

Replication of Diabetogenic and Nondiabetogenic Variants of Encephalomyocarditis (EMC) Virus in ICR Swiss Mice¹ (41819)

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Abstract. The replication of the diabetogenic D (EMC-D) and nondiabetogenic B (EMC-B) variants of encephalomyocarditis virus in various tissues of the murine host was determined. Pancreatic insulin levels were also measured. EMC-D replicated in the spleen, pancreas, heart, lung, and intestines, while EMC-B was limited to the spleen and pancreas. EMC-B interfered with the replication of EMC-D in each of the tissues examined. Insulin levels were initially increased by both viruses. By 4 days postinfection, insulin levels were either normal or undetectable in (EMC-D)-infected animals, but were dramatically elevated in those infected with EMC-B.

The D variant of encephalomyocarditis virus (EMC-D) infects and destroys murine pancreatic β cells, producing a disease syndrome similar to human insulin dependent diabetes mellitus (1, 2). The B variant (EMC-B), which is serologically indistinguishable from EMC-D, is nondiabetogenic and has been shown to protect mice against the production of diabetes by EMC-D (2). Little is known about the replication of these viruses within the murine host. The purpose of this investigation was to determine the tissue tropism of each virus during the course of infection, and to correlate replication within these tissues with serum glucose and pancreatic insulin levels. In this communication, we report that while EMC-D was found to replicate in each of the tissues tested, EMC-B was limited to the spleen and pancreas.

Materials and Methods. *Viruses.* The EMC-B and EMC-D variants were propagated and titrated by methods previously described (3). EMC-B was passed once through L929(L) cells followed by one passage through HeLa cells, while EMC-D was passed five times through L, twice through BHK21, and once again through L cells. This cell-passage schedule resulted in EMC-B stock virus which is nondiabetogenic, produces very small discrete plaques in L cells, and is a good inducer of interferon (IFN). In contrast, the EMC-D

variant is diabetogenic, produces large diffuse plaques in L cells, and does not induce the production of IFN. Each virus was diluted in Hank's balanced salt solution containing 2% calf serum (HBSS). Mice were given a single intraperitoneal injection of the diluted virus (0.2 ml) as indicated. In each instance the doses were; EMC-D = 1.2×10^4 plaque forming units (PFU), EMC-B = 2.0×10^4 PFU.

Animals. Male ICR Swiss mice were purchased from Harlan Laboratories, Indianapolis, Indiana, and were housed in groups of 10. At the time of infection, the animals were 10 weeks of age with an average weight of 35 g.

Glucose tolerance test (GTT). At the times indicated, a nonfasting GTT was done on each of the animals. Each mouse received an ip injection of glucose at a concentration of 2 mg/g body wt. After 1 hr the animals were bled and the serum glucose levels determined using a YSI Model 23A glucose analyzer. It has been shown that nonfasting glucose levels demonstrate a greater sensitivity to reduction of insulin secretion than does fasting glucose (4). Mice with GTT levels at least five standard deviations (SD) above control means were considered to be diabetic.

Preparation of tissues. Tissues collected at 0, 1, 2, 3, 4, and 7 days postinfection, were homogenized and sonicated for 30 sec (Heat Systems Sonicator Model W-220F) to free intracellular virus. Cellular debris was removed by centrifugation (2000g for 15 min), and the

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clarified fluids were then filter-sterilized (Millipore, 0.45 μm) and stored at -70°C until ready to be assayed for viral PFU content.

Insulin levels. Pancreatic homogenates were pooled according to normal or abnormal GTT responses to virus infection and assayed for insulin content using a competitive binding radioimmunoassay procedure (Amersham Corp., Arlington Heights, Ill.).

Virus replication. The relative amount of viral replication in each of the tissues is expressed in terms of replication indexes. The replication index is defined as the number of PFU per gram of tissue divided by the challenge dose of virus.

Results. Mice were divided into four groups of 60 animals each. At -24 hr two groups of animals were infected with EMC-B (2×10^4 PFU per mouse), while the other two groups received HBSS. Twenty-four hours later (0 hr), EMC-D was given to one (EMC-B)-infected group and one HBSS-treated group of mice (1.5×10^4 PFU per mouse). The remaining two groups of animals received HBSS. At 0, 1, 2, 3, 4, and 7 days after infection with EMC-D, serums were assayed for virus content and glucose levels, and virus replication in mouse tissues were determined. In addition, insulin levels in the pancreas were measured at each test period.

Serum glucose levels. The data in Table I show that EMC-B was nondiabetogenic but suggest that animals infected with this virus were hypoglycemic at 2 and 3 days postinfection and that serum glucose levels remained suppressed at Days 4 and 7. In mice infected with EMC-D, hypoglycemia at Days 2 and 3 was followed by hyperglycemia at Days 4 and

7. Preinfection of the animals with EMC-B prevented the production of the diabetic state by EMC-D.

Viremia. The viral PFU content in the serums of infected mice was monitored during the course of infection. Viremia was observed up to 2 days postinfection with peak titers of 3.0×10^2 to 4.0×10^4 PFU/ml (data not shown).

Virus replication in mouse tissues. Figures 1a and b show that both EMC-B and EMC-D were found in high concentration and apparently replicated in pancreatic and splenic tissues. It is also evident that the replication of EMC-D was more pronounced than that of EMC-B, especially at Day 2 in pancreata of animals with hypoglycemia (abnormal GTT). These data also show that the concentration of EMC-D was much greater in the spleen than in the pancreas in mice that did not develop hypoglycemia (Normal GTT). The replication index of EMC-D in these two organs was markedly diminished in animals preinfected with EMC-B.

Figures 1c-e show that EMC-D replicated to a very limited extent in heart, lung, and intestinal tissues. They also indicate that even though EMC-B apparently did not replicate in these tissues, it blocked the multiplication of EMC-D.

Pancreatic insulin levels. Figure 2 depicts the levels of insulin obtained from the pancreata of each group of animals. Both EMC-D and EMC-B increased the levels of insulin 24 hr postinfection. In (EMC-D)-infected mice that developed hypoglycemia by Day 3, and hyperglycemia by Day 4 (abnormal GTT), the concentration of insulin dropped to unde-

TABLE I. GLUCOSE TOLERANCE IN MALE ICR SWISS MICE INFECTED WITH EMC-D AND EMC-B VIRUS

Treatment ^a at:		1 hr GTT mg% (mean) at Day:						
-24 hr	0 hr	0 ^b	1	2	3	4	7	
EMC-B	HBSS	207	179	166	202	199	204	
EMC-B	EMC-D	201	171	190	184	213	231	
HBSS	EMC-D	190	207	140 ^c	203 ^c	419 ^d	418 ^d	
HBSS	HBSS	187 \pm 50	205 \pm 31	227 \pm 28	254 \pm 46	225 \pm 26	223 \pm 38	

^a Male ICR Swiss mice (10 per group) given an intraperitoneal injection (0.2 ml) of either EMC-B (2×10^4 PFU), EMC-D (1.2×10^4 PFU), or Hank's balanced salt solution (HBSS) at the times indicated.

^b Mice were sacrificed immediately after injection of either virus or HBSS at 0 hr.

^c Over 50% of the animals were hypoglycemic (serum glucose levels at least 2 SD below control means).

^d Over 70% of the animals were diabetic (serum glucose levels at least 5 SD above control means).

tectable levels by Day 3. Animals that did not develop the diabetic state, had levels of hormone which were essentially the same as those in uninfected controls. Concentrations of insulin in (EMC-B)-infected mice were essentially normal at 3 days postinfection, but were dramatically elevated at 4 and 7 days. Subsequent experiments have shown essentially the same results (data not shown).

Discussion. The data in this study show that the diabetogenic EMC-D and nondiabetogenic EMC-B differ in their tissue tropism and relative degree of replication in the murine host. While EMC-D was found to replicate to substantial levels in the pancreas and spleen, and to a lesser extent in heart, lung, and intestinal tissues, multiplication of EMC-B was limited to the spleen and pancreas.

The main targets of EMC-D appear to be the pancreas and the spleen where marked virus replication was observed within 48 hr postinfection (Figs. 1a, b). The high concentration of virus in the pancreas at Day 2 correlates with the hypoglycemia evident at this time (Table I). The hypoglycemia state is probably a reflection of beta cell degranulation which results from the replication of this lytic virus (5, 6). Destruction of the beta cells led to the diabetic state seen on Days 4 and 7 (Table I). It is interesting to note that in animals which did not develop an abnormal GTT in response to EMC-D, the replication of the virus was suppressed in pancreatic tissue, but was elevated in the spleen, a lymphocyte-rich organ. This might allow for more efficient clearance of the virus, preventing it from infecting and destroying sufficient numbers of pancreatic β cells to cause the diabetic state.

Previous studies have shown that the production of diabetes by EMC-D can be prevented by prior exposure of the animal to EMC-B (2, 7). This observation was confirmed in the present study (Table I). It has been suggested that this inhibition is due to interferon (IFN) produced in response to EMC-B (2). Subsequent studies, however, indicate that ICR Swiss mice are not protected against (EMC-D)-induced diabetes by IFN, and that in this mouse strain protection by EMC-B is probably due to other undetermined mechanisms (7). One possible mechanism is suggested by the data in Fig. 1a which show that

EMC-B replicates in the pancreas. It is probable that (EMC-B)-infected pancreatic β cells become resistant to superinfection by EMC-D. It is also evident that when compared to that of EMC-D, the replication of EMC-B was very limited in this organ. This observation implies that EMC-B destroys a limited number of pancreatic β cells, which allows the animals to recover and not develop diabetes. The mechanism(s) limiting the replication of EMC-B within the pancreas is not known. Although the IFN system cannot be ruled out, we have been unable to detect its presence in extracts of pancreatic tissue.

It is evident that EMC-B interfered with the replication of EMC-D in each of the tissues examined. The protection of tissues against EMC-D appears to result from more than one mechanism. The spleen, like the pancreas, is probably at least partially protected by the active replication of EMC-B. Protection of heart, lung, and intestinal tissues, which apparently did not support the replication of EMC-B, could be due to the action of IFN. EMC-B has been shown to induce the production of IFN in mice (2), and in the present study we found circulating IFN levels of up to 200 PR₅₀ units in mice infected with this virus (data not shown). The EMC-D variant has been reported to respond to the protective action of IFN (7).

A comparison of pancreatic insulin levels revealed significant differences in the pathological effects between the two viruses (Fig. 2). The variability of the levels of insulin in control animals probably reflects their non-fasting state. The initial increase in pancreatic insulin levels seen at 24 hr postinfection with either virus could result from an increase in the metabolic activity of beta cells as they synthesize viral components and produce mature virions. At 2 and 3 days following virus inoculation, significant decreases in insulin levels were noted in both groups of test animals. By Day 3, insulin was undetectable in (EMC-D)-infected mice which developed diabetes. In contrast, insulin levels were normal in nondiabetic, virus-infected animals, and remained normal throughout the rest of the test period. These observations suggest that the amount of insulin in the pancreata of these mice reflects the extent of beta cell damage. In (EMC-B)-infected animals, pancreatic insulin con-

REPLICATION OF EMC VIRUS

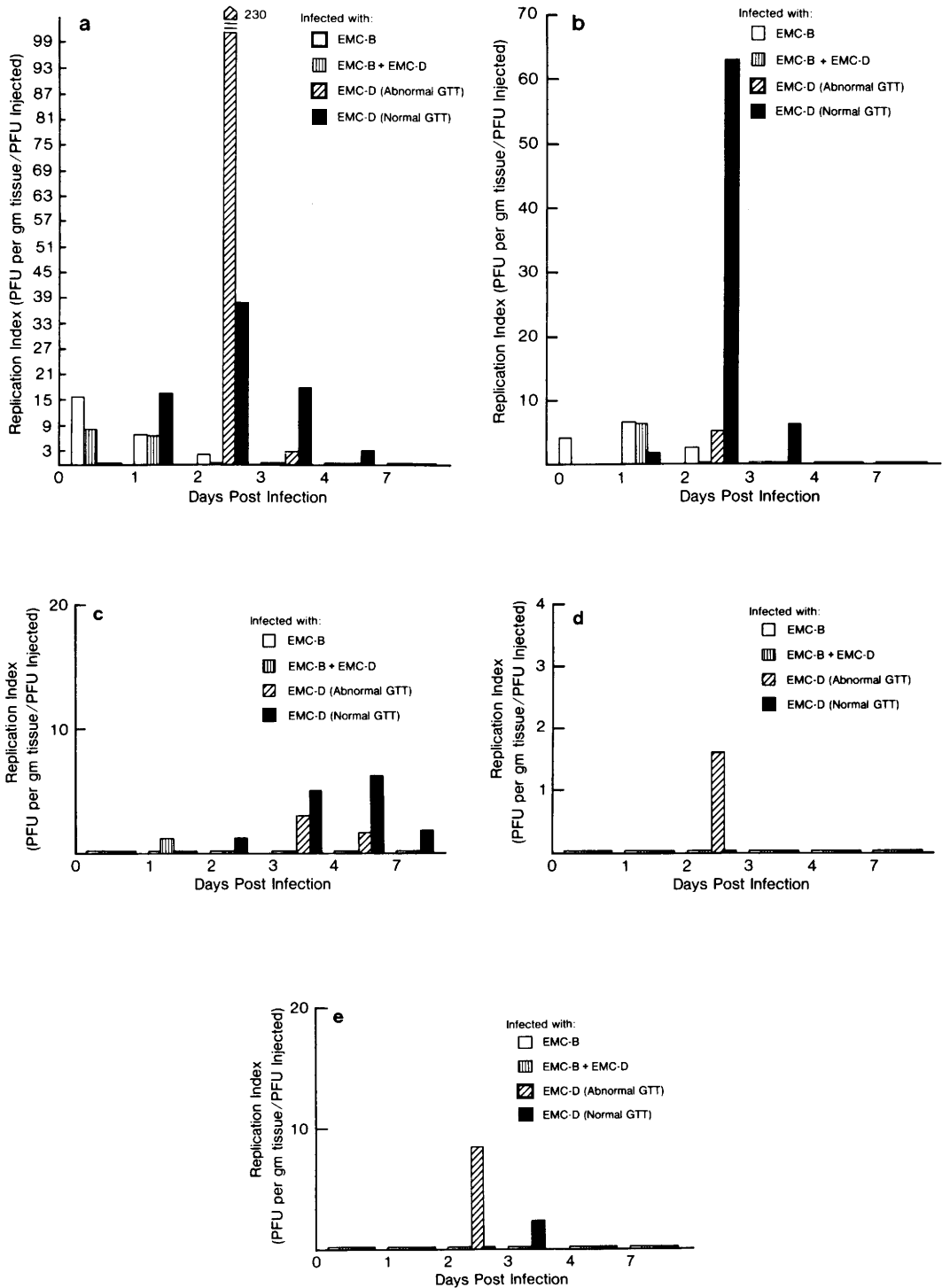


FIG. 1. Virus replication in pancreatic (a), splenic (b), heart (c), lung (d), and intestinal (e) tissues. EMC-B was given 24 hr prior to EMC-D. Tissue extracts were assayed for viral PFU content at 0, 1, 2, 3, 4, and 7 days after infection with EMC-D. Abnormal GTT before Day 4 = hypoglycemia; after Day 3 = hyperglycemia.

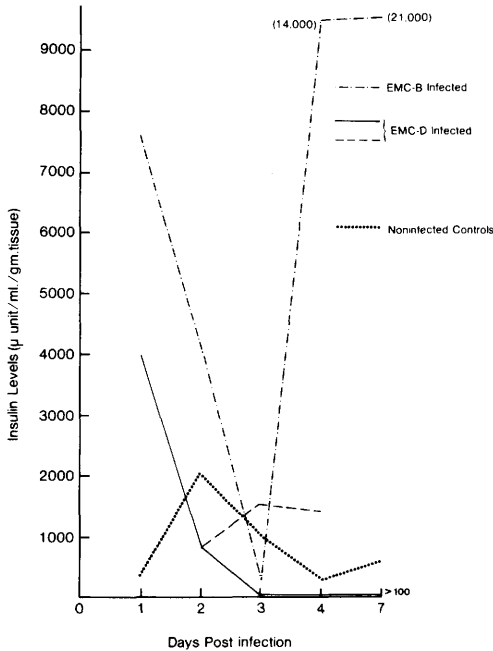


FIG. 2. Pancreatic insulin levels. Mice were infected with either EMC-B or EMC-D and the levels of insulin in pancreatic extracts determined at the times indicated. (EMC-D)-infected animals were separated into those with an abnormal GTT (—) and those with a normal GTT (---).

centration increased dramatically at 4 and 7 days postinfection. This increase may represent an attempt by the β cells to compensate for the viral insult. The high levels of insulin correlates with the chronic state of hypoglycemia seen in animals infected with this virus

(Table I). The mildness of the hypoglycemic state indicates that most of the hormone was retained within the β cells, and that the release mechanism was not impaired.

In vitro systems will be utilized to better identify the mechanisms involved in the observations made in this study.

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