Mouse Models and the Genetics of Diabetes

Is There Evidence for Genetic Overlap Between Type 1 and Type 2 Diabetes?

Edward H. Leiter[1] and Chul-Ho Lee[2]

In humans, both type 1 and type 2 diabetes exemplify genetically heterogeneous complex diseases in which epigenetic factors contribute to underlying genetic susceptibility. Extended human pedigrees often show inheritance of both diabetes types. A common pathophysiological denominator in both disease forms is pancreatic β-cell exposure to proinflammatory cytokines. Hence, it is intuitive that systemically expressed genes regulating β-cell ability to withstand chronic diabetogenic stress may represent a component of shared susceptibility to both major disease forms. In this review, the authors assemble evidence from genetic experiments using animal models developing clearly distinct diabetes syndromes to inquire whether some degree of overlap in genes contributing susceptibility can be demonstrated. The conclusion is that although overlap exists in the pathophysiological insults leading to β-cell destruction in the currently studied rodent models, the genetic bases seem quite distinct. *Diabetes* 54 (Suppl. 2):S151–S158, 2005

The complex of genes in the *HLA* locus (*IDDM1*) contributing the major component of human susceptibility to autoimmune type 1 diabetes clearly provides a starting point for comparison for overlap with major susceptibility contributors to classic type 2 diabetes. Indications of humoral immunity against pancreatic β-cell autoantigens in 10–30% of patients deemed clinically to have type 2 diabetes led to the concept that these patients exhibit latent autoimmune diabetes in adults (LADA), or "type 1.5" diabetes (1). Many LADA patients exhibit decreased frequency of the highest risk HLA class I and class II alleles and increased frequency of HLA alleles conferring strong protection against juvenile-onset type 1 diabetes (1). Although most type 2 diabetes cases clearly are not autoimmune in causation, and thus, specific HLA alleles are not identified as major type 2 diabetes susceptibility contributors, the LADA cases raise the possibility that a subset of non-HLA susceptibility may be shared. Under such circumstances, the absence of the high-risk HLA alleles results in a more slowly progressive disease that only presents in adulthood when pathophysiological stresses on β-cells, including obesity and insulin resistance shared in common with patients with classic type 2 diabetes, become prevalent.

Polymorphisms in the upstream regulatory region of the insulin gene currently represent the strongest non-HLA locus linked to human type 1 diabetes susceptibility (*IDDM2*) (2). The pathogenic mechanism has not been associated with insulin processing, secretion, or action, but rather with the ability to express intrathymically and elicit T-cell tolerance to this major type 1 diabetes autoantigen (3,4). Alleles associated with resistance to type 1 diabetes, on the other hand, have been associated with polycystic ovarian syndrome (5), an insulin resistance syndrome that often leads to type 2 diabetes. Because type 2 diabetes often culminates in insulin deficiency attributed to β-cell failure associated with chronic lipotoxicity, glucotoxicity, and free radical stress (6), it is not surprising that functional changes in genes associated with β-cell glucose sensing and metabolism produce nonautoimmune but insulin-requiring diabetes syndromes. Glucokinase (*GCK*) is an example of one such β-cell essential gene linked to both type 1 and type 2 diabetes (7,8). Although GAD65 is considered a major β-cell autoantigen in type 1 diabetes, genetic association with the gene encoding it (GAD2) has been with obesity, the major risk factor for type 2 diabetes (9). Indeed, when specific candidate genes identified in either type 1 or type 2 diabetes are screened for association/linkage in the other disease form, evidence for overlap is rare (10). Given that human predisposition to obesity and type 2 diabetes is controlled by large numbers of quantitative trait loci (11), and similarly, inheritance of a complex assortment of major histocompatibility complex (MHC) and non-MHC loci are required to increase susceptibility to type 1 diabetes (12), genetically defined strains of inbred mice with known predisposition to either type 1 or type 2 diabetes have been used to model the extent to which genes contributing susceptibility to one form of diabetes might also contribute to the other form. The examination of results of such modeling studies in mice is the subject of this review.

C57BLKS/J AND NOD/Lt: TWO PARADIGM DIABETES-PERMISSIVE INBRED STRAINS FOR COMPARISON OF COMMON SUSCEPTIBILITY GENES

The C57BLKS/J (BKS) strain (originally named C57BL/KsJ) was little studied before the report of a monogenic mutation producing chronic hyperglycemia and β-cell destruction on this genetic background (13). This strain arose through a genetic contamination of the C57BL/6J (B6) strain, probably by DBA/2J (14). The molecular basis

From the [1]The Jackson Laboratory, Bar Harbor, Maine, and the [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.

Address correspondence and reprint requests to Dr. Edward H. Leiter, The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609. E-mail: ehl@jax.org.

Received for publication 30 March 2005 and accepted in revised form 8 April 2005.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

IL, interleukin; LADA, latent autoimmune diabetes in adults; MHC, major histocompatibility complex; ROS, reactive oxygen species; TNF, tumor necrosis factor.

for the "diabetes" mutation entails an abnormal splice junction variant that prevents transcription of a leptin receptor "long" isoform (*Lepr*$^{db\text{-}1J}$) (15). The leptin receptor long form (LEPR-Rb) is a member of the interleukin (IL)-6 family of receptors. Leptin binding elicits receptor dimerization, recruitment, and autophosphorylation of JAK1/JAK2 kinases that, in turn, activate STATs, especially STAT3 and STAT5, by tyrosine phosphorylation (16). Regardless of inbred strain background, homozygous *Lepr*$^{db\text{-}1J}$ mutants are obese and insulin resistant and therefore are generally considered to be a type 2 diabetes model. However, diabetogenesis in this model completely depends on the inbred strain background. BKS-*Lepr*$^{db\text{-}1J}$ mice show early onset of a juvenile hyperglycemia, followed by massive loss of β-cells and islet atrophy within 2 months after puberty. Although transfer of the *Lepr*$^{db\text{-}1J}$ mutation onto the B6 genetic background produces even more extreme obesity and insulin resistance, B6-*Lepr*$^{db\text{-}1J}$ mice develop only a mild and transient diabetes (13). Importantly, the same susceptibility difference distinguishing the BKS from the B6 genetic backgrounds was exactly reproduced when the unlinked recessive *Lep*^{ob} "obese" mutation or its ligand, leptin, was studied on both backgrounds (13). Similarly, the "fat" mutation in the carboxypeptidase E locus, which was not diabetogenic on the HRS/J background, did produce diabetes in males when backcrossed into the BKS strain background (17). What are these genetic background differences eliciting such disparate syndromes? B6 and BKS share genetic identity at ~88% of markers tested. Differences include MHC haplotypes, but this has been excluded as the explanation (18). Diabetes pathogenesis in BKS-*Lepr*$^{db\text{-}1J}$ mice was particularly responsive to dietary carbohydrate (19). Elevated glucose concentrations in vitro induced expression of defective endogenous retroviral genomes in β-cells from wild-type BKS but not B6 islets (20). Male BKS mice were further differentiated from B6 males in terms of their heightened sensitivity to diabetes induced by diabetogens, including alloxan (21) and multiple low doses of streptozotocin (22). The latter represents a model assumed to entail an immune component and has also been associated with induction of retroviral genomes in β-cells (23). Indeed, investigators interested in the immunology of diabetes previously viewed BKS-*Lepr*$^{db\text{-}1J}$ mice as a model of "type 1.5 diabetes" because evidence for both thymus-dependent and humoral reactivity against β-cells was found (rev. in 24). However, combination of this mutation with various immunodeficiency mutations showed that diabetogenesis did not require either T- or B-cell–mediated events (25). Rather, the major differences distinguishing the "type 1.5" diabetes in BKS-*Lepr*$^{db\text{-}1J}$ mice versus the mild remitting type 2 diabetes syndrome in B6-*Lepr*$^{db\text{-}1J}$ mice, other than the ability to express retroviral genetic elements in β-cells, were differences in intracrine sex steroid metabolism, conferring greater androgen sensitivity in BKS mice of both sexes (26,27). This would also account for the differential BKS/B6 male sensitivity to multiple low-dose streptozotocin-induced diabetes.

Whereas it can be debated as to what kind of human diabetes is modeled by BKS-*Lepr*$^{db\text{-}1J}$ mice, there is no question that the NOD mouse clearly models for autoimmune T-cell–mediated type 1 diabetes in humans. The diabetogenic MHC (*H2*^{g7}) of NOD mice, comprising contributions from both class I and class II loci and collectively designated as *Idd1*, is the major genetic contributor to susceptibility. There are well in excess of 20 non-MHC susceptibility linkages defined by segregation analysis following outcross with either related (NON/Lt, ALR/Lt) or unrelated (B6, C57BL/10, C57L, PWK) strains. Homozygous expression of the diabetogenic MHC provides a permissive scaffold onto which variable sets of non-MHC genes contribute in an additive threshold fashion, with both the dietary and microbial environment exerting major influences on disease penetrance (28). An updated listing of the currently known chromosomal regions containing "*Idd*" loci may be found in a recent review (29). The next section examines how the type 2 diabetes–predisposing BKS genome and the type 1 diabetes–predisposing NOD genome interact.

TABLE 1
Comparison of two mouse models of diabetes

	C57BL/KsJ-*db/db*	NOD/Lt
Obesity	Yes	No
Insulin resistance	Yes	No
Age of diabetes onset (weeks)	4–6	12–30
β-Cell necrosis	>90%	100%
Insulitis	No	Yes
Endogenous β-cell retroviruses	Yes	Yes
Sex bias	Male	Female
Diabetes model	Type 2/type "1.5"?	Type 1

NOD AND BKS GENOMES DO NOT SHOW COMPLEMENTATION OF COMMON TYPE 1 DIABETES SUSCEPTIBILITY GENES

Table 1 summarizes similarities and differences of the two different diabetes models discussed above. If the NOD and BKS genomes harbored common susceptibility genes, the cellular level for this shared susceptibility might be the β-cell. NOD β-cells, like BKS β-cells, are permissive for retroviral gene expression (30,31) and males of both strains are very susceptible to insulin-dependent diabetes induced by multiple low-dose streptozotocin treatments (32). NOD β-cells are claimed to exhibit an unusually strong spontaneous "wave" of apoptosis before weaning that triggers insulitis (33). Although increased apoptosis in BKS versus B6 β-cells has not been reported, transplanted BKS islets are more susceptible to glucotoxic stress than are B6 islets transplanted into diabetic (BKS × B6)F1 recipients (34). Accordingly, shortly after an NOD colony was established at The Jackson Laboratory and at the Diabetes Research Institute, Düsseldorf (by Dr. L. Herberg), outcrosses between NOD/Lt and related strains (NON/Lt and SWR/J) as well as completely unrelated strains (BKS, B6, CBA/LsLt) were analyzed. The frequencies of diabetes obtained after outcross and first backcross (to NOD) are summarized in Table 2. All diabetic probands were *H2*^{g7} homozygotes; indeed, none of the approximate 50% of the MHC heterozygotes among first backcross progeny would have been permissive for spontaneous diabetes development. Even though the related strains (NON/Lt, SWR/J) differed from NOD at ~40–50% of polymorphic markers typed, such outcrosses yielded a higher diabetes frequency (among the *H2*^{g7} homozygous segregants) than outcross/backcross with the unrelated strains. BKS as an outcross partner produced the lowest diabetes frequency. Thus, all the genetic and physiological features that rendered this strain highly susceptible to β-cell toxins and to diabetogenic stress exerted by monogenic obesity mutations were not capable of synergizing

TABLE 2
Diabetes incidence in NOD backcrosses

Cross*	Number of diabetic animals/total	Diabetes incidence (%)
NOD/Lt × NON	19/200	9.5
NOD/Lt × SWR/J	10/88	11.4
NOD/Lt × C57BLKS/J	1/115	0.9
NOD/Lt × C57BL/6J†	5/383	1.3
NOD/Shi × CBA/LsLt†	4/179	2.2

*F1 outcross used to produce [F1 × NOD]BC1 offspring. †Done in collaboration with Dr. Lieselotte Herberg, Diabetes Research Institute, Dusseldorf, Germany.

deleteriously with the *Idd* susceptibility loci in the NOD genome. Indeed, after a second backcross (to NOD) was performed, and the diabetogenic $H2^{g7}$ haplotype along with multiple other type 1 diabetes susceptibility loci and albino coat color were fixed, an inadvertent genetic contamination occurred that gave rise to the type 1 diabetes–resistant NOR/Lt strain (35). Genetic analysis of the $H2^{g7}$-identical NOR/Lt, showed that, like its BKS progenitor, it was a recombinant congenic strain with ~88% genomic identity with NOD/Lt. BKS-derived loci on chromosome 1, 2, 4, and 11 are now known to confer this type 1 diabetes resistance (36–38). The one known example of a shared NOD/BKS type 1 diabetes susceptibility allele is the common class I $H2K^d$ allele, a component of the complex *Idd1* locus on chromosome 17. But the BKS genome suppresses any potential type 1 diabetes susceptibility conferred by this shared peptide antigen-presenting molecule. β2-Microglobulin binding to class I chains is required to produce a molecular conformation that can bind and present peptide antigen. BKS mice express a β2-microglobulin ($B2m^b$) allele on chromosome 2, whose protein product differs by only a single amino acid from that of the $B2m^a$ allele expressed by NOD mice. NOR/Lt mice express the $B2m^b$ allele acquired from BKS in the presence of MHC class I genes inherited from NOD. The steric differences in molecular folding produced by the $B2m^a$ and $B2m^b$ isoforms has been shown to control the immunogenicity of NOD β-cells targeted by cytotoxic T-cells (39). In addition to this allotypic $B2m^b/H2K^d$ combination distinguishing BKS from NOD, the disparate BKS MHC class II molecules expressed from its $H2^d$ haplotype would be more than sufficient to suppress generation of autoimmune T-effector cells.

SUPERIMPOSING OBESITY AND INSULIN RESISTANCE ON THE NOD/Lt MOUSE

If the BKS genome does not provide genes capable of interacting productively with the NOD genome to precipitate type 1 diabetes, does the inverse hold? Will the NOD genome be permissive to a type 2 diabetes syndrome? A spontaneously occurring mutation at the leptin receptor locus in the distribution colony of NOD/LtJ mice at The Jackson Laboratory (designated $Lepr^{db\text{-}5J}$) provided the opportunity to assess whether a BKS-like obesity-induced type 2 diabetes ("diabesity") syndrome would result or whether a well-compensated "B6-like" obesity syndrome with mild remitting "diabesity" would develop. Also, how would autoimmune insulitis development respond to the systemic disruptions in the endocrine/metabolic milieu produced by obesity?

The leptin receptor itself is a member of the class I cytokine receptor family typified by the IL-6 receptor, and leptin is a family member of the helical cytokines and has a structure similar to IL-2. Leptin exerts multiple effects on systems biology in addition to its well-known effects on feeding behavior and energy homeostasis. Indeed, this adipokine has been associated with modulation of a wide variety of immune functions in both humans and mice (40,41). Early administration of a high dose of recombinant leptin to NOD females, but not males, drastically accelerated onset of type 1 diabetes so that onset was truly juvenile rather than the customary NOD adult-onset pattern (42). This finding contrasted with a study virtually unknown outside of Japan wherein NOD/Shi mice were made congenic for either the $Lepr^{db\text{-}1J}$ or Lep^{ob} mutations (43). These congenic NOD/Shi mice developed a transitory type 2 diabetes manifested by obesity, hyperinsulinemia, and islet hyperplasia. Importantly, the insulitic process prevailed, producing a life-shortening hypoinsulinemic type 1 diabetes (43). One would have thought that if endogenous leptin was critical to the development of autoimmune T-effectors, then the Japanese investigators would have observed longer-lived obese-diabetic NOD mice—an impression definitely not imparted by their report.

Figure 1*A* compares the standard (lean) NOD/Lt mice with the obese phenotype of NOD-$Lepr^{db\text{-}5J}$/Lt mice. In this regard, the coisogenic mutation (e.g., spontaneously occurring in the NOD genome) produced the same obesity phenotype as the NOD stock congenic for the BKS-derived $Lepr^{db\text{-}1J}$ mutation studied in Japan. However, the disease course taken, particularly in females, was drastically different. Figure 1*B* illustrates the fulminant intra-islet insulitis that predominates in the pancreas of NOD mice approaching clinical type 1 diabetes. Based on the results of the congenic transfer of $Lepr^{db\text{-}1J}$ from the BKS background into the NOD genetic background cited above (43), wherein obesity and insulin resistance were nevertheless accompanied by widespread destructive insulitis, we anticipated that NOD-$Lepr^{db\text{-}5J}$/Lt females would also show a "BKS-like" diabesity with massive β-cell failure and islet atrophy associated with widespread destructive insulitis. However, what has been observed in all NOD-$Lepr^{db\text{-}5J}$ females, and in approximately two-thirds of the mutant males, was a "B6-like" compensatory pattern wherein massive β-cell hyperplasia (Fig. 1*C*) successfully compensated for insulin resistance and brought a juvenile-onset hyperglycemia (initiating between 5 and 7 weeks of age) into complete remission by 12 weeks of age—a time point at which the first wild-type (lean) NOD/Lt females were transiting into clinical type 1 diabetes. Plasma leptin concentrations were 15-fold elevated, and plasma insulin concentrations were ninefold elevated at weaning (5 weeks), gradually declining to threefold higher as hyperglycemia spontaneously remitted. The unrestricted β-cell hyperplasia was equally unexpected based on the published congenic transfer study (43) reporting that destructive insulitis was the prominent histopathological feature. In all NOD-$Lepr^{db\text{-}5J}$/Lt females analyzed histologically, peri-insulitis was present, but failed to infiltrate into the islets.

A somewhat more complex set of diabesity syndromes was observed in NOD-$Lepr^{db\text{-}5J}$/Lt males. In all cases, mutant males, like mutant females, developed obesity and hyperglycemia immediately after weaning, and like females, all of these obese-hyperglycemic males were hyperleptinemic and hyperinsulinemic, living well past 40 weeks

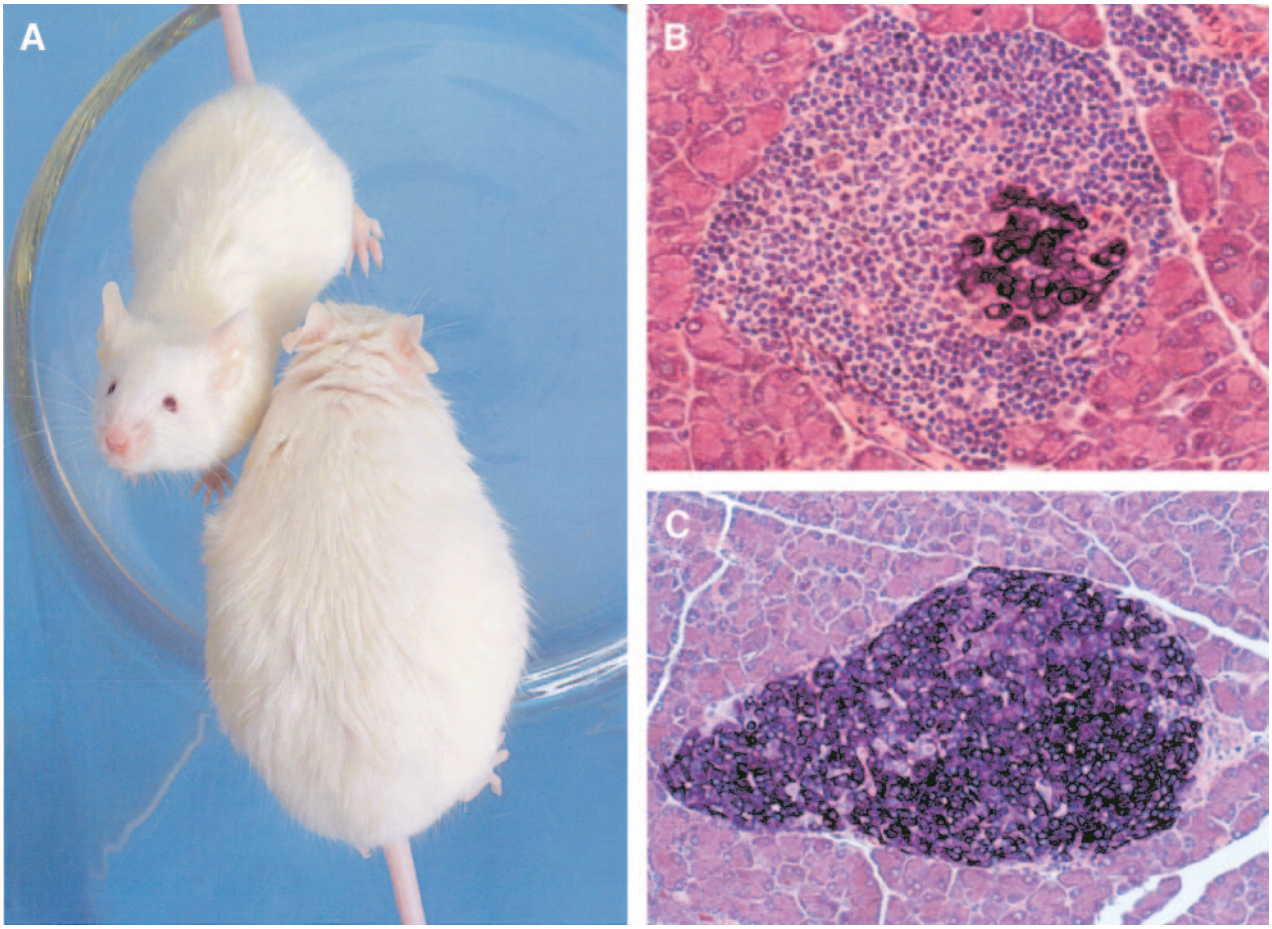

FIG. 1. *A*: An obese NOD-*Lepr*$^{db\text{-}5J}$/Lt mouse and a standard NOD/Lt age-matched control. *B*: Intra-islet invasive insulitis characteristically observed is associated with islet degranulation and β-cell destruction in a lean 25-week-old NOD/Lt female. *C*: Unrestricted hyperplasia of islet β-cells in a 48-week-old NOD-*Lepr*$^{db\text{-}5J}$/Lt female that had undergone spontaneous remission from diabetes. Insulitis, when present, is generally limited to the islet periphery. Aldehyde fuchsin staining of granulated β-cell, 20×.

of age without any requirement for insulin. However, of a group of 17 aging males, only 4 of 17 exhibited full remission from hyperglycemia while remaining obese and moderately hyperinsulinemic (sevenfold higher than normal). Another subset (7/17) remained obese and even more hyperinsulinemic (25-fold elevated), but remained hyperglycemic (mean plasma glucose ~450 mg/dl over a 39-week time span). A third phenotypic class (6/17 males) showed peak mean body weight by 9 weeks and then gradually began to lose weight, with three mice dying between 41 and 49 weeks and the remainder continuing to lose weight, such that mean body weights fell into a normal (nondiabetic lean NOD) range by 41–49 weeks of age. In this subset, plasma glucose gradually increased to a very high concentration (mean >750 mg/dl by 41–49 weeks), with concomitant declines in plasma insulin concentrations from an early hyperinsulinemic range (eightfold above normal) to a value not different from young lean controls. A comparable level of β-cell hyperplasia and hypertrophy characterized the islets of the former two groups (diabetic remitters and intermediate severity diabetics), while the latter class of severely diabetic mutants showed a pattern of β-cell depletion and islet atrophy more reminiscent of BKS-*Lepr*$^{db\text{-}1J}$ mice. In all three male phenotypic classes, intra-islet insulitis was markedly suppressed, as it was in mutant females.

Several explanations can be offered to account for the differences between the Bar Harbor results and those previously reported in Japan, including NOD substrain differences, linked BKS insulitis accelerators in the congenic segment on chromosome 4 (highly unlikely given the results shown in Table 1), or environmental differences. We believe the most likely explanation is the molecular difference between the two mutations. The direct sequencing of *Lepr*$^{db\text{-}5J}$ revealed a G-to-T transversion mutation in exon 13 producing a glycine640valine change in the very distal portion of the extracellular domain immediately adjacent to the transmembrane domain. Glycine at this base position is highly conserved across genera. Structural analysis of the human LEPR extracellular domain suggests that this amino acid change likely could affect the nearby ligand binding domain (44). RT-PCR showed that this mutation did not prevent transcription of a full-length (LEPR-Rb) transcript in the hypothalamus. The *Lepr*$^{db\text{-}1J}$ mutation used to produce the NOD congenic stock in Japan, by contrast, has an insertion that eliminates an essential splice domain required to generate the LEPR-Rb isoform. Thus, partial signaling (e.g., JAK/STAT recruitment and phosphorylation) likely is occurring in the coisogenic *Lepr*$^{db\text{-}5J}$ mutant stock but not the *Lepr*$^{db\text{-}1J}$ congenic stock. It remains to be demonstrated that a reduced level of signaling of leptin through its receptor, or secondary changes in the endocrine/metabolic milieu resulting from impaired signaling, explain the unusual suppression of destructive insulitis. The conclusions reached from this study extend the earlier findings that type 2

diabetes–predisposing factors in the BKS genome do not deleteriously combine with type 1 diabetes–predisposing NOD genes by revealing that a monogenic obesity mutation that promotes β-cell failure in BKS mice actually inhibits invasive insulitis in the NOD genome.

PROINFLAMMATORY CYTOKINES, LIPID MEDIATORS, AND REACTIVE OXYGEN SPECIES: THE MISSING LINK BETWEEN THE GENETICS OF TYPE 1 AND TYPE 2 DIABETES SUSCEPTIBILITY?

One of the unifying themes in diabetes pathophysiology is that proinflammatory cytokines, chemokines, lipid mediators, and reactive oxygen species (ROS) harm β-cells regardless of whether the immune system or obesity and insulin resistance are the primary pathogenetic factors leading to their generation. Common human polymorphisms in the HLA class III region encoding tumor necrosis factor (TNF)-α and TNF-β have been associated with susceptibility to both type 1 and type 2 diabetes (45,46). Similarly, human polymorphism in the IL-6 receptor has been associated with the metabolic syndrome/type 2 diabetes (46,47). TNF-α and IL-6 are representative of a growing number of cytokines frequently found to be elevated in both forms of diabetes. Both IL-6 and TNF-α, as well as leptin, are excellent examples of cytokines/adipokines whose concentrations in blood increase during infection or inflammation. Hyperglycemia, the common pathophysiological feature of both type 1 and type 2 diabetes, can elicit ROS generation and increased oxidative stress systemically and in β-cells in particular (6,48). High concentrations of obesity-associated free fatty acids can compromise insulin action and β-cell function, as well as drive β-cell apoptosis (49). Proinflammatory lipid (eicosanoid) mediators derived from arachidonic acid metabolism have also been associated with β-cell pathogenesis in type 1 diabetes in NOD mice (50) and deterioration of vascular function in *Lepr*^{db} mice (51). Because β-cells are especially sensitive to oxidative stress, genes contributing to maintenance of systemic and β-cell redox potential might be expected to contribute to resistance to both forms of diabetes. We used the ALR/Lt (alloxan-resistant) strain to examine the effects of a genome contributing unusually strong type 1 diabetes–protective free radical detoxification ability when challenged with NOD-derived autoimmune effectors or with the type 2 diabetes/diabesity–promoting polygenes in NZO/Lt (New Zealand Obese) mice.

ALR mice were selected in Japan for resistance to type 1 diabetes elicited by a low dose of the β-cell toxin alloxan (52). A strain selected for sensitivity to the same dose was designated ALS (alloxan sensitive). Given that alloxan is a potent generator of hydroxyl radicals, it was not surprising that the physiological differences distinguishing these two strains primarily entailed the ability to suppress ROS generation and their detoxification if generated (53). This resistance was systemically expressed but was shown to extend to the β-cells both in terms of increased activities of enzymes that generated reduced glutathione and detoxified free radicals once they were generated (54). What was surprising, however, was how well the ALR/Lt defenses responded to autoimmune effectors generated from the very closely related NOD mouse (54). To establish the genetic basis for this systemic ability to dissipate ROS stress elicited by a diabetogenic immune system, reciprocal outcrosses between ALR/Lt and NOD/Lt mice were performed and chromosomal linkages were established.

When NOD/Lt was the female parent, and the diabetes-resistant F1 females were backcrossed to NOD/Lt males, ALR/Lt-derived resistance alleles on chromosomes 3, 8, and 17 were identified. The chromosome 17 gene was tightly linked to the distal end of the MHC and appeared to control β-cell antigen presentation rather than ROS detoxification (55). The linkages to chromosomes 3 and 8, however, were associated with free radical defense and/or dissipation (56,56a). When the outcross was performed differently, so that now ALR/Lt females were crossed to NOD/Lt males, and the diabetes-resistant F1 females backcrossed to NOD/Lt males, too few diabetic probands were generated to allow genetic linkage analysis (57). This extra component of resistance associated with use of ALR/Lt as the maternal parent, coupled with the knowledge that mitochondria, inherited from the maternal parent, are a major source of intracellular ROS, led us to do comparative sequencing of the mitochondrial genomes of ALR and its related strains (NOD, ALS, NON). The ALR mitochondrial genome differed from all other mouse mitochondrial genomes sequenced in showing nonconservative replacement of a leucine with a methionine at amino acid residue 276 of the NADH dehydrogenase subunit 2. This finding that an additional component of diabetes susceptibility was encoded by the mitochondrial genome was particularly relevant because mutations in the human *mtNd2* gene have been associated with both forms of diabetes, with the specific mutation found in the ALR/Lt *mtNd2* allele associated with protection against type 1 diabetes in a Japanese study group (58). With regard to the ALR/Lt mouse, we conjecture that the unusually high expression of nuclear genes whose products are concerned with defense against ROS is a response to an increased proton "leak" from the mitochondrial electron transport chain. In effect, an altered mitochondrial membrane potential may convey an internal "danger signal" conferring constitutive expression of systemic defenses normally upregulated in other mouse strains only after application of ROS stress.

MATCHING THE ALR/Lt GENOME AGAINST POLYGENETIC TYPE 2 DIABETES IN THE NZO/Lt MALE MOUSE

If impaired β-cell function reflects, in part, the consequences of ROS in type 2 diabetes as well as is likely the case in type 1 diabetes, can the strong systemic ability of the ALR/Lt genome to dissipate ROS protect against type 2 diabetes development? Unlike the monogenic obesity elicited by the *Lepr*^{db} mutations described above, obesity-induced type 2 diabetes (diabesity) in NZO/Lt males is polygenic in origin and is driven by the rate of weight gain during the peripubertal period (59). Interestingly, in the same fashion that the BKS-*Lepr*$^{db\text{-}1J}$ diabetic mouse has sometimes been considered a potential model of "type 1.5" diabetes because of potential involvement of the immune system in pathogenesis, similar indications have been observed in NZO/Lt males (60). In addition to inflammatory infiltrates associated with the pancreatic islets of chronically diabetic males (60), the strain produces autoantibodies to the insulin receptor (61). As we found for the immune-associated phenomena in BKS-*Lepr*$^{db\text{-}1J}$ diabetic mice, these immune phenomena in NZO/Lt males were secondary and not primary pathogenic responses (62). Nevertheless, diabetogenesis in this type 2 diabetes model

TABLE 3
Antioxidant defenses and type 2 diabetes

Matings	Number of mice	Body weight (g)	Plasma glucose (mg/dl)
ALR × NZO	13	60.9 ± 2.0	271 ± 46
NZO × ALR	3	66.9 ± 3.0	464 ± 37*
NON × NZO	5	64.9 ± 1.8	348 ± 39
NZO × NON	5	63.4 ± 4.7	342 ± 30

Data were collected from 24-week-old males maintained on an NIH-31 diet containing 4% fat. *Differences between reciprocal outcrosses significant at $P < 0.001$.

is associated with increasingly more severe insulin resistance associated with chronic exposure of β-cells to hyperglycemic and hyperlipidemic environments (59). Data in Table 3 compare outcrosses between NZO/Lt and ALR/Lt or with the related (but ROS-damage susceptible) NON/Lt strain. Consistent with a role for ALR/Lt mitochondrial genomic contributions to the upregulated constitutive defenses against ROS damage, the (ALR × NZO)F1 males showed diminution of type 2 diabetes severity, whereas (NZO × ALR)F1 males generated in the reciprocal outcross (mitochondrial genome from NZO) showed increased obesity and diabesity (e.g., significantly higher plasma glucose concentrations). Regarding the significantly increased body weight in this reciprocal outcross, we have previously shown that the NZO/Lt female contributes an epigenetic factor to diabesity in terms of the nutritional content of milk over the postparturitional period (63). As shown in Table 3, outcross of NON/Lt with NZO in either cross direction failed to retard development of diabesity as effectively as outcross with ALR/Lt under conditions where ALR provided the maternal genomic and epigenetic environment. This rather limited dataset is supportive of the conclusion that genes determining the level of ROS stress are important in both type 1 and type 2 diabetes. However, in the mouse models of each disease form chosen, one might infer that ROS-mediated damage to β-cells may be primary in the type 1 diabetes model, but secondary in the type 2 diabetes model chosen.

CONCLUSIONS

Our hypothesis initially stated that some of the genes contributing to β-cell resistance to the autoimmune stresses underlying type 1 diabetes also contribute to β-cell resistance to the disturbed metabolic milieu engendered by type 2 diabetes. However this hypothesis has been quite difficult to confirm using the available mouse models. Studies with the NOD/Lt mouse suggest that genetic backgrounds predisposing to a type 1.5/type 2 diabetes "metabolic exhaustion" of β-cells actually repress the autoimmune insulitis required for type 1 diabetes development. The best evidence to support the hypothesis comes from analysis of mitochondrial contributions to both forms of diabetes. β-Cell apoptotic death pathways can be activated by signals emanating from mitochondria in type 1 diabetes (64), and cumulative mutations in the mitochondrial genome can lead to increased ROS release accompanied by declining cellular energy production and insulin secretion as β-cells age (65). Thus, strategies that aim to preserve mitochondrial integrity and suppress intracellular generation of ROS should prove useful in preserving β-cell integrity and function (66,67).

ACKNOWLEDGMENTS

The research was supported by grants from the American Diabetes Association and National Institutes of Health Grants DK36175 and DK27722 (E.H.L.) and a fellowship from the Hardt Brothers Foundation (C.-H.L.). Institutional shared services were supported by National Cancer Institute Center Support Grant CA-34196.

The authors gratefully acknowledge contributions by Drs. Clayton Mathews (University of Pittsburgh) and Jing Chen (The Jackson Laboratory) to the research reviewed herein. The expert technical assistance of Peter Reifsnyder, Darcy Pomerleau, Rebecca Bagley, and Pam Stanley are also acknowledged.

REFERENCES

1. Naik RG, Palmer JP: Latent autoimmune diabetes in adults (LADA). *Rev Endocr Metab Disord* 4:233–241, 2003
2. Bennett ST, Lucassen AM, Gough SCL, Powell EE, Undlien DE, Pritchard LE, Merriman ME, Kawaguchi Y, Dronsfield MJ, Pociot F, Nerup J, Bouzekri N, Cambonthomsen A, Ronningen KS, Barnett AH, Bain SC, Todd JA: Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet* 9:284–292, 1995
3. Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C: Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet* 15:289–292, 1997
4. Pugliese A, Miceli D: The insulin gene in diabetes. *Diabete Metab Res Rev* 18:13–25, 2002
5. Waterworth D, Bennett S, Hague S, White D, Gharani N, Batty S, Todd J, McCarthy M, Conway G, Franks S: Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic-ovary-syndrome. *Lancet* 349:986–990, 1997
6. Robertson RP, Harmon J, Tran PO, Poitout V: Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 53 (Suppl. 1):S119–S124, 2004
7. Froguel P, Vaxllaire M, Sun F, Velho G, Zouali H, Butel M, Lesage S, Vionnet N, Clement K, Fougerousse F, Tanizawa Y, Weissenbach J, Beckmann J, Lathrop GM, Passa P, Permutt M, Cohen D: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin dependent diabetes mellitus. *Nature* 356:162–164, 1992
8. Rowe RE, Wapelhorst B, Bell GI, Risch N, Spielman RS, Concannon P: Linkage and association between insulin-dependent diabetes mellitus (IDDM) susceptibility and markers near the glucokinase gene on chromosome 7. *Nat Genet* 10:240–242, 1995
9. Boutin P, Dina C, Vasseur F, Dubois S, Corset L, Seron K, Bekris L, Cabellon J, Neve B, Vasseur-Delannoy V, Chikri M, Charles MA, Clement K, Lernmark A, Froguel P: GAD2 on chromosome 10p12 is a candidate gene for human obesity. *PLoS Biol* 1:E68, 2003
10. Eftychi C, Howson JM, Barratt BJ, Vella A, Payne F, Smyth DJ, Twells RC, Walker NM, Rance HE, Tuomilehto-Wolf E, Tuomilehto J, Undlien DE, Ronningen KS, Guja C, Ionescu-Tiirgoviste C, Savage DA, Todd JA: Analysis of the type 2 diabetes-associated single nucleotide polymorphisms in the genes IRS1, KCNJ11, and PPARG2 in type 1 diabetes. *Diabetes* 53:870–873, 2004
11. Rich SS, Bowden DW, Haffner SM, Norris JM, Saad MF, Mitchell BD, Rotter JI, Langefeld CD, Wagenknecht LE, Bergman RN: Identification of quantitative trait loci for glucose homeostasis: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* 53:1866–1875, 2004
12. Merriman T, Todd J: Genetics of insulin-dependent diabetes: non-major histocompatibility genes. *Horm Metab Res* 28:289–293, 1996
13. Coleman DL: Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* 14:141–148, 1978
14. Naggert JK, Mu M-L, Frankel WF, Paigen B: Genomic analysis of the C57BL/Ks mouse strain. *Mammal Genome* 6:131–133, 1995
15. Lee G, Proenca R, Montez J, Carroll K, Darvishzadeh J, Lee J, Friedman J: Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379:632–635, 1996
16. Baumann H, Morella K, White D, Dembski M, Bailon P, Kim H, Lai C, Tartaglia L: The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci U S A* 93:8374–8378, 1996
17. Leiter EH, Kintner J, Flurkey K, Beamer WG, Naggert JK: Physiologic and endocrinologic characterization of male sex biased diabetes in

C57BLKS/J mice congenic for the fat mutation at the carboxypeptidase E locus. *Endocrine* 10:57–66, 1999
18. Leiter EH, Le PH, Coleman DL: Susceptibility to db gene and streptozotocin-induced diabetes in C57BL mice: control by gender-associated, MHC-unlinked traits. *Immunogenetics* 26:6–13, 1987
19. Leiter EH, Coleman DL, Eisenstein AB, Strack I: Dietary control of diabetes pathogenesis in C57BL/KsJ-db/db diabetes mice. *Metabolism* 30:554–562, 1981
20. Leiter EH, Fewell JW, Kuff EL: Glucose induces intracisternal type A retroviral gene transcription and translation in pancreatic beta cells. *J Exp Med* 163:87–100, 1986
21. Cohn JA, Cerami A: The influence of genetic background on the susceptibility of mice to diabetes induced by alloxan and on recovery from alloxan diabetes. *Diabetologia* 17:187–191, 1979
22. Rossini AA, Appel MC, Williams RM, Like AA: Genetic influence of the streptozotocin-induced insulitis and hyperglycemia. *Diabetes* 26:916–920, 1977
23. Like AA, Rossini AA: Streptozotocin induced pancreatic insulitis: new model of diabetes mellitus. *Science* 193:415–417, 1976
24. Leiter EH, Prochazka M, Coleman DL, Serreze DV, Shultz LD: Genetic factors predisposing to diabetes susceptibility in mice. In *The Immunology of Diabetes Mellitus*. Jaworski MA, Molnar GD, Rajotte RV, Singh B, Eds. Amsterdam, Elsevier, 1986, p. 29–36
25. Leiter EH, Prochazka M, Shultz LD: Effect of immunodeficiency on diabetogenesis in genetically diabetic (db/db) mice. *J Immunol* 138:3224–3229, 1987
26. Leiter E, Chapman H, Falany C: Synergism of obesity genes with hepatic steroid sulfotransferases to mediate diabetes in mice. *Diabetes* 40:1360–1363, 1991
27. Leiter E, Chapman H: Obesity-induced diabetes (diabesity) in C57BL/KsJ mice produces aberrant *trans*-regulation of sex steroid sulfotransferase genes. *J Clin Invest* 93:2007–2013, 1994
28. Serreze DV, Leiter EH: Genes and pathways underlying autoimmune diabetes in NOD mice. In *Molecular Pathology of Insulin-Dependent Diabetes Mellitus* von Herrath M, Ed. New York, Karger, 2001, p. 31–67
29. Leiter EH: Nonobese diabetic (NOD) mice and the genetics of diabetes susceptibility. *Curr Diabetes Rep* 5:141–148, 2005
30. Gaskins HR, Prochazka M, Hamaguchi K, Serreze DV, Leiter EH: Beta cell expression of endogenous xenotropic retrovirus distinguishes diabetes susceptible NOD/Lt from resistant NON/Lt mice. *J Clin Invest* 90:2220–2227, 1992
31. Pak CY, Jun HS, Lee M, Yoon JW: Beta cell-specific expression of retroviral mRNAs and group-specific antigen and the development of beta cell-specific autoimmunity in non-obese diabetic mice. *Autoimmunity* 20:19–24, 1995
32. Leiter EH, Gerling IC, Flynn JC: Spontaneous insulin dependent diabetes mellitus (IDDM) in nonobese diabetic (NOD) mice: comparisons with experimentally-induced IDDM. In *Experimental Models of Diabetes*. McNeill JH, Ed. Boca Raton, FL, CRC, 1999, p. 257–295
33. Trudeau JD, Dutz JP, Arany E, Hill DJ, Fieldus WE, Finegood DT: Neonatal beta-cell apoptosis: a trigger for autoimmune diabetes? *Diabetes* 49:1–7, 2000
34. Leiter EH: Analysis of differential survival of syngeneic islets transplanted into hyperglycemic C57BL/KsJ versus C57BL/6J mice. *Transplantation* 44:401–406, 1987
35. Prochazka M, Serreze DV, Frankel WN, Leiter EH: NOR/Lt: MHC-matched diabetes-resistant control strain for NOD mice. *Diabetes* 41:98–106, 1992
36. Serreze DV, Prochazka M, Reifsnyder PC, Bridgett MM, Leiter EH: Use of recombinant congenic and congenic strains of NOD mice to identify a new insulin dependent diabetes resistance gene. *J Exp Med* 180:1553–1558, 1994
37. Fox CJ, Paterson AD, Mortin-Toth SM, Danska JS: Two genetic loci regulate T cell-dependent islet inflammation and drive autoimmune diabetes pathogenesis. *Am J Hum Genet* 67:67–81, 2000
38. Reifsnyder P, Li R, Silveira P, Churchill G, Serreze D, Leiter E: Conditioning the genome identifies additional diabetes resistance loci in type 1 diabetes resistant NOR/Lt mice. *Genes Immun* 6:528–538, 2005
39. Hamilton-Williams EE, Serreze DV, Charlton B, Johnson EA, Marron MP, Mullbacher A, Slattery RM: Transgenic rescue implicates β2-microglobulin as a diabetes susceptibility gene in NOD mice. *Proc Natl Acad Sci U S A* 98:11533–11538, 2001
40. La Cava A, Matarese G: The weight of leptin in immunity. *Nat Rev Immunol* 4:371–379, 2004
41. Matarese G, Moschos S, Mantzoros CS: Leptin in immunology. *J Immunol* 174:3137–3142, 2005
42. Matarese G, Sanna V, Lechler RI, Sarvetnick N, Fontana S, Zappacosta S, La Cava A: Leptin accelerates autoimmune diabetes in female NOD mice. *Diabetes* 51:1356–1361, 2002
43. Nishimura M, Miyamoto H: Immunopathological influence of the Ay, db, ob and nu genes placed on the inbred NOD background as murine models for human type I diabetes. *J Immunogenet* 14:127–130, 1987
44. Sandowski Y, Raver N, Gussakovsky EE, Shochat S, Dym O, Livnah O, Rubinstein M, Krishna R, Gertler A: Subcloning, expression, purification, and characterization of recombinant human leptin-binding domain. *J Biol Chem* 277:46304–46309, 2002
45. Pociot F, Briant L, Jongeneel CV, Molvig J, Worsaae H, Abbal M, Thomsen M, Nerup J, Cambon-Thomsen A: Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol* 23:224–231, 1993
46. Hamid YH, Urhammer SA, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: The common T60N polymorphism of the lymphotoxin-alpha gene is associated with type 2 diabetes and other phenotypes of the metabolic syndrome. *Diabetologia* 48:445–451, 2005
47. Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, Kristiansen OP, Mandrup-Poulsen T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia* 48:251–260, 2005
48. Elouil H, Cardozo AK, Eizirik DL, Henquin JC, Jonas JC: High glucose and hydrogen peroxide increase c-Myc and haeme-oxygenase 1 mRNA levels in rat pancreatic islets without activating NFkappaB. *Diabetologia* 48:496–505, 2005
49. Kharroubi I, Ladriere L, Cardozo AK, Dogusan Z, Cnop M, Eizirik DL: Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. *Endocrinology* 145:5087–5096, 2004
50. Lety MA, Coulaud J, Bens M, Dardenne M, Homo-Delarche F: Enhanced metabolism of arachidonic acid by macrophages from nonobese diabetic (NOD) mice. *Clin Immunol Immunopathol* 64:188–196, 1992
51. Hatley ME, Srinivasan S, Reilly KB, Bolick DT, Hedrick CC: Increased production of 12/15 lipoxygenase eicosanoids accelerates monocyte/endothelial interactions in diabetic db/db mice. *J Biol Chem* 278:25369–25375, 2003
52. Sekiguchi F, Ishibashi K, Katoh H, Kawamoto Y, Ino T: Genetic profile of alloxan-induced diabetes-susceptible mice (ALS) and resistant mice (ALR). *Exp Anim* 39:269–272, 1990
53. Mathews CE, Leiter EH: Constitutive differences in anti-oxidant defense status distinguishes alloxan resistant (ALR/Lt) and alloxan susceptible (ALS/Lt) mice. *Free Radic Biol Med* 27:449–455, 1999
54. Mathews CE, Graser R, Savinov A, Serreze DV, Leiter EH: The NOD/Lt-related ALR/Lt strain: unusual resistance of beta cells to autoimmune killing uncovers a role for beta-cell expressed resistance determinants. *Proc Natl Acad Sci U S A* 98:235–240, 2001
55. Pomerleau DP, Bagley RJ, Serreze DV, Mathews CE, Leiter EH: Major histocompatibility complex-linked diabetes susceptibility in NOD/Lt mice: subcongenic analysis localizes a component of Idd16 at the H2-D end of the diabetogenic $H2^{g7}$ complex. *Diabetes* 54:1603–1606, 2005
56. Mathews CE, Graser RT, Bagley RJ, Caldwell JW, Li R, Churchill GA, Serreze DV, Leiter EH: Genetic analysis of resistance to type 1 diabetes in ALR/Lt mice, a NOD-related strain with defenses against autoimmune-mediated diabetogenic stress. *Immunogenetics* 55:491–496, 2003
56a. Mathews CE, Suarez-Pinzon WL, Baust JJ, Strynadka K, Leiter EH, Rabinovitch A: Mechanisms underlying resistance of pancreatic islets from ALR/Lt mice to cytokine-induced destruction. *J Immunol* 175:1248–1256, 2005
57. Mathews CE, Leiter EH, Spirina O, Bykhovskaya Y, Gusdon AM, Ringquist SM, Fischel-Ghodsian N: mt-Nd2 allele of the ALR/Lt mouse confers resistance against both chemically-induced and autoimmune diabetes. *Diabetologia* 48:261–267, 2005
58. Uchigata Y, Okada T, Gong JS, Yamada Y, Iwamoto Y, Tanaka M: A mitochondrial genotype associated with the development of autoimmune-related type 1 diabetes. *Diabetes Care* 25:2106, 2002
59. Koza RA, Flurkey K, Graunke DM, Braun C, Pan HJ, Reifsnyder PC, Kozak LP, Leiter EH: Contributions of dysregulated energy metabolism to type 2 diabetes development in NZO/H1Lt mice with polygenic obesity. *Metabolism* 53:799–808, 2004
60. Junger E, Herberg L, Jeruschke K, Leiter EH: The diabetes-prone NZO/Hl strain. II. Pancreatic immunopathology. *Laboratory Invest* 82:843–853, 2002
61. Harrison LC, Itin A: A possible mechanism for insulin resistance and hyperglycaemia in New Zealand obese mice. *Nature* 279:334–336, 1979

62. McInerney MF, Naąjjar SM, Brickley D, Lutzke M, Abou Rjaily GA, Reifsnyder PC, Haskell BD, Flurkey K, Zhang YJ, Pietropaolo SL, Pietropaolo M, Byers JP, Leiter EH: Anti-insulin receptor autoantibodies are detected in type 2 diabetes-prone NZO-Lt mice using a new flow cytometric assay. *Exp Diabesity Res* 5:177–186, 2004
63. Reifsnyder PC, Churchill G, Leiter EH: Maternal environment and genotype interact to establish diabesity in mice. *Genome Res* 10:1568–1578, 2000
64. Sandler S, Andersson AK, Barbu A, Hellerstrom C, Holstad M, Karlsson E, Sandberg JO, Strandell E, Saldeen J, Sternesjo J, Tillmar L, Eizirik DL, Flodstrom M, Welsh N: Novel experimental strategies to prevent the development of type 1 diabetes mellitus. *Ups J Med Sci* 105:17–34, 2000
65. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI: Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300:1140–1142, 2003
66. Wallace DC: Mouse models for mitochondrial disease. *Am J Med Genet* 106:71–93, 2001
67. Mathews CE, Berdanier CD: Noninsulin-dependent diabetes mellitus as a mitochondrial genomic disease. *Proc Soc Exp Biol Med* 219:97–108, 1998