Altered Expression of Diabetes in BB/Wor Rats by Exposure to Viral Pathogens

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Autoimmune diabetes mellitus affects >50% of diabetes-prone BB (DP BB) rats but <1% of diabetes-resistant BB (DR BB) rats. We report an outbreak of spontaneous diabetes among DR BB rats that coincided with serologic evidence of the onset of viral infection. This apparent link between a change in the environment and the expression of diabetes then led us to study the interaction of environmental exposure to viral pathogens in this disorder with virally seropositive and seronegative populations of BB rats and polyinosinic–polycytidylic acid (poly I:C), an interferon inducer known to accelerate diabetes onset in DP rats. We administered a cytotoxic anti-RT6 monoclonal antibody, poly I:C, or both to DR rats. Depletion of the RT6.1$^+$T-lymphocyte population has previously been shown to induce diabetes and thyroiditis in DR rats. RT6 alone did not induce diabetes in seronegative DR rats, and poly I:C was only weakly effective, but nearly all animals given both reagents became diabetic. When given to seropositive DR rats, either reagent alone induced diabetes; when given to non-BB rats, neither agent was effective. Poly I:C also accelerated the onset of DP diabetes to a greater extent in seropositive than in seronegative rats. We conclude that expression of the genetic predisposition to diabetes present in all BB rats depends on cellular factors that include the presence or absence of regulatory (RT6$^+$) T lymphocytes and modulatory environmental factors including exposure to viral pathogens. *Diabetes* 40:255–58, 1991

Diabetes-prone (DP) BB rats are susceptible to autoimmune insulitis, hyperglycemia, and thyroiditis (1). They are lymphopenic and lack RT6$^+$ T lymphocytes (2). Diabetes-resistant (DR) BB rats were derived from the DP line and have normal lymphocyte numbers including RT6.1$^+$ T lymphocytes (3). Diabetes is rare in these rats, but they do possess autoreactive cells; in vivo elimination of RT6.1$^+$ T lymphocytes in 30-day-old animals induces diabetes within 3–4 wk (3).

An unexplained outbreak of spontaneous diabetes among nonlymphopenic DR rats occurred in 1983–1984 (4). In 1989–1990, another self-limited outbreak of diabetes was observed in our breeding colony of DR BB rats. It affected ~20 rats in a room housing ~350. Diabetic animals were neither lymphopenic nor grossly deficient in RT6$^+$ lymph node cells. The course of the outbreak is shown in Fig. 1, which also indicates that it coincided with changes in the viral serology profiles of sentinel rats, heralding new viral pathogens in the colony. Proof that a specific viral pathogen was the cause of the outbreak of diabetes is being sought.

This outbreak suggested a link between changes in the environment (viral infection) and the expression of BB diabetes and prompted us to develop a strategy for analyzing the interaction of environmental and cellular variables in these rats. We used polyinosinic–polycytidylic acid (poly I:C), an agent that increases interferon activity (5) and reportedly accelerates diabetes onset in DP BB rats (6), and we also exploited the availability of distinct populations of BB rats, a viral antibody-free population maintained in a seronegative environment and a seropositive population reared in conventional housing.

RESEARCH DESIGN AND METHODS

DR BB and DP BB/Wor rats were obtained from two colonies at the University of Massachusetts: the National Institutes of Health (NIH)-sponsored BB rat resource colony (7) and a subcolony maintained by us with breeding stock obtained from the NIH colony every three generations. The incidence of diabetes in DP BB/Wor rats is 60–80%. Spontaneous diabetes has occurred in <1% of DR BB/Wor rats. All BB rats express the *RT1^u* MHC haplotype. PVG rats (*RT1^c*) were ob-

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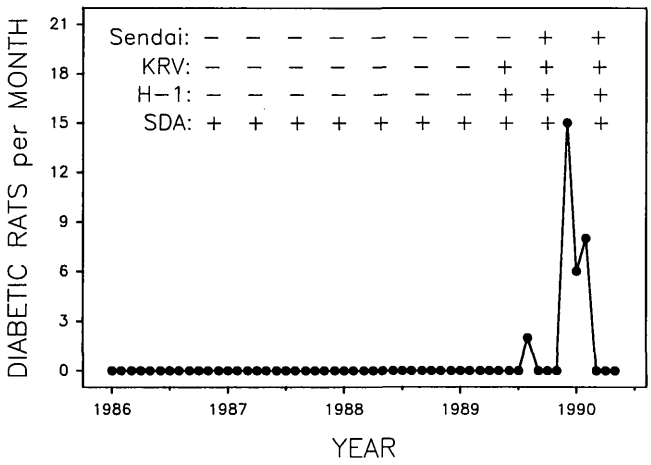

FIG. 1. Viral antibody serology and diabetes incidence in diabetes-resistant subcolony of BB/Wor rats maintained by our laboratory. Each *data point* represents 1 mo of given year. Total census in affected quarters varied but averaged ~350 rats. Serologic information was obtained from sentinel rats maintained for this purpose. Sendai, Sendai virus; KRV, Kilham's rat virus; H-1, Toolan's H-1 virus; SDA, sialodacryoadenitis virus.

tained from our colony; Wistar-Furth (WF, *RT1^u*) and Buffalo (*RT1^b*) rats from the National Cancer Institute (Frederick, MD); and New England Deaconess Hospital (NEDH, *RT1^g*) rats from Simonsen (Gilroy, CA). Animals were tested for glycosuria twice weekly; diabetes was diagnosed if plasma glucose was ≥11.1 mM.

Environments. The NIH BB/Wor rat colony was viral antibody free (VAF; 7). Exposure to environmental pathogens is minimized by access restrictions and sterilization of food and bedding. DP and DR rats from this facility are free of *Mycoplasma pulmonis*, Sendai virus, pneumonia virus of mice (PMV), sialodacryoadenitis virus (SDA), Kilham's rat virus (KRV), Toolan's H-1 virus (H-1), and reovirus type 3 (REO3).

Other animal quarters in our facility were maintained conventionally (8). They were not provided with sterilized food or bedding. Commercial serologic testing (Charles River, Wilmington, MA) revealed the presence of Sendai, SDA, KRV, and H-1 but not PMV or REO3 in these rooms.

For these studies, a special seronegative environment was created. VAF rats obtained from the NIH colony were transferred in covered cages to this room and maintained under filter bonnets. Food and bedding in this and the seropositive rooms were the same. Serologic testing of animals maintained in this room for at least 4 wk revealed none of the seven pathogens listed above.

Reagents and injection protocols. DS4.23 anti-RT6.1 monoclonal antibody (MoAb) was prepared as previously described (3). To deplete DR rats of RT6$^+$ T lymphocytes, 2 ml/rat i.p. unconcentrated hybridoma supernatant was injected 5 times/wk for 4–5 wk. Poly I:C (Sigma, St. Louis, MO) was diluted in phosphate-buffered saline (PBS) and given at a dose of 5 μg/g body wt i.p. 3 times/wk. Control injections were RPMI or PBS in the same volume given on the same schedule; a few controls were uninjected.

Experimental protocols. Two groups of 30-day-old DR rats were tested, one obtained from the NIH colony and kept seronegative and one born, reared, and tested in conventional seropositive housing. Littermates in both groups were randomized to four experimental conditions: anti-RT6, poly I:C, anti-RT6 plus poly I:C, and control. The various agents were administered until rats were 60 days of age. Three groups of 21- to 30-day-old DP rats were tested. VAF animals obtained from the NIH colony were housed in either the seronegative environment described above or in conventional seropositive housing. A third seropositive group was born, reared, and tested in conventional housing. Rats in these groups were randomized to receive poly I:C or vehicle through 120 days of age.

Animals were killed at diabetes onset or at the end of the experiment in 100% CO_2. Lymph node cells expressing the CD4, CD5, CD8, and RT6.1 surface markers were quantified by standard flow microfluorometry as previously described (3,9). Pancreas and thyroid were fixed in Bouin's solution and processed for routine light microscopy. Sections were interpreted by a pathologist unaware of the treatment status of the animals. Parametric data are means ± SE. Nonparametric data were analyzed with Fisher's exact (2-tailed) test or χ^2-test.

RESULTS

The frequency of diabetes, insulitis, and thyroiditis in treated DR-BB/Wor rats is shown in Table 1. Among DR rats born and depleted of RT6$^+$ T lymphocytes in the seropositive environment, the disease rates were comparable to those reported previously (3). A new finding was that poly I:C alone induced diabetes to a similar degree. The combination of both administrations increased this percentage. Among nondiabetic rats given either reagent, insulitis and thyroiditis were common. No controls had diabetes or insulitis, but thyroiditis was present in 2 of 16 controls. Sera from six rats tested positive serologically for Sendai virus, KRV, SDA, and H-1.

The same interventions performed on VAF DR BB/Wor rats yielded a strikingly different outcome (Table 1). Depletion of RT6$^+$ T lymphocytes alone did not induce diabetes or insulitis, and poly I:C alone was only weakly effective. In contrast, the combined regimen induced diabetes in nearly all animals. The effectiveness of the combined regimen was limited to pancreatic autoimmunity; however, no thyroiditis was observed. A sampling of pancreases from diabetic rats in all treatment groups revealed either typical insulitis or end-stage islets. A mild degree of peritoneal and/or exocrine pancreatic inflammation was noted in some but not all animals. Viral seronegativity was confirmed at the conclusion of the experiment in a sample of four rats.

RT6 depletion and poly I:C alone or in combination did not induce diabetes in conventionally housed PVG, Buffalo, or NEDH rats, which, like the DR rats, express the RT6.1 alloantigen (n = 6–11 rats/group). In WAG (n = 6) and WF (n = 10) rats, which being RT6.2$^+$ could not be RT6 depleted with our reagents, poly I:C alone did not induce diabetes. No treated non-BB rats had insulitis or thyroiditis.

Immunophenotypic analysis revealed similar numbers of RT6.1$^+$ lymph node cells in seropositive (70 ± 1%, n = 5) and seronegative (65 ± 4%, n = 5) DR controls. Poly I:C administration did not affect the percentage of RT6.1$^+$ T lymphocytes (data not shown). DS4.23-administered DR rats were depleted of RT6.1$^+$ T lymphocytes to a comparable extent in both seropositive (8 ± 1%, n = 5) and seronegative

TABLE 1
Frequency of diabetes, insulitis, and thyroiditis in diabetes-resistant BB/Wor rats administered anti-RT6.1 antibody, polyinosinic–polycytidylic acid (poly I:C), or both

		Groups			
	Outcome	Anti-RT6	Poly I:C	Both	Neither
NIH colony rats	Diabetes*	0 of 18 (0)†	4 of 18 (22)	17 of 18 (94)	0 of 11 (0)
Seronegative environment	Nondiabetic with insulitis	0 of 16 (0)†	4 of 14 (29)	1 of 1 (100)	0 of 10 (0)
	Diabetes or insulitis*	0 of 16 (0)†	8 of 18 (44)	18 of 18 (100)	0 of 10 (0)
	Thyroiditis	0 of 8 (0)	0 of 10 (0)	0 of 10 (0)‡	0 of 10 (0)
Subcolony rats	Diabetes*	11 of 17 (65)	10 of 17 (59)	13 of 17 (76)	0 of 16 (0)
Seropositive environment	Nondiabetic with insulitis	5 of 6 (83)	2 of 7 (29)	2 of 4 (50)	0 of 16 (0)
	Diabetes or insulitis*	16 of 17 (94)	12 of 17 (71)	15 of 17 (88)	0 of 10 (0)
	Thyroiditis§	3 of 8 (38)	2 of 9 (22)	4 of 7 (57)	2 of 16 (13)

Values in parentheses are percentages. NIH, National Institutes of Health. Paired comparisons are not significant except where noted. Three pancreases from nondiabetic rats were technically unsatisfactory, and the number of rats analyzed with respect to diabetes plus insulitis is sometimes less than for diabetes alone.
*Overall χ^2-test comparing treatment groups was significant ($P < 0.005$).
†$P < 0.001$, ‡$P < 0.02$, vs. corresponding seropositive group.
§Overall χ^2-test showed no differences in thyroiditis.

(10 ± 1%, $n = 5$) rats. Coadministration of poly I:C did not affect the degree of depletion (data not shown). Percentages of $CD4^+$, $CD5^+$, and $CD8^+$ cells were comparable in all control and experimental groups regardless of serologic status (data not shown).

Table 2 shows the frequency of diabetes in poly I:C–administered DP BB rats partitioned according to origin and serologic status. Through 60 days of age, poly I:C accelerated the onset of diabetes, particularly in the VAF group. Through 120 days of age, the cumulative frequency of diabetes in all three groups was statistically similar.

DISCUSSION

These data reveal striking variations in the frequency of diabetes and thyroiditis in BB rats as a function of environmental and immunologic perturbations. We have confirmed the report of Ewel et al. (6) that poly I:C accelerates the onset of DP rat diabetes and have extended it by our observation that this effect is more pronounced in seronegative than in seropositive DP animals. The data do not allow us to define the mechanism that underlies this observation, but it could represent an effect of poly I:C on natural killer (NK) cells. Several studies have suggested that NK cell activity is relatively increased in the DP rat (10,11) and that NK cells may act as β-cytotoxic effectors in these animals (12,13). Because poly I:C reportedly stimulates NK activity in mice (14,15), it is plausible to infer an NK-mediated effect of poly I:C in this study. However, such an explanation does not explain the more pronounced effect of poly I:C observed in animals in a seronegative versus a seropositive environment. Note that this last observation is consistent with a report that spontaneous diabetes also appears earlier in VAF DP than in seropositive DP rats (7).

We have confirmed that depletion of $RT6^+$ T lymphocytes by itself induces diabetes, insulitis, and/or thyroiditis in most seropositive DR animals (3,9). Poly I:C is added to the list of interventions capable of inducing diabetes in DR rats: cyclophosphamide (16), low-dose γ-irradiation (17), and/or media conditioned by spleen cells cultured in the presence of lectin (18).

More important is the difference in outcome of anti-RT6 and poly I:C administration in seronegative DR rats. Depletion of $RT6^+$ T lymphocytes by itself does not induce autoimmune pathology if the depleted DR rats are seronegative. This finding clarifies the report of Like (19) that RT6 depletion induced diabetes in only 2–8% of intraperitoneally treated DR rats. The animals in that study were specific-pathogen–free rats from the NIH colony at the University of Massachusetts, where the animals were also maintained while being treated. Like (19) speculated that peritoneal irritation might be required for the induction of RT6-depletion diabetes and induced peritonitis by injecting autoclaved rat

TABLE 2
Frequency of prior diabetes in diabetes-prone BB/Wor rats given polyinosinic–polycytidylic acid (poly I:C)

		Through 60 days		Through 120 days	
Rat source	Housing	Poly I:C	Control	Poly I:C	Control
NIH colony	Seronegative	7 of 8 (86)*	0 of 7 (0)	8 of 8 (100)	7 of 7 (100)
NIH colony	Seropositive	3 of 11 (27)	0 of 11 (0)	9 of 11 (82)	8 of 11 (73)
Laboratory subcolony	Seropositive	2 of 6 (33)	0 of 6 (0)	3 of 6 (50)	4 of 6 (67)

Values in parentheses are percentages. NIH, National Institutes of Health. The cumulative frequency of diabetes in the same group of rats is shown at 60 and 120 days of age. Seroconversion among NIH-origin rats treated in the seropositive environment was documented at the end of the experiment in 2 animals. For the 6 60-day-old groups, overall χ^2-test = 25.3, df = 5, $P < 0.001$. For the 6 120-day-old groups, overall χ^2-test = 8.1, NS. Comparisons are not statistically significant except as noted.
*$P < 0.002$ vs. control.

feces into DR rats. This by itself produced no hyperglycemia but, in conjunction with RT6 depletion, did increase the frequency of diabetes to 33%. In this study, poly I:C was not associated with florid peritonitis or pancreatitis, and by itself, poly I:C produced diabetes or insulitis in >40% of seronegative DR rats. The data indicate that the serologic status of DR rats is a major determinant of their susceptibility to spontaneous diabetes.

The fact that poly I:C appears to act much as a surrogate for seropositivity in DR rats suggests a potential role for cytokines such as interferon, which is commonly produced in response to viral infection. Poly I:C increases circulating interferon activity (5), but our DP data indicate that the effects of virus exposure and poly I:C administration probably involve more than the generation of a single cytokine. In DR rats, poly I:C facilitated diabetes onset in seropositive animals; in DP rats, it was more effective in seronegative animals. In addition, note that infection with lymphocytic choriomeningitis virus prevents hyperglycemia in DP rats (20) and that poly I:C prevents autoimmune diabetes in the NOD mouse (21). It is also interesting that thyroiditis could not be induced in seronegative animals by 60 days of age, suggesting a specific contribution of environmental pathogen exposure to this process. The state of equilibrium between effector and regulatory populations in these animals is likely to be complexly determined by many factors.

We previously suggested that the induction of autoimmune diabetes in the BB rat is a function of the balance that exists between autoreactive (effector) and regulatory ($RT6^+$) cell populations (1). This concept has been confirmed by the detection of autoreactive cell populations in strains of normal rats by depletion of their $RT6^+$ T lymphocytes followed by the adoptive transfer of spleen cells to athymic recipients (9). The data in this study suggest that environmental factors in general and viral pathogens in particular can perturb the balance between autoreactive cell populations and regulatory cells in the BB rat.

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