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Inhibition of Virus-Induced Diabetes Mellitus by Interferon Is Influenced by the Host Strain[1] (41651)

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Abstract. The diabetogenic variant of encephalomyocarditis virus (EMC-D) induces a diabetes-like syndrome in certain strains of mice. A study was done to determine if virus-induced diabetes could be prevented by interferon (IFN). It was found that the production of diabetes by EMC-D was blocked by either IFN_β or a variety of IFN-inducers in SWR/J, but not ICR Swiss mice. The replication of EMC-D in cell culture was inhibited by IFN_β. It is concluded that the response of pancreatic beta cells to the protective effect of IFN, is probably under genetic control.

Previous studies suggest that the induction of diabetes in mice by encephalomyocarditis (EMC) virus is sensitive to the protective action of interferon (IFN). Gadzik *et al.* (1) reported that a single injection of the interferon-inducer poly rI-rC (poly I:C) protects a significant number of SWR/J male mice against the production of diabetes by the M variant of EMC virus (EMC-M). Yoon *et al.* (2) found that the nondiabetogenic B variant (EMC-B) inhibits the induction of diabetes by the diabetogenic D variant (EMC-D). The same study showed that EMC-B induces the production of high levels of IFN in mice. It was concluded that IFN is at least partially responsible for the inhibitory effect of EMC-B on infection by EMC-D. In the present study, data are presented which show that IFN does not protect all mouse strains against virus-induced diabetes.

Materials and Methods. *Viruses.* The MM (EMC-MM), D (EMC-D), and B (EMC-B) strains of EMC virus were propagated and titrated in L929 (L) cells employing methods previously described (3). Virus dilutions were made in Hank's balanced salt solution containing 2% calf serum (HBSS). Experimental animals were given a single intraperitoneal (ip) injection (0.2 ml) of each virus as indicated. In each instance, the doses were; EMC-D = 1.2×10^4 plaque-forming units (PFU), EMC-MM = 4×10^4 PFU, and EMC-B = 1.2×10^5 PFU.

Animals. Male, ICR Swiss and SWR/J mice were purchased from Harlan Laboratories, Indianapolis, Indiana and Jackson Laboratories, Bar Harbor, Maine, respectively. At the time of inoculation with the virus, the animals were 9–12 weeks of age with an average weight of 27–30 g. Twenty mice per group were used in each of the experiments.

Glucose assay. Seven days after receiving EMC-D, 2 mg glucose/g body weight were given ip to each of the animals. After 1 hr the mice were bled and the serum assayed for glucose content on a YSI Model 23A glucose analyzer. We have found that the 1-hr glucose tolerance (GT) correlates very well with glucose indices as determined by Yoon *et al.* (4). Mice with GT levels at least five standard deviations (SD) above control means were considered to be diabetic.

Interferon inducers. Endotoxin (*Escherichia coli* 0:128:B12 lipopolysaccharide B) was purchased from Difco Laboratories Inc., Polyinosinic polycytidylic acid (poly I:C) was obtained from Sigma Chemical Company, Maleic acid-divinyl ether copolymer (pyran) was generously supplied by Hercules Inc., and tilorone hydrochloride (tilorone) was a gift from the William S. Merrell Company. Each inducer was administered ip in 0.2-ml volumes of HBSS which contained 100 μg of endotoxin, 150 μg poly I:C, 200 μg pyran, or 2.4 mg tilorone, respectively.

Interferon. Concentrated, partially purified L cell IFN_β was prepared by methods previ-

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ously described (5). Interferon activity was determined by the 50% plaque-reduction assay (PR_{50}) technique using EMC-MM as the challenge agent (6). We have determined that each PR_{50} unit by this method is equivalent to 0.5 NIH (G002-904-511) reference units. Mice were given a single ip injection (0.2 ml) of IFN_β at the doses indicated.

Anti-IFN gamma globulin. Anti-IFN_β gamma globulin was extracted from rabbit anti-IFN_β serum according to methods previously described (5). The preparation was used at a 1:10 dilution which we have found to completely neutralize the plaque-reducing ability of 10,000 PR_{50} units of IFN_β.

Results. *Effect of EMC-B, poly I:C, and IFN_β on virus-induced diabetes.* ICR Swiss mice were treated with either EMC-B, poly I:C, or IFN_β (20,000 PR_{50} units/mouse) 24 hr before being challenged with EMC-D. The 1-hr GT was determined on each mouse 7 days later. The results of two separate experiments are shown in Table I. These data indicate that while EMC-B protected the animals against the diabetogenic effects of EMC-D, poly I:C and IFN_β had no effect on the development of the diabetic state.

Comparison of the effects of EMC-B, poly I:C, and IFN_β on infection by EMC-D and EMC-MM. Poly I:C and IFN_β have been shown to protect mice against infection by EMC-MM (5). An experiment was done to determine if EMC-B protected mice against infection by EMC-MM and to verify that our poly I:C and IFN_β preparations had the expected protective properties against this EMC strain of virus. Groups of ICR Swiss mice were treated with either EMC-B, poly I:C, or IFN_β (5000 PR_{50} units/mouse). After 15 min, anti-IFN_β gamma globulin sufficient to neutralize 10,000 PR_{50} units of IFN_β was administered to EMC-B- and IFN_β-treated animals. The mice were infected with EMC-D or EMC-MM 24 hr later. The number of deaths were recorded daily in (EMC-MM)-infected animals for a period of 10 days. The GT was determined 7 days after infection on each animal receiving EMC-D. The results (Table II) show that, as in the previous experiments (Table I), there was no protection by either poly I:C or IFN_β against the diabetogenic effects of EMC-D. They also indicate that the protection by EMC-B was not reversed by anti-IFN_β gamma globulin. In contrast, EMC-B, poly I:C, and IFN_β significantly ($P < 0.001$ in each instance) protected the animals against the lethal effects of EMC-MM. The protection by IFN_β was reversed ($P > 0.05$) by anti-IFN_β gamma globulin, but the antibody had no effect on protection by EMC-B.

Inhibition of EMC-D replication by IFN_β. The experiments above suggested that EMC-D might not be sensitive to the protective action of IFN. An experiment was done to determine if IFN_β interfered with the replication of EMC-D in L cells. Monolayer cultures of L cells seeded into 60-mm plastic dishes 24 hr earlier (1.5×10^6 cells/dish) were treated with various concentrations of IFN_β for 24 hr. The cultures were then washed and challenged with either EMC-D or EMC-MM at a multiplicity of infection of 0.4 PFU/cell. After

TABLE I. EFFECT OF EMC-B, POLY I:C AND IFN_β ON VIRUS-INDUCED DIABETES IN ICR SWISS MICE

	Experiment No. 1		Experiment No. 2	
Treatment[a]	GT[b] mg% (mean)	Diabetic[c] (%)	GT mg% (mean)	Diabetic (%)
EMC-B	204	0	230	0
Poly I:C	516	90	358	50
IFN_β	350	50	454	90
HBSS	307	70	400	70
Uninfected control	184 ± 21	0	190 ± 17	0

[a] Mice were given ip injections of EMC-B (1.2×10^5 PFU), poly I:C (150 μg), IFN_β (20,000 PR_{50} units), or HBSS 24 hr prior to infection with EMC-D (1.2×10^4 PFU).

[b] Seven days after infection, the mice were given an ip injection of glucose (2 mg/g body weight). The animals were bled 1 hr later and the serum assayed for glucose content. GT = glucose tolerance.

[c] Animals with GT at least 5 standard deviations (SD) above control mean, were considered to be diabetic.

TABLE II. EFFECT OF EMC-B, POLY I:C AND IFN_β ON INFECTION OF ICR SWISS MICE BY EMC-D AND EMC-MM

Treatment[a]	EMC-D[b] GT mg% (mean)	EMC-D[b] Diabetic (%)	EMC-MM[c] Deaths (%)
EMC-B	249	20	10
(EMC-B) + anti-IFN_β	237	0	10
Poly I:C	483	70	20
(Poly I:C) + anti-IFN_β	380	50	N/D[d]
IFN_β	396	70	10
(IFN_β) + anti-IFN_β	491	80	60
HBSS	442	90	80
Uninfected control	190 ± 18	0	N/A[e]

[a] Mice received the inducers at the concentrations indicated in Table I. Anti-IFN_β gamma globulin was administered 15 min after the inducer as shown.

[b] EMC-D (1.2×10^4 PFU) given ip 24 hr after initial treatment. One hour GT determined 7 days after infection.

[c] EMC-MM (4×10^4 PFU) given ip 24 hr after initial treatment. Deaths recorded daily for a 10-day period.

[d] N/D = not done.

[e] N/A = not applicable.

a 1-hr adsorption period, the cultures were washed twice with HBSS and fed with Dulbecco's minimal essential medium supplemented with 10% calf serum. The cells and fluids were collected 24 hr later and assayed for total virus yields by methods previously described (7). The results (Table III) show that although apparently not as sensitive to the protective action of IFN_β as EMC-MM, EMC-D replication was inhibited at each of the concentrations of IFN_β tested. As little as 50 PR_{50} units reduced the virus yield by about 97%.

Comparison of protection by IFN_β and IFN-inducers in ICR Swiss and SWR/J mice. Groups of each mouse strain were treated with either poly I:C, pyran, endotoxin, tilorone, IFN_β, or EMC-B 24 hr before infection with EMC-D. The GT was determined 7 days later. The results (Table IV) show that neither IFN_β nor any of the inducers protected the ICR Swiss against the production of diabetes. The protective property of EMC-B against EMC-D in this mouse strain was again evident. The data also show, that with the exception of endotoxin, each of the inducers and IFN_β, significantly protected ($P < 0.05$ in each instance) SWR/J against the production of diabetes.

TABLE III. REPLICATION OF EMC-D AND EMC-MM IN IFN_β-TREATED L CELLS.

IFN_β concentration[a] (PR_{50} units)	24 hr virus yields[b] EMC-D	EMC-MM
0	7.5×10^7	4.4×10^6
10	3.9×10^7	4.0×10^4
50	2.0×10^6	<100
100	5.5×10^4	<100
1000	6.0×10^3	<100
2000	<100	<100

[a] Confluent monolayer cultures of L cells were treated for 24 hr with IFN_β at the concentrations indicated.

[b] IFN_β-treated cultures were infected with either EMC-D or EMC-MM (0.4 PFU cell). Cells and supernatant fluids were collected 24 hr after virus infection and assayed for PFU content.

Discussion. The data presented in this communication show that in ICR Swiss male mice, the production of diabetes in response to infection by EMC-D was not blocked by either IFN_β or a variety of IFN inducers. The replication of EMC-D however, was shown to be inhibited by IFN_β (Table III); which agrees with previous reports showing that EMC viruses are sensitive to the protective action of IFN (6, 8, 9). Although IFN did not protect the animals against diabetes, it did protect

TABLE IV. EFFECT OF IFN AND INDUCERS ON INFECTION BY EMC-D IN ICR SWISS AND SWR/J MICE

Treatment[a]	ICR-Swiss GT mg% (mean)	ICR-Swiss Diabetic (%)	SWR/J GT mg% (mean)	SWR/J Diabetic (%)
Poly I:C	360	50	252	0
Pyran	307	40	295	20
Endotoxin	331	50	359	50
Tilorone	378	60	288	10
L-IFN	396	70	202	0
EMC-B	230	0	198	0
HBSS	409	50	468	60
Uninfected control	207 ± 15	0	216 ± 28	0

[a] Procedures were identical to those outlined in Tables I and II. Inducer concentrations were: poly I:C 150 μg, pyran 200 μg, endotoxin 100 μg, tilorone 2.4 mg, and EMC-B 1.2×10^5 PFU. L-IFN dose was 5000 PR_{50} units.

them against the lethal effects of EMC-MM. In contrast to the results observed with ICR Swiss mice, with the exception of endotoxin, IFN_β and each of the inducers effectively protected SWR/J mice against the diabetogenic effects of EMC-D. Endotoxin is a poor inducer of IFN and it has been suggested that its protective action against viral infections is by a mechanism(s) other than IFN (5). These results are in agreement with those reported by Gadzik *et al.* (1), which show that SWR/J mice are protected against EMC-induced diabetes by Poly I:C.

These observations imply that in ICR Swiss mice IFN_β and the IFN produced in response to the inducers, did not reach the beta cells. Alternatively, the beta cells in this mouse strain might not be sensitive to the protective action of IFN. The differences seen in the two mouse strains suggest that protection by IFN_β or IFN inducers against the diabetogenic effects of EMC-D might be under genetic control.

We have confirmed the observation reported by Yoon *et al.* (2), that EMC-B blocks the induction of diabetes by EMC-D. They concluded that this inhibition is at least partially mediated by the IFN induced by EMC-B. In their study, SJL/J mice given 5×10^5 PFU of EMC-B produced approximately 600 PR_{50} units of circulating IFN over a 5-day period. In our system, the animals produced between 500 and 1000 PR_{50} units of IFN in response to 1.2×10^5 PFU of EMC-B (data not shown). However, since exogenous IFN was not effective in protecting ICR Swiss mice, coupled with the observation that anti-IFN_β gamma globulin did not reverse the protective effect of EMC-B, it is likely that another type of interference is involved. For example, EMC-B being antigenically very similar to EMC-D (2), could occupy the receptor sites on the pancreatic beta cells needed by EMC-D to initiate infection. It has been shown that EMC-B is nondiabetogenic (2) which implies that it does not destroy beta cells. However, this would not preclude the possibility that the virus adsorbs to the cells. Another possibility is a nonlytic infection of beta cells by EMC-B making them refractory to superinfection with EMC-D. The inability of anti-IFN_β gamma globulin to reverse the protective effect of EMC-B against EMC-MM suggests that the same interference mechanism(s) which protects the animals against EMC-D is (are) also involved in preventing infection by EMC-MM.

The questions raised in the present study can be resolved by use of an *in vitro* system consisting of pancreatic beta cells in relatively pure culture. We are in the process of establishing such a system in our laboratory.

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