Virus-induced Diabetes in Autoimmune New Zealand Mice

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SUMMARY

Infection of autoimmune New Zealand mice with the D variant of encephalomyocarditis (EMC) virus results in beta-cell damage and clinical diabetes. The induction of diabetes in parental NZB and NZW strains was independent of sex. However, the susceptibility to virus-induced diabetes in their F_1 offspring was sex dependent. This susceptibility was significantly higher in male (NZB × NZW) F_1 mice as compared with female F_1 mice. Castration of male F_1 mice significantly reduced the susceptibility to diabetes. These results suggest that parental NZB and NZW strains have recessive genes at different loci which do not allow sex hormones to influence the susceptibility to diabetes. It is concluded that both the genetic background of the host and sex hormones influence the development of virus-induced diabetes in autoimmune New Zealand mice. DIABETES 32:755–759, August 1983.

Viruses and autoimmunity are both implicated in the pathogenesis of insulin-dependent diabetes mellitus (IDDM).[1] Numerous case reports of the onset of certain viral infections and the subsequent development of diabetes, the recent isolation of viruses from acute diabetic patients, and the development of diabetes in mice by those viruses strongly suggest that viruses may play an etiologic role in some cases of IDDM.[2–5] Immune processes also are believed to contribute to the pathogenesis of IDDM.[6–8] IDDM is often associated with autoimmune endocrinopathies. Anti-islet cell-mediated autoimmunity is predominantly found in young patients with recent-onset IDDM.[9,10] Higher incidences of autoantibodies against thyroid and nucleic acids are also observed.[11,12]

Encephalomyocarditis (EMC) virus, Coxsackie virus B4, and reovirus type 1 and 3 are capable of inducing diabetes in mice by infecting and destroying pancreatic beta-cells. These viruses proved to be useful models for the study of genetic susceptibility, environmental factors, and immune mechanism.[13–16]

New Zealand Black (NZB) and its F_1 hybrid (NZB × NZW F_1) mice have been extensively studied as an animal model of autoimmune diseases.[17–19] The NZB strain develops Coombs positive autoimmune hemolytic anemia and its F_1 hybrid (NZB × NZW) is used as a model for systemic lupus erythematosus. Recently, NZB and (NZB × NZW) F_1 mice were found to have lymphocytic infiltration of the pancreatic islets and abnormal glucose tolerance.[20] Another closely related New Zealand mouse strain, the New Zealand Obese (NZO), was found to spontaneously develop adult-onset obese type diabetes combined with autoimmune disease.[21]

This investigation was initiated to see whether the prediabetic state and the autoimmune disease would contribute to the susceptibility to diabetes or alter the disease. We found a remarkable pattern of inheritance of sex hormonal modulation of susceptibility to virus-induced diabetes.

MATERIALS AND METHODS

Animals. NZB, NZW, BALB/c, and C3H/HeN mice and their crosses were obtained from the breeding colonies at the National Institutes of Health, Bethesda, Maryland. SJL/J mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). Mice were maintained on Purina NIH mouse ration containing 5% fat and 23.5% protein. Except where noted, 5–8-wk-old mice were used. The data presented are from three separate experiments. There was good correlation between the results.

Virus. The preparation and source of the D variant of EMC virus are described elsewhere.[22] Except where noted, mice were inoculated intraperitoneally with 3×10^5 plaque-forming units (PFU) of virus. Virus titer was determined by plaque

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TABLE 1
Glucose indices of various strains of mice infected with the D variant of encephalomyocarditis (EMC) virus

Strain	Sex	No. mice		Glucose index (M ± SD)	
		Uninfected	Infected	Uninfected	Infected
NZB	M	10	50	155 ± 36	221 ± 54
	F	10	70	169 ± 13	219 ± 88†
NZW	M	10	20	141 ± 14	223 ± 75
	F	10	10	146 ± 18	226 ± 75†
(NZB × NZW) F_1	M	40	60	157 ± 23	316 ± 103*
	F	40	60	146 ± 11	173 ± 58‡

*The male (NZB × NZW) F_1 group had a significantly greater glucose index ($P < 0.005$) than parental male NZB or male NZW mice.
†Female NZB and NZW groups not different from male controls of the same strain ($P > 0.10$).
‡Female (NZB × NZW) F_1 mice significantly less than male littermates ($P < 0.001$).

assay on secondary mouse embryo (SME) cells.[22] The same virus preparation was used throughout the experiments.

Detection of viral antigens by direct immunofluorescence. Antiserum to EMC virus was prepared in SJL/J mice and labeled with fluorescein isothiocyanate (FITC) as described previously.[23] Cryostat sections (5 μm thick) of tissue from uninfected and infected animals were fixed in acetone for 5 min at room temperature, flooded with FITC-labeled anti-EMC serum, incubated at 4°C overnight, and examined by fluorescence microscopy.

Glucose and insulin assays. Blood samples were collected by retro-orbital venous plexus puncture. Glucose levels were measured by the glucose-oxidase method.[24] Blood glucose tolerance tests were measured by obtaining samples 60 min after intraperitoneal injection of 2 mg glucose/g weight. Nonfasting (NF) glucose levels were measured 7 and 14 days after infection, and glucose tolerance tests (GTT) were performed 10 and 17 days after infection. These four values were then combined to give the glucose index as previously described.[25] The mean glucose index of 80 uninfected mice was 162 ± 16 mg/dl. Any mouse with a glucose index greater than 210 mg/dl, which is 3 SD above the mean of uninfected controls, was considered to be diabetic. The concentration of insulin in the pancreas of the infected and uninfected mice was measured by radioimmunoassay.[26]

Neutralization test. Approximately 100 PFU of EMC virus was incubated with an equal volume of serial twofold dilutions of serum from infected mice for 45 min at room temperature. The reaction mixture was then plated on SME cells, and the number of plaques were counted 3 days later.[22] Neutralization titers were expressed as the reciprocal of the highest dilution of serum that inhibited plaque formation by 50%.

Islet cell antibodies. Pancreas from uninfected mice were frozen on dry ice and then cut. The cryostat sections (5 μm in thickness) were incubated with various dilutions of sera from diabetic or normal mice and stained with fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse IgG (heavy

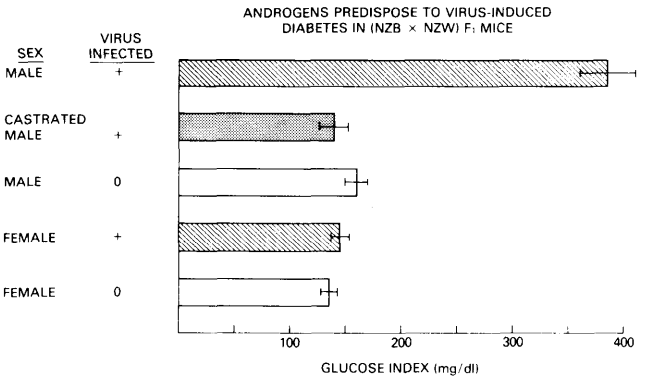

FIGURE 1. The effect of castration on the induction of diabetes in (NZB × NZW) F_1 males infected with the D variant of EMC virus. Sham castrated mice were used as either positive (infected) or negative (uninfected) controls. Vertical bars delimit the standard errors of the means. There was an average of 35 mice per group.

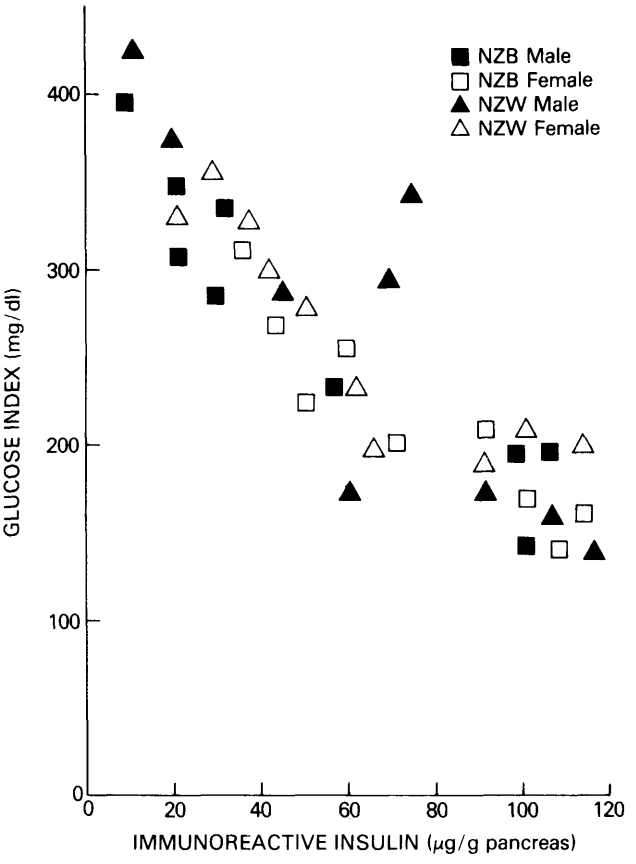

FIGURE 2. Relationship of concentration of blood glucose (GI) in the plasma to the concentration of immunoreactive insulin (IRI) in the pancreas after infection of NZB and NZW mice with 3 × 10^5 PFU of the D variant of EMC virus. There was a strong inverse correlation between GI and IRI ($P < 0.001$).

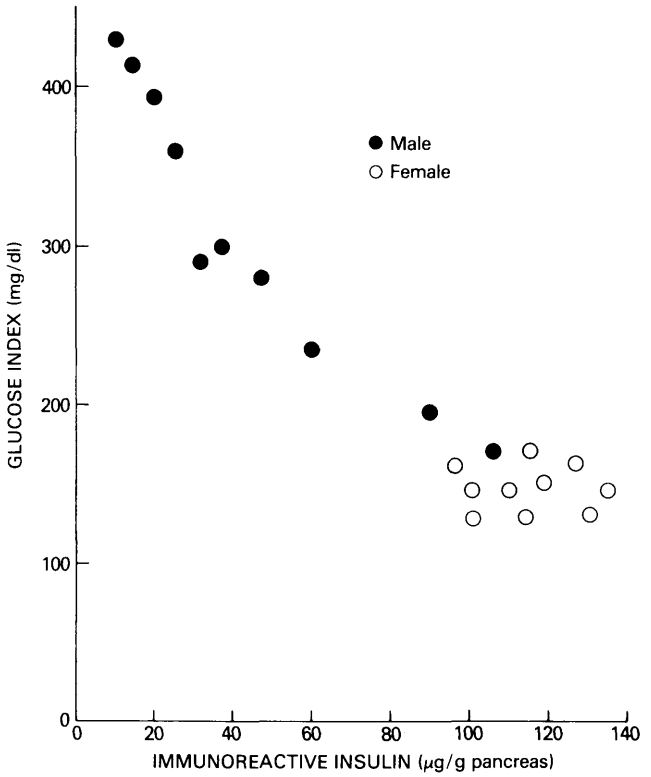

FIGURE 3. Relationship between glucose index (GI) and pancreatic immunoreactive insulin in (NZB × NZW) F_1 mice. There was a strong inverse relationship between the two measures in males ($P < 0.001$); however, the females all had normal measures of both.

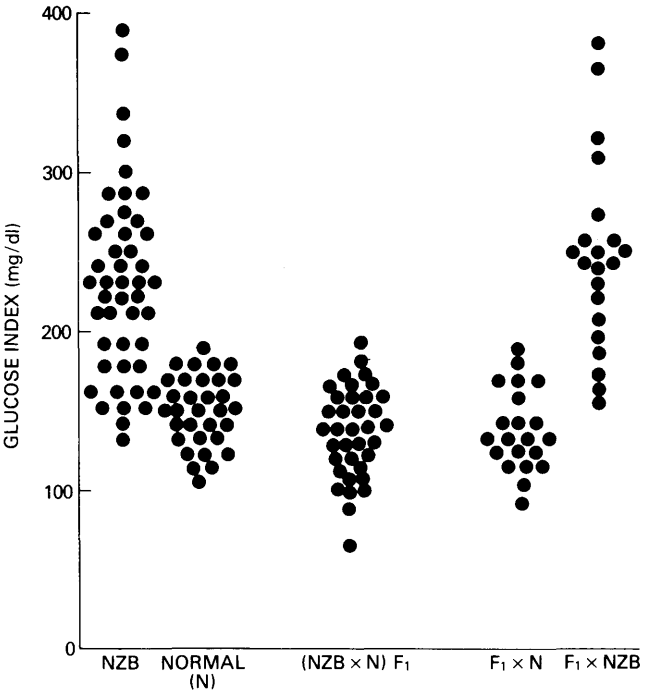

FIGURE 4. The glucose indices for each male mouse from the experiments for the F_1 generation and backcrosses. The normal mice were BALB/c and C3H/HeN. The F_1 generation includes (NZB × C3H) F_1 and (NZB × BALB) F_1 mice. Only NZB and backcrosses to NZB had abnormal glucose indices indicating a recessive form of inheritance.

and light chain; Miles Laboratories, Inc., Elkhart, Indiana) at 4°C overnight.[27] Background staining was assessed by using normal mouse sera.

Surgery. Twenty-day-old mice were anesthetized intraperitoneally (i.p.) with sodium pentobarbital. Testes and epididymes were removed through a scrotal incision. Skin was closed with 6-0 silk sutures. Sham operations consisted of exposing the organs without removing them.

RESULTS

Induction of diabetes and the effect of sex. NZB and NZW parental strains and their F_1 offspring proved to be susceptible to EMC virus-induced diabetes (Table 1). Glucose indices of the infected mice were significantly higher ($P < 0.001$) than those of uninfected mice. In the parental strains, the induction of diabetes was independent of sex. In contrast, male F_1 mice were much more susceptible to the induction of diabetes than were their female littermates ($P < 0.001$) (Table 1). This difference was attributable to the sex hormone differences in the F_1 mice. Castration of male F_1 mice prevented the development of diabetes (Figure 1).

Studies of pancreatic immunoreactive insulin (IRI). Twenty days after infection, the concentration of IRI in the pancreas was determined and plotted against the concentration of glucose in the blood. In the parental NZB and NZW strains, the concentration of IRI in the pancreas of mice infected with the D variant was inversely related to the glucose level (Figure 2). This was true of both sexes (Figure 2). There also was an inverse relationship between blood glucose and IRI concentration in male F_1 mice; however, female F_1 mice all had normal amounts of IRI (≥80 μg/g) and normal glucose levels (Figure 3).

Pancreatic beta-cell damage. Islets from animals infected with EMC virus revealed infiltration of lymphocytes and disruption of the architecture of the islets of Langerhans. (Data not shown.) When stained with FITC-labeled antibody to EMC virus, viral antigens were seen in the cytoplasm of beta-cells within 3 days after infection. (Data not shown.) Since there is a strong correlation between viral antigen in islets and infiltration by inflammatory cells,[28] it is not possible to say whether the inflammatory cells or the preceding viral infection is responsible for the islet cell failure and the development of diabetes.

Genetic studies. In view of the finding that (NZB × NZW) F_1 males had significantly greater glucose levels after EMC infection than did either parental strain (Table 1), additional genetic studies were performed. The frequency distributions of the glucose indices of NZB males and their male offspring are plotted in Figure 4. The male F_1 hybrids resulting from the cross between susceptible autoimmune NZB and normal resistant C3H and BALB/c mice were resistant to the development of EMC virus-induced diabetes (Figure 4). Backcrosses to the normal resistant parent were also resistant.

$$\underset{\text{Resistant}}{\text{NZB } (R^aR^a, R^BR^B)} \times \underset{\text{Resistant}}{\text{NZW } (R^AR^A, R^bR^b)}$$
$$\underset{\text{Sensitive}}{(\text{NZB} \times \text{NZW})\ F_1\ (R^AR^a, R^BR^b)}$$

FIGURE 5. Genetics of androgen resistance and sensitivity in New Zealand mice. Resistance occurs when either the A locus or the B locus is homozygous for resistance, designated by a capital letter.

When the F_1 hybrids were crossed with the susceptible NZB parents, many of the offspring had elevated glucose levels after infection with EMC virus (Figure 4).

Immune responses. Neutralizing viral antibody could be detected within 1 wk after infection (mean titer: 160), increased slightly at 14 and 31 days after infection (mean titer: 640), and then slightly declined (mean titer at 60 days = 320). There was no significant difference between the sexes in NZB, NZW, or (NZB × NZW) F_1 mice in the development of anti-viral antibody. Sera of New Zealand mice (NZB, NZW, and F_1) were tested for autoantibodies against islet cells. Islet cell antibody was not detected in sera of mice infected with the D variant of EMC virus at 7, 14, 21, 30, and 60 days.

DISCUSSION

The present studies extend our information regarding EMC-induced diabetes in mice.[29] First, the autoimmune New Zealand strains were susceptible to virus-induced diabetes. Second, males and females of parental NZB and NZW strains were equally affected, whereas males are usually much more susceptible than females.[29-31] Third, the equal susceptibility of the two parental strains was not inherited by their F_1 offspring. In the (NZB × NZW) F_1 mice, males, but not females, were susceptible to virus-induced diabetes. Finally, the sex differences were found to be related to androgens in the F_1 mice in that castration of the males rendered them insusceptible.

Very few mouse strains have been shown to be susceptible to the development of EMC virus-induced diabetes. Both the SJL and New Zealand susceptible mice share autoimmune features, suggesting that a predisposition to autoimmunity might be critical to the development of this disorder.[32] These additional New Zealand strains may allow further study of virus-induced diabetes.

The genetics of the EMC virus-induced diabetes was most exceptional and unexpected. Previous study of two susceptible strains (e.g., SWR and SJL) led to F_1 male offspring that had milder diabetes than either parental strain.[29,30] In contrast, in the present study, the (NZB × NZW) F_1 males had more severe EMC virus-induced diabetes than did either parental strain. This result suggests that each parent may have contributed susceptibility. However, a single recessive gene has been suggested for the susceptibility to EMC-induced diabetes[29] and is supported by the present studies of mating NZB mice with insusceptible normal strains. Therefore, another mechanism must be invoked. A clue comes from the equal susceptibility of male and female parental NZB and NZW strains to EMC-induced diabetes but a marked susceptibility in male, but not female, F_1 mice. These findings suggest that a gene for androgen insensitivity is present in the parental NZB and NZW strains. However, if the gene were at the same locus in the two strains, the F_1 mice would be expected also to share in a lack of sex difference. The finding of a marked sex difference between male and female (NZB × NZW) F_1 mice, but not in either parental strain, suggests that different loci are involved in the two parental strains. Moreover, both must be recessive. A diagram indicating these genetic patterns is shown in Figure 5.

The above possibility is supported by previous findings of androgen insensitivity of NZB mice with regard to anti-T cell autoantibodies.[33] In other words, in another system the parental NZB strain demonstrates a lack of sensitivity to sex hormones, but its F_1 offspring do have normal sensitivity to sex hormones.[33]

The studies of EMC virus-induced diabetes in the New Zealand strains raise interesting questions regarding the basis for the susceptibility. Some clue may be found in the sensitivity of the parental NZB and NZW females but not the (NZB × NZW) F_1 females. Recent studies have suggested that maturation of T cells within the thymus is dependent upon sex hormones and that the (NZB × NZW) F_1 mice demonstrate this sensitivity quite well.[34] It appears that estrogens lead to relatively greater maturation of helper cells and relatively poorer maturation of suppressor cells. In contrast, the androgens lead to greater maturation of suppressor cells and poorer maturation of helper cells. Thus, it might be expected that androgens would tend to alter the susceptibility to virus-induced diabetes by an action on the immune system. Castration of the male F_1 mice abolished their susceptibility, suggesting that androgens influence the susceptibility to a greater extent than estrogens. Moreover, it is known that the parental strains do not have the normal androgen sensitivity manifested by the F_1 mice with regard to either EMC-induced diabetes or other immune functions.[33] Therefore, it appears that both genetic susceptibility to virus-induced diabetes and sex hormones may determine whether or not diabetes will occur. In strains that are not susceptible to the virus, diabetes will not occur. In strains that are susceptible to the virus, diabetes will occur; however, in a subset of those strains there is normal sex hormone sensitivity. In those strains, males are susceptible and females are resistant. In other strains that do not manifest normal sensitivity to sex hormones, both males and females are susceptible. Further study of the relationships between the viral infection in susceptible strains, sex hormone sensitivity, and the immune system should provide even greater insights into the pathogenetic mechanisms in virus-induced diabetes.

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