

RS Tirabassi*, DL Guberski*, DA Winans*, JH Leiff‡ and JP Mordes‡
*Biomedical Research Models, Inc and ‡University of Massachusetts Medical School

Abstract

Viral infection may play an important role in the pathogenesis of human type 1 diabetes. We previously reported that MHC congenic (*RT1u/u^a*) LEW.1WR1 rats in the BRM colony develop spontaneous autoimmune diabetes at a low frequency (~2%) and are also exquisitely susceptible to the induction of diabetes in response to environmental perturbants. In particular, LEW.1WR1 rats are highly susceptible to induction of diabetes after viral infection. The parvovirus, Kilham rat virus (KRV; 1×10^7 PFU) induces diabetes in approximately 40% of animals, and infection with rat cytomegalovirus (RCMV; 1×10^5 PFU) induces diabetes with varying incidence (20-65%). Co-infection of weanling LEW.1WR1 rats with KRV and RCMV induces diabetes in up to 100% of animals. We previously demonstrated that infection of LEW.1WR1 dams with both KRV and RCMV prior to pregnancy ("maternal immunization") protected pups from diabetes induced by inoculation of both viruses. To extend these studies further, we tested whether maternal immunization with RCMV alone would protect pups from RCMV-induced diabetes or KRV-induced diabetes. While we observed 62% induction of diabetes in pups from naïve females, none of the pups weaned from the immunized females developed diabetes (0/26). We further observed that maternal immunization with RCMV did not protect pups from KRV-induced diabetes – 62% of the pups weaned from RCMV-immunized females developed diabetes after KRV infection. These data demonstrate that maternal immunization with multiple viruses protects weanlings from virus-induced diabetes. The observed protection is virus-specific.

Background

Type 1 diabetes mellitus (T1D) is an autoimmune disease that remains exceedingly difficult to study in children. Despite intensive research in children and animal models, the cause of T1D remains unknown, and there are as yet no safe and effective methods for its prevention. Expression of T1D in humans is clearly modified by environmental perturbation. Among individuals with high risk HLA alleles, only about 1 in 15 children in the general population and 1 in 5 with a first degree relative with T1D will develop disease. Additionally, the observation that the concordance for T1D among identical twins is approximately 40% supports the theory of an environmental trigger.

Among candidate perturbants, strong evidence suggests that viral infection is the most important, particularly in populations in which the prevalence is increasing. The association was first noted in epidemiological studies and has been confirmed in more recent investigations. Viruses associated with human T1D include measles, rubella, mumps and cytomegalovirus. Several searches for evidence of direct viral infection of islet cells have yielded consistently negative results. Viral induction of autoimmunity has thus been proposed to involve several other mechanisms including bystander activation, molecular mimicry, and/or T cell activation by viral superantigen.

We have recently described a new and powerful rat model of environmental induction of diabetes that arose in a major histocompatibility complex (MHC) congenic LEW rat. Autoimmune diabetes occurs in adolescent LEW.1WR1 rats in viral-antigen free housing at a rate of ~2%. The disease is characterized by hyperglycemia, glycosuria, ketonuria and polyuria. Both sexes are affected, and islets of acutely diabetic rats exhibit insulinitis and are devoid of beta cells whereas alpha cell populations are spared. The peripheral lymphoid phenotype is normal, including the fraction of ART2+ regulatory T cells. We have demonstrated that the frequency of diabetes in the LEW.1WR1 rat is dramatically increased by immunological perturbation of innate or adaptive immunity. Treatment of young rats with the Treg-depleting anti-ART2.1 monoclonal antibody (mAb) increases the frequency of diabetes to about 50%. Combined treatment with anti-ART2.1 mAb and the toll-like receptor 3 ligand, poly I:C increases the frequency of diabetes to 100%. We have also documented that LEW.1WR1 rats develop autoimmune diabetes in response to infection with several viral agents. Kilham rat virus (KRV) and rat cytomegalovirus (RCMV) both induce diabetes in ~40% of the animals.

In the studies presented here, we demonstrate that maternal immunization can prevent diabetes induced by either one or two concurrent infections in weanling LEW.1WR1 rats. These data suggest that vaccination strategies directed at candidate viruses could be successful in preventing juvenile diabetes.

Methods

LEW.1WR1 Rats

- *MHC haplotype RT1^{u/a}
 - ↳ Shares MHC Class II u haplotype common to all diabetic rats
- *2% incidence spontaneous diabetes
- *Abrupt onset of hyperglycemia, glycosuria, proteinuria and ketonuria
- *Normal immune cell profile
- *Exquisitely sensitive to environmental induction of diabetes (Mordes, et al. 2005)
 - ↳ Low dose TLR 3 ligand poly I:C (100%)
 - ↳ Regulatory T cell depletion (46%)
 - ↳ Induction of diabetes by multiple viruses

20-25 day old male and female BBDR/Wor or LEW.1WR1 rats were injected i.p. with the agents listed in Table 1. Following treatment, animals were tested for glycosuria three times a week. Blood glucose was determined for animals with positive urine glucose tests. Animals with blood glucose levels >250mg/dL were considered diabetic. Rats were sacrificed shortly after diabetes onset and pancreatic sections from representative animals were analyzed to confirm diagnosis.

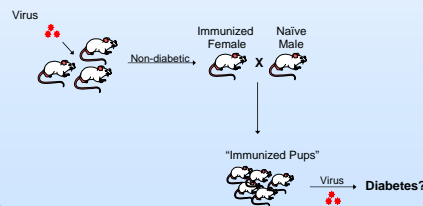
Table 1: Agents Used in this Study

Treatment	Regimen
Toll-like receptor 3 (TLR 3) ligation	1µg/gm body weight Poly I:C; 3x
Kilham Rat Virus (KRV)	10 ⁷ PFU i.p.
Rat Cytomegalovirus (RCMV)	10 ⁴ PFU i.p.
Coxsackie B4 (CoxB4)	10 ⁸ PFU i.p.
Vaccinia Virus (VV)	10 ⁶ PFU i.p.
TLR 3 ligation + infection	Poly I:C injection days -3, -2, and -1 prior to infection

Maternal Immunization Experiments

As shown in Figure 1, 20-25 day old male and female LEW.1WR1 rats were injected i.p. with either RCMV or KRV + RCMV. Animals were observed for diabetes onset for 40 days. Infected, non-diabetic "immunized" female rats were bred to naïve males. The pups resulting from these matings were subsequently infected with either RCMV alone, KRV alone or KRV + RCMV at 20-25 days of age. At the same time, naïve control weanlings were infected with the same dose of virus.

Figure 1: Outline of Maternal Immunization Protocol



Results

Table 2: Multiple Environmental Perturbants Alter the Incidence of Diabetes in LEW.1WR1 Pups

Treatment	N	Diabetic
Poly I:C	14	0 (0%)
RCMV	38	14 (37%)
CoxB4	18	0 (0%)
VV	10	0 (0%)
Poly I:C+RCMV	29	3 (10%)
Poly I:C + CoxB4	6	1 (17%)
Poly I:C + VV	5	4 (80%)
RCMV + KRV	45	34 (76%)
RCMV + CoxB4	8	0 (0%)

Figure 2: Viral Antigen is not Detected in Pancreatic Islets Isolated from RCMV-infected Diabetic LEW.1WR1 Pups

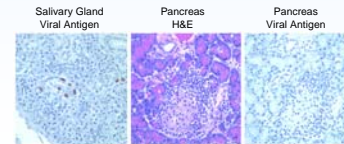


Figure 2: Detection of viral antigen in salivary gland and pancreas from virally infected diabetic animals. Salivary glands and pancreata were isolated from diabetic animals 2 days after onset of hyperglycemia. The organs were fixed in 10% formalin, embedded in paraffin and processed for immunohistochemistry using an antibody that recognizes the RCMV early antigen. Serial sections were also stained with hematoxylin and eosin (H&E). Viral antigen (brown staining) was readily detected in salivary glands of infected animals while no viral antigen was detected in pancreata from the same animals.

Figure 3: Maternal Immunization Protects Weanling LEW.1WR1 Rats from Virus-Induced Diabetes

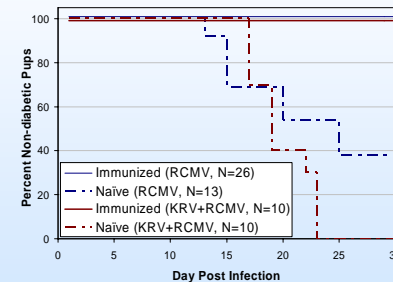


Figure 3: Kaplan Meier plot of results from maternal immunization experiments. Naive pups (dashed lines) or pups from non-diabetic "immunized" female rats (diamonds and solid line) were infected with either RCMV (blue) or KRV+RCMV (red) at 20-25 days of age. Animals were held for 40 days post infection. Average latency to onset of diabetes in naive pups was 19 days (range 13-25) post infection for RCMV and 19.8 days (range 17-23) for KRV+RCMV. Both males and females were equally susceptible to diabetes induction.

Table 3: Maternal Immunization is Effective and Specific in Protection from Virus-Induced Diabetes

Maternal Infection	Progeny Infection	N	Diabetic
None	RCMV	13	8 (62%)
None	KRV	8	3 (38%)
None	RCMV+KRV	10	10 (100%)
RCMV	RCMV	26	0 (0%)
RCMV	KRV	13	8 (62%)
RCMV+KRV	RCMV+KRV	11	0 (0%)

Conclusions

- *The LEW.1WR1 rat is a new flexible model of both spontaneous and environmentally-induced diabetes
- *LEW.1WR1 rats reveal the potential of cytomegalovirus to induce diabetes
- *Diabetes may be preventable by vaccines that target candidate pathogens
- *The LEW.1WR1 rat model is ideally suited to test diabetes vaccination strategies
- *Maternal immunization can provide effective and specific protection from virus-induced diabetes in weanling rats

References

- *Mordes, J.P. et al. 2005. LEW.1WR1 rats develop autoimmune diabetes spontaneously and in response to environmental perturbation. *Diabetes*. 54:2727-2733.
- *Tirabassi, RS., et al. 2005. Maternal immunization protects weanling LEW.1WR1 rats from cytomegalovirus-induced diabetes. *Diabetes*. 54:Supplement 1:A94.
- *Tirabassi, RS., et al. 2004. Rat strain-specific susceptibility to environmental induction of type 1 diabetes. *Diabetes*. *Diabetes*. 53:Supplement 2:A301.

Acknowledgements

- *Many thanks to the technical and husbandry staffs at BRM and UMass
- *Special thanks to Drs. William Shek and Joan Flanagan at Charles River Laboratories
- *Dr. Jan Luuk Hillebrands from the University Medical Center Groningen, Groningen, The Netherlands kindly provided the antibody specific for the RCMV early antigen
- *This work was supported by a grant from the National Center for Research Resources (DLG)