Original Article

Novel Leptin Receptor Mutation in NOD/LtJ Mice Suppresses Type 1 Diabetes Progression

I. Pathophysiological Analysis

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A spontaneous single-base mutation in the leptin receptor of type 1 diabetes–prone NOD/LtJ mice (designated as *Lepr*$^{db\text{-}5J}$) produced a glycine640valine transversion in the extracellular domain. All mutant mice became obese and hyperinsulinemic at weaning, with 70–80% developing early-onset hyperglycemia. However, these obese diabetic mice continued to gain weight without insulin therapy. Spontaneous diabetes remission was observed in all obese females and a subset of obese males. Insulitis was largely limited to islet perimeters, with intraislet insulitis infrequently observed. In 17 obese males (age 39 weeks), we observed phenotypic heterogeneity, including full remission from hyperglycemia (24%), intermediate hyperglycemia with elevated body weight (41%), and severe hyperglycemia and weight loss (35%). The remitting normoglycemic and intermediate hyperglycemic phenotypes were associated with extensive β-cell hyperplasia. Unlike the extensive intraislet insulitis present in diabetic lean NOD/Lt mice, the severe obese diabetic phenotype was associated with islet atrophy without extensive intraislet insulitis. These results indicated that the manipulation of the leptin/leptin receptor axis may provide a novel means of downregulating autoimmunity in type 1 diabetes and confirmed a role for leptin as a mediator in the development of this disease in NOD mice. *Diabetes* 54:2525–2532, 2005

Type 1 diabetes is characterized by the autoimmune destruction of pancreatic β-cells that secrete insulin, resulting in insulin deficiency and hyperglycemia. The NOD mouse strain has been intensively studied as a type 1 diabetes model, with most studies to date focusing on aspects related to the immune response (1) rather than the endocrine system (2).

Leptin is a pleiotropic adipokine associated with the regulation of feeding behavior and energy metabolism (3), reproduction (4), hematopoiesis (5), angiogenesis (6), and bone metabolism (7). Leptin also appears to be an immunomodulatory molecule (8–10). For example, leptin promotes the proliferation and secretion of interleukin (IL)-2 by naive CD4+ T-cells but not by memory T-cells in humans or mice (8). Conversely, when human memory T-cells were examined, leptin was observed to inhibit IL-2 and -4 production while stimulating the secretion of γ-interferon (IFN-γ) (11). These results suggest that the modulation of leptin signaling through its receptor might provide a novel means of downregulating a T helper 1 cell–biased immune response. In support of this hypothesis is the finding from one study that postnatal administration of recombinant leptin precipitates early type 1 diabetes onset (by age 7 weeks) in ~85% of treated NOD females (12). Indeed, the authors of that study suggested a leptin-induced T helper 1 cell deviation based on changes in IFN-γ mRNA staining in spleens.

Previous studies have investigated the effects of transferring the original *Lepr*$^{db\text{-}1J}$ mutation (essentially a null mutation in the leptin receptor long isoform [Rb] that contains the intracellular JAK/STAT signaling domain) onto the NOD genetic background (13). Severe insulitis and type 1 diabetes developed in this congenic stock by age 3–6 months, although there was transitory type 2 diabetes manifested by obesity, hyperinsulinemia, and islet hyperplasia (13). Here, we report the effect of a novel point mutation in the extracellular domain of the NOD leptin receptor (designated as *Lepr*$^{db\text{-}5J}$) that unexpectedly produced a very different phenotypic effect on the course of autoimmune diabetes development. NOD/LtJ mice of both sexes co-isogenic for the *Lepr*$^{db\text{-}5J}$ mutation developed juvenile obesity and type 2 diabetes within 2 weeks of weaning. In sharp contrast to the previous report involving the *Lepr*$^{db\text{-}1J}$ mutation, the type 2 diabetes syndrome imposed by the *Lepr*$^{db\text{-}5J}$ mutation suppressed intraislet insulitis, allowing compensatory β-cell hyperplasia and spontaneous remission from hyperglycemia in female *Lepr*$^{db\text{-}5J}$ mice and a subset of the males.

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IFN-γ, γ-interferon; IL, interleukin; Rb, leptin receptor long isoform.

RESEARCH DESIGN AND METHODS

Mice, genetic mapping, and sequencing. In 2003, animal care technicians at The Jackson Laboratory (Bar Harbor, ME) noticed that a small number of NOD/LtJ mice in the foundation colony became obese within 1 week of weaning. Matings established between young obese mice and lean littermates showed a clear recessive mode of phenotypic inheritance. It was determined that the mutation was co-isogenic on the NOD/LtJ background by genotyping for all the known markers for NOD *Idd* loci using 24 polymorphic microsatellite markers (14). Meanwhile, initial mapping was performed by an outcross of obese NOD/LtJ × lean C57BL/6J mice, with both a backcross of F1 males to obese NOD/LtJ females to generate 86 first backcross (N2) progeny as well as an F1 intercross to produce 167 F2 progeny. All mice were maintained in a specific pathogen-free research animal facility at The Jackson Laboratory and allowed free access to NIH-31 pellet diet (6% fat; PMI, St. Louis, MO) and acidified drinking water.

From the N2 mice, 20 obese mice, age 5 weeks and weighing 6–10 g more than littermates, and 20 lean mice were selected for genotypic analysis. DNA was isolated from tail tips of these mice by phenol-chloroform extraction, and equal amounts of DNA from each donor were aliquoted into obese and lean pools. We used 72 SSLP markers (MIT MapPairs primers; Research Genetics, Huntsville, AL) distinguishing C57BL/6J and NOD/Lt mice, spaced ~20–30 cM apart through the mouse genome, for the PCR genotyping of the pooled DNA samples. PCR was performed for 35 cycles under the following conditions: 30 s at 94°C, 30 s at 55°C, and 40 s at 72°C. Polymorphisms were detected by electrophoresis of the PCR products on 4% Nusieve 3:1 agarose gels in 0.5× Tris-borate EDTA running buffer for 50 min at 400 V, with the gels stained by ethidium bromide and photographed under ultraviolet light.

After initial linkage between the obese phenotype and NOD/LtJ alleles of MIT markers on chromosome 4 was confirmed, further recombination mapping was performed using the individual DNA extracted from all N2 and F2 mice, studies that indicated that the mutation was located in a genetic region between *D4Mit175* (49.6 cM) and *D4Mit331* (50.8 cM). In this interval (1.2 cM), the leptin receptor gene (*Lepr*) was considered as a strong candidate responsible for the obesity mutation. Using the National Center for Biotechnology Information nucleotide database (access. no. U46135) and Ensembl mouse genome server, primer pairs were designed to amplify the coding region of *Lepr*. The PCR products were purified by QIAquick PCR purification columns (Qiagen, Chatsworth, CA) and directly sequenced with an ABI Prism 3700 DNA analyzer. The presence of transcripts specific for the intracellular domain in the long form of the receptor (LEPR-Rb) was assessed by RT-PCR. RNA was extracted from the hypothalamus of wild-type and obese NOD mice and treated with DNAse I (Ambion, Austin, TX); the cDNA was synthesized by reverse transcriptase (Invitrogen, Carlsbad, CA). Using primer sets (forward, 5′-GGTTGGATGAGCTTTTGGAA-3′; reverse, 5′-TCCTGGAGGATCCTGATGTC-3′), PCR was performed for 35 cycles under the following conditions: 30 s at 94°C, 30 s at 58°C, and 40 s at 72°C. The products were run on 1% agarose gel in 0.5× Tris-borate EDTA running buffer for 45 min at 100 V, and the gels were imaged as described above.

Allelic complementation test. To examine whether the mutation producing obesity in NOD/LtJ mice showed allelic complementation with the original *Lepr*$^{db\text{-}1J}$ mutation, heterozygous carriers were obtained from the mating pair between an obese NOD/LtJ female and a NOD/Lt male. The known heterozygous carriers were mated with C57BLKS/J (BKS)-*Lepr*$^{db\text{-}1J}$ mice, and the ratio of obese to lean phenotype was observed in their offspring.

Phenotypic assessment. Both sexes of obese and lean littermates produced in our colony were used for phenotypic analysis. Body weights were measured every 2 weeks, beginning at age 5 weeks and continuing to age 39 weeks. Plasma glucose levels in obese mice were determined every 2 weeks beginning at age 5 weeks (Glucose II Analyzer; Beckman Instruments, Fullerton, CA). The diabetes status of wild-type lean littermates was monitored by testing for glycosuria (Diastix; Bayer, Elkhart, IL). Lean diabetic mice were killed after 2 weeks of consecutive positive tests for glycosuria. A cohort of 4 of the obese females and 13 of the obese males were maintained to age 41–49 weeks and subjected to necropsy. Mice with plasma glucose concentrations consistently ≥200 mg/dl were defined as hyperglycemic. Plasma insulin and leptin concentrations at age 5–21 weeks were detected by multiplex analysis (Linco, St. Louis, MO), and serum insulin and leptin levels were measured by cardiac puncture in the necropsied 41- to 49-week-old mice by a rat insulin radioimmunoassay kit and a mouse leptin radioimmunoassay kit (Linco), respectively. Total serum cholesterol and triglycerides were measured using a Synchron 5 chemistry analyzer (Beckman). When mice were age 15 weeks, dual-energy X-ray absorptiometry (Piximus; LUNAR Instruments, Madison, WI) was used to determine the percentage of body fat; the values are represented as the ratio of total fat to body weight, with the head area being excluded from these analyses. Food consumption (grams per day) was measured at age 5 weeks in males and females by calculating the difference in the weight of the diet in the food hopper before and after 3–4 days minus the wastage (sifted from litter and weighed), divided by number of mice in the pen and then divided by the number of days.

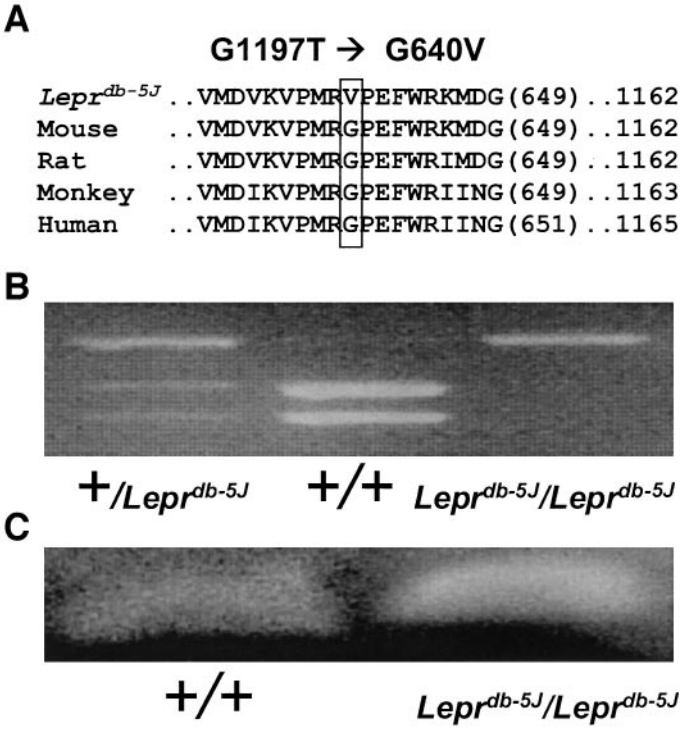

FIG. 1. A novel mutation in the leptin receptor gene of NOD-*Lepr*$^{db\text{-}5J}$/Lt mice. *A*: The alignment shows a G1197T missense mutation in the *Lepr* gene, resulting in a glycine640valine change, as indicated with the open box. *B*: Elimination of the *Hae* III restriction enzyme site by the leptin receptor mutation provides a genotyping tool. *C*: Hypothalamic LEPR-Rb expression by RT-PCR in NOD/Lt and NOD-*Lepr*$^{db\text{-}5J}$/Lt mice confirms expression of a full-length receptor.

Histology. At the time they were killed, the animals' pancreas, liver, and kidney tissues were removed and fixed in Bouin's solution. Three separate levels within each pancreas were sectioned so that separate islets were profiled on each section. Pancreatic β-cells were stained by aldehyde fuchsin, and sections were counterstained with hematoxylin and eosin. Individual islets were examined for the degree of insulitis and were graded as follows: 0, normal; 1, peri-insulitis; 2, infiltration ≤25% of islet mass; 3, infiltration of 25–75% of islet mass; and 4, islet destruction. Liver and kidney tissues were stained by periodic acid Schiff reagent.

Statistical analysis. Student's *t* tests for paired data were performed to assess the significance of differences in comparisons of body weight, plasma glucose, and blood chemistry data at single time points. $P < 0.05$ was considered statistically significant in group comparisons.

RESULTS

Genetic analysis. Test crosses with BKS-+/*Lepr*$^{db\text{-}1J}$ mice showed allelic complementation to produce obesity in 25% of the offspring. Sequencing confirmed that the new obesity mutation indeed entailed the leptin receptor (*Lepr*) locus. The direct sequencing of *Lepr* revealed a G→T transversion mutation at position 640 encoding a valine instead of a glycine, with the latter representing a highly conserved residue in mouse, rat, monkey, and human (Fig. 1*A*) and eliminating a *Hae* III restriction enzyme site present in the wild-type receptor (Fig. 1*B*). In contrast to the *Lepr*$^{db\text{-}1J}$ mutation (where insertion is in a splice domain that produces truncation of the intracellular portion of the receptor), the altered amino acid in the new mutation, designated as *Lepr*$^{db\text{-}5J}$, resides in the very distal portion of the extracellular domain. Located between

fibronectin-III binding domains and immediately adjacent to the transmembrane domain of the receptor, the mutation did not prevent the production of a full-length transcript in the hypothalamus (Fig. 1*C*). Evidence that at least a partial function of this mutant receptor is retained was suggested by the finding that the mutant mice could be successfully mated to lean littermates of the opposite sex. Meanwhile, co-isogenicity of the mutation on a pure NOD background was confirmed by genotyping for NOD alleles to all the known microsatellite markers for NOD "*Idd*" loci (data not shown).

Phenotypic analysis. The first phenotypic manifestation in NOD-*Lepr*$^{db\text{-}5J}$ homozygotes of both sexes was an approximate 6- to 10-g greater weight gain compared with wild-type littermates within 1 week of weaning (Fig. 2). All females continued to show a rapid weight gain up to ~15 weeks, at which time weights stabilized around 40 g (Fig. 2*A*). Analysis by dual-energy X-ray absorptiometry of an obese female at age 15 weeks showed a 40.5% carcass fat content compared with the 19.9% observed in a lean littermate. NOD-*Lepr*$^{db\text{-}5J}$ homozygous males also exhibited the marked weight gain profile from weaning (Fig. 2*B*). At age 15 weeks, the carcass fat of an obese male was 33% compared with 14.7% in a lean male. However, after the onset of puberty, two phenotypes were noted. Postpubertal weight gains continued in 11 of 17 (64.7%) males, but 6 of 17 (35.3%) males showed a persistent decrease in body weight, beginning at age 7–9 weeks and continuing to age 39 weeks (Fig. 2*B*). The early weight gains were associated with hyperphagia; food consumption in 5-week-old mutant mice was approximately twice that of lean controls (Fig. 2*C*). Plasma insulin and leptin concentrations rose well above those in lean controls as the obese mutants aged (Table 1). Despite early increases in plasma insulin, hyperglycemia developed in most obese mice within 2 weeks of weaning. At age 5–7 weeks, 4 of 5 obese females (80.0%) and 12 of 17 obese males (70.6%) were hyperglycemic (≥200 mg/dl), and most of them remained hyperglycemic through the peripubertal period (range 253–686 mg/dl at age 13 weeks). The obese *Lepr*$^{db\text{-}5J}$ mice developing juvenile-onset hyperglycemia did not require insulin therapy for long-term survival to age 39 weeks and beyond, whereas the wild-type NOD/Lt mice developing abrupt, adult-onset hyperglycemia rapidly lost weight without insulin treatment and required euthanasia (Fig. 3).

In one unexpected finding, unlike the previous report that NOD mice congenic for the *Lepr*$^{db\text{-}1J}$ allele eventually succumb to type 1 diabetes (13), the hyperglycemia in all *Lepr*$^{db\text{-}5J}$ females and in a subset of the males remitted to normoglycemia with increasing age, whereas both sexes of lean wild-type littermates showed a constant increase of type 1 diabetes in a time-dependent manner (Fig. 4). Spontaneous diabetes remission in *Lepr*$^{db\text{-}5J}$ females was not accompanied by losses in body weight (Fig. 2*A*). The combination of body weight and plasma glucose identified three phenotypes in *Lepr*$^{db\text{-}5J}$ males by age 39 weeks (Fig. 4). A group of 6 of 17 males (35%) depicted in Fig. 2*B* as exhibiting age-associated gradual declines in mean body weight showed unabated, severe hyperglycemia over time (mean plasma glucose = 581 ± 56 mg/dl at 39 weeks); 3 of the 6 males in this weight-losing phenotypic class died at age 41–49 weeks. Of the remaining 11 males that maintained elevated body weights (Fig. 2*B*), 4 showed a complete remission from diabetes comparable with that observed in females (35% of total group of 17 males; mean plasma glucose 145 ± 15 mg/dl at age 39 weeks). This remitting cohort accounted for the age-associated decrease in diabetes incidence depicted in Fig. 4*B*. The remaining 7 males maintaining an elevated mean body weight but exhibiting lower mean plasma glucose at age 39 weeks (368 ± 55 mg/dl)) were still hyperglycemic at necropsy (41%). At age 41–49 weeks, one of the seven males in this group died. The data in Table 1 provide longitudinal plasma insulin and leptin concentrations for the various subsets of aging *Lepr*$^{db\text{-}5J}$ mice and serum

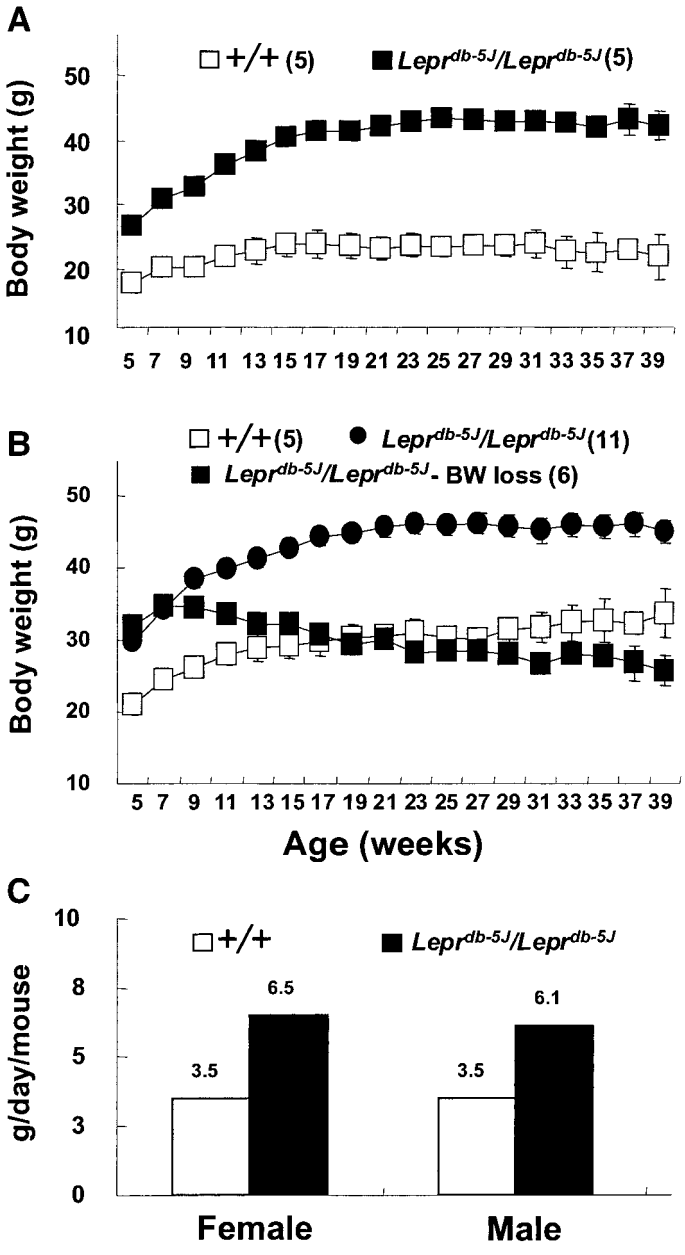

FIG. 2. Increased weight gain distinguishes NOD-*Lepr*$^{db\text{-}5J}$/Lt mutant females (*A*) and males (*B*) from wild-type mice. However, mutant males fell into two classes: those that continued to manifest increased weight gain and those that failed to maintain increased weight compared with wild type (*B*). Mutant mice of both sexes exhibited increased food consumption at age 5 weeks (*C*). (*n*), number of mice observed. BW, body weight.

TABLE 1
Comparative insulin and leptin levels

Phenotypes	Insulin (ng/ml)				Leptin (ng/ml)			
	Age 5 weeks	Age 9–13 weeks	Age 15–21 weeks	Age 41–49 weeks	Age 5 weeks	Age 9–13 weeks	Age 15–21 weeks	Age 41–49 weeks
Females								
Lean, nondiabetic ($n = 3$)	3.5 ± 0.6	1.9 ± 0.3	3.2 ± 0.6	NT	6.0 ± 2.9	3.4 ± 1.0	3.3 ± 2.2	NT
Obese, diabetes remission ($n = 4$)	27.6 ± 10.8*	14.3 ± 8.0*	12.4 ± 6.8*	5.9 ± 1.2†	40.9 ± 12.4‡	16.0 ± 5.5‡	47.4 ± 10.7‡	49.0 ± 17.5†
Males								
Lean, nondiabetic ($n = 3$)	2.7 ± 0.7	2.2 ± 0.8	2.1 ± 0.9	NT	1.9 ± 0.6	3.4 ± 0.8	5.8 ± 2.7	NT
Obese, diabetic, body weight loss ($n = 3$–6)*	16.5 ± 3.7*	3.3 ± 1.1	2.0 ± 0.9	1.8 ± 0.8	33.5 ± 9.7*	8.8 ± 0.8*	7.0 ± 3.2	17.4 ± 4.4
Obese, diabetic, no body weight loss ($n = 6$–7)*	27.1 ± 6.7*	35.1 ± 4.3*	24.9 ± 5.1*	49.4 ± 4.6†	47.7 ± 7.5*	71.6 ± 30.7*	82.9 ± 31.7*	54.9 ± 16.4†
Obese, diabetes remission ($n = 4$)	34.5 ± 4.5*	26.3 ± 2.7*	32.8 ± 5.5*	14.4 ± 6.9†	51.1 ± 28.6*	33.1 ± 9.8*	52.3 ± 2.8*	23.4 ± 8.8†

Data are means ± SE. Data for ages 5–21 weeks were obtained from plasma and those for ages 41–49 weeks were obtained from serum. *$P < 0.05$ vs. same-age lean mice. †Three males from the obese, diabetic, body weight loss group and one male from the obese, diabetic, no body weight loss group died at age 41–49 weeks. ‡$P < 0.05$ vs. 9-week-old lean mice. NT, not tested.

FIG. 3. Comparison of survival rates in lean NOD/Lt and obese NOD-*Lepr*$^{db\text{-}5J}$/Lt females (*A*) and males (*B*). As noted in RESEARCH DESIGN AND METHODS, lean diabetic mice had to be killed shortly after diabetes detection, whereas obese mice did not. (*n*), number of mice observed.

levels at necropsy at age 41–49 weeks. Mutant females that had remitted to normoglycemia still exhibited significantly elevated concentrations of insulin and leptin. The phenotypic variance in males was reinforced by differences in plasma insulin and leptin concentrations, distinguishing mutant males that had gone into a period of protracted weight loss combined with increasingly more severe hyperglycemia from the other two classes (obese with no weight loss and intermediate hyperglycemia and obese with no weight loss and full remission). The obese males exhibiting weight loss coupled with sustained severe hyperglycemia exhibited declines from a hyperinsulinemic range (16.5 ng/ml) at age 5 weeks to a concentration at necropsy (1.8 ng/ml) not different from that of young lean nondiabetic male controls. In both groups of mutant males maintaining elevated body weights, insulin concentrations remained elevated, with the fully remitting group showing the less extreme hyperinsulinemia. It was not surprising that leptin concentrations were significantly elevated in all obese groups. Interestingly, a major difference distinguishing all of these obese NOD-*Lepr*$^{db\text{-}5J}$ mutants from BKS mice homozygous for the *Lepr*$^{db\text{-}1J}$ mutation was the absence of a significant increase in serum lipids in the former mice (data not shown).

Histopathology. In both sexes of 8-week-old NOD-*Lepr*$^{db\text{-}5J}$ homozygotes, the pancreatic islets showed a similar pattern of normal-size islets containing markedly

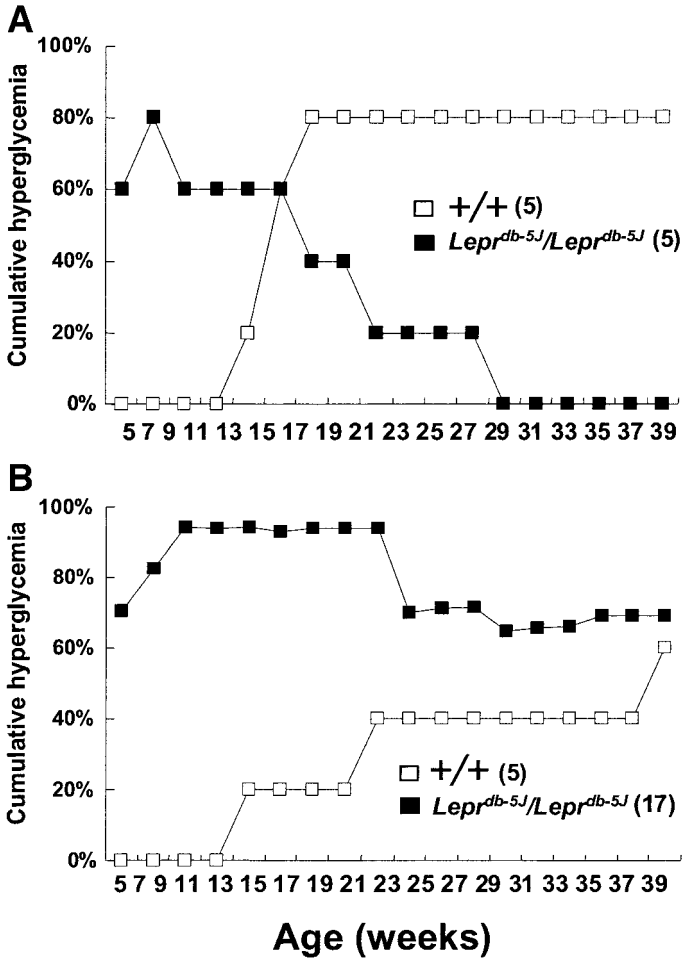

FIG. 4. Cumulative diabetes frequencies show juvenile-onset hyperglycemia in obese NOD-*Lepr*$^{db\text{-}5J}$/Lt females that spontaneously remit (*A*). Obese males also show juvenile onset, with a later remission in a subset, thus explaining the age-related drop in frequency (*B*). In contrast, lean NOD females and males showed a later (postpuberty) onset that was life shortening.

degranulated β-cells, thereby reflecting the early hypersecretory stress (Fig. 5*A*). Perivascular/periductular leukocytic infiltrates were clearly present, but intraislet infiltrates were not observed. Temporal changes in islet numbers and β-cell mass produced two different morphologies that were consistent with whether animals had maintained high body weight with or without remission from intermediate levels of hyperglycemia or had reverted from maintenance of high body weight to a pattern of weight loss correlated with increasingly more severe plasma glucose concentrations. Mice in the former two categories exhibited extremely hyperplastic islets containing well-granulated β-cells, with insulitis relegated to the islet perimeters (peri-insulitis) (Fig. 5*B*). In addition to the hypertrophied and hyperplastic islet masses, numerous small β-cell clusters were distributed throughout centroacinar regions of exocrine lobules. Intraislet leukocytic infiltration was not common in either the large or the small islet masses, although peri-insular aggregates at islet poles were frequently observed. In some obese hyperglycemic males without weight loss, a pattern of partial degranulation was observed in hyperplastic islets (Fig. 5*C*). The pancreases of males exhibiting unrestrained, severe, chronic hyperglycemia and gradual age-associated weight loss showed islets reduced in both size and number of granulated β-cells (Fig. 5*D*). However, these islets undergoing atrophy nevertheless did not resemble typical "end-stage" islets in standard NOD/Lt lean mice in that many of the atrophic islets in the former were still minimally associated with intraislet insulitis, although extensive perivascular/periductular lymphoid cell accumulations were common. This remarkable suppression of intraislet insulitis is summarized by the semiquantitative assessment of comparative insulitis scores distinguishing lean and obese mice (Fig. 6). Fatty deposits were noted in all pancreases regardless of diabetes outcome. No significant pathology was observed in liver and kidney tissues except for occasional mild fatty change and glomerular expansion in diabetic males.

DISCUSSION

Matarese et al. (12) were the first to report that type 1 diabetes in NOD mice could be modulated by stimulation through the leptin/leptin receptor axis, showing that postnatal administration of recombinant leptin abruptly precipitated juvenile-onset of diabetes by age 7 weeks in about 85% of treated NOD females. Our results demonstrating that a spontaneous mutation in the leptin receptor resulted in suppressed development of intraislet insulitis in the NOD model are consistent with evidence of an important role for the leptin/leptin receptor axis in the pathogenesis of type 1 diabetes. Adoptive transfer experiments have shown that T-cells from young NOD-*Lepr*$^{db\text{-}5J}$ donors fail to transfer type 1 diabetes into NOD-*Rag* recipients over a 13-week period in which all recipients of wild-type T-cells developed diabetes (C.-H.L., E.H.L., unpublished observations). Activation of β-cell destructive insulitis was also suppressed when irradiated NOD-*Lepr*$^{db\text{-}5J}$ recipients were reconstituted with nominally diabetogenic NOD bone marrow. Because hyperinsulinemia development is an early event in the obese mutant mice, induction of tolerance to insulin is a formal possibility. However, this seems unlikely as we have observed insulin autoantibodies in these animals (C.-H.L., E.H.L., unpublished observations). Although human CD4+ T-cells express the long Rb signaling isoform of the leptin receptor, we have found only the short (Ra) isoform to be highly expressed on purified NOD/Lt T- and B-cells by RT-PCR under conditions that did not detect the Rb isoform. However, more recently, increasing template RNA, annealing temperature, extension time, and cycle number has permitted the detection of low concentrations of Rb in purified NOD CD4+ T-cells.

In the present study, the surprising features of NOD-*Lepr*$^{db\text{-}5J}$ mutants included the suppressed development of invasive intraislet insulitis and the strong β-cell hyperplastic response, producing long-term survival without insulin treatment in the face of chronic hyperglycemia although pancreatic lymphocyte accumulation occurred in perivascular and periductular areas. Numerous studies have indicated that NOD females are at a higher risk for developing type 1 diabetes than are NOD males. In our colony, 90–100% of NOD/Lt females developed type 1 diabetes by age 24 weeks whereas only 50–70% of NOD/Lt males developed diabetes by age 30 weeks. The suppression of

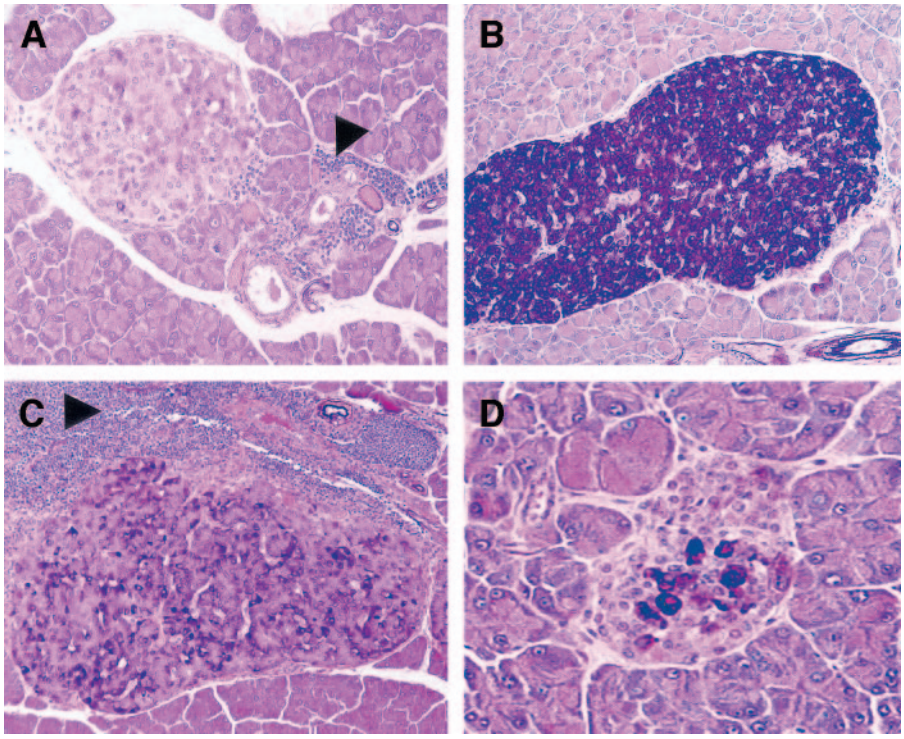

FIG. 5. Pancreatic islet histopathology with age and clinical phenotypes in NOD-*Lepr*$^{db\text{-}5J}$/Lt mice (aldehyde fuchsin staining). *A*: Normal-sized islet in an 8-week-old obese male with heavily degranulated β-cells and peri-insulitis (*arrowhead*; plasma glucose [PG] = 673 mg/dl; plasma insulin [PI] = 14.6 ng/ml, magnification ×20). *B*: Extensive β-cell hyperplasia in a 49-week-old obese male in diabetes remission. β-cells are heavily granulated and the islet is free of leukocytic infiltrate (PG = 163 mg/dl; PI = 21.3 ng/ml; magnification ×10). *C*: Hyperplastic islets with extensive β-cell degranulation in a 25-week-old obese diabetic male with peri-insulitis (*arrowhead*; PG = 298 mg/dl; PI >110 ng/ml; magnification ×10). *D*: Atrophic islet in a 43-week-old obese diabetic male with weight loss. Only a few residual granulated β-cells remain, but insulitis is absent, suggesting β-cell exhaustion rather than autoimmune destruction (PG = 936 mg/dl; PI >2.6 ng/ml; magnification ×10).

destructive insulitis in NOD-*Lepr*$^{db\text{-}5J}$ females is therefore particularly impressive, allowing for the type 2 diabetes syndrome (customarily more severe in males than in females) to transit into full remission. The remission in all mutant females and in >33% of the obese males was associated with β-cell hyperplasia and islet volume expansion that relegated the insulitic infiltrate to the perivascular poles of the islets. In the ~33% of the obese hyperglycemic males that lost weight over a protracted period and showed a marked reduction in plasma insulin, peri-insulitis was a feature of the islet histopathology at necropsy, but the overall islet appearance was more reminiscent of β-cell exhaustion and islet atrophy described for BKS- *Lepr*$^{db\text{-}1J}$ mice (15) rather than typical "end-stage" NOD islet histopathology.

The ability of the *Lepr*$^{db\text{-}5J}$ mutation to produce an obesity/insulin resistance syndrome capable of suppressing insulitic destruction of β-cells was quite unexpected, given the report from Japan (13) that congenic introduction of the *Lepr*$^{db\text{-}1J}$ mutation failed to suppress this autoimmune destruction. Similarly, sustained insulin secretion and extended lifespan was reported when a mutation in the extracellular domain of the rat leptin receptor (*Lepr*^{fa}) was introgressed into the autoimmune diabetes-prone BB/Wor rat, despite ongoing insulitis (16). On the other hand, leptin administration to SJL/J females before and after induction of experimental autoimmune encephalomyelitis exacerbated disease severity (17). More recently, this SJL/J disease exacerbation has been correlated with a leptin-associated reduction in T-regulatory CD4+CD25+ cells (18). The unique location of the *Lepr*$^{db\text{-}5J}$ mutation in the extracellular domain coupled with the finding that the NOD-*Lepr*$^{db\text{-}5J}$ mice are reproductively competent, suggests that the mutation retains some intracellular JAK/STAT signaling capacity, but that this is reduced due to either impaired ligand binding or receptor dimerization. The salient difference between the NOD co-isogenic *Lepr*$^{db\text{-}5J}$ mutant receptor studied herein versus the NOD mice congenic for the *Lepr*$^{db\text{-}1J}$ mutation studied in Japan (13) is the absence of the intracellular signaling domain in the latter (and introduction of the BKS-derived flanking genome on chromosome 4). It seems unlikely that BKS (rather than NOD) alleles on chromosome 4 are the reason for the development of destructive insulitis in the congenic NOD-*Lepr*$^{db\text{-}1J}$ mouse but not the NOD-*Lepr*$^{db\text{-}5J}$ co-isogenic mouse carrying only NOD alleles on chromosome 4. The differential effects of the two mutations on the immune system are particularly hard to interpret in view of our finding that the two mutations complement each other in producing an obesity phenotype in *Lepr*$^{db\text{-}5J}$/*Lepr*$^{db\text{-}1J}$ mice. When NOD mice were outcrossed with *Mus spretus*, a wild mouse strain and backcross segregants (to NOD) were phenotyped, a non−major histocompatibility complex, obesity-associated type 2 diabetes rather than a type 1 diabetes syndrome predominated, especially in males (19). β-Cell hyperactivity may increase autoantigen presentation (20) and thereby drive the autoimmune attack. Hence, if the *Lepr*$^{db\text{-}5J}$ Rb retains some signaling capacity that is completely absent in *Lepr*$^{db\text{-}1J}$ mice, modification of β-cell activity might be expected to be less severe in the former. However, as shown in Table 1, plasma insulin concentrations were comparably elevated in NOD-*Lepr*$^{db\text{-}5J}$ males that underwent complete remission versus males that remained hyperglycemic without weight loss. It is reasonable to suppose that the degree of insulin resistance at the late stages of the syndrome in males determined whether complete β-cell exhaustion and severe type 2 diabetes or complete remission developed. Clearly, further study is required to understand why an intact leptin receptor promoted type 1 diabetes, a signaling defective receptor (*Lepr*$^{db\text{-}1J}$) failed to abrogate insulitic destruction of β-cells, and yet a receptor with potentially partial signaling

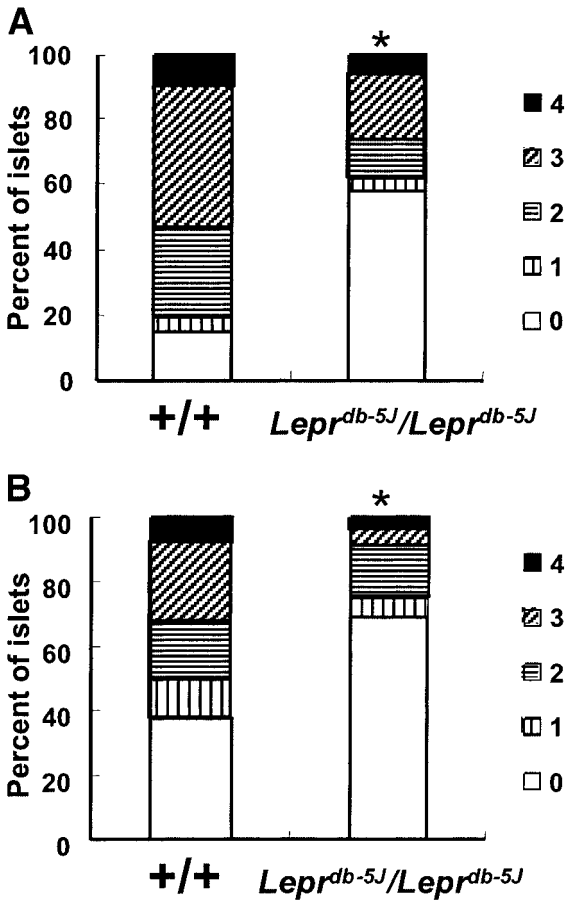

FIG. 6. Pancreatic insulitis is suppressed in obese NOD-*Lepr*$^{db\text{-}5J}$/Lt females age 43–45 weeks; $n = 4$; *A*) and obese males (age 41–49 weeks; $n = 13$; *B*) compared with lean wild-type nondiabetic females (age 12 weeks; $n = 3$) and wild-type nondiabetic males (age 22 weeks; $n = 3$). As noted in RESEARCH DESIGN AND METHODS, the insulitis of each individual islet was graded on a 0–4 scale: 0, normal; 1, peri-insulitis; 2, infiltration ≤25% of islet mass; 3, infiltration of 25–75% of islet mass; and 4, islet destruction. Liver and kidney tissues were stained by periodic acid Schiff reagent. *$P < 0.001$ vs. lean wild-type mice.

capacity (*Lepr*$^{db\text{-}5J}$) produced an endocrine and metabolic milieu that suppressed destructive insulitis.

Type 1 diabetes in NOD mice is a complex process resulting from immune dysregulation at multiple levels, including defective maturation and function of macrophages (21) and dendritic cells (22), low natural killer and natural killer T-cell activity (22,23), deficiencies in regulatory $CD4^+CD25^+$ T-cell subsets (24), and defective induction of central and peripheral T-cell tolerance (25,26). Comparison of these immune phenotypes in NOD versus NOD-*Lepr*$^{db\text{-}5J}$ mice will permit new insights into the role of leptin signaling in cueing the cytopathic function of autoimmune T-effector cells.

In summary, we have identified a spontaneous novel leptin receptor mutation (*Lepr*$^{db\text{-}5J}$) producing obesity and type 2 diabetes in NOD/Lt mice that has the unexpected effect of suppressing invasive intraislet insulitis. The altered endocrine and metabolic milieu generated in these mice produces a remarkable degree of β-cell hyperplasia. Thus, the new model will be useful not only to dissect the interaction between the endocrine and immune systems in type 1 diabetes–prone mice but also to understand the trophic factors that are generated to produce such a robust proliferation of β-cells.

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