Original Article

LEW.1WR1 Rats Develop Autoimmune Diabetes Spontaneously and in Response to Environmental Perturbation

John P. Mordes,[1] Dennis L. Guberski,[2] Jean H. Leif,[1] Bruce A. Woda,[3] Joan F. Flanagan,[2] Dale L. Greiner,[1] Edward H. Kislauskis,[2] and Rebecca S. Tirabassi[2]

We describe a new rat model of autoimmune diabetes that arose in a major histocompatibility complex congenic LEW rat. Spontaneous diabetes in LEW.1WR1 rats (*RT1*$^{u/u/a}$) occurs with a cumulative frequency of ~2% at a median age of 59 days. The disease is characterized by hyperglycemia, glycosuria, ketonuria, and polyuria. Both sexes are affected, and islets of acutely diabetic rats are devoid of β-cells, whereas α- and δ-cell populations are spared. The peripheral lymphoid phenotype is normal, including the fraction of ART2$^+$ regulatory T-cells. We tested the hypothesis that the expression of diabetes would be increased by immunological perturbation of innate or adaptive immunity. Treatment of young rats with depleting anti-ART2.1 monoclonal antibody increased the frequency of diabetes to 50%. Treatment with the toll-like receptor 3 ligand polyinosinic:polycytidylic acid increased the frequency of diabetes to 100%. All diabetic rats exhibited end-stage islets. The LEW.1WR1 rat is also susceptible to collagen-induced arthritis but is free of spontaneous thyroiditis. The LEW.1WR1 rat provides a new model for studying autoimmune diabetes and arthritis in an animal with a genetic predisposition to both disorders that can be amplified by environmental perturbation. *Diabetes* 54: 2727–2733, 2005

From the [1]Diabetes Division, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts; [2]BioMedical Research Models, Worcester, Massachusetts; and the [3]Department of Pathology, University of Massachusetts Medical School, Worcester, Massachusetts.

Address correspondence and reprint requests to Dennis L. Guberski, BioMedical Research Models, 67 Millbrook St., Suite 422, Worcester, MA 01606. E-mail: dguberski@biomere.com.

Received for publication 1 March 2005 and accepted in revised form 3 June 2005.

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

IFA, incomplete Freund's adjuvant; mAb, monoclonal antibody; MHC, major histocompatibility complex; PE, phycoerythrin; poly I:C, polyinosinic:polycytidylic acid; TCR, T-cell receptor; TLR, toll-like receptor; Tregs, regulatory T-cells.

Type 1A diabetes comprises ~10% of all diabetes mellitus, and its prevalence is increasing (1). It results from inflammatory infiltration of the islets of Langerhans, leading to selective destruction of β-cells (2). There is general agreement that the disease is autoimmune in origin (2), and it often occurs in people in whom other autoimmune diseases are present (3). It is heritable, associated with the major histocompatibility complex (MHC), T-cell dependent, and ameliorated by immunosuppression. Unfortunately, type 1A diabetes remains refractory to prevention (4–6) by methods other than immunosuppression (7). This refractoriness may in part result from the possibility that type 1 diabetes is caused by nongenetic environmental factors operating in a genetically susceptible host (8,9). The disease may therefore be due to interaction with the environment of alleles at many loci (10). Analysis of such interactions in humans is exceptionally difficult given the outbred nature of the population and the randomness of environmental events in the lives of children.

To complement human studies, investigators continue to need animal models that can be tested, biopsied, and autopsied. We report a new model of type 1 diabetes, the LEW.1WR1 rat. These animals are of unusual interest because they develop autoimmune diabetes both spontaneously at a rate of ~2% and in response to immunological perturbation at a rate that can reach 100%.

RESEARCH DESIGN AND METHODS

Inbred MHC-congenic LEW.1WR1 rats (*RT1 A^{u}B/D^{u}C^a*, *ART2a*) were obtained in 1989 from the Hanover Institute, Hanover, Germany. They have subsequently been maintained in a closed colony by sibling mating, initially at the University of Massachusetts Medical School (Worcester, MA) and thereafter at the facilities of BioMedical Research Models (Worcester, MA; http://www.biomere.com). Rats from this colony are designated LEW.1WR1/Wor//Brm rats. BBDR/Wor//Brm rats (*RT1 A^{u}B/D^{u}C^u*, *ART2a*) were also obtained from BioMedical Research Models. Animals were housed in a shower-in barrier facility, and periodic testing of sentinel rats was performed to assure the absence of the common rodent viruses and other pathogens, which are listed in the online appendix (supplemental Table 4 [available at http://diabetes.diabetesjournals.org]). Animals were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committees of both the University of Massachusetts Medical School and BioMedical Research Models and in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 1996). All rats were provided with

autoclaved laboratory Rat Chow (Purina 7012) and acidified drinking water ad libitum.

Reagents. Hybridoma cells secreting the DS4.23 rat anti-rat ART2.1 monoclonal antibody (mAb) (IgG2b) are maintained by the National Cell Culture Center (Minneapolis, MN). DS4.23 mAb was produced as tissue culture supernatant and purified by affinity chromatography at the National Cell Culture Center. The concentration of contaminating endotoxin in the purified supernatant was determined commercially (Charles River Endosafe, Charleston, SC) and was <10 U/mg mAb. Intraperitoneal injection of the DS4.23 mAb depletes essentially all circulating ART2.1$^+$ T-cells (11). Antibodies to the αβ T-cell receptor (αβ TCR, clone R73), CD4 (clone OX-35), CD8α chain (clone OX-8), appropriate isotype control antibodies (mouse IgG1, mouse IgG2a, and rat IgG2b), and phycoerythrin (PE)- or CyChrome-conjugated streptavidin were purchased from BD Biosciences PharMingen (San Jose, CA).

The toll-like receptor (TLR) 3 ligand (12) polyinosinic:polycytidylic acid (poly I:C) was purchased from Sigma (St. Louis, MO; lot 51K4100), dissolved in Dulbecco's PBS, sterile filtered, and stored at −20°C until used. The concentration of contaminating endotoxin (lipopolysaccharide [LPS]) was <50 U/mg (Charles River Endosafe). Purified LPS, a ligand of TLR4 (13,14) was purchased from Sigma (L-2654). In one experiment, this preparation of LPS was further purified by phenol extraction to remove residual contaminating lipopeptides and TLR2-activating activity as described previously (15,16).

Treatment protocols

TLR ligation and regulatory T-cell (Treg) depletion. Diabetes induction protocols used poly I:C, LPS, and DS4.23 mAbs administered either separately or in combination. Rats of either sex were 28–32 days of age when treated. DS4.23 mAb was injected intraperitoneally at a dose of 0.025 mg five times weekly as described previously (17). Poly I:C was injected intraperitoneally at a dose of 1.0 μg/g body wt, three times per week as described (17). The dose of poly I:C was selected on the basis of a preliminary titration experiment. When administered as monotherapy to BBDR rats for a total of 40 days, 1 μg/g did not induce diabetes (n = 5; Fig. 2, *inset*), whereas 2.5 μg/g induced diabetes in five of six animals. LPS was injected intraperitoneally as described in RESULTS. Doses of LPS were based on a preliminary titration experiment demonstrating that 100 μg was the maximum nonlethal dose tolerated by LEW.1WR1 rats. Treatments were continued for 40 days or until diabetes onset.

Detection of diabetes and ketonuria and treatment of diabetes. Animals were screened three times weekly for glycosuria (CliniStix; Bayer HealthCare, Diabetes Care Division, Elkhart, IN). The diagnosis of diabetes in glycosuric rats was established on the basis of a plasma glucose concentration >250 mg/dl (11.1 mmol/l/l). Measurements were performed using either a GM7 Analyzer (Analox Instruments, London, UK) or an Accu-Chek Active meter (Roche Diagnostics, Indianapolis, IN). Ketonuria was measured using KetoStix (Bayer). Selected diabetic animals were treated with insulin to prevent ketoacidosis. To minimize the risk of hypoglycemia, doses used were not sufficient to normalize glycemia fully. Insulin was administered either as a daily injection of short-acting PZI insulin (IDEXX Pharmaceuticals, Greensboro, NC) or by subcutaneous implantation of sustained-release insulin pellets (Linplant; LinShin, Ontario, Canada).

Induction of collagen-induced arthritis. Susceptibility to collagen-induced arthritis was assessed in two trials that used slightly different protocols. In trial 1, 32- to 46-day-old LEW.1WR1 rats of either sex were randomized into two groups. Those in the experimental group were injected at the base of the tail with 200 μg of bovine type II collagen (Chondrex, Redmond, WA) emulsified in incomplete Freund's adjuvant (IFA; Chondrex) according to the manufacturer's instructions. The volume of the injection was 200 μl. No booster injections were given. Control animals were treated with IFA alone. In trial 2, 42- to 48-day-old rats of either sex were randomized into two groups. Experimental animals were injected at the base of the tail with 100 μg of predissolved type II bovine collagen in IFA (Chondrex) prepared according to the manufacturer's instructions. A second identical injection was given 8 days later. Control animals were injected with IFA alone on the same schedule. Severity of arthritis was scored subjectively every 3 days on a scale of 0–3 as follows: 0, no clinically apparent abnormality; 1, slight swelling of paw, some immobility of hind limbs; 2, moderate swelling, significant immobility of hind limbs; 3, severe swelling, hind limbs immobile. Latency to onset of disease was defined as the first of 2 consecutive days on which grade one or higher swelling was observed.

Immunophenotyping and cell counts. Flow microfluorometry was used to quantify expression of surface markers on freshly harvested spleen and lymph node cells as described (18). DS4.23 anti-ART2.1 mAb was biotin conjugated using BIOTIN-X-NHS (Calbiochem, La Jolla, CA). Other antibodies were either directly conjugated with fluorochromes (fluorescein isothiocyanate, PE, or CyChrome) or used as biotin conjugates followed by PE- or CyChrome streptavidin. Samples were fixed in a final concentration of 1% paraformaldehyde in PBS and analyzed using a FACScan (Becton Dickinson, Franklin Lakes, NJ). A minimum of 30,000 viable cells in each sample was analyzed. The lymphocyte fraction was gated on the basis of forward- and side-light scatter. Total spleen cell counts were determined using a Coulter counter (Beckman Coulter, Fullerton, CA).

Histology. After the diagnosis of diabetes or at the conclusion of an experiment, rats were killed, and pancreases were removed and fixed in 10% buffered formalin. Paraffin-embedded sections of pancreas were sectioned at 4-μm intervals and stained with hematoxylin-eosin or processed for immunoperoxidase histochemistry with antibodies specific for insulin and glucagon (Dako, Carpinteria, CA). A qualified evaluator (B.A.W.) who was not informed of the donor's glycemic status scored the tissues. On the basis of histological appearance, pancreases were graded on a scale of 0 to 4+ as follows: 0, no inflammatory mononuclear cell infiltration of any islets; 1+, small numbers of mononuclear cells infiltrating into islets with preservation of islet architecture or mononuclear cell infiltration only at the periphery of the islet ("peri-insulitis"); 2+, moderate numbers of infiltrating mononuclear cells with preservation of islet architecture; 3+, large numbers of mononuclear cells with most islets affected and distortion of islet architecture; and 4+, florid infiltration and distorted islet architecture or classical end-stage islets with or without residual inflammation.

Statistics. Statistical comparisons of diabetes-free survival were performed using the method of Kaplan and Meier (19); when necessary, analyses were stratified by sex (20). The equality of nondiabetic survival distributions for animals in different treatment groups was tested by log-rank statistic (21). Average latency to onset of diabetes is presented as the median. Other parametric data are presented as arithmetic means ± 1 SD. Groups of three or more means were compared using one way ANOVA and the Scheffé procedure for a posteriori contrasts (20). P values <0.05 were considered statistically significant. The Fisher exact statistic was used to analyze 2 × 2 tables.

TABLE 1
Frequency of spontaneous diabetes in LEW.1WR1 rats

Year	2000	2001	2002	2003	2004	Total
Males	6	6	9	8	0	29
Females	4	6	17	14	5	46
Age at onset (days)	49–74	50–79	50–80	49–86	46–76	75

No diabetes was observed in the closed colony of LEW.1WR1 rats at BRM from the time of acquisition in 1989 until 2000. Beginning in February 2000, and continuing to the present, spontaneous diabetes has been detected periodically, as indicated. The exact incidence of diabetes cannot be calculated because the size of this commercial breeding colony varies continuously and animals are removed at different ages, but the 75 diabetic animals detected represent ~2% of the total number of LEW.1WR1 rats weaned in the colony from 2000 through 2004.

RESULTS

Spontaneous autoimmune diabetes. No evidence of diabetes was observed in the closed BioMedical Research Models colony of LEW.1WR1 rats from the time of acquisition in 1989 until 2000. Beginning in February, 2000, and continuing to the present, spontaneous diabetes has been detected periodically. A total of 75 cases of diabetes was detected in the production line of LEW.1WR1 rats between February 2000 and December 2004 (Table 1). Among these, 29 (39%) were male and 46 (61%) female (Table 1). Median age at onset was 59 days (range, 49–86). Mean plasma glucose concentration at diagnosis was 535 mg/dl (range, 258–798).

The 75 diabetic animals in Table 1 represent ~2% of the ~3,000 animals produced during that period. Because animals are removed from the production line at various ages for shipment and culling, and because at different times animals of one sex may have been removed preferentially, these data provide only an approximation of cumulative frequency and sex distribution. However, the

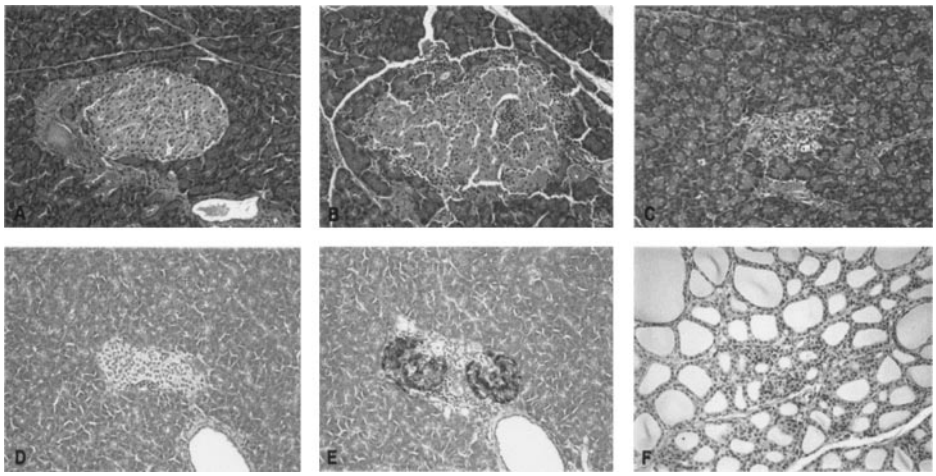

FIG. 1. Islet and thyroid pathology in LEW.1WR1 rats. *A*: Normal islet from a 120-day-old nondiabetic animal. *B*: 3+ insulitis in a 120-day-old nondiabetic animal. *C*: End-stage islet with minimal residual inflammatory infiltrate from a spontaneously diabetic animal obtained 2 h after detection of hyperglycemia. *D* and *E*: Immunohistochemical analysis of adjacent sections of an islet obtained from an animal that had been diabetic for 2 days. *D*: Absence of detectable insulin-containing cells. *E*: Abundant glucagon-containing cells. *F*: Histologically normal thyroid gland obtained from the same animal. *A–C* and *F*: Hematoxylin-eosin. Final magnification in all panels, ×133. A color version of this figure is available online (supplemental Fig. 3).

cumulative frequency of diabetes was also assessed in a smaller sample consisting of 150 rats used as breeders. In this sample, we observed three diabetic rats, consistent with our initial estimate that diabetes occurs in ~2% of LEW.1WR1 rats before 120 days of age.

The diabetic syndrome observed in these animals had the following clinical characteristics: abrupt onset of polyuria and weight loss, increased water consumption, 4+ glycosuria, and large amounts of urinary ketones. Some diabetic animals were treated with exogenous insulin and showed rapid clinical improvement, including resolution of ketonuria, reduction in plasma glucose concentration, and weight gain. In accordance with Animal Care and Use (ACUC) protocols, rats with new onset diabetes were either killed or treated with insulin; for this reason, the natural history of the untreated disease cannot be described.

In an attempt to increase the frequency of spontaneous diabetes in the LEW.1WR1 colony, selected diabetic males were mated with diabetic females. Both sire and dam were treated with insulin. A total of 165 progeny were followed through 120 days of age for onset of diabetes. Among them, one male and three females (2.4%) became diabetic.

Pathology of spontaneously diabetic and nondiabetic rats

Islet histopathology. Histological studies were performed on 26 nondiabetic and 10 spontaneously diabetic animals. Among the nondiabetic pancreases, 24 showed no pathology (Fig. 1*A*); one from a female showed 1+ insulitis and one from a male showed 3+ insulitis (Fig. 1*B*). Four of the 10 diabetic rats were studied within several hours to 2 days after the diagnosis of diabetes, and all revealed end-stage insulitis. The islets were distorted and reduced in size, and few infiltrating lymphocytes were present, even in specimens obtained shortly after diagnosis (Fig. 1*C*). Immunohistochemical staining of specimens obtained shortly after diagnosis revealed that residual islets contained few if any insulin-positive cells (Fig. 1*D*), whereas glucagon-containing cells (Fig. 1*E*) and somatostatin-containing cells (not shown) were abundant.

Six pancreas specimens were obtained from insulin-treated animals 4 to 7 months after the onset of diabetes. These also revealed end-stage islets that were shrunken; they were entirely free of inflammatory infiltration. There was no peri-insulitis in any specimen. There was minimal evidence of focal exocrine pancreatitis in one and some evidence of periductular inflammation in a second.

Other tissues. Among the 10 diabetic animals, there was no evidence of lymphocytic thyroiditis in any specimen (Fig. 1*F*). For studies of other tissues, specimens were obtained from one rat that was acutely diabetic, one that had been diabetic for 4 months, and one that had been diabetic for 6 months. All specimens of stomach, small intestine, and salivary glands were within normal limits. All liver specimens were free of inflammation, but the acute and 4-month specimens showed evidence of fatty infiltration, consistent with poorly controlled diabetes. Two of the three colon specimens were normal; the sample from the acutely diabetic animal showed minimal mucosal inflammation.

Immunological features. The LEW.1WR1 rat is not lymphopenic; total spleen cell counts ($\times 10^6$) were 504 ± 80 in LEW.1WR1 rats ($n = 3$) compared with 300 ± 58 in BBDR rats ($n = 3$). Table 2 shows the comparative phenotypic profiles of LEW.1WR1, BBDR, and LEW rats at 35–42 days of age. ANOVAs revealed a small number of statistically significant differences among strains in several tissues. These included differences in the percentages of TCR^+ cells, TCR^+CD8^+ cells, TCR^+CD4^+ cells, and $CD4^+$ cells expressing CD25 (Table 2). However, these differences were not present in all lymphoid tissues, and none was suggestive of consistent or important biological differences among the strains. With one exception, there were no statistically significant differences with respect to putative regulatory T-cells expressing either the $CD4^+CD25^+$ or the $CD4^+ART2.1^+$ phenotype. The exception was a lower percentage of $CD25^+$ cells in the $CD4^+$ population in the mesenteric lymph nodes of LEW.1WR1 rats (7 ± 1%) as compared with either BBDR (9 ± 1%) or LEW (10 ± 1%) animals ($P < 0.05$ for both comparisons).

Autoimmune diabetes after immunological perturbation

Frequency of diabetes: poly I:C plus anti-ART2.1 mAb. It is known that among rats expressing the *RT1B/D^u* class II MHC haplotype, several express autoimmune diabetes after treatment with either the TLR3 ligand poly I:C (22) or poly I:C in combination with Treg depletion

TABLE 2
Immunophenotype of LEW.1WR1 rats

Tissue	Phenotype (%)	Strain		
		LEW.1WR1	BBDR	LEW
Spleen	TCR^+	36 ± 5	35 ± 4	45 ± 5*
	TCR^+ $CD8^+$	12 ± 2	12 ± 1	16 ± 3*
	TCR^+ $CD4^+$	23 ± 4	21 ± 4	26 ± 1
	TCR^+ $ART2.1^+$	27 ± 8	25 ± 9	30 ± 4
	$CD25^+$ in the $CD4^+$ population	8 ± 3	9 ± 2	11 ± 2
Cervical lymph nodes	TCR^+	66 ± 5	72 ± 4	61 ± 3†
	TCR^+ $CD8^+$	23 ± 3	21 ± 1	16 ± 2*
	TCR^+ $CD4^+$	38 ± 5	48 ± 4‡	36 ± 2
	TCR^+ $ART2.1^+$	40 ± 5	39 ± 6	36 ± 2
	$CD25^+$ in the $CD4^+$ population	9 ± 1	10 ± 1	9 ± 1
Mesenteric lymph nodes	TCR^+	71 ± 14	76 ± 8	71 ± 2
	TCR^+ $CD8^+$	21 ± 6	22 ± 7	17 ± 1
	TCR^+ $CD4^+$	48 ± 10	52 ± 5	46 ± 3
	TCR^+ $ART2.1^+$	50 ± 12	47 ± 7	45 ± 3
	$CD25^+$ in the $CD4^+$ population	7 ± 1§	9 ± 1§	10 ± 1§
Pancreatic lymph nodes	TCR^+	71 ± 9	78 ± 1	75 ± 6
	TCR^+ $CD8^+$	20 ± 3	25 ± 3‡	16 ± 1
	TCR^+ $CD4^+$	49 ± 10	50 ± 11	55 ± 6
	TCR^+ $ART2.1^+$	43 ± 8	42 ± 7	33 ± 10
	$CD25^+$ in the $CD4^+$ population	10 ± 4	13 ± 5	10 ± 1

Data are means ± SD of positively stained cells from five to six individual rats. Nondiabetic, unmanipulated LEW.1WR1, BBDR, and LEW rats 35–42 days of age were processed for immunophenotyping as described in RESEARCH DESIGN AND METHODS. The LEW.1WR1 and BBDR rats were of both sexes; the LEW rats were all male. Statistical analysis by one-way ANOVA revealed a number of statistical differences for various phenotypes scattered through the dataset, but there were no consistent differences that appeared to indicate biological differences among the strains. The ART2 surface alloantigen in the rat was formerly designated RT6. *$P < 0.05$ vs. BBDR and LEW.1WR1; †$P < 0.05$ vs. BBDR; ‡$P < 0.05$ vs. LEW and LEW.1WR1; §$P < 0.05$ vs. both other strains. No other pairwise comparisons among the three strains were statistically significant.

(17,23). We hypothesized that the rate at which autoimmune diabetes is expressed in the LEW.1WR1 rat would be increased after immunomodulatory perturbation. As shown in Fig. 2, no spontaneous diabetes was diagnosed in a sample of 27 LEW.1WR1 rats observed through 120 days of age. Among these animals, only two (8%) revealed any evidence of insulitis (grades 1 and 3). In contrast, when LEW.1WR1 rats were treated with both poly I:C and anti-ART2.1 mAbs, diabetes occurred in 96% of animals within 40 days of starting treatment ($n = 24$; median latency, 15 days; range, 12–21 days; $P < 0.0001$). Consistent with previous reports (17,23), we also observed diabetes in 100% of a sample of BBDR rats treated with poly I:C and anti-ART2.1 mAb (Fig. 2, *inset*; $n = 12$; median latency, 16 days; range, 13–25 days). Most diabetic rats in this study exhibited end-stage insulitis; the mean insulitis score was 3.9.

Frequency of diabetes: poly I:C or anti-ART2.1 mAb alone. When treated with poly I:C alone, 100% of LEW.1WR1 rats became diabetic with kinetics similar to those in rats treated with both poly I:C and anti-ART2.1 mAbs (Fig. 2; median latency, 15 days; range, 11–34; P = N.S. vs. LEW.1WR1 rats given both mAbs and poly I:C). This finding was surprising because we had established this dose of poly I:C as insufficient to induce diabetes in the BBDR rat (Fig. 2, *inset*). Also surprising was our observation that treatment with anti-ART2.1 mAb as monotherapy induced diabetes in 46% of treated animals ($n = 24$, median time to onset 29 days, range 24–38, $P < 0.0005$ vs. untreated controls). This treatment fails to induce diabetes in BBDR rats housed in barrier facilities (24). Among 11 LEW.1WR1 rats treated with anti-ART2.1 mAb alone that did not become diabetic and for which technically satisfactory histology was available, eight were normal, and the remaining three had insulitis of an average grade of 3.3. There was no statistically significant effect of sex on the frequency of diabetes in any treatment group.

Frequency of diabetes: LPS. In three independent trials, LEW.1WR1 rats were treated with the TLR4 ligand LPS. In trial 1, LPS was given at a dose of either 2 μg/g ($n = 8$) or 4 μg/g ($n = 8$) body wt on 5 consecutive days, and none became diabetic. A dose of 8 μg/g was lethal to three of seven animals, and the surviving four were nondiabetic. In trial 2, LPS was given at a dose of 100 μg three times weekly through 70 days of age, and none of 10 rats developed diabetes. In trial 3, phenol-extracted LPS was given at a doses of 2, 4, or 8 μg/g body wt three times weekly for 40 days. In this trial, one of six animals treated with 2 μg/g LPS became diabetic after 36 days, and two of six treated with 4 μg/g LPS became diabetic within 23–26 days. None of the three rats treated with 8 μg/g became diabetic. Histological study of pancreases from trial three revealed 3+ or 4+ insulitis in the three diabetic rats and either 1+ ($n = 11$) or no ($n = 1$) insulitis in the nondiabetic rats.

Collagen arthritis. Type 1 diabetes often occurs together with other autoimmune disorders, and both BBDR (25) and standard LEW (26) rats are susceptible to collagen-induced arthritis. As shown in Table 3, LEW.1WR1 rats are also highly susceptible to this disorder. More than 80% of animals treated with either of two induction protocols developed significant joint swelling within 2 weeks of the injection of type II collagen in IFA. In both trials, both males (18 of 21 overall) and females (21 of 25 overall) were

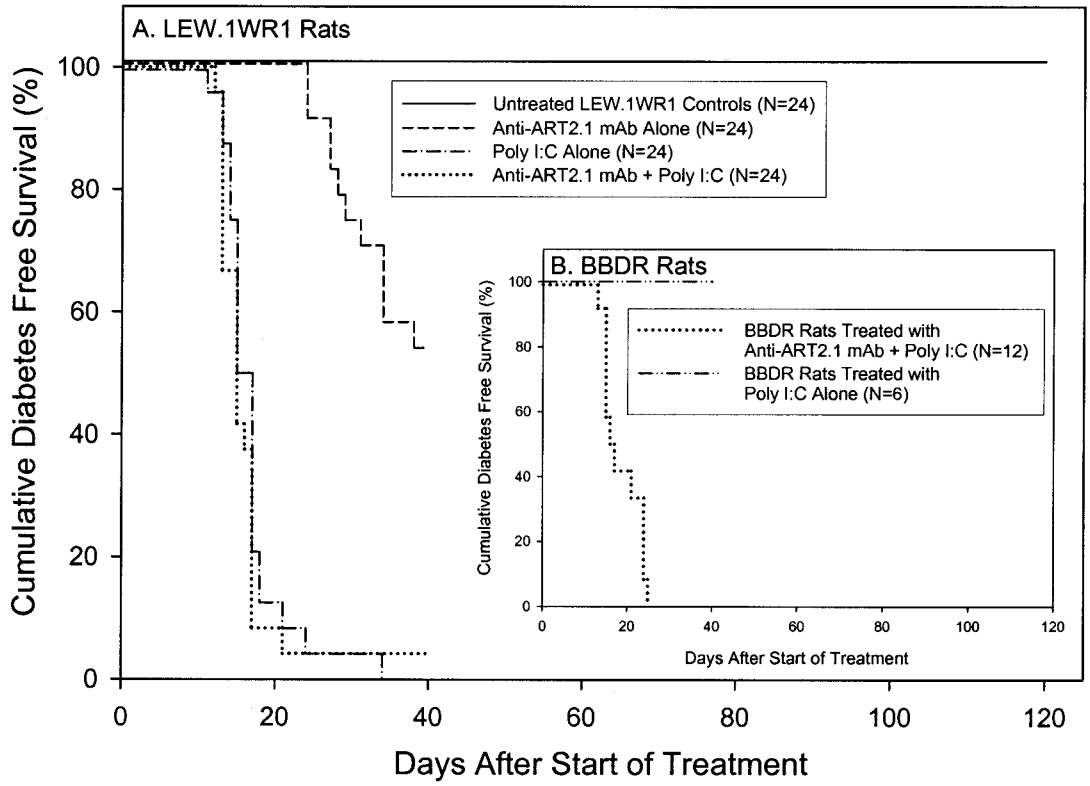

FIG. 2. Frequency of diabetes in treated and untreated rats. LEW.1WR1 (*A*) and BBDR (*B*) 28- to 32-day-old rats of either sex were either untreated or injected intraperitoneally with anti-ART2.1 mAb (five times per week), with poly I:C (1.0 μg/g, three times per week), or with both reagents as described in RESEARCH DESIGN AND METHODS. Treatment was continued for 40 days or until onset of diabetes. Untreated rats were observed through 120 days of age. The dose of poly I:C was selected on the basis of the preliminary titration experiment (*inset*) showing that, as monotherapy, it does not induce diabetes in BBDR rats. The same preparation of poly I:C was used to treat both strains. Overall log-rank statistic for the LEW.1WR1 rat dataset adjusted for sex = 105.8; df = 3; $P < 0.0001$.

found to be equally susceptible. No animals treated with IFA alone developed evidence of disease. One animal in the first trial became diabetic during the period of observation.

DISCUSSION

These data suggest that LEW.1WR1 rats will be a valuable addition to the repertoire of animals that can be used to model human type 1A diabetes mellitus. They have a normal immunophenotype, but in barrier housing, a small but consistent percentage of them develop ketosis-prone diabetes mellitus at a developmental stage corresponding to adolescence. Diabetic animals require exogenous insulin and respond rapidly to therapy.

Both sexes are affected, but spontaneous diabetes was observed more frequently in females (61%) than in males (39%). That diabetes in NOD mice is more frequent among females than among males is well known (27), but our observation in the LEW.1WR1 rat could simply reflect the fact that more females than males are present in commercial breeding facilities. Definitive ascertainment of sexual bias will have to await further study.

The histological results reveal classical inflammatory insulitis in newly diagnosed diabetic animals and "end-stage" islets in animals with chronic diabetes. There was no evidence of the "peri-insulitis" lesion that is characteristic of the islets of NOD mice but not of humans with type 1A diabetes. Immunohistochemistry confirmed that insulin-containing cells were largely absent in the islets of both acutely and chronically diabetic animals.

Histological study of a sample of other tissues for

TABLE 3
Frequency and severity of collagen-induced arthritis in LEW.1WR1 rats

Trial	Treatment	*n*	*n* (%) with arthritis	Median (range) latency to arthritis onset (days)	Mean (±SD) maximal severity score
1	Collagen + IFA	24	20 (83)*	10 (10–12)	2.8 ± 0.6
	IFA alone	7	0 (0)	—	0
2	Collagen + IFA	19	22 (86)*	14 (12–14)	3.0 ± 0.0
	IFA alone	8	0 (0)	—	0

LEW.1WR1 rats 32–48 days of age of either sex were randomized and injected with either type II collagen emulsified in IFA or IFA alone as described in RESEARCH DESIGN AND METHODS. Animals in trial 1 received a single injection; those in trial 2 received two injections separated by 8 days. Arthritis severity was scored on a scale of 0–3 as described in RESEARCH DESIGN AND METHODS. Time to onset was defined as the first of 2 consecutive days on which grade 1 or higher swelling was observed. *$P < 0.001$ vs. IFA alone group (Fisher exact statistic).

evidence of inflammatory lesions was largely negative. Unlike many BB rats, NOD mice, and humans with type 1A diabetes, LEW.1WR1 rats showed no evidence of lymphocytic thyroiditis. Whether a longer period of observation or exposure to dietary iodide (28) would engender thyroiditis, however, remains unknown. All specimens of stomach, small intestine, liver, and salivary glands were normal.

Another important characteristic of the LEW.1WR1 rat is its normal immunophenotype. There is no evidence of the lymphopenia or severe deficiencies in $CD4^+$, $CD8^+$, and $ART2^+$ cells that are characteristic of the BBDP rat. The percentages of $CD4^+$, $CD8^+$, and $ART2^+$ cells in LEW.1WR1 rats are comparable with those observed in the ancestral LEW rat and the BBDR rat.

As is true for all but one rat model of type 1 diabetes, the class 2 MHC haplotype of the LEW.1WR1 is *RT1B/D*^{u} (29). The majority of diabetes-susceptible rats are *RT1*^{u} at both class I and class II loci. These include the BBDP and BBDR (30), WF (31), and KDP (32) strains. The complete haplotype of the LEW.1WR1 rat is *RT1 A*^{u}*B/D*^{u}*C*^{a}, also designated *RT1*^{r4} (29). Interestingly, another recently described rat strain that also exhibits spontaneous autoimmune diabetes, the LEW.1AR1/Ztm-*iddm* strain has the *RT1 A*^{a}*B/D*^{u}*C*^{u} (*RT1*^{r2}) MHC haplotype (33,34). These observations further confirm the critical importance of the *u* allele of *RT1 B/D* and confirm that diabetes susceptibility is preserved in the presence of non-*u* class I alleles at either the A or C locus (35,36). The one reported non-*RT1 B/D*^{u} rat with diabetes susceptibility is the *RT1*^{c} PVG rat. Diabetes occurs in PVG rats after treatment with thymectomy and sublethal irradiation (37,38).

What is perhaps most noteworthy in the LEW.1WR1 rat as a model of type 1 diabetes are the graded increases in penetrance of disease that are observed after various forms of immunological perturbation. Whereas the cumulative frequency of spontaneous diabetes is 2–3% in unmanipulated animals, the frequency of diabetes increased to up to 100% in animals treated with the TLR3 ligand poly I:C. Treg depletion alone induced diabetes in fewer (46%) treated animals, and the TLR4 ligand LPS induced diabetes in only 3 of 48 animals (6%). It is interesting to note, however, that neither Treg depletion alone (24) nor LPS alone (39) is reported to induce diabetes in BBDR rats. These are, however, historical comparisons, and it will be important in the future to determine relative susceptibility to induced diabetes in the two strains directly. Susceptibility of LEW.1WR1 rats to diabetes induced by high-dose (7.5 μg/g) poly I:C had been noted previously (22), but at that time, spontaneous diabetes in these animals was unknown, and no further studies were performed.

The data reveal interesting differences between the LEW.1WR1 rat and the BBDR and LEW.1AR1/Ztm-*iddm* strains. BBDR rats do not develop spontaneous diabetes and, in barrier facilities, depletion of $ART2.1^+$ Tregs alone fails to induce disease. In addition, we document here that a low dose of the TLR3 ligand poly I:C that was ineffective in inducing diabetes in BBDR rats was 100% effective in the LEW.1WR1 rat. In the case of the LEW.1AR1/Ztm-*iddm* rat, which develops diabetes spontaneously in ~20% of cases, treatment of nondiabetic animals with poly I:C reportedly does not induce the disease (34). Interestingly, however, insulitis in the LEW.1AR1/Ztm-*iddm* rat at the time of diagnosis appears more intense than that in the LEW.1WR1 animal (33,34).

There remain many unanswered questions about the LEW.1WR1 rat. Autoantibody production, the composition of the islet infiltrate, the cytokine responses that characterize the infiltrating cells, and especially the changes in the antigen-presenting cell and Treg populations that may occur in the draining pancreatic lymph nodes have not been analyzed. Whether LEW.1WR1 rat T-cells are capable of the adoptive transfer of disease or whether spontaneous disease can be prevented by immunosuppression or immunomodulation (e.g., costimulatory blockade) is not yet known.

Another intriguing question is why spontaneous diabetes appeared in the colony after 10 years of inbreeding. A similar event occurred in the German LEW.1AR1/Ztm-*iddm* rat colony. Whether these observations represent new genetic mutations or can in some way be related to the increasing incidence of human type 1 diabetes are tantalizing subjects for further study. Another characteristic of the LEW.1WR1 rat under investigation in our laboratory is its response to viral infection. Preliminary data suggest that the penetrance of diabetes in these animals is increased by exposure to Kilham rat virus (40), as it is in the BBDR rat (41).

A final noteworthy finding in the present study is the susceptibility of LEW.1WR1 rats to collagen-induced arthritis. In this regard, they are similar to BBDR rats (25,42,43) and the parental LEW rat (26), which are comparably susceptible. Rheumatoid arthritis occasionally occurs in patients and families with type 1 diabetes (44), and genetic analyses in both the rat and human suggest that some susceptibility loci for both diseases are in linkage disequilibrium (45,46). LEW.1WR1 rats are also susceptible to experimental allergic encephalomyelitis (47). The basis for these comorbidities is not known.

In their aggregate, the characteristics of the LEW.1WR1 rat suggest that it will be useful as a model system in which to test the hypothesis that human type 1 diabetes is caused by nongenetic environmental factors operating in a genetically susceptible host to initiate a destructive immune process (8,9). The LEW.1WR1 and other newer rat models of autoimmune diabetes suggest a promising "environmental genetics" approach to modeling human type 1A diabetes.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Center Grant DK32520. J.P.M. has received National Institutes of Health Grants DK49106 and DK25306. D.L.G. has received National Institutes of Health Grant DK49106. D.G. has received U.S. Public Health Service Grants 1R43K60374 and 1R41RR019260.

We thank Drs. Eugene Handler and Aldo Rossini for reading the manuscript.

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