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 orthologues of human non–major histocompatibility complex (MHC) 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 BBDP 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 $RT1^u$ class II allele as BBDR) are susceptible to infection with KRV but are completely resistant to both spontaneous and virus-induced diabetes (21).

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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 *Iddm4*), 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.*Iddm14*^{DR} 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 RT1A^{u}B/D^{u}C^{a} (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 (RT1A^{u}B/D^{u}C^{a}, 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, sterile filtered, and stored at −20°C until used. Contaminating endotoxin concentration was <50 units/mg (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 (10^7 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., J.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 glycosuria (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-μ 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 (Hanover, 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 T_{melt} 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), *IFN-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 7900 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 prepared 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

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

	Genotype*	Number	Days to onset
Diabetic rats	*Iddm14* W/W	0	NA
	Iddm14 L/W	36	16.6 ± 2.8
	Iddm14 L/L	21	15.8 ± 2.8
	Total	57	
Nondiabetic rats	*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 × 3 test).

Iddm14 allele from the LEW.1WR1 parent ($P = 1.5 \times 10^{-11}$, Fisher 2 × 3 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 $Iddm14^L$ allele ($P = 0.434$).

A new locus, *Iddm37*, enhances penetrance of *Iddm14*. As we observed for the (BBDR × WF) F2 cross (25), not all (LEW.1WR1× WF) F2 rats with diabetogenic *Iddm14* alleles became sick during the 40-day observation period. Only 41% (36 of 91) of heterozygotes and 57% (21 of 37) of $Iddm14^{L/L}$ 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 ≥10.5; "significant" linkage, an LRT ≥17.5; and "highly significant" linkage, an LRT ≥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 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 $Iddm37^{L/L}$ (46%), or

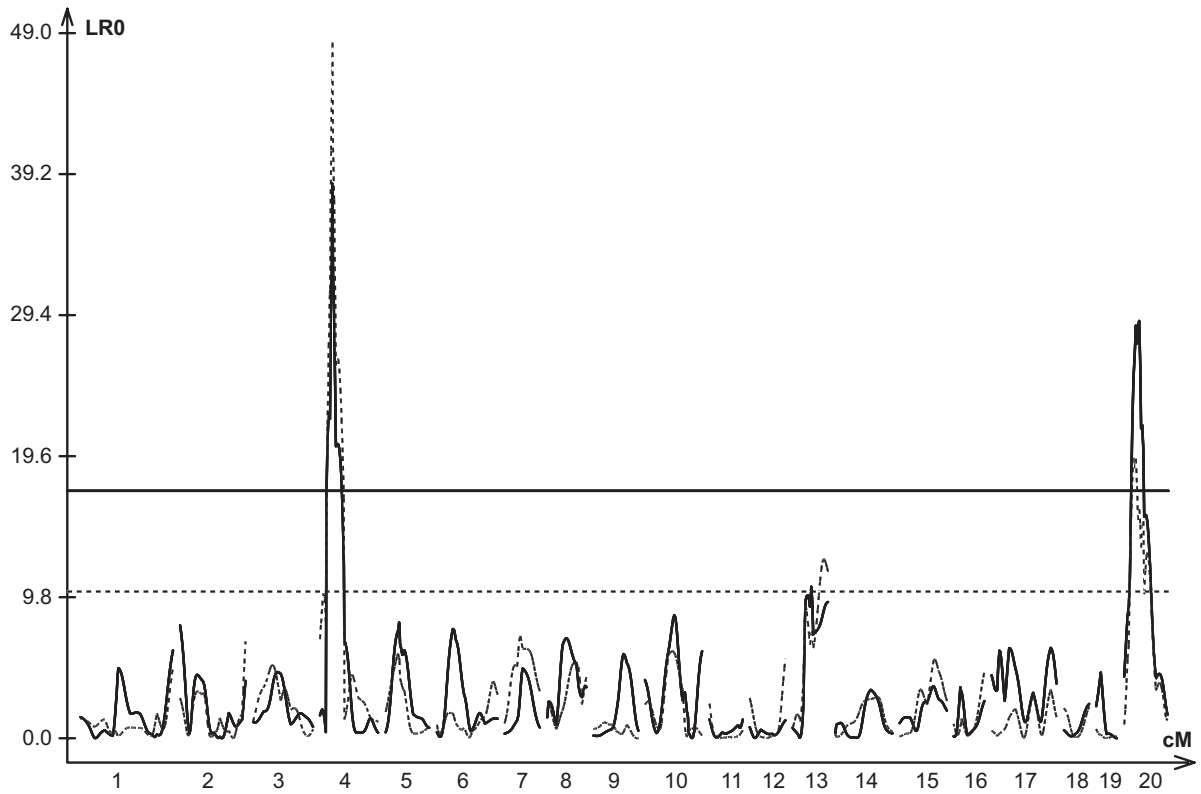

FIG. 1. Composite interval analysis of linkage to the diabetes subphenotypes of insulitis and latency to onset in 182 (LEW.1WR1 × WF) F2 rats was performed as described in RESEARCH DESIGN AND METHODS. The QTLs for insulitis are depicted by solid lines and the QTLs for latency by dashed lines; the LRT 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 insulitis, *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).

TABLE 2
Permissive LEW.1WR1 alleles at *Iddm37* increase diabetes susceptibility in F2 rats that bear permissive alleles at *Iddm14*

	Iddm37 L/L			*Iddm37* L/W			*Iddm37* W/W		
	Diabetic	Well	Total	Diabetic	Well	Total	Diabetic	Well	Total
Iddm14 L/L or L/W	12 (46%)	14	26	37 (63%)	22	59	8 (19%)	35	43
Iddm14 W/W	0	11	11	0	30	30	0	12	12
Total	12	25	37*	37	52	89†	8	47	55‡

Among 182 (LEW.1WR1 × 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 *Iddm37*L alleles. In the absence of *Iddm37*, the diabetogenicity of *Iddm14* is substantially less powerful. *Iddm37*, therefore, conditions the penetrance of *Iddm14*. *The significance of *Iddm14* in the *Iddm37*L/L cohort is $P = 0.006$. †The significance of *Iddm14* in the *Iddm37*L/W cohort is $P < 0.0001$. ‡There is no significance of *Iddm14* in the *Iddm37*W/W cohort, $P = 0.106$.

Iddm37$^{L/W}$ (63%) animals compared with *Iddm37*$^{W/W}$ rats (19%, $P < 0.0001$, χ^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 *Iddm24*$^{L/L}$ rats to 17.6 ± 2 days in *Iddm24*$^{W/W}$ 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

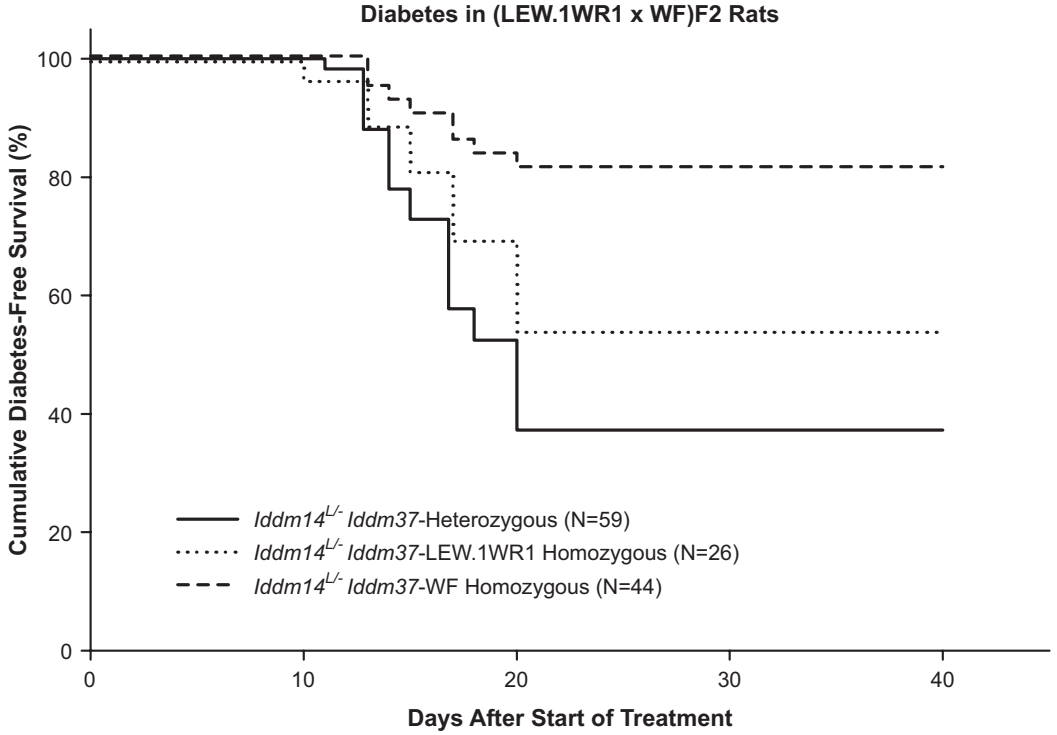

FIG. 2. (LEW.1WR1 × WF) F2 rats were subjected to the diabetes induction protocol described in RESEARCH DESIGN AND METHODS. Diabetes was observed to occur only in those 129 animals that expressed at least one LEW.1WR1-derived allele of *Iddm14* (*Iddm14*$^{L/-}$; RESULTS). Shown is a Kaplan-Meier diabetes-free survival plot for the these 129 rats stratified according to *Iddm37* genotype. The figure shows that the presence of one or two copies of *Iddm37*-L favors development of diabetes. Comparisons: overall, the three groups are highly significantly different by log-rank analysis, $P < 0.001$; *Iddm37* heterozygotes vs. *Iddm37* L/L, P = NS; *Iddm37* heterozygotes vs. *Iddm37* W/W, $P < 0.001$; *Iddm37* L/l vs. *Iddm37* W/W, $P = 0.015$.

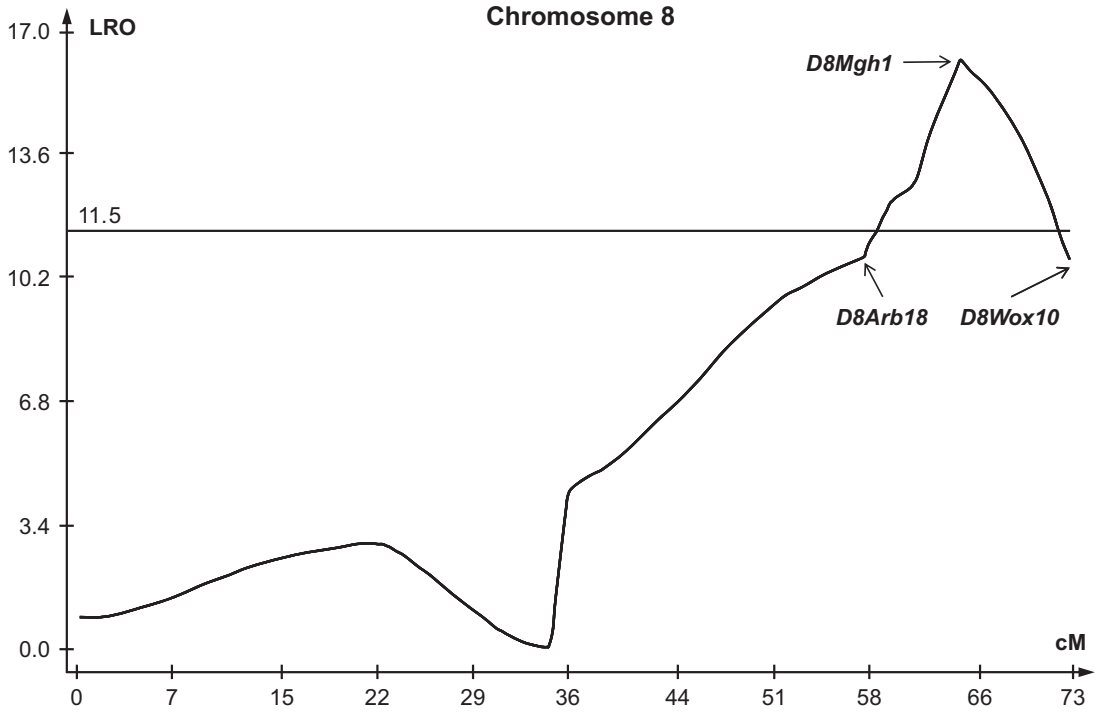

FIG. 3. Latency among sick animals is linked to a QTL on distal chromosome 8. The likelihood ratio statistic of this QTL, located in the interval that contains the *Iddm24* locus (38), achieves independent significance for linkage in the present KRV-induced diabetes study. The horizontal line indicates a "suggestive" cutoff threshold.

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.1*A*, LEW.1WR1) and the "L" haplotype (LEW, WF, LEW.1*W*) (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 NH_2-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 at 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. 5*A*). 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. 5*B*). Thus, the allelic coding region polymorphisms seen in the LEW.1WR1 and LEW rat *UBD* genes, and not differ-

TABLE 3
SNPs in *Iddm37* in four rat strains

	Position	LEW.1WR1	WF	LEW.1W	LEW.1A	Donor to LEW.1WR1	Background strain (LEW)	SNP
ENSRNOSNP2803333	520,944	*C*	G	G	*C*	*LEW.1A*	G	Noncoding
D20Wox18	698,147	*150*	155	155	*150*	*LEW.1A*	150	—
D20Got3	1,402,527	*150*	170	170	*150*	*LEW.1A*	170	—
UBD (SNP 22)*	1,476,122	*C*	A	A	*C*	*LEW.1A*	A	UBD coding*
UBD (SNP 21)	1,476,176	*A*	A	A	*A*	*LEW.1A*	T	UBD noncoding
UBD (SNP 20)	1,476,198	*C*	T	T	*C*	*LEW.1A*	T	UBD noncoding
UBD (SNP 19)	1,476,363	*T*	C	C	*T*	*LEW.1A*	C	UBD noncoding
UBD (SNP 18)*	1,476,529	*G*	A	A	*G*	*LEW.1A*	A	UBD coding*
UBD (SNP 17)	1,476,872	*C*	DEL	DEL	*C*	*LEW.1A*	DEL	UBD noncoding
UBD (SNP 16)	1,476,873	*G*	A	A	*G*	*LEW.1A*	A	UBD noncoding
UBD (SNP 15)	1,476,897	*G*	A	A	*G*	*LEW.1A*	A	UBD noncoding
UBD (SNP 14)	1,476,926	*G*	A	A	*G*	*LEW.1A*	A	UBD noncoding
UBD (SNP 13)	1,477,157	*A*	G	G	*A*	*LEW.1A*	G	UBD noncoding
UBD (SNP 12)	1,477,164	*G*	A	A	*G*	*LEW.1A*	A	UBD noncoding
UBD (SNP 11)	1,477,184	*C*	T	T	*C*	*LEW.1A*	T	UBD noncoding
UBD (SNP 10)	1,477,191	*C*	T	T	*C*	*LEW.1A*	T	UBD noncoding
UBD (SNP 9)	1,477,224	*T*	C	C	*T*	*LEW.1A*	C	UBD noncoding
UBD (SNP 8)	1,477,230	*G*	A	A	*G*	*LEW.1A*	A	UBD noncoding
UBD (SNP 5)	1,477,370	*A*	C	C	*A*	*LEW.1A*	C	UBD noncoding
UBD (SNP 4)	1,477,524	*C*	T	T	*C*	*LEW.1A*	T	UBD noncoding
UBD (SNP 3)	1,477,687	*T*	C	C	*T*	*LEW.1A*	C	UBD noncoding
UBD (SNP 2)	1,477,714	*T*	C	C	*T*	*LEW.1A*	C	UBD noncoding
UBD (SNP 1)	1,477,718	*G*	A	A	*G*	*LEW.1A*	A	UBD noncoding
D20Rat47	1,516,333	131	135	135	131	*LEW.1A*	131	—
D20Rat46	3,039,000	*160*	174	170	*160*	*LEW.1A*	150	—
ENSRNOSNP2803355	3,067,787	*G*	A	A	*G*	*LEW.1A*	G	Flot1 noncoding
AIF1	3,714,566	*T*	C	C	*T*	*LEW.1A*	C	AIF1 coding
ENSRNOSNP2803365	3,816,348	*T*	C	C	*T*	*LEW.1A*	C	Clic1 noncoding
rs8154092	3,955,709	*A*	C	C	*A*	*LEW.1A*	A	Hspa1b noncoding
rs8154089	3,956,875	*C*	T	T	*C*	*LEW.1A*	-	Hspa1 coding
SNP 3956957	3,956,957	*T*	G	G	*T*	*LEW.1A*	-	—
Hsp70	3,957,645	*C*	-	G	*C*	*LEW.1A*	-	Unknown
rs8163033	3,964,007	*C*	T	T	*C*	*LEW.1A*	T	Unknown
rs13456239	3,999,462	*T*	C	C	*T*	*LEW.1A*	T	Unknown
rs13456241	3,999,719	*A*	T	T	*A*	*LEW.1A*	A	Unknown
ENSRNOSNP2803370	4,071,738	G	G	G	A	LEW.1W	A	C2 noncoding
RT1D	4,636,358	RT1u	RT1u	RT1u	RT1a	LEW.1W	RT1L	RT1 class II
RT1B	4,730,559	RT1u	RT1u	RT1u	RT1a	LEW.1W	RT1L	RT1 class II
ENSRNOSNP2803385	4,924,504	C	C	C	T	LEW.1W	T	Col11a2 coding
RT1A	5,056,771	RT1u	RT1u	RT1u	RT1a	LEW.1W	RT1L	RT1 class I
ENSRNOSNP2803403	6,156,925	G	A	G	A	LEW.1W	G	Anks1a noncoding
ENSRNOSNP2803427	8,182,794	C	C	C	A	LEW.1W	A	Mdga1 noncoding
ENSRNOSNP2803448	9,643,317	G	G	G	A	LEW.1W	G	Rsph1 noncoding
ENSRNOSNP2803464	10,602,251	C	C	C	T	LEW.1W	T	Nnp1 coding
Icos-ligand	10,981,192	A	A	A	C	LEW.1W	C	Icos-ligand Intron
AIRE	10,985,967	C	C	C	T	LEW.1W	T	AIRE coding
ENSRNOSNP2803475	11,528,162	C	C	C	T	LEW.1W	T	RGD1311257 coding
D20Rat3	11,900,000	138	146	138	158	LEW.1W	158	—
D20Rat 10	34,000,000	162	156	162	162	LEW	162	—

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 ~4 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.

ences in UBD-inducing cytokines, are associated with statistically significant differences in *UBD* gene transcript levels. In addition, TNF-α is thus unlikely to be a strong candidate gene for *Iddm37*.

DISCUSSION

These data shed new light on the genetics of autoimmune disease in the rat models of human type 1 diabetes. They

"A" HAPLOTYPE (LEW.1WR1 and LEW.1A)

MASCVCVV**R**SEQWPLMTFDTTMSDKVKKINEHIRSQTKVSVQDQILLLDSKILKPHRALS
SYGIDKENTIHLTLKVVKPSDEELPLSLVESGDEGQRHLLRVRRSSSVAQVKEMIENVTAVP
PKKQIVNCNGKRLEDGKIMADY**H**IKSGSLLFLTAHCIGG

"L" HAPLOTYPE (WF, LEW.1W, and LEW)

MASCVCVV**H**SEQWPLMTFDTTMSDKVKKINEHIRSQTKVSVQDQILLLDSKILKPHRALS
SYGIDKENTIHLTLKVVKPSDEELPLSLVESGDEGQRHLLRVRRSSSVAQVKEMIENVTAVP
PKKQIVNCNGKRLEDGKIMADY**N**IKSGSLLFLTAHCIGG

FIG. 4. Predicted amino acid sequences of UBD coding regions derived from cDNA sequences. Amino acid substitutions in the "A" haplotype (LEW.1WR1 and LEW.1A) versus the "L" haplotype (LEW.1W, WF, and LEW) are shown in bold underlined text. The two amino acid changes were the result of two polymorphic nucleotides: R to H from CGT to CAT and H to N from CAC to AAC.

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 × WF intercross, as they are already known to do in BBDR × WF 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 β (*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 × WF progeny was *Iddm20* on chromosome 17 (25);

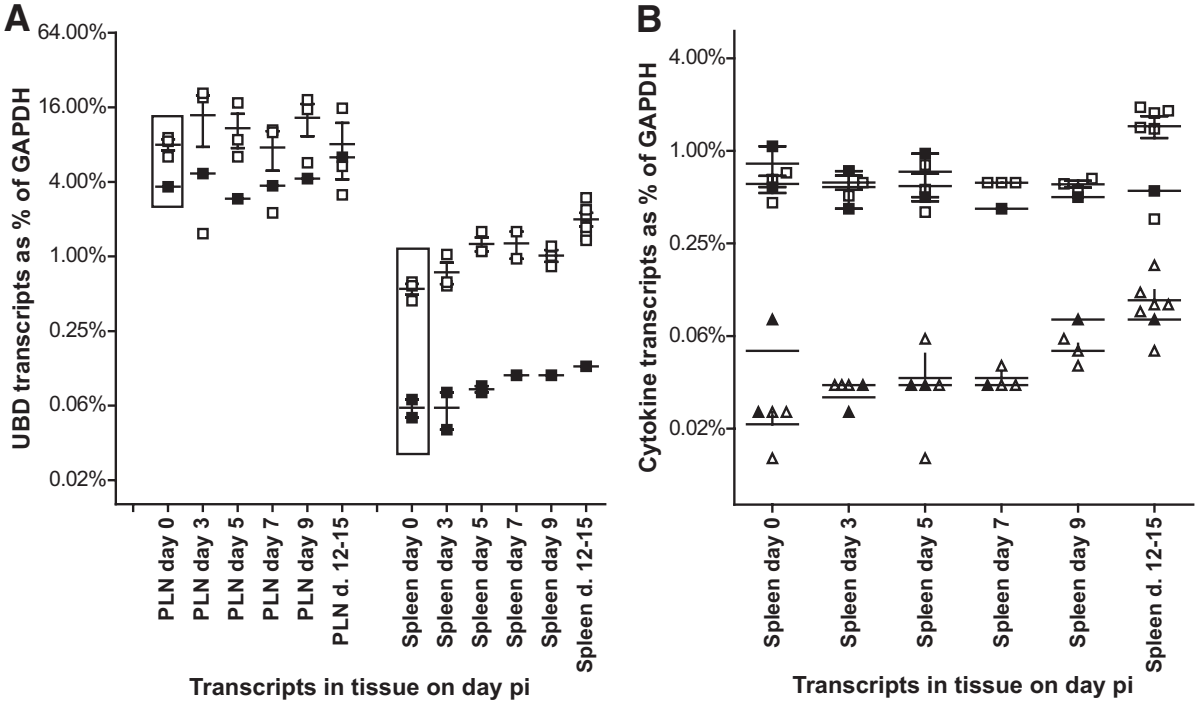

FIG. 5. UBD and cytokine expression in PLN and spleen of LEW and LEW.1WR1 rats, as assessed by RT-PCR. Rats were either untreated or injected with chronic poly I:C alone three times weekly as described in RESEARCH DESIGN AND METHODS. LEW.1WR1 rats typically become diabetic on days 10–14 when treated with this protocol. Graphs show mRNA expression levels in rats that received no poly I:C (day 0) and in rats that had been started on the diabetes induction protocol 3, 5, 7, 9, or 12–15 days before tissue harvest. All values are expressed as a percent of GAPDH transcripts measured in the same sample (means ± SE). *A*: Relative expression of UBD in tissues collected from one to six animals per strain at each time point. Boxed comparisons show day 0 (no poly I:C) values of each sample. On day 0, the spleens from untreated LEW.1WR1 rats have significantly elevated UBD expression compared with LEW ($P = 0.0063$, t test). UBD expression in treated rats over all days is significantly different between strains in both PLN and spleen ($P = 0.004$; Mann-Whitney test). UBD expression was also significantly higher as a function of the duration of poly I:C treatment in spleens ($P = 0.003$ in LEW.1WR1, $P = 0.009$ in LEW; linear regression) but not in PLN (P = not significant). □, LEW.1WR1 UBD; ■, LEW UBD. *B*: Cytokine mRNA expression in spleens taken from the same animals. Although IFN-γ levels increase with time ($P = 0.02$, ANOVA), the levels of IFN-γ and TNF-α do not differ between strains ($P = 0.72, 0.32$) either before or after poly I:C exposure, suggesting that the differences in UBD expression are not due to differences in inducing cytokine levels in each rat. □, LEW.1WR1 TNF; ■, LEW TNF; △, LEW.1WR1 IFN-γ; ▲, LEW IFN-γ.

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* L/L 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 *Idd24*, which maps between *Lta* (35.3 MB) and *D17Mit105* (41.4 MB) on chromosome 17. The mouse *Idd24* 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 *MAS1L*. Studies have revealed that siblings who have inherited shared *UBD/MAS1L* 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 *MAS1L* 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 BBDP 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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REFERENCES

1. Todd JA. From genome to aetiology in a multifactorial disease, type 1 diabetes. BioEssays 1999;21:164–174
2. Hawa MI, Beyan H, Buckley LR, Leslie RDG. Impact of genetic and non-genetic factors in type 1 diabetes. Am J Med Genet 2002;115:8–17
3. Åkerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. Am J Med Genet 2002;115:18–29
4. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF, Payne F, Lowe CE, Szeszko JS, Hafler JP, Zeitels L, Yang JH, Vella A, Nutland S, Stevens HE, Schuilenburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, Wallace C, Howson JM, Guja C, Ionescu-Tîrgovişte C. Genetics of Type 1 Diabetes in Finland, Simmonds MJ, Heward JM, Gough SC; Wellcome Trust Case Control Consortium, Dunger DB, Wicker LS, Clayton DG. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857–864
5. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:645–646
6. Filippi CM, Von Herrath MG. Viral trigger for type 1 diabetes: pros and cons. Diabetes 2008;57:2863–2871
7. Anderson MS, Bluestone JA. The NOD mouse: a model of immune dysregulation. Annu Rev Immunol 2005;23:447–485
8. Mordes JP, Poussier P, Rossini AA, Blankenhorn EP, Greiner DL. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. In *Animal Models of Diabetes: Frontiers in Research*. 2nd ed. Shafrir E, Ed. Boca Raton, CRC Press, 2007, p. 1–39
9. Rainbow DB, Esposito L, Howlett SK, Hunter KM, Todd JA, Peterson LB, Wicker LS. Commonality in the genetic control of type 1 diabetes in humans and NOD mice: variants of genes in the IL-2 pathway are

associated with autoimmune diabetes in both species. Biochem Soc Trans 2008;36:312–315
10. Serreze DV, Leiter EH. Genes and pathways underlying autoimmune diabetes in NOD mice. In *Molecular Pathology of Insulin Dependent Diabetes Mellitus*. Von Herrath MG, Ed. New York, Karger Press, 2001, p. 31–67
11. Atkinson M, Leiter EH. The NOD mouse model of insulin dependent diabetes: as good as it gets? Nat Med 1999;5:601–604
12. Hermitte L, Vialettes B, Naquet P, Atlan C, Payan M-J, Vague P. Paradoxical lessening of autoimmune processes in non-obese diabetic mice after infection with the diabetogenic variant of encephalomyocarditis virus. Eur J Immunol 1990;20:1297–1303
13. Wilberz S, Partke HJ, Dagnaes-Hansen F, Herberg L. Persistent MHV (mouse hepatitis virus) infection reduces the incidence of diabetes mellitus in non-obese diabetic mice. Diabetologia 1990;34:2–5
14. Oldstone MBA. Viruses as therapeutic agents: I. Treatment of nonobese insulin-dependent diabetes mice with virus prevents insulin-dependent diabetes mellitus while maintaining general immune competence. J Exp Med 1990;171:2077–2089
15. Serreze DV, Ottendorfer EW, Ellis TM, Gauntt CJ, Atkinson MA. Acceleration of type 1 diabetes by a coxsackievirus infection requires a preexisting critical mass of autoreactive T-cells in pancreatic islets. Diabetes 2000;49:708–711
16. Horwitz MS, Krahl T, Fine C, Lee J, Sarvetnick N. Protection from lethal coxsackievirus-induced pancreatitis by expression of gamma interferon. J Virol 1999;73:1756–1766
17. Imagawa A, Hanafusa T, Tamura S, Moriwaki M, Itoh N, Yamamoto K, Iwahashi H, Yamagata K, Waguri M, Nanmo T, Uno S, Nakajima H, Namba M, Kawata S, Miyagawa J, Matsuzawa Y. Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type I diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. Diabetes 2001;50:1269–1273
18. LeCompte PM, Gepts W. The pathology of juvenile diabetes. In *The Diabetic Pancreas*. Volk BW, Wellmann KF, Eds. New York, Plenum Press, 1977, p. 325–363
19. Wallis RH, Wang K, Marandi L, Shieh E, Ning T, Chao G, Sarmiento J, Paterson AD, Poussier P. Type 1 diabetes in the BB rat: a polygenic disease. Diabetes 2009;58:1007–1017
20. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE, Welsh RM. Induction of type 1 diabetes by Kilham's rat virus in diabetes resistant BB/Wor rats. Science 1991;254:1010–1013
21. Zipris D, Hillebrands JL, Welsh RM, Rozing J, Xie JX, Mordes JP, Greiner DL, Rossini AA. Infections that induce autoimmune diabetes in BBDR rats modulate $CD4^+CD25^+$ T cell populations. J Immunol 2003;170:3592–3602
22. Mordes JP, Guberski DL, Leif JH, Woda BA, Flanagan JF, Greiner DL, Kislauskis EH, Tirabassi RS. LEW.1WR1 rats develop autoimmune diabetes spontaneously and in response to environmental perturbation. Diabetes 2005;54:2727–2733
23. Mordes JP, Bortell R, Blankenhorn EP, Rossini AA, Greiner DL. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. ILAR J 2004;45:278–291
24. Zipris D, Lien E, Xie JX, Greiner DL, Mordes JP, Rossini AA. TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. J Immunol 2005;174:131–142
25. Blankenhorn EP, Rodemich L, Martin-Fernandez C, Leif J, Greiner DL, Mordes JP. The rat diabetes susceptibility locus *Iddm4* and at least one additional gene are required for autoimmune diabetes induced by viral infection. Diabetes 2005;54:1233–1237
26. Hedrich HJ. Mutant genes and polymorphic loci of the laboratory rat. In *Genetic Monitoring of Inbred Strains of Rats*. 1st ed. Hedrich HJ, Ed. Stuttgart, Gustav Fischer Verlag, 1990, p. 289–406
27. Wurst W, Rothermel E, Gunther E. Genetic mapping of C4 and Bf complement genes in the rat major histocompatibility complex. Immunogenetics 1988;28:57–60
28. Ellerman KE, Like AA. Susceptibility to diabetes is widely distributed in normal class II^u haplotype rats. Diabetologia 2000;43:890–898
29. Tirabassi RS, Flanagan JF, Kislauskis EH, Mordes JP, Greiner DL, Leif JH, Guberski DL. Rat strain specific susceptibility to environmental induction of type 1 diabetes (Abstract). Diabetes 2004;53(Suppl. 2):A301
30. Tirabassi RS, Guberski DL, Leif JH, Winans DA, Mordes JP. Maternal immunization protects weanling LEW.1WR1 rats from cytomegalovirus-induced diabetes (Abstract). Diabetes 2005;54(Suppl. 1):A94
31. Mordes JP, Leif J, Novak S, DeScipio C, Greiner DL, Blankenhorn EP. The *iddm4* locus segregates with diabetes susceptibility in congenic WF.*iddm4* rats. Diabetes 51:3254–3262
32. National Institutes of Health. *Guide for the Care and Use of Laboratory Animals*. Washington, DC, Department of Health, Education, and Welfare, 1985 [NIH publ. no. 78-23]
33. Wang S, Basten CJ, Zeng Z-B. Windows QTL Cartographer 2.5. [article online], 2009. Available from http://statgen.ncsu.edu/qtlcart/WQTLCart.htm. Accessed 15 January 2009
34. Doerge RW, Churchill GA. Permutation tests for multiple loci affecting a quantitative character. Genetics 1996;142:285–294
35. Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, Welsh KI. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 1995;46:355–367
36. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–481
37. Martin A-M, Maxson MN, Leif J, Mordes JP, Greiner DL, Blankenhorn EP. 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. Diabetes 1999;48:2138–2144
38. Wallis RH, Wang K, Dabrowski D, Marandi L, Ning T, Hsieh E, Paterson AD, Mordes JP, Blankenhorn EP, Poussier P. A novel susceptibility locus on rat chromosome 8 affects spontaneous but not experimentally induced type 1 diabetes. Diabetes 2007;56:1731–1736
39. Wurst W, Rothermel E, Gunther E. Genetic-mapping of C-4 and Bf complement genes in the rat major histocompatibility complex. Immunogenetics 1988;28:57–60
40. Aly TA, Baschal EE, Jahromi MM, Fernando MS, Babu SR, Fingerlin TE, Kretowski A, Erlich HA, Fain PR, Rewers MJ, Eisenbarth GS. Analysis of single nucleotide polymorphisms identifies major type 1A diabetes locus telomeric of the major histocompatibility complex. Diabetes 2008;57:770–776
41. Aly TA, Ide A, Jahromi MM, Barker JM, Fernando MS, Babu SR, Yu L, Miao D, Erlich HA, Fain PR, Barriga KJ, Norris JM, Rewers MJ, Eisenbarth GS: Extreme genetic risk for type 1A diabetes. Proc Natl Acad Sci U S A 2006;103:14074–14079
42. Raasi S, Schmidtke G, de Giuli R, Groettrup M. A ubiquitin-like protein which is synergistically inducible by interferon-gamma and tumor necrosis factor-alpha. Eur J Immunol 1999;29:4030–4036
43. Mordes JP, Cort L, Norowski E, Leif JH, Fuller J, Lernmark Å, Greiner DL, Blankenhorn EP. Analysis of the *Iddm14* rat diabetes susceptibility locus in multiple rat strains: identification of a susceptibility haplotype in the Tcrb-V locus. Mamm Genome 2009;20:162–169
44. Fuller JM, Kwitek AE, Hawkins TJ, Moralejo DH, Lu W, Tupling TD, MacMurray AJ, Borchardt G, Hasinoff M, Lernmark A. Introgression of F344 rat genomic DNA on BB rat chromosome 4 generates diabetes-resistant lymphopenic BB rats. Diabetes 2006;55:3351–3357
45. Deruytter N, Boulard O, Garchon HJ. Mapping non-class II H2-linked loci for type 1 diabetes in nonobese diabetic mice. Diabetes 2004;53:3323–3327
46. Kalveram B, Schmidtke G, Groettrup M. The ubiquitin-like modifier FAT10 interacts with HDAC6 and localizes to aggresomes under proteasome inhibition. J Cell Sci 2008;121:4079–4088
47. Chiu YH, Sun Q, Chen ZJ. E1-L2 activates both ubiquitin and FAT10. Mol Cell 2007;27:1014–1023
48. Davis CT, Blankenhorn EP, Murasko DM. Genetic variation in the ability of several strains of rats to produce interferon in response to polyriboinosinic-polyribocytodilic acid. Infect Immun 1984;43:580–583
49. Ebstein F, Lange N, Urban S, Seifert U, Kruger E, Kloetzel PM. Maturation of human dendritic cells is accompanied by functional remodelling of the ubiquitin-proteasome system. Int J Biochem Cell Biol 2009;41:1205–1215
50. Bates EE, Ravel O, Dieu MC, Ho S, Guret C, Bridon JM, Ait-Yahia S, Briere F, Caux C, Banchereau J, Lebecque S. Identification and analysis of a novel member of the ubiquitin family expressed in dendritic cells and mature B cells. Eur J Immunol 1997;27:2471–2477
51. Gomez-Martin D, Diaz-Zamudio M, Alcocer-Varela J. Ubiquitination system and autoimmunity: the bridge towards the modulation of the immune response. Autoimmun Rev 2008;7:284–290