Rapid Publications

Effect of Genetic Obesity in Mice on the Induction of Diabetes by Encephalomyocarditis Virus

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SUMMARY

The "M" variant of the encephalomyocarditis (EMC) virus causes a diabetes-like disease in some, but not all, strains of mice. The genetic basis for either resistance or susceptibility to the diabetogenic effect of the virus is not known. After infection with EMC, C57BL/6 mice seldom develop hyperglycemia and the insular lesions are subtle. To explore the possible effects of metabolic influences on the viral susceptibility of the islets, we studied C57BL/6 mice that were carriers of the ob gene. After virus inoculation, obese homozygous C57BL/6-ob/ob mice consistently developed hyperglycemia during the acute stages of infection, whereas nonobese littermates did not. Infection induced more severe lesions in the pancreatic islets of obese mice than in islets of the lean littermates. These studies suggest that the functional activity of the beta-cells influences the severity of the viral injury to the beta-cell, and the consequent occurrence of diabetes. DIABETES *30*:451–454, May 1981.

A diabetes mellitus-like disease develops in some, but not all, strains of mice infected with the "M" variant of the encephalomyocarditis (EMC) virus.[1–3] Necrotizing lesions and mononuclear cell inflammation of the islets accompany the metabolic abnormalities and are attributable to viral replication in the beta-cells.[4] The features of this experimental model of human diabetes have been recorded in several recent publications.[5,6]

The susceptibility of mice to the diabetogenic effects of EMC virus is influenced by one or more recessive genes.[1,7] The basis for either the susceptibility or resistance of different strains of mice is uncertain, although Yoon et al.[8] suggested that the density of viral receptors on the beta-cell is a critical, genetically influenced determinant. Studies in our laboratory[9,10] and elsewhere[11] argue against this hypothesis.

Mice of both diabetes-susceptible and -resistant strains sustain systemic infections after inoculation of EMC virus. The time course of the infection appears to be comparable, but mortality differs dramatically from one strain to another. Although the majority of a group of animals of a susceptible strain develop hyperglycemia, its severity and duration are variable. Similarly, resistance to the diabetogenic effect of the virus is relative, since a few infected animals become hyperglycemic during the acute stages of infection and others fail to tolerate a carbohydrate challenge normally during convalescence.[1,6] Although beta-cell lesions can be demonstrated in the islets of resistant strains, they are not prominent and relatively few cells appear to be infected.[1,7] These observations suggest that host factors may influence the severity of the beta-cell injury and the consequent diabetes.

Using mice of the diabetes-resistant C57BL/6 strain that are carriers of the ob gene[12] we undertook studies to determine whether an increase in the metabolic activity of the beta-cells would affect susceptibility to viral injury. During adulthood, homozygotic ob/ob animals develop obesity and hyperglycemia associated with insular hyperplasia and hyperinsulinemia, whereas heterozygotes and mice lacking the gene fail to exhibit these changes. In the experiments reported here we compared the metabolic and pathologic changes induced by the M variant of EMC virus in obese (ob/ob) and lean (ob/+ and +/+) C57BL/6 mice.

MATERIALS AND METHODS

Animals. Male, 10–17-wk-old, C57BL/6-ob/ob mice and lean (ob/+, +/+) colonymates were obtained from Jackson Laboratories (Bar Harbor, Maine). The lean homozygous (+/+) and heterozygous (ob/+) animals are indistinguishable phenotypically and therefore are denoted hereafter as ?/+. Mice were housed individually in shoebox-sized cages and maintained at about 24°C on a fixed 12-h light and dark

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cycle with free access to Purina laboratory rodent chow (protein 23%, fat 4.5%) and water. Phenotypically obese and nonobese animals were allocated randomly to study groups and only mice weighing ±2 SD of the group mean were used. Animals were weighed at weekly intervals during the course of experiments.

Virus. The origin and properties of the M variant of EMC have been described elsewhere.[5,13] The virus inoculum was prepared as a 10% suspension of homogenized heart tissue using balanced salt solution as a diluent. Animals were infected intraperitoneally with approximately 32 tissue culture (L-929 cells) infectious dosages (50% effective) in 0.1 ml.

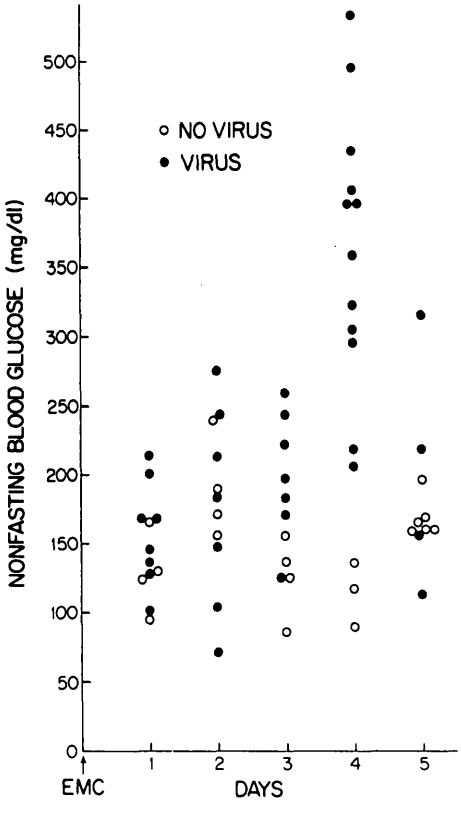
Blood glucose. To reduce the adverse effects of handling and anesthesia, the experimental groups (control and infected) were subdivided and bled in the nonfasting state before and on alternate days after infection. Blood was collected in heparinized microhematocrit capillary tubes from the retro-orbital venous plexus after light ether anesthesia. Samples were held at 4°C and analyzed the same day by the glucose-oxidase method using a glucose analyzer (YSI Model 23A, Yellow Springs, Ohio). Nonfasting blood glucose (NFBG) values greater than ±2 SD from the mean of control, uninfected mice were considered abnormal.

RESULTS

Experiments of two types were carried out. In the first, animals were observed to monitor mortality consequent to infection. In the second, groups of lean and obese mice were bled on alternate days to determine blood glucose concentrations.

Obese mice were 25–30 g heavier than the mean of their littermates by 10 wk of age and blood glucose concentrations were substantially higher (132 ± 25 versus 84 ± 11 mg/dl). Three days after virus inoculation, infected obese animals exhibited signs of illness and the majority (18/20) either died spontaneously or were killed in a moribund state before the sixth day of the experiment. Signs of illness were more subtle in the lean mice and only a few (3/20) died. The deaths of these animals occurred 5–16 days after inoculation. Body weight decreased approximately 3.5% in both the lean and obese animals during the acute stages of infection.

FIGURE 1. Effect of EMC infection on nonfasting blood glucose concentrations in C57BL/6-ob/ob mice. The group mean for uninfected ob/ob controls was 154 ± 36 mg/dl.



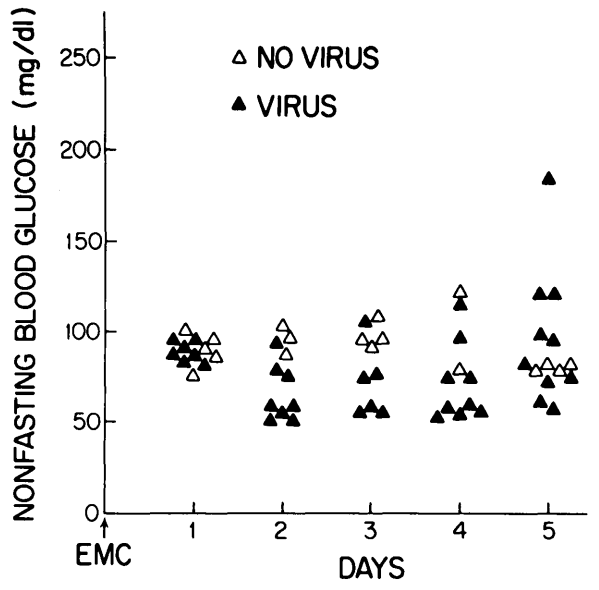


FIGURE 2. Effect of EMC infection on nonfasting blood glucose concentrations in C57BL/6-?/+ mice. The group mean for uninfected ?/+ controls was 91 ± 14 mg/dl.

Since the results of blood glucose determinations in studies using 10- and 17-wk-old mice were similar, the data from experiments using animals of these two ages are combined in Figures 1 and 2. As can be seen in Figure 1, infected obese animals became hyperglycemic 3–5 days after inoculation. In contrast, the lean mice failed to develop elevations of blood glucose during the initial 4 days of the experiments and only one of the 10 animals was hyperglycemic on the fifth day.

Microscopic examination on the pancreas revealed interstitial inflammation in and around the islets of both lean and obese mice (Figures 3C and D). However, insulitis was more prominent in the obese animals. Coagulative necrosis also was a distinct feature of many of the islets in these mice, whereas it was not observed in the insular tissue of the lean mice. Insular lesions in the pancreatic tissue of the ?/+ mice were limited either to individual beta-cells or focal areas in the islets (Figure 3D).

DISCUSSION

The genetic basis for either the susceptibility or resistance of various mouse strains to the diabetogenic effect of the M variant of EMC has not been defined. Yoon et al.[8] proposed that the density of viral receptors on the plasma membrane of the beta-cell accounts for these differences, but studies in our laboratory fail to support this conclusion. We found that cultured beta-cells from both susceptible and resistance strains undergo cytolysis to a similar extent when infected in vitro.[9] Moreover, islets in the pancreas of resistant strains are infected by the virus and degranulate in vivo, al-

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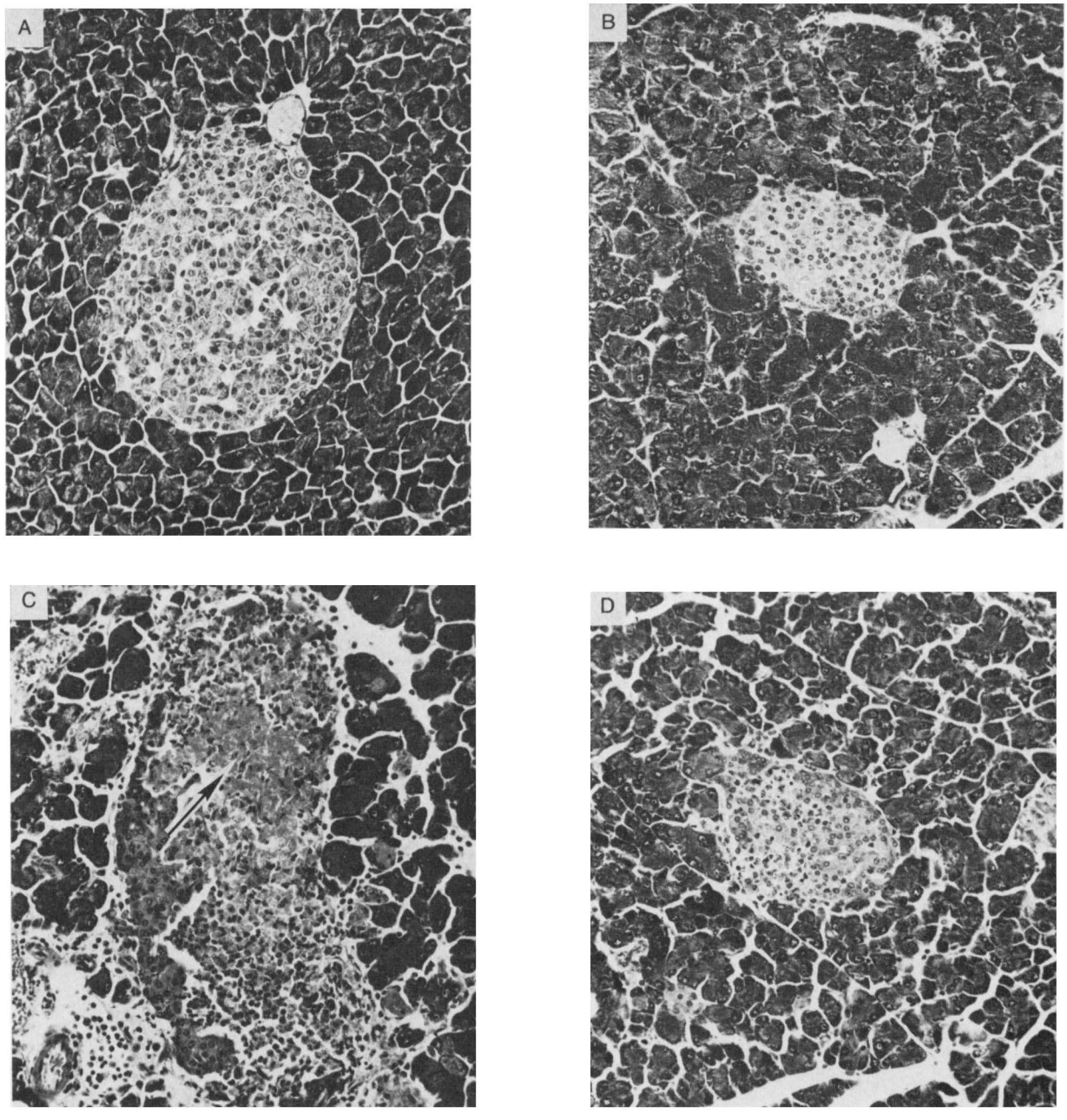

FIGURE 3. Lesions induced by EMC virus in the pancreatic islets of 10-wk-old ob/ob mice and ?/+ colonymates. (A) Hyperplastic islet of uninfected ob/ob mouse. Note the dilated and congested sinusoids. (B) Islet of uninfected ?/+ mouse. (C) Hyperplastic islet of ob/ob mouse 4 days after virus inoculation. Note the prominent cellular infiltrate and coagulative necrosis of beta-cells (arrow). (D) Islet of ?/+ mouse 4 days after virus inoculation. Note the localized infiltrates of mononuclear cells. Islet cell necrosis is present but focal and less severe. Tissues were fixed in Bouin's solution and 5-μm sections stained with hematoxylin and eosin (783×).

beit to a lesser extent than the islets of susceptible strains. These observations suggest that host factors, rather than the intrinsic viral susceptibility of the beta-cell, are important. Interestingly enough, the islets of both resistant and susceptible strains of animals develop coagulative necrosis when corticosteroids are administered after virus inoculation. Recent studies by Dafoe et al.[11] provide additional insight. In their experiments, islets from diabetes-resistant strains were implanted into the progeny of F_1 crosses between susceptible and resistant animals that had been made diabetic with streptozotocin. When the transplant recipients were infected with EMC virus the grafted beta-cells underwent necrosis.

The studies reported here were undertaken to determine whether resistance to EMC-induced beta-cell injury could be overcome when islets were metabolically stimulated by obesity. Homozygous carriers of the ob gene develop insulin resistance and hyperglycemia during adulthood, and the islets become strikingly hyperplastic. We speculated that either the increased metabolic activity of the beta-cells or the accompanying insular hyperplasia (or both effects) increase the susceptibility of the beta-cells to infection and the associated viral injury. Our observations are consistent with the hypothesis, but fail to differentiate between these two possibilities.

It might be asked whether the ob gene that conveys the propensity to obesity is linked to an undefined gene af-

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fecting viral susceptibility. Although this possibility cannot be excluded, it seems a relatively remote consideration. Our studies with a nongenetic murine model of obesity support this conclusion. We investigated the influence of gold thioglucose-induced obesity on the diabetogenic effect of the M variant of EMC virus in diabetes-resistant strains of mice. This chemical causes hypothalamic lesions and, as a result of the associated hyperphagia, obesity develops and islets of Langerhans undergo hyperplasia. When infected with EMC virus, the obese resistant animals became diabetic, as was found with the model of genetic obesity described in this report.

The M variant of EMC virus produces a diabetes-like disease in the murine model described here that strikingly resembles insulin-dependent diabetes in man. Metabolic factors would appear to be important determinants affecting the development of the disease. On the basis of the evidence provided here, it is reasonable to speculate that the susceptibility of human beta-cells to etiologic environmental insults could be influenced by host factors.

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