

Rat Models of Type 1 Diabetes: Genetics, Environment, and Autoimmunity

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Abstract

For many years, the vast amount of data gathered from analysis of nonobese diabetic (NOD) and congenic NOD mice has eclipsed interest in the rat for the study of type 1 diabetes. The study of rat models has continued, however, and recently there has been a reanimation of interest for several reasons. First, genetic analysis of the rat has accelerated. *Ian4L1*, *cblb*, and *Iddm4* are now known to play major roles in rat autoimmunity. Second, rats are amenable to study the interactions of genetics and environment that may be critical for disease expression in humans. Environmental perturbants that predictably enhance the expression of rat autoimmune diabetes include viral infection, toll-like receptor ligation, and depletion of regulatory T cell populations. Finally, data generated in the rat have correctly predicted the outcome of several human diabetes prevention trials, notably the failure of nicotinamide and low dose parenteral and oral insulin therapies.

Key Words: animal model; autoimmunity; BB rat; immunogenetics; rat; translational research; type 1 diabetes

Introduction

Type 1 diabetes comprises ~10% of all diabetes mellitus, and its prevalence is increasing (Green et al. 2001). Affected individuals require lifelong injections of insulin for survival (Rossini et al. 2003). The disease results from inflammatory infiltration of the islets of Langerhans (insulinitis) and selective destruction of insulin-producing beta cells (Atkinson and Eisenbarth 2001). Generally considered to be autoimmune in origin, type 1 diabetes often occurs in persons who also suffer from other autoimmune diseases of the gut and thyroid. It is strongly associated with the major histocompatibility complex (MHC¹), is T cell

dependent, and is ameliorated by immunosuppression. Despite a wealth of information, however, type 1 diabetes remains refractory to prevention (Allen et al. 1999; Lampeter et al. 1998; Skyler et al. 2002) by methods other than unacceptably toxic immunosuppression (Parving et al. 1999).

To circumvent the ethical and logistical constraints inherent in studying type 1 diabetes in outbred populations of humans exposed at random to chemical and microbiological agents, investigators continue to rely on animal models that can be readily tested, biopsied, and autopsied. It is possible to breed these models to study and manipulate inheritance and to test for their response to environmental agents.

Two species have provided extensive data relevant to spontaneous human type 1 diabetes—the rat and the mouse. The most widely used model is the nonobese diabetic (NOD¹) mouse (reviewed in Serreze and Leiter 2001). Literally thousands of studies have illuminated the complex genetics and immunology of these animals whose disease involves at least 27 genetic loci and a large number of immunological defects (Serreze and Leiter 2001). They have, however, proven disappointing as models in two respects. First, immunomodulatory interventions, other than systemic immunosuppression, that readily prevent diabetes in these mice (Atkinson and Leiter 1999) have to date proven inefficacious in humans (Allen et al. 1999; Lampeter et al. 1998; Skyler et al. 2002). Second, with respect to the environment, they model type 1 diabetes poorly because the overwhelming majority of perturbants, including virus infection (Hermitte et al. 1990; Leiter 1998; Oldstone 1990; Wilberz et al. 1991), reduce the frequency of diabetes and often prevent it entirely (Atkinson and Leiter 1999).

Although the original NOD mouse and various NOD congenic mice continue to provide a wealth of valuable information, there is growing recognition of the need to continue to study alternative yet complementary systems (Greiner et al. 2001). Rats provide one such system. The BB rat (reviewed in Mordes et al. 2001) is the oldest, best known, and most extensively studied strain, but a predisposition to autoimmune beta cell destruction is common to

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¹Abbreviations used in this article: BB/Wor rat, BB Worcester rat; BBDP rat, diabetes-prone BB rat; BBDR, diabetes-resistant BB rat; BCG, Bacille

Calmette-Guérin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; GAD, glutamic acid decarboxylase; IFN, interferon; KRV, Kilham rat virus; LETL rat, MHC, major histocompatibility complex; NK, natural killer; NOD mouse, nonobese diabetic mouse; poly I:C, poly I:C: polyinosinic:polycytidylic acid; RCMV, rat cytomegalovirus; TLR, toll-like receptor; Treg, regulatory T cell; VAF, viral antibody-free; WF rat, Wistar Furth rat.

several rat strains (Table 1). Signature features of rat models of type 1 diabetes that are stressed in this review are highlighted in Tables 2 and 3. Rat autoimmune diabetes may occur “spontaneously” in viral antibody-free (VAF¹) housing, as is the case with the NOD mouse. Alternatively, diabetes may appear in response to some form of immunological perturbation, which is generally not the case in mouse models (Table 1).

Spontaneous Autoimmune Diabetes in the Rat

The principal animal models of spontaneous type 1-like diabetes in the rat include the following: (1) the diabetes-prone BB rat, (2) the LETL rat, (3) the KDP rat, and (4) the congenic LEW rat. Some of the salient characteristics of these models are listed in Table 2, with corresponding characteristics of the human disease.

Diabetes-prone BB Rat

BB rats are the most extensively studied rat model of type 1 diabetes. They are derived from a Canadian colony of outbred Wistar rats in which spontaneous hyperglycemia and ketoacidosis occurred in the 1970s. Affected animals were the founders for two colonies that were later used to establish all other BB rat colonies. One colony established in Worcester, Massachusetts (BB/Wor¹), has been inbred, and the spontaneously diabetic animals are formally designated Worcester diabetes-prone BB rats (BBDP/Wor¹) (see www.biomere.com). From a second colony in Ottawa, Canada, “BBdp” rats are outbred. Several of the tertiary BB

rat colonies have given rise to immunologically and genetically distinct BB rat substrains (Prins et al. 1991), including BB/OK, BB/Pfd, and BB.SHR rats (Klötting et al. 1998; Kovács et al. 2001; Mathieu et al. 1994; Schröder et al. 2002). Rats of Worcester origin sold by a major European vendor, M&B (formerly Møllegaard), are designated BB/Mol or BB/Wor//Mol (Mordes et al. 2001). Results of studies that use BB rats of different origins may not be directly comparable (Mordes et al. 2001). In this article, the term “BB rat” is used when referring to findings that appear to apply generically to all rats derived from the original founders; more specific designations are used in reference to findings that may be substrain specific.

Clinical and Immunological Pathology

Both inbred BBDP/Wor rats and outbred BBdp rats of both sexes develop pancreatic insulinitis that is rapidly followed by selective destruction of beta cells and frank diabetes between 50 and 90 days of age (Guberski 1994). It is noteworthy that the natural course of insulinitis in the spontaneously diabetic BB rat (Figure 1) is different from that of the NOD mouse. In the rat, there is no significant or persistent infiltration adjacent to the islet (“peri-insulinitis”) before progression to frank insulinitis and overt diabetes. Insulinitis in BB rats is morphologically similar to that observed in human type 1 diabetes and features a predominance of Th1-type lymphocytes (Kolb et al. 1996; Zipris et al. 1996).

After the onset of hyperglycemia, residual “end-stage” islets are small, distorted, and composed predominantly of nonbeta cells. Unless treated with exogenous insulin, hyperglycemic BB rats quickly progress to fatal diabetic ketoacidosis. BBDP/Wor rats also develop spontaneous

Table 1 Rat models of type 1 diabetes^{a,b}

Type	Strain	MHC	Reference (see text)
Spontaneous	BBDP and BBdp	<i>RT1^{u/u/u}</i>	Mordes et al. 2001
	LETL	<i>RT1^{u/u/u}</i>	Kawano et al. 1991
	KDP	<i>RT1^{u/u/u}</i>	Yokoi et al. 2002
	LEW.1AR1/Ztm-iddm	<i>RT1^{u/u/a}</i>	Lenzen et al. 2001
Induced	BBDR	<i>RT1^{u/u/u}</i>	Mordes et al. 2001
	PVG	<i>RT1^{c/c/c}</i>	Penhale et al. 1990
	PVG. <i>RT1^u</i>	<i>RT1^{u/u/u}</i>	Ellerman and Like 2000
	PVG.R8	<i>RT1^{a/u/u}</i>	Ellerman and Like 2000
	WAG	<i>RT1^{u/u/u}</i>	Ellerman and Like 2000
	WF. <i>iddm4^d</i> congenic	<i>RT1^{u/u/u}</i>	Mordes et al. 2002
Both	LEW.1WR1	<i>RT1^{a/u/u}</i>	Mordes et al. 2003a
Transgenic	None	NA	NA

^aModels of autoimmune diabetes in the rat. All models are characterized by the presence of pancreatic insulinitis. The major histocompatibility locus (MHC) in the rat is designated *RT1* and the haplotypes are designated *RT1 A*, *B/D*, and *C*. *RT1 A* and *RT1 C* are class I loci; the class II loci are in linkage disequilibrium and designated *B/D*. With the exception of the PVG model induced by the combination of irradiation and thymectomy, all rat models of autoimmune diabetes express the *RT1 B/D^u* class II allele. Methods of induction include regulatory T cell depletion, toll-like receptor (TLR) ligation, and certain viral infections.

^bBBDP (inbred) BBdp (outbred) rats from different colonies; NA, not applicable.

Table 2 Comparative clinical and genetic features of spontaneous autoimmune diabetes: Characteristics of spontaneous autoimmune diabetes mellitus in humans, NOD mice, and three rat model systems^a

	Human	NOD mouse	BBDP/Wor	LEW.1AR1/ Ztm-iddm	KDP
Age at onset:	Adolescence	Up to 6 mo	7-14 wk	6-12 wk	8-16 wk
Ketosis:	Severe	Mild	Severe	Severe	Severe
Insulin deficiency:	Severe	Mild to severe	Severe	Severe	Severe
Associated autoimmune diseases:	Thyroiditis, celiac disease, vitiligo, PA, polyendocrine syndromes	Sialadenitis, thyroiditis	Thyroiditis	Thyroid, adrenal, salivary glands free of infiltration	Thyroid and kidney infiltrates present
Autoantibodies:	Insulin, GAD, ICA, ICSA, BSA, CPH, EC, IA-2, IAA	Insulin, GAD, ICA	ICA present; GAD and IAA are controversial	ICA and antibodies to GAD, IA-2 not found	Unknown
MHC genes:	HLA-DQ and DR	Unique I-A ^{g7} Absent I-E	<i>RT1^{u/u/u}</i>	<i>RT1^{u/u/a}</i>	<i>RT1^{u/u/u}</i>
Non-MHC genes:	<i>CTLA-4</i> . At least 2 loci, perhaps ≥ 16	$\beta 2$ microglobulin; possibly <i>CTLA-4</i> ; ≥ 27 loci	>3 loci. <i>Ian4L1</i> mutation causes lymphopenia; <i>Iddm4</i>	At least 1 recessive locus	<i>Cblb</i>
Gender effect:	M = F	F > M	M = F	M = F	M = F
Response to general immunosuppression:	Cyclosporine prolongs endogenous insulin production if given at onset	Cyclosporine, tacrolimus prevent diabetes	Cyclosporine, tacrolimus, thymectomy, ALS, radiation prevent diabetes	Unknown	Unknown
Response to environmental perturbation	Diet and viral infection implicated in pathogenesis, but no definitive proof	More than 150 interventions prevent disease	LCMV prevents disease; Certain diets and bacterial vaccines reduce diabetes frequency	Unknown	Unknown

^aALS, anti-lymphocyte serum; BSA, bovine serum albumin; CPH, carboxypeptidase H; CTLA-4, cytotoxic T-lymphocyte antigen 4; DR, diabetes resistant; EC, endothelial cell surface; GAD, glutamic acid decarboxylase; HLA, human leukocyte antigen; IAA, insulin autoantibodies; ICA, islet cell autoantibodies; ICSA, islet cell surface antibodies; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; PA, pernicious anemia.

lymphocytic thyroiditis but not clinical hypothyroidism (Rajatanavin et al. 1991).

The most obvious and problematic immunopathology in all spontaneously diabetic BB rats is profound T cell lymphopenia (Elder and Maclaren 1983; Yale et al. 1985). This condition is characterized by a severe reduction in the number of CD4⁺ T cells (Jackson et al. 1981) and nearly complete absence of the CD8⁺ T cell subset (Jackson et al. 1983; Poussier et al. 1982; Woda et al. 1986). The rats are also severely deficient in ART2⁺ T cells. ART2 is a rat maturational T cell alloantigen with nicotinamide adenine dinucleotide glycohydrolase activity that appears to identify cells with immunoregulatory properties (Bortell et al. 1999). The phenotypic T cell abnormalities in BBDP/Wor rats can be attributed to a severely reduced life span of periph-

eral T cells. The majority of cultured BBDP/Wor T cells undergo apoptosis within 24 hr (Ramanathan et al. 1998), and recent thymic emigrants in vivo exhibit a preapoptotic $\alpha\beta$ TCR^{low}B220⁺CD4⁺CD8⁻ phenotype and undergo apoptosis in the liver (Iwakoshi et al. 1998). The presence of lymphopenia does not in itself confer susceptibility to autoimmunity in the rat (Joseph et al. 1993), but spontaneous diabetes in BBDP/Wor rats requires that they be lymphopenic (Awata et al. 1995). This pathology is not characteristic of either NOD mice or humans with type 1 diabetes, and it has compromised the acceptability of diabetes-prone BB rats as a model for the human disease.

Neonatal thymectomy, injections of antilymphocyte serum, and depletion of CD8⁺ T cells (but not natural killer [NK¹] cells) all prevent the disease, as do many standard

Table 3 Comparative features of diabetic syndromes with autoimmune features in rats and one mouse system: Clinical and genetic features of some induced autoimmune diabetes syndromes^a

	BBDR rat	LEW.1WR1 rat	PVG/c rat	<i>RT1^u</i> Strains: YOS, WF, PVG. <i>RT1^u</i> , WF. <i>Iddm4^d</i> , <i>PVGR8</i>	Low-dose STZ mouse
Methods of Induction:	ART2 ⁺ Treg depletion TRL Ligation (poly I:C) Kilham rat virus; Combination treatments	ART2 ⁺ Treg depletion TLR Ligation (poly I:C) Virus: KRV; RCMV Combination treatments	Thymectomy plus Irradiation	Poly I:C; poly I:C plus Treg depletion; poly I:C plus KRV; thymectomy plus irradiation	Multiple subdiabetogenic doses of streptozotocin
Latency to onset:	2-4 wk	2-4 wk	3-8 wk	2-4 wk	10-14 days
Ketosis:	Severe	Severe	Severe	Severe	None
Associated autoimmune diseases:	Thyroiditis; collagen-induced arthritis	Collagen-induced arthritis	Thyroiditis	Not known	None
Autoantibodies:	Endothelial cell	Not known	Not known	Not known	Not known
MHC genes:	<i>RT1^{w/u/u}</i>	<i>RT1^{w/u/a}</i>	<i>RT1^{c/c/c}</i>	<i>RT1^{w/u/u}</i>	None
Non-MHC genes:	<i>Iddm4</i> and others	Not known	Not known	Strain specific	Strain specific
Gender effect:	M = F	M = F	M > F	M = F	M > F

^aLow-dose STZ, rodent treated with multiple subdiabetogenic doses of streptozotocin. KRV, Kilham rat virus; MHC, major histocompatibility complex; RCMV, rat cytomegalovirus; TLR, toll-like receptor; Treg, regulatory T cell; poly I:C, polyinosinic:polycytidylic acid.

immunosuppressive drugs and immunomodulatory modalities (Mordes et al. 2001). Spontaneous diabetes in BB rats is prevented or retarded by tumor necrosis factor- α (Sato et al. 1990), lymphotoxin (Takahashi et al. 1993), interferon

(INF¹)- α (Sobel et al. 1998a), and IFN- γ (Nicoletti et al. 1998; Sobel and Newsome 1997), but paradoxically anti-IFN- γ is also effective (Nicoletti et al. 1997). Treatment with interleukin-2 is not effective (Burstein et al. 1987).

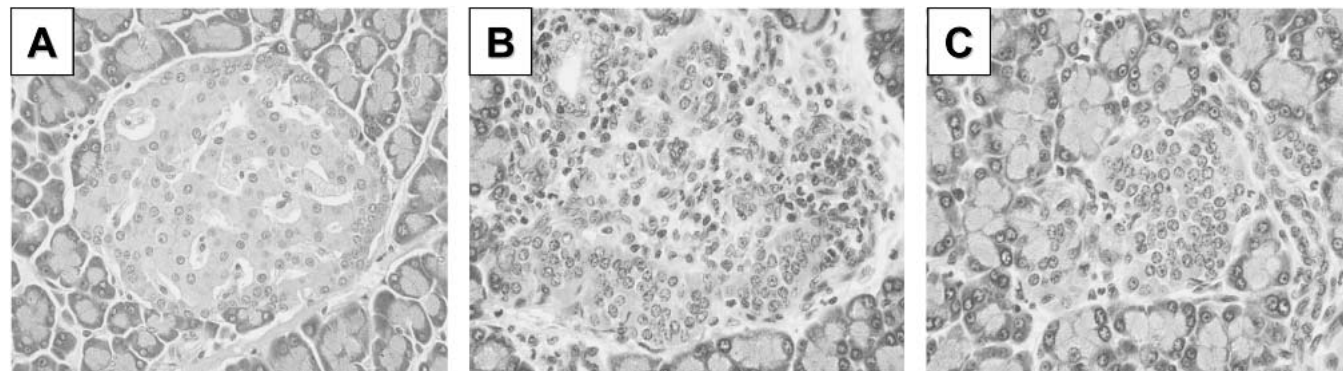


Figure 1 Hematoxylin and eosin stained sections of BBDR/Wor rat pancreatic islets of Langerhans. (A) Normal islet from a young adult animal before the onset of disease. (B) Insulinitis lesion, extensively infiltrated by mononuclear cells. (C) End-stage islet from an animal with longstanding diabetes that had been treated with a daily injection of exogenous insulin to prevent ketoacidosis. The islet is shrunken and its architecture has been distorted; immunohistochemical staining would demonstrate the selective absence of insulin-containing beta cells from the islet with preservation of alpha, delta, and pancreatic polypeptide cells (Nakhoda et al. 1977). Phenotyping of the cells that comprise the infiltrate in panel B would reveal the presence of CD4⁺ and CD8⁺ T lymphocytes, natural killer (NK) cells, B lymphocytes, and macrophages (Hosszufalusi et al. 1993), in addition to the presence of Th1-type cytokines (Zipris et al. 1996). There is no evidence of any intrinsic abnormality of the beta cells themselves. Comparable pathology is observed in the islets of humans (Itoh et al. 1993; LeCompte and Gepts 1977). Photomicrographs courtesy of Michael C. Appel, Ph.D., University of Massachusetts Medical School, Worcester, MA. Original magnification of all images $\times 200$.

Mitogen-activated spleen cells from acutely diabetic donors accelerate disease in young diabetes-prone BB rats and transfer it to MHC-compatible naive recipients. Diabetes-prone BB rats from some (Ziegler et al. 1994) but not all (Davenport et al. 1995; Mackay et al. 1996) colonies circulate anti-islet cell and anti-glutamic acid decarboxylase (GAD¹) autoantibodies. Antibodies directed against wheat globulin are also detectable (MacFarlane et al. 2003). This finding is of interest for several reasons: (1) dietary wheat protein has been associated with a high rate of diabetes in Ottawa BBdp rats; (2) there is a high prevalence of celiac disease among humans with type 1 diabetes (MacFarlane et al. 2003); and (3) early cereal consumption has been identified as a potential environmental precipitant of human type 1 diabetes (Norris et al. 2003).

Genetics

As is indicated in Table 1, the appearance of autoimmune diabetes in nearly all rats appears to require at least one gene associated with the rat MHC. The primary components of the rat MHC, designated *RT1*, include two class I loci, *A* and *C*, and two class II loci in linkage disequilibrium, designated *B/D* (Gunther and Walter 2001). In the BB rat, the *RT1 B/D* region is now designated *Iddm1*, and expression of diabetes requires the presence of at least one class II *RT1 B/D*^u allele (Colle 1990; Fuks et al. 1990). As suggested by Table 1, the presence of a class II *RT1 B/D*^u haplotype appears in part to render many rat strains susceptible to autoimmune diabetes (Ellerman and Like 2000). The class II allele in the BBdp rat is not a unique isoform; *u* alleles from normal rat strains also confer susceptibility when moved to the BBdp strain (Colle et al. 1986). The enrichment for DQβ chain alleles with uncharged amino acids at position 57 characteristic of Caucasian humans (Nepom et al. 1996) and of NOD mice (Corper et al. 2000) is not found in the BB rat. The region around position 57 of the class II β chains of normal LEW and BUF rats is identical to that found in BBDP/Wor rats (Chao et al. 1989).

The lymphopenia of the BBDP/Wor rat is due to a mutation in a recessive gene designated *Ian4L1* or *Iddm2* (Hornum et al. 2002; MacMurray et al. 2002). This gene encodes the mitochondrial membrane protein Ian4. Recent data have shown that the absence of Ian4 in T cells causes mitochondrial dysfunction, increased mitochondrial levels of stress-inducible chaperonins, and T cell-specific spontaneous apoptosis (Pandarpurkar et al. 2003). T cell activation and caspase 8 inhibition both prevented apoptosis, whereas transfection of T cells with Ian4-specific siRNA recapitulated the apoptotic phenotype. The findings establish Ian4 as a novel, tissue-specific regulator of mitochondrial integrity.

In analyses of (BBDP/Wor × WF) × WF rats, which do not become spontaneously diabetic but do become diabetic in response to perturbation (*v.i.*), a locus was mapped on chromosome 4 (*Iddm4*) with significant linkage to diabetes (Martin et al. 1999a,b). The diabetogenic allele of *Iddm4* has been confirmed, and the data suggest that it is a major,

but incompletely penetrant, non-MHC determinant of autoimmune diabetes in the *RT1*^u BBDP/Wor rat (Mordes et al. 2002).

The existence of one or more rat diabetes resistance genes has been inferred from crosses between diabetes-prone BB and resistant non-BB rats (Colle et al. 1992; Hornum et al. 2001; Jacob et al. 1992). Using the BB/OK rat and crosses to several strains including the DA and the spontaneously hypertensive SHR rat, Klötting and colleagues have also reported several other disease-modifying loci (Klötting and Kovacs 1998a,b; Klötting et al. 1998, 2001; Van den Brandt et al. 1999).

Pathogenesis

The inciting event that leads to beta cell autoreactivity in the spontaneously diabetic BB rat is not known but probably involves the presentation of autoantigen by *RT1*^u molecules. The identity of the primary autoantigen or autoantigens is unknown (Mordes et al. 2001). Substantial data document that the immunopathogenic process in the BBDP/Wor rat originates at the level of bone marrow progenitor cells (Naji et al. 1983; Nakano et al. 1988). Reciprocal bone marrow and thymus transplants have been used to reveal the presence of a thymic microenvironmental defect of bone marrow origin (Georgiou and Bellgrau 1989; Georgiou et al. 1988), leading to the hypothesis that intrathymic antigen-presenting cells are abnormal in BBDP/Wor rats. This abnormality appears to generate a limited peripheral TCR β chain variable region repertoire associated with diabetogenicity (Gold and Bellgrau 1991). Additional abnormalities in populations of NK cells, NK T cells, and intraepithelial lymphocytes (reviewed in Mordes et al. 2001) have also been observed, but their contribution to pathogenesis is unclear.

Translational Modeling Power

The classic method for ameliorating incipient type 1 diabetes is to use cyclosporine to induce generalized immunosuppression, an approach effective in both diabetes-prone BB rats (Laupacis et al. 1983; Like et al. 1983) and NOD mice (Mori et al. 1986). In the rodent trials, primary prevention was almost uniformly successful, therapy could be brief, and no toxicities were reported. In human clinical trials, cyclosporine was documented clearly to ameliorate type 1 diabetes and preserve insulin secretory capability when given promptly after onset (Mahon et al. 1993); however, disease usually recurred when therapy was stopped, and long-term results were disappointing (De Filippo et al. 1996). Therapy was sometimes complicated by early (Feutren and Mihatsch 1992; Rodier et al. 1991) and late (Parviny et al. 1999) drug-induced nephrotoxicity.

Modest doses of parenteral (Atkinson et al. 1990) or oral (Zhang et al. 1991) insulin at an early age prevent diabetes in NOD mice, suggestive of the induction of tolerance. Parenteral insulin also prevents diabetes in diabetes-prone BB

rats (Gotfredsen et al. 1985), but the intervention requires relatively substantial doses of insulin leading to beta cell involution. Oral tolerance with insulin in diabetes-prone BB rats with or without adjuvant is ineffective (Mordes et al. 1996b) and may exacerbate the disease (Bellmann et al. 1998). Results in humans to date suggest that neither parenteral (Skyler et al. 2002) nor oral (Chaillous et al. 2000) insulin prevents or delays diabetes onset.

Nicotinamide prevents diabetes in NOD mice (Elliott et al. 1993) but not diabetes-prone BB/Mol rats (Hermitte et al. 1989) or, to date, in humans (Lampeter et al. 1998; Philips and Scheen 2002). GAD and bovine serum albumin (BSA¹) are candidate autoantigens in humans and are effective in the prevention of NOD mouse diabetes (Atkinson and Leiter 1999), but ineffective in the BB/Wor/Mol rat (Petersen et al. 1997). A number of bacterial vaccines impair the development of Th1-type inflammatory responses and prevent diabetes, including the following: Bacille Calmette-Guérin (BCG¹) (Yagi et al. 1991) and complete Freund's adjuvant (CFA¹) (Qin et al. 1993) in the NOD mouse; CFA (Sadelain et al. 1990) and OK-432 (Satoh et al. 1988) in BB rats. BCG in humans is ineffective (Allen et al. 1999); no data are available for the other reagents.

Certain defined diets and diets low in essential fatty acids reduce the prevalence of disease in BBDP/Wor rats but do not prevent it (Mordes et al. 2001). Proteins present in cow's milk are hypothesized to play a role in the pathogenesis of type 1 diabetes in humans, but it is not yet known whether elimination of these proteins from the diets of children at risk will reduce the frequency of diabetes. Although dietary modification can reduce spontaneous diabetes expression in BBDP/Wor rats, the agent of protection is not elimination of cow's milk protein (Malkani et al. 1997). The addition of BSA or intact milk protein does not abrogate the effectiveness of a protective diet. Interestingly, feeding lipopolysaccharide, a TLR4 ligand, to neonatal BBdp rats increases insulinitis severity and the cumulative frequency of diabetes (Scott et al. 2002).

A preventive strategy unique to the spontaneously diabetic BB rat model is transfusion of CD4⁺ART2⁺ T cells to overcome the effects of lymphopenia (Burstein et al. 1989). There is no documented human equivalent of the ART2⁺ T cell. The primate equivalent of the rat ART2 gene is a pseudogene (Haag et al. 1994). BBDP/Wor rat diabetes can also be prevented by intrathymic transplantation of islets (Posselt et al. 1992) and low doses of the TLR3 ligand polyinosinic:polycytidylic acid (poly I:C¹) (Sobel et al. 1998b), but high dose poly I:C accelerates diabetes (Sobel et al. 1994). The relevance of these preventive strategies to human diabetes is not yet clear.

Cure of BB rat diabetes has been achieved with islet transplantation plus either immunosuppression (Dugoni and Bartlett 1990) or co-stimulatory blockade (Kover et al. 2000). The latter finding is of particular interest in light of the resistance of the NOD mouse to tolerance-based transplantation (Markees et al. 1999) and the fact that this resistance is genetically distinct from its susceptibility to

autoimmune diabetes (Pearson et al. 2003). Arguably the BB rat may be a preferable small animal model for the study of islet transplantation tolerance induction.

Spontaneous diabetes in BBDP/Wor rats occurs in more than half of animals housed in "conventional" vivaria (Guberski 1994), whereas diabetes is uncommon in NOD mice exposed to most microbiological agents (Serreze and Leiter 2001). Like the NOD mouse, however, diabetes does occur earlier and with a higher frequency in VAF vivaria than in less clean environments. Deliberate infection with lymphocytic choriomeningitis virus prevents diabetes, perhaps by deleting effector T cells (Dyrberg et al. 1988), but infection with Kilham rat virus (KRV¹) and other viruses does not affect disease frequency or age at onset (Guberski et al. 1991).

Spontaneously Diabetic LETL and KDP Rats

The first rat model of spontaneous autoimmune diabetes without lymphopenia was the LETL rat (Kawano et al. 1991). Disease in these animals initially occurred at a rate of 15 to 20%. Subsequently two substrains were established (Komeda et al. 1998). In the nonlymphopenic KDP substrain, the cumulative frequency of diabetes is ~70%, and all rats have mild to severe insulinitis at 120 to 220 days of age. They also exhibit lymphocytic infiltration of thyroid and kidney, but little more has been reported regarding their immunological phenotype. The Komeda nondiabetic substrain is disease free. Genetic analysis indicated that diabetes in the KDP rat required the presence of the *RT1 B/D^u* class II MHC and at least one additional recessive gene. The diabetogenic allele at that genetic locus has been identified as a nonsense mutation in *Cblb*, a member of the *Cbl/Slit* family of ubiquitin-protein ligases (Yokoi et al. 2002). Transgenic complementation significantly reduced diabetes frequency in rescued animals, and it was hypothesized that in the KDP rat *Cblb* functions as a negative regulator of autoimmunity. *Cblb* is a major susceptibility gene for type 1 diabetes in the rat and has been designated *Iddm3* for this purpose. *Cblb* is one of few non-MHC genes definitively linked to autoimmune diabetes in any strain or species. *Cblb* has not been linked to diabetes in the BB rat. Whether impairment of the *Cblb* signaling pathway contributes to human type 1 diabetes is as yet unknown.

MHC Congenic LEW Rats with Spontaneous Diabetes

Another new rat model of type 1 diabetes arose through a spontaneous mutation in the MHC congenic LEW.1AR1 strain (Lenzen et al. 2001). The MHC haplotype (*RT1 A^aB/D^uC^u*) of these animals includes a class II *u* allele. These models are designated LEW.1AR1/Ztm-iddm rats. The cumulative frequency of diabetes is ~20% by 2 mo of age, and both sexes are affected equally. Diabetic animals are keto-

nuric. They are not lymphopenic, and they express normal numbers of ART2⁺ T cells. Islets of affected animals are infiltrated with B and T lymphocytes, macrophages, and NK cells, and it appears that beta cells die via apoptosis.

Interestingly, spontaneous diabetes began to appear at about the same time in a different colony of MHC congenic LEW.1WR1 rats. The MHC haplotype (*RT1.A^uB/D^uC^u*) of these animals again includes a class II *u* allele, but the class I haplotype is different from that of the LEW.1AR1/Ztm-iddm rat. In the colony of LEW.1WR1 rats maintained at BRM, Inc. (Worcester, MA), spontaneous diabetes was absent from acquisition in 1989 and until 1999. It now occurs with a low frequency (~5%) but, as is discussed below, the frequency of diabetes can be increased by exposure to environmental perturbation (Mordes et al. 2003a).

Induced Autoimmune Diabetes in the Rat

Reasons for Inducing Diabetes

Many lines of evidence, including a concordance rate in monozygotic twins that averages only ~50% (Kyvik et al. 1995; Redondo et al. 2001), suggest that type 1 diabetes is caused by nongenetic environmental factors operating in a genetically susceptible host to initiate a destructive immune process (Åkerblom et al. 2002; Hawa et al. 2002). Strong evidence suggests that among the many candidate perturbants, viral infection is the most important (Pietropaolo and Trucco 1996; Yoon and Jun 2000), particularly in populations in which the incidence of diabetes is increasing (Laron 2002). In addition, many human type 1 diabetes susceptibility genes may operate only in the context of environmental perturbation, amplifying the immune response and the rate of disease progression (Hawa et al. 2002). It is thought that disease is due to interaction with the environment of alleles at many loci that are scattered throughout the genome (Todd 1999). The need to surmount these complexities has animated interest in appropriate and tractable inbred animal model systems that, as may children, develop autoimmune diabetes in response to environmental interactions.

Induced Type 1 Diabetes

Nontransgenic mouse models of induced type 1 diabetes are few, and most are not widely used. Immunization with a peptide fragment of a heat shock protein induces a type 1 diabetes-like syndrome in normal mice (Elias et al. 1995) as can certain orally ingested autoantigenic peptides (Blanas et al. 1996). As noted previously, NOD mice model interactions with the environment poorly because the great majority of perturbants, including viral infection, reduce the frequency of diabetes and often prevent it entirely (Atkinson and Leiter 1999). A toxin-inducible model is the mouse treated with multiple small doses of streptozotocin (Mordes et al. 2000). Because this induction method can lead to

diabetes in NOD-severely compromised immunodeficient ("SCID") mice that have no functional lymphocytes, its relevance as a model of type 1 diabetes has been questioned (Gerling et al. 1994).

In contrast, susceptibility to induced autoimmune diabetes in the rat appears to be relatively common. As noted in Table 1, with one exception the rat models all share the class II *u* MHC allele. Activated spleen cells from *RT1^c* PVG rats depleted of regulatory T cells (Treg cells) are capable of the adoptive transfer of diabetes (McKeever et al. 1990). Diabetes also occurs in PVG rats after treatment with thymectomy and sublethal irradiation (Penhale et al. 1990; Stumbles and Penhale 1993), whereas other rat strains including the *RT1^u* WAG rat are resistant. The thymectomy plus irradiation induction system in PVG rats is exceptional with respect both to MHC and to the intensity of the induction process; it is seldom used today. The remaining systems, which continue in use, employ viral infection, toll-like receptor (TLR¹) ligation, Treg depletion, and combinations of these perturbants.

Immunological Perturbation and Diabetes Induction in the Diabetes-resistant BB Worcester (BBDR/Wor¹) Rat

BBDR/Wor rats comprise a distinct inbred strain of BB rats (Guberski 1994; Mordes et al. 2001). They were derived from BBDP/Wor forebears at the 5th generation of inbreeding by selection for the absence of disease. They share the *RT1^u* MHC haplotype of the spontaneously diabetic BB rat and are not lymphopenic because the breeding process at that time selected against the recessive *Ian4L1* mutation. They circulate normal numbers of CD4⁺, CD8⁺, and ART2⁺ T cells and never become spontaneously diabetic in VAF vivaria (Butler et al. 1991).

Treg Depletion

As noted above, spontaneous diabetes in lymphopenic BBDP/Wor rats can be prevented by transfusion with CD4⁺ART2⁺ Treg cells. Conversely, BBDR/Wor rats housed in conventional, non-VAF conditions become diabetic when treated with a depleting anti-ART2⁺ antibody (Greiner et al. 1987). Cyclophosphamide (Like et al. 1985) and low-dose irradiation (Handler et al. 1989) can also precipitate diabetes in this animal under these conditions, but whether the mechanism involves Treg populations is unknown.

In VAF conditions, BBDR/Wor rats rarely become diabetic when treated with a depleting anti-ART2⁺ antibody alone. In this case, coadministration of an immune system activator like poly I:C, a synthetic double-stranded polyribonucleotide, is required (Guberski et al. 1991; Thomas et al. 1991).

It has been known for some time that poly I:C alone or in synergy with Treg depletion can induce diabetes in the BBDR/Wor rat. It is now known that poly I:C binds to TLR3 (Alexopoulou et al. 2001) and acts much as a “viral-mimetic” agent (Doukas et al. 1994). Treatment of BBDR/Wor rats with low doses of poly I:C (5 $\mu\text{g/g}$) induces diabetes in ~20% of animals (Thomas et al. 1991). Higher doses of poly I:C (10 $\mu\text{g/g}$) induce diabetes in nearly all BBDR/Wor rats (Sobel et al. 1992). Parenthetically, high-dose poly I:C accelerates diabetes in the BBDR/Wor rat (Ewel et al. 1992; Sobel et al. 1995), an effect associated with increased levels of IFN- α (Sobel et al. 1994). At lower doses, it is protective (Sobel et al. 1998b).

Viral Infection

In contrast to viral infection in NOD mice, infection of the BBDR/Wor rat with KRV induces diabetes. Naturally occurring infection in a closed colony of inbred animals affected ~1% of one generation before its eradication by Caesarian rederivation of the colony (Guberski et al. 1991). Intentional infection (10^7 plaque-forming units of KRV) induces diabetes in ~30% of BBDR/Wor rats (Guberski et al. 1991). Infection is associated with the development of pancreatic insulinitis, but not with infection of islet cells themselves or with the development of exocrine pancreatitis (Brown et al. 1993). The latter characteristic distinguishes this analytic system from that of encephalomyocarditis virus in mice, which appears not to have a primary autoimmune component to pathogenesis but to be largely due to the rapid destruction of beta cells by the replication of the virus within them (Jun and Yoon 2001). The ability to induce autoimmune diabetes in BBDR/Wor rats is virus specific; infection with the closely homologous parvovirus H-1, which is ~98% identical to KRV at the level of DNA, fails to induce diabetes despite the induction of robust cellular and humoral immune responses (Zipris et al. 2003).

The mechanism by which KRV induces diabetes is not yet established. The virus does infect T and B lymphocytes (McKisic et al. 1995) but does not cause the severe T lymphopenia that is characteristic of the spontaneously diabetic BB rat. Infected lymphocytes are phenotypically normal but have diminished proliferation and cytolytic responses (McKisic et al. 1995). Yoon and colleagues hypothesized that diabetes might be the result of molecular mimicry. This hypothesis was disconfirmed in studies in which BBDR/Wor rats were given injections of viral vectors encoding KRV proteins (Chung et al. 2000). No diabetes occurred despite the generation of cellular and humoral immune responses to those proteins. More recently, it has been suggested that KRV may induce diabetes in the BBDR/Wor rat by altering the immunoregulatory environment of these animals, specifically by reducing the frequency of CD4⁺CD25⁺ Treg cells (Zipris et al. 2003).

Noninfectious environmental perturbants can synergize with KRV to induce diabetes. Depletion of ART2⁺ Treg cells in the BBDR/Wor rat synergizes with KRV infection and leads to a high frequency of autoimmune diabetes (Ellerman et al. 1996). The combination of KRV infection and a brief course of poly I:C (one incapable of inducing diabetes by itself) induces diabetes in 100% of BBDR/Wor rats (Ellerman et al. 1996; Zipris et al. 2003). Poly I:C ligation of TLR3 is known to lead to a pleiotropic immune response and antigen-presenting cell activation that could clearly “disequilibrate” an immune system genetically predisposed to autoimmunity. Consistent with this view, it is known that macrophage depletion renders BBDR/Wor rats resistant to the induction of diabetes in response to KRV plus poly I:C (Chung et al. 1997).

Summarizing these data, KRV but not H-1 by itself can induce diabetes in resistant BBDR/Wor rats; KRV and H-1 both induce strong immune responses; but only KRV decreases splenic Treg populations in a virus-specific manner. The data demonstrate that activation of the innate immune system may synergize with certain viruses and greatly increase the penetrance of diabetes in these susceptible animals. This kind of analysis may help unravel the puzzling possible role of viral infection in type 1 diabetes and provide better understanding as to why identical twins and triplets can be discordant for the disease.

Genetic Analysis

The susceptibility of BBDR/Wor rats to induced autoimmune diabetes has proven useful in the identification of disease susceptibility loci. In analyses of (BBDR/Wor \times WF) \times WF backcross rats, none became spontaneously diabetic whereas ~25% did so after treatment with Treg depletion and poly I:C. Genome-wide scan and an identity-by-descent analysis of these animals revealed that they share the genetically dominant diabetes susceptibility locus designated *Iddm4* that is also critical for diabetes in BBDR/Wor rats (Martin et al. 1999b; Mordes et al. 2002). The BB/Wor-origin allele at *Iddm4* has 79% sensitivity and 80% specificity in prediction of diabetes in rats that are segregating for this locus. Comparably treated MHC-identical WF rats (*Iddm4*^{wt}) resist diabetes induction. A WF rat congenic for the BB/Wor-derived allele at *Iddm4* (WF.*Iddm4*^d) has been developed for further analysis of this locus and identification of the gene (Martin et al. 1999b; Mordes et al. 2002). Interestingly, preliminary data suggest that WF.*Iddm4*^d congenic rats that become diabetic in response to Treg depletion plus poly I:C do not become diabetic after KRV infection plus poly I:C (Mordes et al. 2003b). A genome-wide scan of (BBDR/Wor \times WF)F₂ rats has preliminarily suggested that diabetes is induced by KRV in BBDR/Wor rats only if the BB-origin alleles of *Iddm4* and at least one additional susceptibility gene are present.

BBDR/Wor rats induced to become diabetic develop hyperglycemia under well-controlled circumstances and within a defined time frame, usually 2 to 4 wk. Accordingly, they provide an opportunity to assess preventive intervention strategies. Few such studies have been reported to date, but it is known that intrathymic islet transplantation (Battan et al. 1994) and hydrolyzed casein diets (Malkani et al. 1997) do not prevent induction of diabetes in animals treated subsequently with Treg depletion and poly I:C. Intervention based on the administration of parenteral insulin is effective in similarly treated BBDR/Wor rats, but only at high doses of insulin sufficient to cause beta cell involution (Gottlieb et al. 1991). Rats treated in this way that remain nondiabetic still exhibit thyroiditis and insulinitis and harbor autoreactive cells that can transfer diabetes to naive recipients. Diets low in essential fatty acids do prevent disease in Treg-depleted BBDR/Wor rats (Lefkowitz et al. 1990).

Immunological Perturbation and Diabetes Induction in Other Rat Strains

TLR Ligation

Susceptibility to induced autoimmune diabetes appears to be common in rat strains that express a class II μ MHC haplotype (Table 1). Some of the characteristics of these systems are summarized in Table 2. Although the doses and duration of treatment were variable among the studies, the importance of the class II haplotype and the diversity of susceptible strains are apparent. It is interesting to note that in the case of LEW.1AR1/Ztm-iddm rats, some of which develop spontaneous autoimmune diabetes, treatment of nondiabetic animals with poly I:C fails to induce disease (Lenzen et al. 2001), whereas the LEW.1WR1 rat is susceptible (Ellerman and Like 2000). The mechanisms underlying these observations are unknown, but clearly a diverse array of background genes in these strains must modulate the basic susceptibility conferred by the *RT1 B/D μ* MHC. The data are particularly intriguing in light of the increasing incidence of type I diabetes in humans, which some believe to be linked to environmental modulation of innate immune responses—the so-called “hygiene hypothesis” (Bach 2002).

Viral Infection

KRV induces insulinitis and autoimmune diabetes in LEW.1WR1 rats (Ellerman et al. 1996). Preliminary data suggest that LEW.1WR1 rats, like BBDR/Wor rats, also resist diabetes induction when treated with H-1 or vaccinia (Mordes et al. 2003a). Surprisingly, however, and unlike BBDR/Wor rats, LEW.1WR1 rats developed autoimmune diabetes after infection with rat cytomegalovirus (RCMV¹). No rats had exocrine pancreatitis. The underlying mecha-

nisms and susceptibility genes that account for this differential autoimmune response to infection are unknown. Comparative study of the BBDR/Wor and LEW.1WR1 systems should be of particular interest in light of recent data suggesting a possible role for cytomegalovirus in human type 1 diabetes (Hiemstra et al. 2001). The combination of KRV infection and a brief course of poly I:C (one incapable of inducing diabetes by itself) induces diabetes in 100% of BBDR/Wor and LEW.1WR1 rats and a fraction of PVG.*RT1 μ* rats (Ellerman et al. 1996; Zipris et al. 2003).

One Working Hypothesis of Autoimmune Diabetes in the Rat

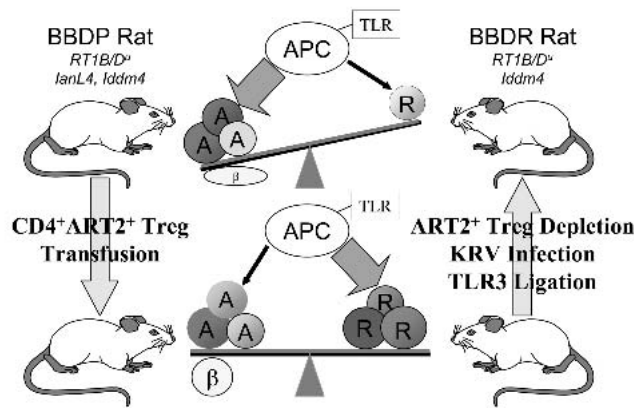
The enlarging repertoire of rat strain resources for the study of autoimmune diabetes allows for the expansion and strengthening of a working hypothesis of autoimmune diabetes that was originally derived from studies in the BB/Wor rat (Mordes et al. 1996a). As depicted schematically in Figure 2, this hypothesis holds that diabetes in the rat results from an imbalance between (1) beta cell-cytotoxic effector cells, and (2) regulatory cells that normally prevent disease. The hypothesis predicts the existence of at least two defects.

The first defect is genetic, involves the class II μ allele of the MHC, and leads to the generation of autoreactive effector cells, not only in the BB rat but also in several other rat strains (Table 1). The second defect leads to amplification of the autoreactive population and/or to a regulatory cell deficiency. The second defect can be genetic or acquired. In the diabetes-prone BB rat, this defect is congenital lymphopenia, which can be overcome by the transfusion of CD4⁺ART2⁺ Treg cells. Strengthening and expanding this concept is the KDP rat (Yokoi et al. 1997) in which a loss of function mutation in the *Cblb* gene appears to predispose to abnormal T cell activation (Yokoi et al. 2002). In terms of our hypothesis, the KDP rat combines an MHC-dependent genetic predisposition with a “disequilibrating” genetic defect leading to autoreactive T cell activation.

Alternatively, the second defect can be environmental. The array of relevant environmental perturbants and the number of rat strains that they affect is growing and includes the following: KRV infection in BBDR/Wor and LEW.1WR1 rats, RCMV infection in LEW.1WR1 rats, TLR3 ligation in an array of rats (Table 3), and synergistic combinations of these agents that have only begun to be studied.

In their aggregate, the available data gathered from the study of newer rat models of autoimmune diabetes suggest a promising “environmental genetics” approach to modeling type 1 diabetes. This approach may allow for coordinated analysis of the influence of genetic makeup on the balance hypothesis. It may also enable us to coordinate data on the initiation of autoimmune processes with the response of the individual to environmental agents that perturb the immune system in a diverse array of rat strains.

Autoimmune Diabetes



Tolerance in the Presence of Genetic Susceptibility

Figure 2 The balance hypothesis of autoimmune diabetes based on studies of diabetes-prone (BBDP) and diabetes-resistant (BBDR) BB rats. (Top) Imbalance between autoreactive (A) and regulatory (R) cell populations leading to destruction of pancreatic beta cells. (Bottom) A balance between the forces described above. BBDR and BBDR rats share a genetic susceptibility to autoimmunity based on the presence of the *RT1 B/D^u* MHC class II allele and the *Iddm4* locus. APC, antigen-presenting cell; TLR, toll-like receptor; Treg, regulatory T cell; KRV, Kilham rat virus. For the BBDR rat (left), the presence of an additional mutation in the *Ian4L1* gene causes lymphopenia and an imbalance between autoreactive and regulatory cell populations, leading to diabetes. Transfusions of MHC-compatible lymphocytes restore immunological balance and prevent diabetes. For the BBDR rat (right), autoreactive cells are present but do not lead to the expression of diabetes unless the balance is perturbed. Perturbants that lead to diabetes include regulatory cell depletion with anti-ART2.1 monoclonal antibody, infection with Kilham rat virus, which can decrease regulatory cell numbers, and injection of the TLR3 ligand poly I:C, which may alter the immunological balance by activating APCs.

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