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Short Communications

Testosterone Effect on Experimental Diabetes Mellitus in Encephalomyocarditis (EMC) Virus Infected Mice

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Summary. A diabetes mellitus-like disease occurs in male DBA/2 mice infected with the M variant of the encephalomyocarditis virus. Female mice of this strain sustain systemic infection, but rarely exhibit hyperglycaemia. The diabetogenic effects of the virus were studied in 3 groups of adult DBA/2 males-castrates, castrates treated with testosterone, and sham-operated controls. After infection, pancreatic insulin concentrations decreased precipitously to approximately 10% of control values in intact males and castrates treated with testosterone; hyperglycaemia occurred concomitantly in both groups. In contrast, untreated castrates failed to develop hyperglycaemia and the effect on the insulin content of the pancreas was less striking.

Key words: Encephalomyocarditis virus, diabetes, testosterone, mice, immunological reactive insulin, islets of Langerhans.

The M variant of EMC virus induces a diabetes mellitus-like disease in several strains of mice [1–7]. In most of these strains, hyperglycaemia is observed in infected male animals, but occurs inconsistently, or not at all, in females [1, 8]. The studies reported here were undertaken to determine the influence of male sex hormone on the diabetogenic effect of the virus.

Materials and Methods

Animals

Male, 10-week-old, DBA/2J mice, weighing 20–25 g were housed in individual cages on a twelve hour light-dark cycle and provided with water and Purina Lab Chow ad libitum. Glycosuria was not present in mice before infection.

Mice were inoculated routinely by the subcutaneous route with 20–30 PFU of the M variant. The source and properties of this variant of EMC have been described [5, 9].

Biochemical Studies

Glucose determinations were carried out in duplicate on heparinized whole blood using a glucose oxidase micro-method. Glycosuria was assessed with glucose-oxidase, chromogen-impregnated paper strips. Pancreatic IRI was determined on extracted homogenates of tissue [11] using solid-phase radioimmunoassay. (Phadebas Insulin Test, Pharmacia).

Testosterone

Testosterone enanthate was diluted in sesame oil, and mice were injected with 1 mg in 0.1 ml subcutaneously [12] at intervals of seven days. Controls received an equal volume of sesame oil. This hormone dosage was shown to be physiologically effective by Browning et al. [13]. Using the submaxillary gland bioassay of Junquera et al. [14] we found it to be effective in DBA/2 mice.

Experimental Design

Two studies were performed. In the first mice were allocated to three groups of approximately 30 each. Animals in two groups were castrated, and members of the third were sham-operated. One group of castrates was given testosterone, while animals in the other two groups received sesame oil. Seven days later, the mice were inoculated with virus. Urine was checked for glycosuria on days 1 through 7 and on alternate days for the next 2 weeks. Survivors were sacrificed on day 21.

The second experiment was similar to the first except that 5 to 7 animals from each group were killed at 2-day intervals for the first 2 weeks of the experiment to assay blood glucose and pancreatic IRI contents. To avoid selection mice were assigned to designated groups for sacrifice at specific times after virus inoculation. On day 21, 5 surviving animals were selected randomly for sacrifice. Uninfected control animals were studied on days 7 and 15.

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Table 1. Glycosuria and mortality in EMC-infected mice

	n	Duration of glycosuria			Numbers with glycosuria (% total)	Number dead (% total)
		7 days	8–14 days	15–21 days		
Sham	24	5	0	6	11 (45%)	5 (21%)
Castrate	28	1	0	1	2 (7%)	1 (4%)
Castrate & testosterone	26	8	3	7	18 (69%)	6 (23%)
					$p < 0.005$	$p < 0.10$

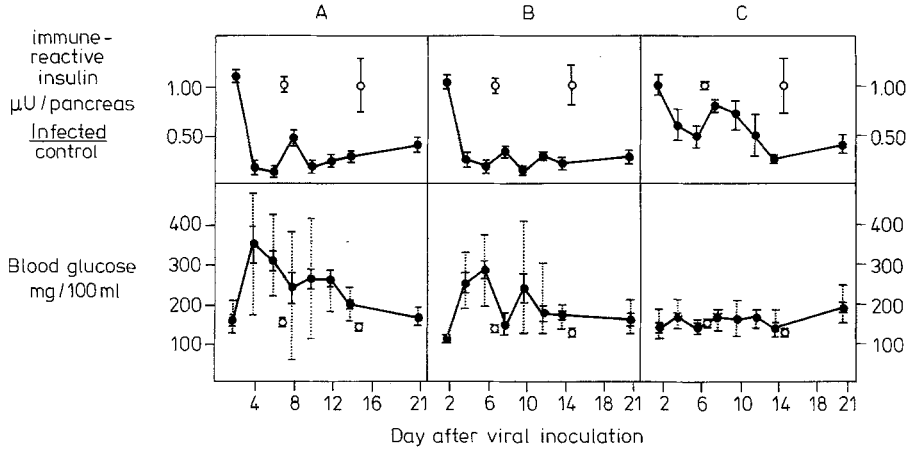

Fig. 1. Response of 10-week-old, DBA/2 male mice to infection with the M variant of EMC virus. Sham-operated (A), castrates treated with testosterone (B), and castrates without treatment (C). Determinations at days 7 and 15 (unconnected circles and boxes) indicate values obtained on uninfected control animals, treated as described above. Solid bars indicate SEM; dotted bars for glucose determinations indicate range. Each point represents 5 to 7 animals

Results

Glycosuria occurred frequently and mortality was high in both the sham-operated and castrate-testosterone-treated animals after virus inoculation (Table 1). Since several mice died early in the course of the experiment without glycosuria, it was not possible to calculate an absolute incidence of glycosuria in the study groups.

Results of studies on animals killed at 2-day intervals after virus inoculation are summarized in Figure 1. The severity of the hyperglycaemia in infected mice was variable. Nonetheless, the mean blood glucose concentrations in the sham-operated, testosterone-treated castrate groups were elevated substantially during the first 2 weeks of the experiment. Significant alterations in blood glucose concentration were not detected in the untreated castrates.

An abrupt decrease in the mean pancreatic IRI was noted early in the course of the infection in both the sham-operated and testosterone-treated castrates. In contrast, a modest reduction in pancreatic IRI occurred as the infection progressed in the untreated castrates.

Discussion

The effect of sex on the susceptibility of mice to EMC virus was studied by Friedman et al. [15]. They showed that the virus was more lethal for males than for females and male castrates. Testosterone increased the mortality of females and of both male and female castrates when administered before virus inoculation.

Using the M variant of EMC, Boucher et al. [16] noted that hyperglycaemia was more severe in male DBA/2 mice than in females. Moreover, blood glucose concentrations in male castrates were substantially lower than in non-castrates. These workers failed to observe differences between intact males and castrates in the distribution of viral antigen in the beta cells and the severity of histologically recognizable insular damage. Our unpublished studies confirm this latter observation.

It is clear that the depletion of pancreatic insulin in sham-operated males and in testosterone-treated castrates occurs early in the course of infection and is substantially greater than in castrates. Since testosterone does not appear to affect the susceptibility of beta cells to the virus, the basis for its effect on viral induced degranulation is unclear.

In our studies, aldehyde-fuchsin stained histologic sections of the pancreas failed to define the impact of infection on beta cell granulation. Thus, assays of pancreatic IRI provide a more meaningful assessment of the insulin reserves of the pancreas.

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