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Glucose Tolerance and Pancreatic Ultrastructure in Mice with Long-Term Diabetes Induced by EMC Virus (M Variant) (38518)

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The M variant of the encephalomyocarditis (EMC) virus causes severe morphological lesions in the heart and in the endocrine pancreas of infected mice (1–6). Damage to the islets of Langerhans is often severe enough to induce a diabetes mellitus-like syndrome (2, 4, 6–8). Thus far, only short-term observations comprising the first few weeks after infection have been recorded. The present study extends the time of observation to a full year.

Material and Methods. A total of 50 male, 12–16 wk old CD-1 mice, procured from Charles River Breeding Laboratories, Wilmington, MA, were utilized. Their weights ranged from 25 to 40 g, with most weights falling between 30 and 38 g. The mice were housed in cages each containing four animals and were given Purina mouse chow and tap water *ad libitum*.

We are indebted to Dr. John E. Craighead, Department of Pathology, College of Medicine, University of Vermont, for having supplied us with the M variant of the EMC virus. Virus for subcutaneous injection was assayed prior to inoculation utilizing mouse embryo fibroblasts and L-2 cells (9). Based upon the results of the plaque assay, the virus pool concentrate was diluted to yield either 20 or 80 plaque-forming units (PFU) per injectable dose.

Forty-two mice were injected subcutaneously with 0.1 ml of the suspension containing the virus; the remaining eight animals received 0.1 ml of physiological saline solution each and served as controls. Repeated glucose tolerance tests were performed on all mice surviving the acute phase following viral infection. For each such test 100 mg of glucose, as a 50% solution in water, were injected intraperitoneally, and blood for glucose determination (10) was withdrawn from the orbital plexus one, 2 and 5 hr later.

Two of the mice were sacrificed, by cervical dislocation, 4 and 7 days after the inoculation of the virus in order to confirm the efficacy of the viral infection by assessing the extent of the morphological damage in heart and pancreas. All other animals were killed eleven to 12 mo after infection. Their pancreases were removed and processed for light and electron microscopic study with methods detailed previously (4).

Results. Of 42 infected mice, two were sacrificed during the acute stage of the experiment. The morphologic changes observed in their hearts and pancreases corresponded to those recorded in earlier reports (1–6). Thirteen additional animals expired spontaneously, most between the third and fourteenth day after inoculation. Twenty-seven mice survived until the conclusion of the experiment. Of eight uninfected control animals, one died prematurely.

The blood glucose values measured during the acute phase followed the biphasic pattern described earlier (4): They peaked on the second or third day after infection whereas a second, less pronounced rise took place during the second week. After the third or fourth wk of the experiment, the fasting blood glucose values of all but three animals had returned to normal or near normal (110 mg/100 ml, or lower). Only three animals maintained persistently high fasting blood glucose values (above 150 mg/100 ml), and only these three mice displayed unequivocally diabetic glucose tolerance curves even after a full year of observation (Fig. 1). Two of the three animals had been infected with a dose of 80 PFU while the third had received 20 PFU.

The light and electron microscopic examination of the endocrine pancreas 11 or 12 mo after infection failed to reveal evidence of islet cell damage or beta cell hyperfunction. The beta cells were well granulated, in-

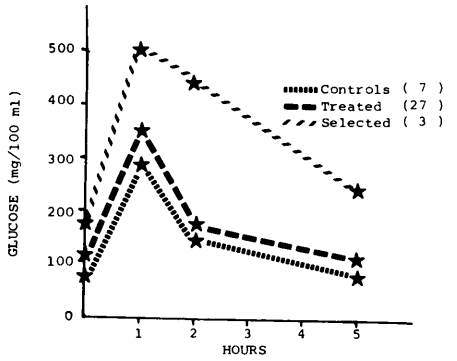

FIG. 1. Glucose tolerance curves (combined averages) 11–12 mo after infection, for seven untreated controls, all infected and surviving animals (n = 27), and three infected mice with a persisting diabetic response.

cluding those of the three animals with abnormal glucose tolerance curves.

Discussion. It has been established by previous work in various laboratories (1–8) that young male CD-1 mice are highly susceptible to an infection with the M variant of the EMC virus. The large majority of all infected animals exhibit a characteristic, biphasic hyperglycemic response during the acute phase of the experiment. At the same time, severe morphologic lesions are demonstrable in the islets of Langerhans; nevertheless, such alterations are usually selective, for damaged and intact beta cells are often seen to coexist side-by-side (4). Many animals do not survive the acute stage of the infection.

The present study shows that of those that do survive for as long as a full year, most do so with complete recovery of their functional and morphological parameters. Islet cell reserves in these animals are obviously of sufficient magnitude to compensate, first by functional adaptation of undamaged beta cells (4) and later probably through neogenesis of islet cells, for the losses sustained during the initial phase of the infectious process.

In a minority of cases (here, 3 of 27 mice, or 11%), functional recovery fails to occur, and impaired glucose tolerance persists for a lengthy period of time, perhaps indefinitely. One would expect to find morphological evidence of cellular hyperfunction in the beta cells of these animals, such as degranulation, vesiculation of the units of the endoplasmic reticulum, and prominence of the Golgi apparatus. Why such changes were not demonstrable in this small group of mice remains, at present, undetermined, but the mere fact that animals with unequivocally diabetic glucose tolerance curves fail to display concomitant endocrine pancreatic alterations is in itself remarkable. In this context, it should perhaps be remembered that human maturity onset diabetes, in many cases, is also accompanied by a conspicuous lack of morphologically obvious changes in the islets of Langerhans. Since the possibility exists that human diabetes mellitus may at times be induced by viruses (11), the findings discussed above assume added significance.

Summary. Of 27 young male CD-1 mice infected with the M variant of the encephalomyocarditis (EMC) virus and surviving for 11 or 12 mo, all but three had normal glucose tolerance curves, and all displayed normal islet cell morphology, at the time of sacrifice, in spite of an initial hyperglycemic response. Three animals maintained diabetic glucose tolerance curves one year after infection but they, too, failed to show significant morphological alterations in their pancreatic beta cells.

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