

# Diabetes-Prone and Diabetes-Resistant BB Rats Share a Common Major Diabetes Susceptibility Locus, *iddm4*

## Additional Evidence for a "Universal Autoimmunity Locus" on Rat Chromosome 4

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Diabetes-prone (DP) BB rats develop autoimmune type 1 diabetes spontaneously. At least five loci are linked to disease expression: the major histocompatibility complex (*iddm2*), two susceptibility loci (*iddm4*, *iddm5*), and, possibly, a resistance locus (*iddm3*). Spontaneous disease also requires homozygosity for *lyp/iddm1*, which causes lymphopenia. It has not been determined whether *lyp/iddm1* is required for predisposition to diabetes autoimmunity in addition to being required for its spontaneous expression. We analyzed backcross rats segregating for diabetes but not lymphopenia using Wistar-Furth (WF) and diabetes-resistant (DR) BB animals. The latter are nonlymphopenic (*lyp*<sup>+/+</sup>) and develop diabetes only in response to immunological perturbants. Treatment of (DR-BB × WF)F<sub>1</sub> × WF animals (all *lyp*<sup>+/+</sup>) using a standard induction protocol caused type 1 diabetes in 58% of progeny. Expression of type 1 diabetes was strongly linked to *iddm4*. The results suggest that *lyp/iddm1* does not determine the predisposition to autoimmunity in BB rats and that *iddm4* is a major diabetogenic locus in both DP- and DR-BB animals. The *iddm4* gene maps to a region containing several major autoimmunity loci, including *aia2*, *aia3*, and *cia3*. We propose that BB rat diabetes requires 1) class II RT1<sup>u</sup> (*iddm2*) for susceptibility, 2) additional loci for disease initiation and progression in response to perturbants, and 3) *lyp* for spontaneous disease. *Diabetes* 48:2138–2144, 1999

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DP, diabetes-prone; DR, diabetes-resistant; LOD, logarithm of odds; mAb, monoclonal antibody; MHC, major histocompatibility complex; poly I:C, polyinosinic-polycytidylic acid; VAF, viral antibody-free; WF, Wistar-Furth.

**D**iabetes-prone (DP) BB rats develop spontaneous autoimmune type 1 diabetes and are used to model human type 1 diabetes (1). More than 90% of viral antibody-free (VAF) DP-BB rats develop the disease (1,2), which can be prevented by immunosuppression (1,3). Pancreatic insulinitis (4) and islet autoantibodies (5) are observed in diabetic DP-BB rats, and their spleen cells can adoptively transfer the disease (6). DP-BB rats are also T-cell lymphopenic, a characteristic not shared by humans with type 1 diabetes (1). They are severely deficient in CD8<sup>+</sup> and RT6<sup>+</sup> T-cells (2) and in NK T-cells (7).

DR-BB rats are histocompatible with DP-BB rats, from which they were derived by selective breeding for absence of spontaneous diabetes (3). Under VAF conditions, they never develop spontaneous type 1 diabetes (8). DR-BB rats are nonlymphopenic and immunocompetent (1,8). DR-BB rats do, however, harbor autoreactive cells, which can be detected by transfer of their splenocytes (9) or thymocytes (10) to histocompatible nude recipients.

As is true of type 1 diabetes in humans (11), type 1 diabetes in BB rats is familial, but the mode of inheritance is non-Mendelian (12–16). In both species, it is most clearly associated with certain major histocompatibility complex (MHC) haplotypes (17–20). Genome-wide searches for human type 1 diabetes genes have reported more than 18 chromosomal regions with linkage to the disease (11,21–23). In the BB rat, fewer diabetes-modifying loci have been identified. One gene, designated *iddm2*, is linked to the rat MHC. Expression of diabetes is independent of the class I haplotype, but it requires at least one class II RT1<sup>u</sup> allele (12,24–26).

An autosomal recessive locus termed *lyp* (or *iddm1*) causes T-cell lymphopenia in DP-BB rats (13,27,28). It has been shown in multiple genetic crosses that deficiency in peripheral T-cells is necessary, but not sufficient, for the expression of spontaneous type 1 diabetes in BB rats (13,27–30). The *lyp/iddm1* locus has been mapped to chromosome 4 (RNO4) (13,31).

Additional type 1 diabetes-modifying genes have been identified in (DP-BB × WF)F<sub>1</sub> × Wistar-Furth (WF) backcross progeny (32). These RT1<sup>u</sup> animals are nonlymphopenic

(*lyp*<sup>+/+</sup> or *lyp*<sup>+/lyp</sup>) and free of spontaneous autoimmune diabetes, but they develop the disease in response to treatment with polyinosinic-polycytidylic acid (poly I:C) and a cytotoxic anti-RT6.1 monoclonal antibody (mAb). This treatment had previously been shown to induce type 1 diabetes in DR-BB rats (33). The first locus, designated *iddm4*, was significantly linked to both insulinitis and diabetes (32); *iddm4* is located on RNO4 and maps ~1.3–4 cM proximal to the *lyp/iddm1* gene.

The susceptibility of both DR-BB rats and (DP-BB × WF)<sub>1</sub> × WF backcross progeny to the induction of type 1 diabetes led us to hypothesize that the DR-BB rat (derived from DP forebears) also shares the diabetogenic allele of *iddm4*. If so, susceptibility to type 1 diabetes in the DR-BB rat should segregate with *iddm4* on chromosome 4. If this could be demonstrated, it would be an important observation because it would exclude any absolute requirement for *lyp/iddm1*, either homozygous or heterozygous, for diabetes susceptibility in BB rats (28). The present studies were therefore undertaken 1) to determine if *iddm4* is required for the induction of type 1 diabetes in DR-BB backcross progeny; 2) to prove that *lyp/iddm1* and *iddm4* are distinct loci; and 3) to exclude any possible confounding effects of heterozygous expression of *lyp/iddm1* on diabetes expression. To achieve these goals, we tested a cohort of (DR-BB × WF)<sub>1</sub> × WF animals for susceptibility to the induction of autoimmune diabetes. WF rats are nonlymphopenic (*lyp/iddm1*<sup>+/+</sup>) and RT1<sup>u</sup>, but are resistant to the induction of autoimmune diabetes with poly I:C (34 and J.P.M., unpublished observations). We found that susceptibility to the induction of type 1 diabetes segregated in the backcross and was due to the action of *iddm4*. The results establish that 1) *lyp/iddm1* is not required for the latent predisposition to autoimmunity in BB rats, and 2) *iddm4* is a major diabetogenic locus not only in DP-BB, but also DR-BB rats.

## RESEARCH DESIGN AND METHODS

**Animals.** DP- and DR-BB/Wor rats were obtained from the VAF colony maintained at the University of Massachusetts Medical School, Worcester. Animals from this colony express the RT6.1 alloantigen (also called ART2 [35]) and the RT1<sup>u</sup> MHC haplotype (8). All animals used were certified to be serologically free of Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, rat corona virus, Kilham rat virus, HI (Toolan's virus), GD7, Reo-3, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, mouse adenovirus, Hantaan virus, and *Encephalitozoon cuniculi*.

WF rats (RT1<sup>u/u</sup>) were purchased from Harlan Sprague Dawley, Indianapolis, IN. WF rats express the RT6.2 alloantigen (36). (DR-BB/Wor × WF)<sub>1</sub> and (DR-BB/Wor × WF)<sub>1</sub> × WF backcross rats were bred at the University of Massachusetts, Worcester. In addition, a small backcross of 40 RT6.1\* (DP-BB × WF)<sub>1</sub> × WF rats was generated to confirm the location of the previously reported *iddm4* locus (32) and to serve as a positive control for diabetes induction in the (DR-BB × WF)<sub>1</sub> × WF backcross.

Animals were maintained in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 1996) and the guidelines of the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School.

**Induction of diabetes.** The DS4.23 hybridoma secreting the rat anti-RT6.1 mAb is maintained in our laboratory (33). This mAb has been documented to be cytotoxic and to deplete RT6<sup>+</sup> T-cells in vivo (37). To induce the expression of autoimmunity, (DR-BB × WF)<sub>1</sub> × WF rats 30 days of age were first screened for the expression of RT6.1 on T-cells by flow cytometry as previously described (32). It was necessary to do so because of the lack of a depleting anti-RT6.2 mAb comparable with the depleting DS4.23 anti-RT6.1 mAb used in all prior analyses of diabetes induction in the RT6.1\* DR-BB rat.

The diabetes-induction protocol was performed on RT6.1\* backcross animals starting at 30 days of age as previously described (32,33). Briefly, unconcentrated

hybridoma supernatant was injected intraperitoneally, 2 ml/rat five times weekly for 4 weeks. Poly I:C (Sigma, St. Louis, MO) was diluted in phosphate-buffered saline and given at a dose of 5 µg/g body weight intraperitoneally three times weekly. More than 90% of VAF DR-BB rats treated in this way develop diabetes within 30 days (33). Animals were tested for glycosuria twice weekly (Tes-Tape; Eli Lilly, Indianapolis, IN). Diabetes was diagnosed on the basis of glycosuria and two plasma glucose concentrations >250 mg/dl on different days. Animals were monitored for hyperglycemia through 70 days of age.

**DNA samples and microsatellite primers.** Genomic DNA was extracted from livers that were snap frozen on dry ice as previously reported (32). All microsatellite primers can be obtained from Research Genetics (Huntsville, AL). Their general map locations were found to be consistent with those reported by Jacob et al. (38), Wilder and Remmers (39), and Bihoreau et al. (40 and <http://www.well.ox.ac.uk/pub/genetics/ratmap>). Polymorphic primers were tested on both diabetic and nondiabetic progeny from the backcrosses.

**Radioactive polymerase chain reaction analysis.** Primers detecting polymorphic microsatellites were end-labeled using [<sup>32</sup>P]ATP, and used in a polymerase chain reaction to amplify products that were resolved by polyacrylamide gel electrophoresis as previously described (32). Sizes of the products amplified by each primer pair were estimated by comparison to the published sizes as reported by Jacob et al. (38) and Research Genetics (Huntsville, AL).

**Data analysis.** The probability of linkage between marker loci and the presence of diabetes was analyzed using the nonparametric  $\chi^2$  statistic for the qualitative trait of diabetes. Certain 2 × 2 tables were analyzed using Fisher's exact statistic (41). Parametric data were compared using one-way analyses of variance and the least significant differences procedure for *a posteriori* contrasts (42). Logarithm of odds (LOD) scores for the linkage of the diabetes trait to markers on chromosome 4 were calculated using MapManager QT software (Version b21, K. Manly, Roswell Park Memorial Cancer Institute, Buffalo, NY). The marker order shown is that generated by MapManager, which identifies the most likely among permuted orders by minimizing the number of double crossovers (<http://mcbio.med.buffalo.edu/MMM/MMMAlgorithms.fm3.html>).

## RESULTS

**Frequency of type 1 diabetes.** We first observed that type 1 diabetes can be induced in (DR-BB × WF)<sub>1</sub> rats. The cumulative frequency of type 1 diabetes was 100% in both DR-BB and (DR-BB × WF)<sub>1</sub> animals (Table 1), a frequency similar to that reported previously for DR-BB rats in which diabetes was induced under VAF conditions using the same protocol (33). The cumulative frequency of diabetes in the positive control (DP-BB × WF)<sub>1</sub> × WF backcross was 75%, a frequency comparable with that observed in the (DR-BB × WF) × WF backcross progeny (58%).

**Latency to onset of type 1 diabetes.** Statistical analysis of the DR-BB, (DR-BB × WF)<sub>1</sub>, and (DR-BB × WF) × WF backcross groups revealed significant differences in latency to onset of diabetes ( $F_{2,57} = 18.9$ ,  $P < 0.001$ ). Latency in the (DR-BB × WF) × WF backcross progeny was significantly delayed ( $P < 0.001$ ) in comparison with both the DR-BB and (DR-BB × WF)<sub>1</sub> groups (Table 1). Latency to onset in the F1 cohort was also longer than that observed in the DR-BB group, but this difference did not achieve statistical significance ( $P = 0.1$ ). The latency in (DR-BB × WF) × WF backcross progeny

TABLE 1  
Frequency and latency to onset of type 1 diabetes in DR-BB, (DR-BB × WF)<sub>1</sub> and (DR-BB × WF) × WF backcross rats

|                           | Frequency (%) of type 1 diabetes | Latency to onset (days) |
|---------------------------|----------------------------------|-------------------------|
| DR-BB                     | 12/12 (100)                      | 11 ± 1                  |
| (DR-BB × WF) <sub>1</sub> | 11/11 (100)                      | 15 ± 5                  |
| (DR-BB × WF) × WF         | 29/50 (58)                       | 22 ± 7*                 |

\* $P < 0.001$  vs. DR-BB and (DR-BB × WF)<sub>1</sub>.

TABLE 2  
Genetic map of the *iddm4* region based on DR- and DP-BB backcrosses

| Marker               | Total cM | DP backcross 1<br>( <i>P</i> value) | DP backcross 2<br>( <i>P</i> value) | DR backcross<br>( <i>P</i> value) | Total<br><i>P</i> value | <i>iddm</i><br>locus | LOD<br>score | Marker identity<br>(DP vs. DR) |
|----------------------|----------|-------------------------------------|-------------------------------------|-----------------------------------|-------------------------|----------------------|--------------|--------------------------------|
| <i>D4Rat9</i>        | 9.5      | 0.640                               | —                                   | —                                 | —                       | —                    | —            | =                              |
| <i>D4Arb25</i>       | 19.0     | 0.330                               | —                                   | NP                                | —                       | —                    | —            | —                              |
| <i>D4Mit9</i>        | 36.6     | 0.046                               | 0.042                               | 0.0005                            | 0.00013                 | <i>iddm4</i>         | 1.7          | =                              |
| <i>D4Wox10</i>       | 41.8     | 0.007                               | 0.010                               | 0.0002                            | 0.000008                | <i>iddm4</i>         | 2.7          | =                              |
| <i>D4Arb11</i>       | 42.4     | 0.007                               | 0.003                               | —                                 | —                       | —                    | —            | =                              |
| <i>D4Mgh16</i>       | 43.0     | 0.007                               | 0.003                               | 0.0002                            | 0.000008                | <i>iddm4</i>         | 3.0          | =                              |
| <i>D4Rat20</i>       | 43.0     | 0.007                               | —                                   | —                                 | —                       | —                    | —            | =                              |
| <i>D4Rat18</i>       | 44.3     | 0.007                               | —                                   | NP                                | —                       | —                    | —            | =                              |
| <i>D4Rat135</i>      | 44.3     | 0.007                               | —                                   | —                                 | —                       | —                    | —            | =                              |
| <i>D4Arb29</i>       | 46.2     | <u>0.0012</u>                       | 0.0020                              | 0.0002                            | 0.000002                | <i>iddm4</i>         | 3.8          | =                              |
| <i>D4Arb9</i>        | 48.7     | <u>0.0012</u>                       | <u>0.0003</u>                       | <u>0.00008</u>                    | <u>0.0000001</u>        | <i>iddm4</i>         | 4.7          | =                              |
| <i>D4Mit3</i>        | 48.7     | <u>0.0012</u>                       | <u>0.0003</u>                       | <u>0.00008</u>                    | <u>0.0000001</u>        | <i>iddm4</i>         | 4.7          | =                              |
| <i>D4Mit5</i>        | 50.0     | <u>0.0012</u>                       | 0.0035                              | 0.0002                            | 0.000002                | <i>iddm1</i>         | 3.5          | —                              |
| <i>D4Mgh24</i>       | 50.0     | <u>0.0012</u>                       | 0.0035                              | 0.0002                            | 0.000002                | <i>iddm1</i>         | 3.5          | —                              |
| <i>D4Arb30</i>       | 50.6     | <u>0.0012</u>                       | 0.0060                              | 0.0002                            | 0.000004                | <i>iddm1</i>         | 3.2          | —                              |
| <i>D4Rat29</i>       | 51.5     | <u>0.0012</u>                       | —                                   | NP                                | —                       | <i>iddm1</i>         | —            | —                              |
| <i>D4Wox21 (Npy)</i> | 53.4     | 0.0046                              | 0.016                               | NP                                | —                       | <i>iddm1</i>         | —            | —                              |
| <i>D4Rat 38</i>      | 62.0     | 0.04                                | 0.02                                | 0.005                             | 0.0002                  | <i>iddm1</i>         | 1.6          | —                              |
| <i>D4Arb24</i>       | 67.5     | 0.505                               | —                                   | NP                                | —                       | —                    | —            | —                              |
| <i>D4Mit17</i>       | 79.7     | 0.484                               | 0.583                               | NP                                | —                       | —                    | —            | —                              |

Genetic map of the *iddm4* region of chromosome 4 based on analyses of two (DP-BB × WF)<sub>F1</sub> × WF backcrosses and one (DR-BB × WF)<sub>F1</sub> × WF backcross. The first DP-BB backcross has been reported elsewhere (32). The second DP-BB backcross and the DR-BB rat backcross are described in RESEARCH DESIGN AND METHODS. The identity of DP and DR-BB alleles (= or ) was assessed for all primers shown. “Total cM,” “Total *P* value,” and “LOD score” refer to the map and the significance of linkage with type 1 diabetes when data generated by the three backcrosses are pooled and analyzed as a single set. Underlined *P* values indicate regions of strongest significance in each population. NP, not polymorphic in this cross. Map location of *lyp/iddm1* taken from Bieg et al. (31).

(22 ± 7 days) was similar to that observed in the identically treated (DP-BB × WF)<sub>F1</sub> × WF animals (25 ± 8 days).

**Diabetes segregates with *iddm4* in (DR-BB × WF) × WF backcross animals.** DR-BB backcross progeny were scored for markers linked to *iddm4* (32, Table 2). Of the 29 diabetic backcross animals, 22 (76%) were heterozygous for the DR-derived alleles of *D4Arb9* and *D4Mit3* that are centered in the interval that defines *iddm4* (32). Seven animals (24%) homozygous for WF-origin markers linked to *iddm4* became diabetic. In the nondiabetic cohort, 4 of 20 animals were heterozygous for the DR-derived allele and 16 of 20 were homozygous for the WF-derived allele. Analysis of the entire (DR-BB × WF)<sub>F1</sub> × WF backcross cohort with multiple markers on chromosome 4 reveals significant linkage of type 1 diabetes susceptibility to *iddm4* with maximum likelihood of linkage at *D4Arb9/D4Mit3* (*P* = 0.00008, Table 2).

In the (DP-BB × WF)<sub>F1</sub> × WF confirmatory backcross performed in the present study, *iddm4* was strongly linked to diabetes incidence (Table 2); 20 of 30 diabetic rats were heterozygous for DP-derived *iddm4* alleles, and 10 of 10 healthy rats were *iddm4*<sup>w/w</sup> (*P* = 0.0003).

Considering the two backcrosses reported here and our original (DP-BB × WF)<sub>F1</sub> × WF backcross (32) together, the presence of *iddm4*<sup>d/-</sup> (*D4Mit3/D4Arb9*) was associated with diabetes in 61 of 98 (62%) of all progeny tested. The absence of *iddm4*<sup>d</sup> was associated with the absence of diabetes in 51 of 70 (73%) of progeny.

**The *iddm4* interval on chromosome 4.** A panel of informative microsatellite markers on RNO4 was used to verify the location of *iddm4* in each of the crosses we have studied. The

map location of *iddm4* on chromosome 4 was identical in all three independent segregating populations: our original (DP-BB × WF)<sub>F1</sub> × WF backcross (32), the (DR-BB × WF)<sub>F1</sub> × WF backcross reported here, and the confirmatory (DP-BB × WF)<sub>F1</sub> × WF backcross also reported here.

Because *iddm4* is found in each of the three crosses at the identical location, the combined progeny can be used to estimate the overall linkage of *iddm4* to diabetes. For the 158 backcross rats from both DR-BB and DP-BB populations, linkage of *D4Arb9/D4Mit3* (*iddm4*) to diabetes is highly significant (*P* = 1 × 10<sup>-7</sup> in a 2 × 2 contingency test). The significance of this linkage drops by a factor of 20 at the *D4Mgh24* locus (*P* = 0.000002) and by a factor of 40 at the *D4Arb30* locus (*P* = 0.000004). In all three crosses, *D4Arb9* and *D4Mit3* segregated together with no recombinants. LOD scores calculated for each polymorphic marker that was typed in all three backcrosses further confirm the influence of *iddm4* on diabetes expression (Table 2). The maximum support interval of *iddm4* is proximal to the *lyp/iddm1* gene region (31 and Table 2).

***iddm4* is found in a cluster of loci common to the DP-BB and DR-BB strains.** Our data demonstrate that *iddm4* alleles have the same phenotype in both DR-BB and DP-BB rats, whereas their *lyp/iddm1* alleles are different. This observation suggests that *iddm1* and *iddm4* have different genetic origins. To determine if this is the case, we analyzed the likely genetic origin of 20 markers on chromosome 4 in the region of *lyp/iddm1* and *iddm4* in the DP- and DR-BB/Wor rats used in our studies. Our results indicate that *lyp/iddm1* resides in a gene cluster containing nine markers

that, like *lyp/iddm1* itself, differ between DP-BB and DR-BB (Table 2). There were no exceptions to the non-DP-BB ancestral origin of this cluster in the 30 cM interval examined in the DR-BB rat. In contrast, *iddm4* in the DR-BB rat resides in a 12 cM cluster of 11 markers, 9 of which exhibit DP-BB ancestral origin and are now common to both DP- and DR-BB rats. The probability of this marker distribution is 0.0003 (Fisher's exact statistic).

## DISCUSSION

We have analyzed rats segregating for diabetes but not lymphopenia using WF and DR-BB animals. We first observed that both DR-BB and (DR-BB  $\times$  WF) $F_1$  animals are uniformly susceptible to type 1 diabetes, suggesting that the majority of diabetogenic genes derived from the DR-BB parent are likely to act in a dominant fashion. This inference was confirmed by the observation that approximately half of (DR-BB  $\times$  WF)  $\times$  WF backcross progeny (all *lyp*<sup>+/+</sup>) became diabetic. Our data on latency to onset of diabetes suggest that the addition of genes from the nondiabetic WF background hinders the induction of type 1 diabetes in both DR- and DP-BB backcross progeny.

In the experiments reported here, the frequency of diabetes in control (DP-BB  $\times$  WF) $F_1$   $\times$  WF backcross animals was higher than that observed in our earlier study (32). Genetic factors, environmental factors, or both could account for this discrepancy. The WF rats in the two studies were obtained from different vendors. No genetic difference was noted for any of the limited number of microsatellite markers tested on WF rats from both vendors, but it is possible that they differ at other loci. The relatively high frequency of disease progression in the present backcrosses could reflect either a difference in environment (43) or a more effective immunostimulatory induction protocol. We have noted that different lots of poly I:C can vary with respect to their ability to induce type 1 diabetes in DR-BB rats (J.P.M., unpublished observations).

The expression of diabetes in (DR-BB  $\times$  WF) $F_1$   $\times$  WF rats is strongly linked to the *iddm4* locus. It is notable that the phenotype of *iddm4*<sup>d/w</sup> revealed in our previous analysis of a (DP-BB  $\times$  WF) $F_1$   $\times$  WF backcross (32) was a predisposition to insulinitis. In that study, linkage of *iddm4* to insulinitis was significant, whereas linkage to diabetes incidence was less robust. We interpret the available data to suggest that when environmental conditions favor disease progression, *iddm4*<sup>d/w</sup> can act to cause both insulinitis and overt diabetes.

We have also determined that *iddm4* is found in a cluster of loci common to both DP- and DR-BB rats. All BB rats are derived from an outbred colony of Wistar rats (3). The DR-BB/Wor rat line used in this study was derived from DP-BB/Wor forebears at the fifth generation of inbreeding by selection for absence of spontaneous diabetes (44). Because DP-BB and DR-BB rats have this close genetic relationship, many, but not all, genes would be expected to be the same in both strains. It is estimated that the DR-BB/Wor rat shares ~85% of its genome with the DP-BB/Wor animal (38). We hypothesized that *iddm4* would be found in a nonsegregating cluster of ancestral markers that are shared by both DP- and DR-BB rats, whereas *lyp/iddm1* would be found in a cluster that segregated and became fixed in the DP-BB rat. This approach has been useful in dissecting the origin and location of diabetes-modifying loci in the nonobese diabetic mouse (45).

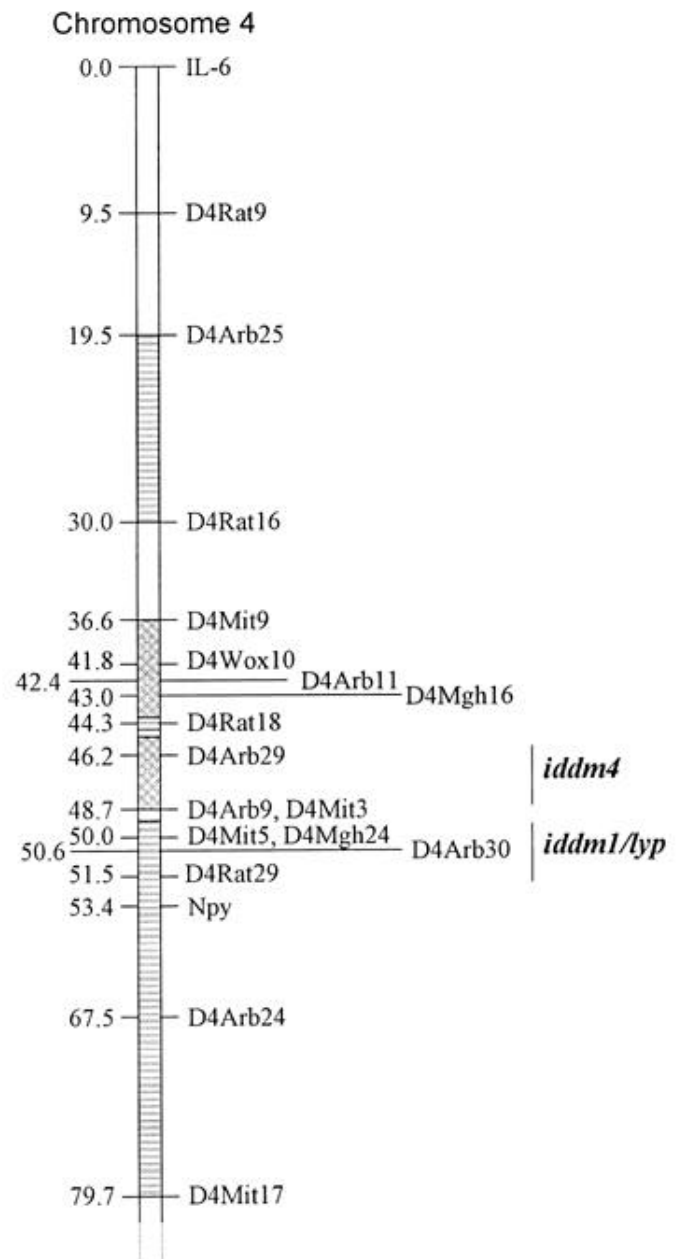


FIG. 1. Map of rat chromosome 4 indicating the positions of *iddm4* and *lyp/iddm1*. Lightly shaded segments indicate contiguous regions of chromosome 4 where marker allele sizes are identical between the DR-BB and DP-BB rats used in this report. Dark shaded areas indicate regions of chromosome 4 where DR-BB and DP-BB alleles are different from one another. *D4Mit3* differs between DR-BB and DP-BB and marks a break point between an identical region and a polymorphic region. Not drawn to scale.

This hypothesis was confirmed by our analysis showing that *lyp/iddm1* in the DR-BB rat resides in a gene cluster containing markers that, like *lyp/iddm1* itself, differ between DP- and DR-BB rats. In contrast, *iddm4* in the DR-BB rat resides in a cluster of markers that are of DP-BB ancestral origin and remain common to both DP- and DR-BB rats.

Our data establish that *iddm4* is functionally independent of, and genetically proximal to, *lyp/iddm1*. The lymphopenia (*lyp/iddm1*) gene has been mapped to rat chromosome 4

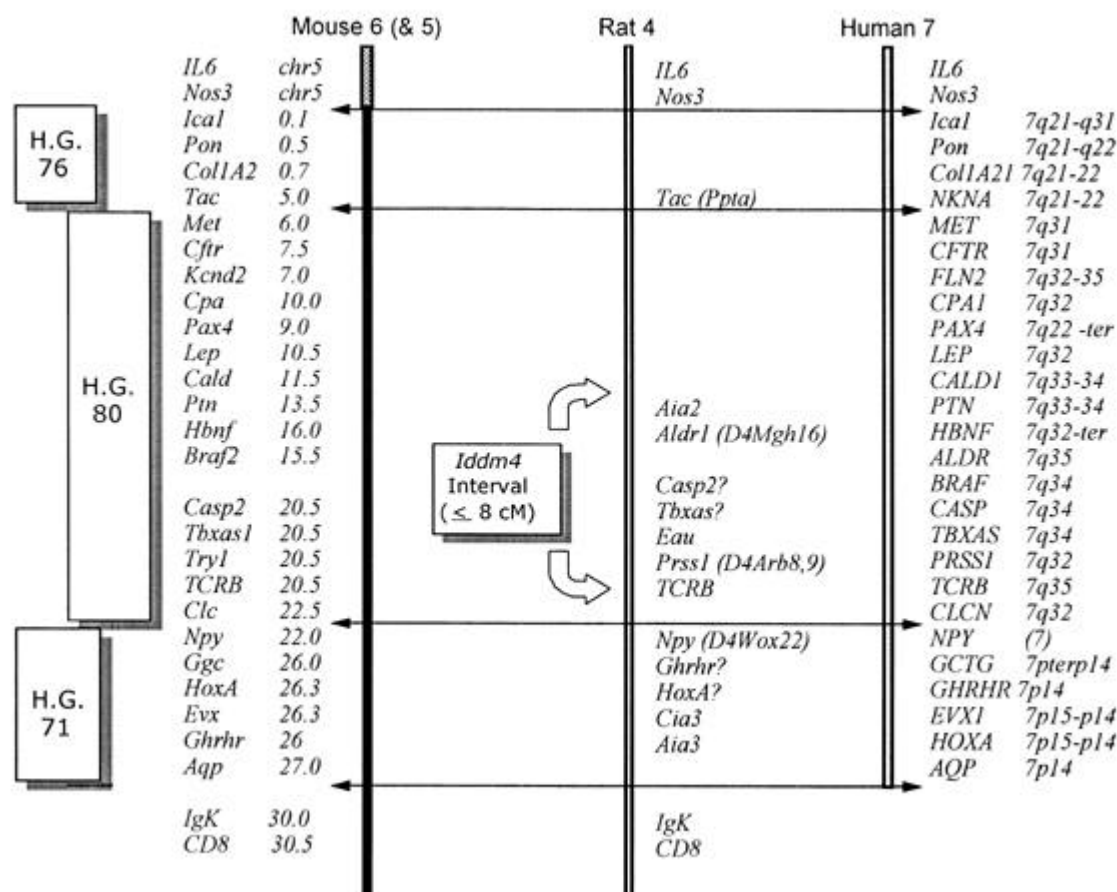


FIG. 2. Syntenic maps of mouse chromosome 6 (and 5), rat chromosome 4, and human chromosome 7. H.G., homology group. Not drawn to scale.

and resides on a 4 cM segment between *D4Mit6* and *D4Mit7(Npy)* (38,46), or between *D4Mit5/D4Mgh24* and *D4Mit24(Npy)* (31). We propose that *iddm4* resides near *D4Arb9* in a 3 cM interval between *D4Arb29* and *D4Mgh24*. Both map location and clustering predict that *iddm4* has the same allele in both DR- and DP-BB rats.

The results from this and previous backcross studies suggest that expression of disease in the BB rat involves several genetic elements. The first genetic component maps to the MHC (25). All rats in our studies express the RT1<sup>u</sup> haplotype, which is strongly associated with susceptibility to autoimmunity (34,47,48). A second component is *iddm4*, which, because it maps to the identical region on rat chromosome 4 in both DR- and DP-BB rats (Fig. 1), is highly likely to be the same gene in both sublines. The *iddm4* gene is associated with an early event in the pathogenesis of type 1 diabetes, perhaps the generation of insulinitis (32).

A third component is *lyp/iddm1*. In contrast to *iddm2* and *iddm4*, neither homozygosity nor heterozygosity at the *lyp/iddm1* locus is associated with the predisposition to diabetes in BB rats. No animals in the (DP-BB × WF)<sub>F1</sub> × WF backcross were homozygous for *lyp/iddm1*, and in the present study, all DR-BB backcross animals were homozygous for wild-type alleles at *lyp/iddm1*. Heterozygosity for *lyp/iddm1* is unlikely to account for the chromosome 4-linked diabetes phenotype seen in (DP-BB × WF) backcross animals, because the same phenotype is seen in the (DR-BB × WF) backcross rats. We propose that the role of *lyp/iddm1* in dia-

betes is to cause lymphopenia, which, due to the absence of RT6<sup>+</sup> regulatory cells from birth (2), unmasks the genetic predisposition to autoreactivity in DP-BB rats that is associated with *iddm2* and *iddm4*.

Our data are consistent with the concept that *iddm4* could be a "universal" autoimmunity susceptibility gene. The *iddm4* gene is located in a region containing several major autoimmunity loci in the rat. The *Cia3* locus contributes significantly to susceptibility to autoimmune collagen arthritis (49). A closely linked and perhaps identical locus, *Aia3*, is associated with adjuvant-induced arthritis (50). Nearby autoimmunity genes on chromosome 4 include *Aia2* and a locus involved in susceptibility to experimental autoimmune uveitis (51).

Figure 2 shows the syntenic maps of mouse chromosome 6 and 5, rat chromosome 4, and human chromosome 7. Proximal rat chromosome 4 comprises an aggregate of three separate homology groups: HG76, HG80, and HG71. HG80 is most likely to contain *iddm4*. At least 16 known genes or gene families and at least two autoimmunity genes (50) have been localized to HG80. These findings raise the possibility that *iddm4* is the same as one of these autoimmunity genes. There is a distinct break in the homology groups proximal to the *Npy* locus contained in HG71. This observation is consistent with the independent derivation of this region in the DR and DP rat strains we have observed (Table 2). The HG71 interval is likely to contain the *lyp/iddm1* locus but not the *iddm4* locus.

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