ORIGINAL ARTICLE

Virus-Induced Autoimmune Diabetes in the LEW.1WR1 Rat Requires *Iddm14* and a Genetic Locus Proximal to the Major Histocompatibility Complex

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OBJECTIVE—To identify genes that confer susceptibility to autoimmune diabetes following viral infection in the LEW.1WR1 rat.

RESEARCH DESIGN AND METHODS—About 2% of LEW.1WR1 rats develop spontaneous autoimmune diabetes. Immunological perturbants including viral infection increase both the frequency and tempo of diabetes onset. To identify diabetes susceptibility genes (LEW.1WR1 × WF), F2 rats were infected with Kilham rat virus following brief pretreatment with polyinosinic:polycytidylic acid. This treatment induces diabetes in 100% of parental LEW.1WR1 rats and 0% of parental WF rats. Linkage to diabetes was analyzed by genome-wide scanning.

RESULTS—Among 182 F2 rats, 57 (31%) developed autoimmune diabetes after a mean latency of 16 days. All diabetic animals and ~20% of nondiabetic animals exhibited pancreatic insulitis. Genome-wide scanning revealed a requirement for the *Iddm14* locus, long known to be required for diabetes in the BB rat. In addition, a new locus near the RT1 major histocompatibility complex (MHC) was found to be a major determinant of disease susceptibility. Interestingly, one gene linked to autoimmune diabetes in mouse and human, *UBD*, lies within this region.

CONCLUSIONS—The *Iddm14* diabetes locus in the rat is a powerful determinant of disease penetrance in the LEW.1WR1 rat following viral infection. In addition, a locus near the MHC (*Iddm37*) conditions diabetes susceptibility in these animals. Other, as-yet-unidentified genes are required to convert latent susceptibility to overt diabetes. These data provide insight into the polygenic nature of autoimmune diabetes in the rat and the interplay of genetic and environmental factors underlying disease expression. *Diabetes* 58:2930–2938, 2009

The precise cause of human type 1 diabetes is unknown but may involve the interaction of genetic susceptibility alleles at many loci with the environment (1). In particular, viral infection has been proposed as the “trigger” of autoimmune destruction of pancreatic β-cells (2,3). Much effort has gone into identifying both the host genes and microbial agent(s) whose combined effects lead to disease. Identification of human type 1 diabetes genes has proven difficult, however, because disease-associated alleles are common, and even the highest-risk genotypes confer only modest risk of disease (4,5). Studying environmental factors is also difficult because the human population is exposed randomly to microbial agents, and the role of viral infection in the disease has remained controversial (6).

In contrast, animal models can be inbred and tested in controlled environments. Two widely used models of autoimmune diabetes are nonobese diabetic (NOD) mice (7) and BB rats (8). More than 30 mouse loci are linked to diabetes, some of which are orthologous of human non–MHC-linked genetic loci identified by genome-wide association studies (GWASs) (9,10). With respect to the environment, however, NOD mice may model type 1 diabetes poorly (11) because most viral infections reduce disease frequency (12–14). Only Coxsackie virus accelerates disease in NOD mice (15), but it is associated with exocrine pancreatitis (16), which is uncharacteristic of human type 1 diabetes (17,18). For these reasons, the NOD mouse has not been informative for modeling the virus-diabetes relationship or the genetic basis for this interaction.

The relevance of autoimmune diabetes in spontaneously diabetic BBDR rats to human disease has been questioned because they are congenitally lymphopenic. Nonetheless, recent studies show that diabetes susceptibility in this animal is linked to MHC- and non–MHC-linked genetic loci that are orthologous to human GWAS loci (19). BBDR and LEW.1WR1 rats, which rarely become spontaneously hyperglycemic, share many of these loci and are susceptible to viral triggering of type 1 diabetes (8,20–22). Susceptibility to triggering by specific viruses in these rats is variable, and linkage studies will be helpful for dissecting the genetic basis for the virus-diabetes relationship.

BBDR rats (23) never develop spontaneous hyperglycemia in clean housing but readily develop diabetes after infection with Kilham rat virus (KRV), a parvovirus (20). Natural infection induces diabetes in ~1% of animals; injection of virus induces diabetes in 30–40% (20). Infection with KRV following brief pretreatment with three nondiabetogenic doses of polyinosinic:polycytidylic acid (poly I:C), a ligand of Toll-like receptor 3, leads to diabetes in 100% of animals (21). Poly I:C is a ligand of Toll-like receptor 3 and by itself is nondiabetogenic at this dose (21). KRV does not infect β-cells, and susceptibility is virus specific; H-1, which is 98% sequence identical, never induces diabetes (21,24). WF rats (bearing the same high-risk RT1α class II allele as BBDR) are susceptible to infection with KRV but are completely resistant to both spontaneous and virus-induced diabetes (21).
We have used the BBDR model to map loci important for predisposition to virus-induced diabetes ("Iddm" loci) using linkage assessment in F2 rats (25). One locus, Iddm14 (formerly Iddm9), is a dominant factor in KRV-induced diabetes in the BBDR strain (25). A second locus, Iddm20, on chromosome 17, is required for full penetrance of Iddm14 (25). Importantly, congenic WF Iddm14DR rats that bear only the BB origin allele of Iddm14 are resistant to KRV-induced diabetes (25).

LEW.1WR1 rats are genetically distinct from BBDR rats (26). They bear an unusual MHC recombinant haplotype RT1^A/B/D^C^0 (27) that includes the class II "u" alleles that are generally required for autoimmune diabetes in the rat (28). They develop spontaneous diabetes at a low rate (−2.5%) but readily develop diabetes during chronic treatment with poly I:C (22). They also become diabetic following exposure not only to KRV but also cytomegalovirus and vaccinia virus (29,30).

In the present report, we have determined the genetic control of virus-induced diabetes in the LEW.1WR1 rat strain. Despite the genetic divergence of the BB and LEW parental strains, we show that both rats require permissive alleles at Iddm14. However, unlike BBDR rats, LEW.1WR1 rats also require an MHC-linked gene for KRV-induced diabetes. Taken together, these studies illustrate the genetic complexity of susceptibility to autoimmune diabetes in the rat.

**RESEARCH DESIGN AND METHODS**

LEW.1WR1 (RT1^A/B/D^C^0, ART2a) rats were obtained from Biomedical Research Models (Worcester, MA). WF.ART2 rats (RT1u/u, ART2a) were developed by us and obtained from the colony at the University of Massachusetts Medical School; they differ from ordinary WF animals in that they express the BB origin "a" rather than the "b" allotype of the ART2 T-cell alloantigen (25,31). WF.ART2a rats do not develop diabetes either spontaneously or in response to KRV infection (25). WF.ART2 animals are referred to as WF in this report. (LEW.1WR1 × WF) F2 rats were bred by us, housed in viral antibody–free conditions, confirmed monthly to be serologically free of rat pathogens (31), and maintained according to guidelines of the institutional animal care and use committees of the University of Massachusetts, Biochemical Research Models, and the Guide for the Care and Use of Laboratory Animals (32).

**Induction of diabetes.** Diabetes was induced using KRV (University of Massachusetts) propagated in normal rat kidney cells (ATCC CRL-6509) as described (25). Poly I:C (Sigma, St. Louis, MO) was dissolved in Dulbecco's PBS, sterilized, and stored at −20°C until used. Continuing use of poly I:C concentration was <50 units/ng (Charles River Endosafe, Charleston, SC). Rats 21–25 days of age of either sex were injected intraperitoneally with poly I:C (1 µg/g body wt) on days −3, −2, and −1 and with KRV (10^6 plaque-forming units) on day 0. We have previously observed that this short course of poly I:C does not itself elicit diabetes but does increase the frequency of diabetes in KRV-treated LEW.1WR1 rats from 40 to 100% (R.S. Tirabassi, D.L.Gr., E.P.B., J.H. Leif, B.A. Woda, Z. Lui, D.A. Winans, D.L.Gu., I.P.M., unpublished data). LEW.1WR1 rats injected chronically with poly I:C (1 µg/g three times per week) become diabetic within 2 weeks (22); we used this protocol to elicit diabetes in rats intended for certain gene expression studies. Animals were screened three times weekly for glycosemia (Tes-Tape; Eli Lilly, Indianapolis, IN) for 40 days. Diabetes was diagnosed on the basis of plasma glucose concentrations >250 mg/dl (OneTouch Ultra Glucometer; Lifescan, Milpitas, CA).

**Histology.** To quantify insulitis, pancreata were harvested and fixed in 10% formalin; 10–µm sections were stained with hematoxylin and eosin and scored by light microscopy on a scale of 0 to 4+ as described (31).

**Genotyping and linkage.** DNA samples were prepared from tail snips or liver samples and genotyped using microsatellite markers as described (25,31). LEW.1W and LEW.1A genomic DNA samples were the gift of Dr. Dirk Wedekind (Max-Planck-Institut, Germany). Genotypes for 133 evenly distributed microsatellite markers were collected in the Map Manager QT program and exported for further analysis to Windows Quantitative Trait Locus (qTL) Cartographer, version 2.5 (33). Severity of insulitis and days to onset (nondiabetic rats being scored as 41 days) were used as the quantitative subphenotypic parameters of diabetes in this QTL analysis. Composite interval mapping was performed using model 6 to identify QTL peaks with a significant likelihood ratio test (LRT) statistic. Permutation analyses to establish thresholds of significance were performed using Map Manager QT, set for 1,000 permutations of the data (34).

**Sequence analyses.** High-fidelity Taq polymerase (Platinum PCR Supermix High Fidelity; Invitrogen) was used to amplify genomic DNA or cDNA, and these products were used as sequencing templates. Primers for amplification were designed using the BN rat genome sequence (available at genome.ucsc.edu) and Primer 3 software (available at http://frodo.wi.mit.edu/primer3).

In addition to full sequences, single nucleotide polymorphisms (SNPs) were typed on chromosome 20 using the phototyping technique (35). Two alternate allele-specific SNP primers and one common opposite-strand primer were designed for each SNP using Primer 3 to have a Tm melting temperature of 58–60°C, where the allele-specific primer has a 3’ nucleotide that matches one of two SNP alleles. SNP haplotypes were assembled in an Excel database and used to delineate the breakpoints of the recombinant chromosome 20 in LEW.1WR1 rats. Only SNPs that distinguish the two LEW.1WR1 parental strains, LEW.1A and LEW.1W (26), are shown in this report.

**Gene expression data.** cDNA was prepared from rat spleen and mesenteric lymph node RNA using the Omniscript RT Kit 200 (Qiagen). Primers for rat UBD (diubiquitin), TNF (tumor necrosis factor), IPN-γ (interferon-γ), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were designed from rat genomic sequences as above (online appendix Table 1 [available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0387/DC1]). Quantitative real-time PCR was performed using SYBR Green PCR mix on an ABI 7000 sequence detector.

**Data analysis.** Life tables were analyzed using the method of Kaplan and Meier and log-rank statistics (36). Parametric data are given as arithmetic means ± SD. Two-group comparisons used unpaired t tests, and comparisons of three or more groups used ANOVA. Real-time quantitative PCR data were analyzed with the Mann-Whitney test and ANOVA. Two-tailed P values <0.05 are considered statistically significant. Genetic interaction between two QTL was assessed using one-way ANOVA adding in each factor (QTL) sequentially.

**RESULTS**

Autoimmune diabetes in (LEW.1WR1 × WF) F2 rats. In a preliminary study, 97% of LEW.1WR1 rats (29 of 30) developed diabetes after a 3-day low-dose pretreatment with poly I:C followed by 10^7 plaque-forming units of KRV. None of six rats became diabetic after this brief course of poly I:C alone. We then tested (LEW.1WR1 × WF) F1 rats using the same protocol, and 44% (26/59) became diabetic with an average latency of 15.5 days.

To generate a linkage map of Iddm loci, we then created a cohort of 182 (LEW.1WR1 × WF) F2 rats and, again, treated them with the same induction protocol. Of these, 57 became diabetic (31%; 27% of females and 36% of males, P = 0.22). Average latency to diabetes among sick animals was statistically similar in male and female rats (16.4 ± 2.7 and 16.2 ± 2.9 days, respectively; P = not significant).

Pancreata obtained from 175 (LEW.1WR1 × WF) F2 rats were scored for insulitis. Among diabetic rats, mean histological score was 3.7 ± 0.5 with no scores <3+. Among nondiabetic rats, the mean insulitis score was 0.6 ± 0.8 (range 0 to 2+; P < 0.0001 vs. diabetic rats); only 19.5% (25 of 128) had insulitis with scores of 1+ to 2+.

The Iddm14 locus is a significant determinant of diabetes in (LEW.1WR1 × WF) F2 rats. We predicted that Iddm14 and/or Iddm20 would be important for diabetes induction by KRV following poly I:C in LEW.1WR1 rats, just as they are required for KRV-induced BBDR diabetes (25). Therefore, we generated genomic DNA samples from 182 (LEW.1WR1 × WF) F2 animals and genotyped them for markers that identify the Iddm14 and Iddm20 intervals on chromosomes 4 and 17, respectively. Iddm14 (as marked by its canonical microsatellite marker D4Arb9 [37]) was highly significantly linked to diabetes in this cohort. All 57 diabetic F2 rats bore at least one
Iddm14 allele from the LEW.1WR1 parent \((P = 1.5 \times 10^{-11}, \text{Fisher} \times 3 \text{ exact probability test})\). There was no linkage to the Iddm20 QTL on chromosome 17 \((25)\), which segregated randomly in this cohort with respect to diabetes in both the total F2 \((P = 0.493)\) and in those F2 rats bearing at least one copy of the susceptible Iddm14 allele \((P = 0.434)\).

**A new locus, Iddm37, enhances penetrance of Iddm14.** As we observed for the (BBDR/H11003 WFlF) F2 cross \((25)\), not all (LEW.1WR1/H11003 WFlF) F2 rats with diabetogenic Iddm14 alleles became sick during the 40-day observation period. Only 41% \((36 \text{ of } 91)\) of heterozygotes and 57% \((21 \text{ of } 37)\) of Iddm14L homozygous rats were diabetic, indicating that another gene (that is not Iddm20 [25] on chromosome 17) is required for diabetes in this strain combination (Table 1). We therefore conducted a full genome scan of F2 to find this QTL.

Permutation analysis of the dataset revealed that “suggestive” linkage for a QTL required an LRT \(\geq 10.5\); “significant” linkage, an LRT \(\geq 17.5\); and “highly significant” linkage, an LRT \(\geq 25.7\). Our first QTL, Iddm14, has a remarkably high LRT of 49 (Fig. 1), and it is therefore a highly significant predictor of diabetes in this strain combination. A second QTL was found on chromosome 20 (Fig. 1), and we provisionally designate it Iddm37. It is a significant predictor of diabetes and severity of insulitis in this F2 cohort (LRT = 19.5 and LRT = 29, respectively).

**Iddm37 acts by modifying the penetrance of Iddm14.** The Iddm14 locus was significantly linked to increased diabetes incidence only when rats carried at least one LEW.1WR1-derived allele of D20Rat47, which, at \(2.8 \text{ Mb}\) from the top of chromosome 20, serves as the closest-linked marker for Iddm37. Among 128 rats bearing a susceptible allele of Iddm14, there was a significant increase in diabetes frequency in Iddm37L/L \((46\%)\), or

| Genotype | Number | Days to onset |
|----------|--------|---------------|
| Iddm14 W/W | 0 | NA |
| Iddm14 L/W | 36 | 16.6 ± 2.8 |
| Iddm14 L/L | 21 | 15.8 ± 2.8 |
| Total | 57 | |
| Iddm14 W/W | 54 | NA |
| Iddm14 L/W | 55 | NA |
| Iddm14 L/L | 16 | NA |
| Total | 125 | |

Data are means ± SD, unless otherwise indicated. The 182 (LEW.1WR1 × WF) F2 rats described in RESEARCH DESIGN AND METHODS were divided into diabetic and nondiabetic categories after a 40-day observation period. Within each group, animals were subcategorized according to their inheritance of Iddm14 alleles: Iddm14 W/W, homozygous for WF-derived alleles at Iddm14; Iddm14 L/W, heterozygous; and Iddm14 L/L, homozygous for LEW.1WR1-derived alleles at Iddm14. Shown in the final column are the mean latencies to diabetes in each cohort. NA, not applicable (not diabetic).

*Significance of linkage of diabetes to Iddm14 is \(P = 1.5 \times 10^{-11}\) (Fisher exact probability \(2 \times 3\) test).

**TABLE 1**

Iddm14 is required but not sufficient for diabetes expression in the (LEW.1WR1 × WF) F2 progeny

**FIG. 1.** Composite interval analysis of linkage to the diabetes subphenotypes of insulitis and latency to onset in 182 (LEW.1WR1 × WF) F2 rats was performed as described in RESEARCH DESIGN AND METHODS. The QTLs for insulitis are depicted by solid lines and the QTLs for latency by dashed lines; the LRT scale is on the y-axis. Suggestive (10.2) and significant (17.2) LRT score cutoffs are indicated by horizontal lines. The peaks of each of the latency QTLs (on chromosome 4, D4Arb9 and D4Got43; and on chromosome 20, D20Rat47) correspond to an LRT of 48.3 and 19.5, respectively (the equivalent LOD scores are 10.52 and 4.53, respectively). For insulinis, Iddm14 is linked with an LRT of 38.5 at the peak marker and Iddm37 an LRT of 29.01 (LOD scores of 8.38 and 6.31, respectively).
Iddm37L/W (63%) animals compared with Iddm37W/W rats (19%, \( P < 0.0001 \), \( \chi^2 \) test) (Table 2). Thus, while diabetes was absolutely dependent on the presence of at least one LEW.1WR1-derived allele of Iddm14, diabetes penetrance was significantly enhanced in those rats that also bore LEW.1WR1 alleles at Iddm37 (Fig. 2).

No other QTLs were significantly linked to diabetes incidence, latency, or insulitis in the genome scan of the whole F2 cohort (Fig. 1). However, among the diabetic animals, we noted a significant linkage of latency to Iddm24 (Fig. 3), a diabetes latency QTL originally discovered in a cross between WF and BBDP rats (38). In both crosses, Iddm24 was marked by microsatellite markers at 110–120 Mb distal to the centromere. Among diabetic rats, WF-derived alleles of Iddm24 were protective when homozygous and prolonged latency from 14.5 ± 2 days in Iddm24L/L rats to 17.6 ± 2 days in Iddm24W/W rats (\( P = 0.0056 \)).

### Candidate genes in the Iddm37 locus.

Iddm37 is located in the extended RT1C region of the MHC, which is known to differ among LEW.1W, LEW.1A, WF, and LEW.1WR1 rats (39). Because LEW.1WR1 rats bear a recombinant MHC haplotype, it remained to be determined which of three independent MHC-linked haplotypes (LEW.1A, LEW.1W, or the LEW background) contributed the diabetes-promoting allele that we identified as the Iddm37 QTL. The original mapping of LEW.1WR1 suggested that the RT1C region in LEW.1WR1 rats was donated by LEW.1A. To confirm this, we conducted an extensive SNP analysis of the chromosome 20 region

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**TABLE 2**

Permissive LEW.1WR1 alleles at Iddm37 increase diabetes susceptibility in F2 rats that bear permissive alleles at Iddm14

| Iddm14 L/L or L/W | Diabetic | Well | Total |
|-------------------|----------|------|-------|
| Iddm37 L/L or L/W | 12 (46%) | 14   | 26    |
| Iddm37 W/W        | 0        | 11   | 11    |
| Total             | 12       | 25   | 37*   |

| Iddm14 L/L or L/W | Diabetic | Well | Total |
|-------------------|----------|------|-------|
| Iddm37 L/L or L/W | 37 (63%) | 22   | 59    |
| Iddm37 W/W        | 0        | 30   | 30    |
| Total             | 37       | 52   | 89†   |

| Iddm14 L/L or L/W | Diabetic | Well | Total |
|-------------------|----------|------|-------|
| Iddm37 L/L or L/W | 8 (19%)  | 35   | 43    |
| Iddm37 W/W        | 0        | 12   | 12    |
| Total             | 8        | 47   | 55‡   |

Among 182 (LEW.1WR1 x WF) F2 rats subjected to our diabetes induction protocol as described in RESEARCH DESIGN AND METHODS, we analyzed 129 that carried at least one LEW.1WR1 origin allele at Iddm14 (either Iddm14 L/L or Iddm14 L/W, row 3) or two WF origin alleles (row 4). These two genetic categories were then subdivided according to their Iddm37 genotype. The columns present the number and percent of diabetic and nondiabetic animals for each of six compound genotypes. The table shows that although Iddm14 was required for diabetes, its effect on susceptibility to diabetes was dependent on the presence of Iddm37L alleles. In the absence of Iddm37, the diabetogenicity of Iddm14 is substantially less powerful. Iddm37, therefore, conditions the penetrance of Iddm14. *The significance of Iddm37 in the Iddm37L/L cohort is \( P = 0.006 \). †The significance of Iddm14 in the Iddm37L/W cohort is \( P < 0.0001 \). ‡There is no significance of Iddm14 in the Iddm37W/W cohort, \( P = 0.106 \).
containing Iddm37. SNPs representing alleles from LEW.1WR1 rats and their three parental strains were identified. The strain distribution pattern of SNP alleles for genes in the RT1C region indicates that the majority of LEW.1WR1 alleles are of LEW.1A origin, consistent with the original mapping (Table 3).

The Iddm37 region includes the gene for diubiquitin (UBD), a candidate gene of interest because it resides in a region that is homologous to mouse and human diabetes-promoting intervals (40,41). We therefore sequenced the complete UBD gene in the strains we studied. This resulted in the identification of two major coding region haplotypes of UBD that we designate the “A” haplotype (LEW.1A, LEW.1WR1) and the “L” haplotype (LEW, WF, LEW.1W) (Fig. 4). The two UBD haplotypes contain extensive SNPs in the two exons and single UBD intron (Table 3), and there is a simple-sequence length polymorphism upstream of the 5’ end of UBD. However, only two UBD SNPs result in nonsynonymous changes. One substitution of histidine for arginine in the NH2-terminal domain at amino acid 9 (SNP no. 18) (Table 3) is conservative in that other mammalian UBD sequences include either amino acid at this residue (online appendix Table 2). Interestingly, the LEW.1WR1 and LEW.1A strains have another SNP (no. 22) (Table 3) that encodes an amino acid change in the COOH-terminal of UBD protein (changing an asparagine to a histidine at residue 145 [N145H]). This residue is not seen in any other mammalian UBD sequence available for comparison (online appendix Table 2). Indeed, the peptide sequence containing amino acid 145N in the rat UBD-W haplotypes is conserved in rat, mouse, C. elegans, and S. cerevisiae; human and porcine UBD have glycine in this position.

Expression of UBD in diabetic versus nondiabetic animals. In a pilot study designed to quantify UBD expression during the course of diabetes onset, we took advantage of the fact that diabetes can be induced in 100% of LEW.1WR1 rats by chronic treatment with poly I:C alone (22). LEW.1WR1 rats were injected with poly I:C (1 μg/g three times weekly), and, as expected (22), 100% (12/12) developed hyperglycemia between days 12 and 14. As would be predicted by the fact that they do not have an RT1u class II haplotype (28), no identically treated LEW control rats developed diabetes. In a separate experiment, LEW.1WR1 and LEW rats were either untreated or treated with poly I:C three times weekly as described above. Spleens and pancreatic lymph nodes (PLNs) were collected from one to six LEW.1WR1 and LEW rats on day 0 (before any poly I:C) and on days 3, 5, 8, 10, and 12 after the first injection of poly I:C. The level of UBD mRNA as a percentage of a control gene product, GAPDH, was assessed by quantitative real-time RT-PCR.

UBD mRNA expression in PLN cells and spleen was higher in LEW.1WR1 rats than in LEW rats at every time point studied (Fig. 5A). In the case of spleen cells, UBD mRNA levels were lower overall than in PLN cells, but they increased progressively during treatment with poly I:C. In the PLN cells, levels did not increase appreciably during the course of poly I:C injections. Our observation that UBD mRNA expression was higher in LEW.1WR1 than in LEW rats at every time point including on day 0 suggests that there is a genetic predisposition to higher UBD levels in LEW.1WR1 rat lymphoid tissues.

To lend support to this hypothesis, we next excluded the possibility that the substantially lower UBD mRNA expression in LEW rat tissues was due to the absence of IFN-γ or TNF-α, known inducers of UBD (42). Simultaneous testing of the cDNA from the same rat spleen samples showed comparable levels of IFN-γ and TNF-α transcripts in both LEW and LEW.1WR1 samples (Fig. 5B). Thus, the allelic coding region polymorphisms seen in the LEW.1WR1 and LEW rat UBD genes, and not differ-

FIG. 3. Latency among sick animals is linked to a QTL on distal chromosome 8. The likelihood ratio statistic of this QTL, located in the interval that contains the Iddm24 locus (38), achieves independent significance for linkage in the present KRV-induced diabetes study. The horizontal line indicates a “suggestive” cutoff threshold.
SNPs were identified in the Iddm37 and linked RT1 loci in LEW.1WR1 (RT1Aub/DuCa), LEW.1W (RT1Aub/DuCu), LEW.1A (RT1Aab/DaCa), and WF (RT1Aub/DuCu) rats by sequencing and phototyping as described in Research Design and Methods. Column 7 indicates the donor of each allele to the LEW.1WR1 rat. The polymorphic nucleotides at each position are listed. The right-most column gives the gene, if any, in which the SNP is located and whether it is known to be a coding region polymorphism. Starred coding nucleotides result in amino acid changes in UBD. Microsatellite markers were scored for size, and the length in nucleotides is given. RT1 alleles are given for reference, and deletions are indicated as “DEL.” The horizontal line at 698,147 Mb indicates the breakpoint for RT1C. RT1C is an old term for a region that contains many genes, and it is proximal to this breakpoint. UBD and other genes in LEW.1WR1 in this region are derived from LEW.1A, not LEW.

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DISCUSSION

These data shed new light on the genetics of autoimmune disease in the rat models of human type 1 diabetes. They
further strengthen the already strong case for Iddm14 as a susceptibility locus. In addition, they identify a new locus, Iddm37, which plays a role in susceptibility to disease that is triggered by the diabetogenic KRV parvovirus in LEW.1WR1 rats.

Insulitis and diabetes both segregate with Iddm14 in the LEW.1WR1/H11003WF intercross, as they are already known to do in BBDR/H11003WF crosses (25). This observation makes Iddm14 one of the most consistent and powerful diabetes susceptibility loci observed in analyses in which WF or F344 rat strains are used as the nondiabetic partner strain (43,44). Strictly speaking, of course, we cannot claim that the specific susceptibility gene or genes associated with the Iddm14 region in BB and LEW.1WR1 rats are identical until they have been cloned, but the peak marker of this susceptibility locus in both strains is the same. Candidates for Iddm14 include the T-cell receptor Tcrb-V chain variable region gene family, especially TcrbV13. Interestingly, LEW.1WR1 rats have the same Tcrbv13 allele as BBDR rats (43).

The QTL most clearly responsible for enhancing diabetes penetrance among rats that had both permissive Iddm14 alleles and insulitis in the KRV-exposed BBDR/H11003WF progeny was Iddm20 on chromosome 17 (25);
however, this locus appears not to play a role in the susceptibility of LEW.1WR1 × WF progeny. The chromosome 17 Iddm20 QTL is relatively weak, and studies in WF.chr17-DR congenic rats have found that Iddm20 is recessive in the first three backcross generations (J.P.M., E.P.B., unpublished data). LEW.1WR1 × WF progeny require a locus on chromosome 20 for full diabetes penetrance in Iddm14-permissive animals. The frequency of diabetes in Iddm37 heterozygous (LEW.1WR1 × WF) F2 progeny exceeded that of both Iddm37 LAL homozygotes and Iddm37 W/W homozygotes in this intercross (Table 2). We therefore expect that Iddm37 will be genetically dominant in congenic rats bearing LEW.1WR1-derived alleles at Iddm14 and Iddm37.

Iddm37 represents a new and interesting QTL in rats because it is syntenic with known diabetes-associated regions in both mouse and human genomes. In mice, three H2-linked QTLs were observed in a study of NOD.B6 congenics (45), including Idd124, which maps between Lta (35.3 MB) and D17Mit105 (41.4 MB) on chromosome 17. The mouse Idd124 QTL has not yet been confirmed, but the interval contains the mouse UBD gene at 37.3 Mb.

Iddm37 is also syntenic with the high-risk locus identified in a scan of HLA-linked SNPs inherited by descent (IBD) in siblings of diabetic children in the DAISY study (41). By eliminating genes that have shared alleles in the linkage disequilibrium–limited HLA region, and would thus have no differential effect on diabetes in this cohort, only two genes remained as candidates in the supported interval, UBD and MASIL. Studies have revealed that siblings who have inherited shared UBD/MASIL alleles IBD with the diabetic proband have a 65% likelihood of developing type 1 diabetes; the likelihood in HLA-matched but not IBD-matched siblings was only 15% (40,41). Of two candidate loci, only UBD is syntenic with the Iddm37 QTL in rats, as rat MASIL is on chromosome 1.

UBD is a member of the family of ubiquitin-like proteins. It has several unusual properties including two ubiquitin-like domains. In most genera, these domains are conserved and direct repeats of one another. In mammals, they are homologous but nonidentical. UBD has functions that include directing proteins to the proteasome or to the aggresome (46) using E1 (47), E2, and E3 molecules that are different from the usual ubiquitin pathway members. In addition, UBD is inducible in the setting of inflammation. IFN-γ and TNF-α are cytokines known to increase the expression of UBD (42). The finding that UBD gene expression is upregulated in LEW.1WR1 rats after stimulation with poly I:C, and not in LEW rats, was somewhat unexpected because LEW rats are known to be highly sensitive to the cytokine-inducing effect of poly I:C (48). Direct measurement of TNF-α and IFN-γ transcripts showed that these cytokines were present in both of the strains we studied, but only in LEW.1WR1 was UBD upregulated. UBD upregulation in LEW.1WR1 rats may therefore reflect a different response to the stimulant at the level of the UBD gene. It is formally possible that upregulation of UBD in LEW.1WR1 rats exposed to poly I:C is a result rather than a cause of the inflammation that will ensue in the diabetes-prone strains, but the significantly higher levels of UBD transcripts present in LEW.1WR1 tissues before poly I:C injection and before hyperglycemia argue against this possibility.

A more intriguing hypothesis is that the UBD allele from LEW.1WR1 is causally associated with the failure of tolerance leading to autoimmune diabetes in this rat in response to environmental stimulants such as Toll-like receptor ligation or viral infection. UBD plays an important role in dendritic cell maturation (49,50) and thus has a potential role in generating autoreactive T-cells that recognize self-peptides. It may also control key signaling molecules in T-cells (51). In future studies, it will be of interest to analyze dendritic cell and T-cell functionality in rat strains that carry each of the UBD alleles that we have identified. Similarly, it will be of interest to determine diabetes susceptibility in other RT1u rat strains as a function of UBD genotype.

In conclusion, we have formally confirmed the role of Iddm14 in diabetes susceptibility in a third rat strain, with LEW.1WR1 joining the previously described BBBD and BBDR strains. In addition, we describe a new locus, Iddm37, that is important for KRV-triggered diabetes in the LEW.1WR1 rat, which can now be used to model an important human syntenic QTL identified in the DAISY cohort. Together with other recent reports of new genetic loci in both spontaneous (19) and induced (25) diabetes, our data highlight the growing relevance of rat models to the study of human type 1 diabetes.

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No potential conflicts of interest relevant to this article were reported.

The designation Iddm37 has been submitted to rat genome database (RGD) and assigned RGD ID 2305926.

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