA and BB family lines lack the 2- and 7-kb *Bam*HI fragments that are present in nonlymphopenic DR lines and four of the lymphopenic DP family lines (BE, NB, PA, and BC). Although this deletion has not been associated directly with the occurrence or absence of diabetes, genetic differences in the MHC might explain why the progeny of only the BB × NLD matings resulted in F_2 NLD animals.

The absence of lymphopenia among F_1 progeny and its reappearance in ~25% of F_2 progeny confirm the recessive nature of the lymphopenia gene (10). The presence of normal percentages of $CD8^+$ and $RT6.1^+$ T-lymphocytes in F_1 animals and nonlymphopenic F_2 rats and their absence among lymphopenic parental and lymphopenic F_2 rats indicate that the expression of these T-lymphocyte defects segregates with the recessive lymphopenia gene and together may be responsible for the profound T-lymphocyte deficiency. The reappearance of diabetes among 84% (102 of 121) of F_2 lymphopenic and 1.4% (5 of 354) of F_2 nonlymphopenic progeny also confirms the strong correlation between diabetes and lymphopenia (12). Because we did not examine parental or F_2 NLD for the presence of $RT6.1^+$ T-lymphocytes, we have no data concerning the association of this lymphocyte subset with nonlymphopenic diabetes.

The distribution of the ages at onset of diabetes among parental (historic) LD rats shows that 95% of these animals were detected after 71 days of age. In contrast, 94% of NLD animals were detected before 70 days of age. At the initiation of these breeding experiments, we speculated that the earlier age at onset of diabetes observed among NLD rats may have been due to the absence of lymphopenia and the presence of a more aggressive autoimmune β-cell–destructive process. However, these experiments suggest that the age at onset of diabetes among BB/Wor rats is under genetic control and independent of genes that control lymphopenia. Part of the evidence for this conclusion is given in Table 4, which reveals that F_2 and intercross LD rats carry a gene(s) for early onset. Among historic parental LD rats, only 5% of diabetics were detected before 71 days of age, and 30% were detected after 99 days of age. In contrast, among F_2 animals, these values were 35 and 7% (χ^2 = 77.8, $P < .001$), indicating that the entire curve of the age at onset of diabetes had been shifted. Because selection for early onset of diabetes in the DP lines was previously ineffective, we assume that the outcross of NLD to DP must have contributed a gene(s) for early onset. This conclusion is reinforced by the onset data among intercross progeny that were almost as early as among F_2 rats (χ^2 = 0.9, P = .95). This suggests that the age at onset of diabetes is a heritable trait, because early onset was transferred to the next generation. Further evidence that F_2 LD rats differed from parental LD rats was shown by the age-at-onset results for the backcross, where the onset of diabetes was significantly earlier than among parental LD rats and significantly later than among F_2 and intercross rats. The progeny of this backcross were probably composed entirely of heterozygotes with early gene(s) from NLD rats and late genes from DP rats. This was confirmed by the observation that 82% of the backcross diabetic rats were detected between 71 and 99 days. These data and the ease of transfer suggest that a single gene may control the inheritance of early-onset diabetes.

ACKNOWLEDGMENTS

We acknowledge Paul D. McGill and Tracy Hessel-Martin for expert technical assistance and Lorna C. Urbsis for preparation of the manuscript.

This work was supported by U.S. Public Health Service Grant DK-19155 and Contract DK7-2287.

W.K. is the recipient of a Juvenile Diabetes Foundation Career Development Award. Product support was provided by Eli Lilly Company (Indianapolis, IN) and Becton Dickinson Corporation (Mountain View, CA).

This work was presented in part at the 22nd annual meeting of the European Association for the Study of Diabetes, Rome, Italy, September 1986.

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