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Effect of Interferon on Encephalomyocarditis Virus Infection of Cultured Mouse Pancreatic B Cells

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Summary. The M variant of encephalomyocarditis virus (EMC) produces a disease similar to human insulin-dependent diabetes mellitus in some but not all strains of mice. This diabetogenic M variant was found to induce fivefold more interferon than non-diabetogenic strains of EMC in cultures of mouse L 929 fibroblasts. When interferon induced by the M variant was added to monolayers of B cells from both diabetes-'susceptible' CD-1 mice and 'resistant' C_{57} bl/6 mice before EMC infection, B cell damage and virus replication were delayed. In addition, viral production in B cell cultures from C_{57} bl/6 mice was reduced five- to tenfold. A similar effect was not found when cultures from CD-1 mice were treated with interferon. Thus, interferon might play an important role in modulating the severity of the initial infection of B cells.

Key words: Encephalomyocarditis virus, interferon, diabetes, B cell monolayer culture.

The M variant of encephalomyocarditis (EMC) virus produces a disease resembling human insulin-dependent diabetes mellitus in some but not all strains of mice. Hyperglycaemia occurs during the acute stages of infection and for varying periods thereafter. These metabolic features are accompanied by degranulation and cytolysis of B cells, in proportion to the degree of glucose intolerance [1, 2].

Expression of the diabetogenic effect of EMC virus is influenced by one or more genes having a recessive mode of inheritance [3–5]. This genetically mediated effect on the occurrence of diabetes is relative since both diabetes-'susceptible' and 'resistant' strains develop islet lesions during the course of a systemic infection [6, 7].

Cultures of B cells prepared from mice that are 'susceptible' or 'resistant' to the diabetogenic effect of EMC virus promptly undergo cytolysis when infected in vitro [8]. Thus, it appears unlikely that differences among strains can be attributed solely to the intrinsic susceptibility of B cells to infection. Genetic influences on the defence mechanisms of the intact animal, however, could account for these differences.

This study was undertaken to determine whether the diabetogenic M variant of EMC differs from other antigenically similar viruses in its ability to induce interferon release by cells. The effect of interferon on EMC infection in B cell cultures from 'susceptible' and 'resistant' strains of mice was also documented.

Materials and Methods

Pancreatic Monolayer Cultures

Monolayer cultures of B cells were prepared from the pancreatic tissue of 4–6 week-old male inbred C_{57} bl/6 and outbred CD-1 mice. The isolated pancreases were dissociated in a phosphate-buffered 0.85% saline solution containing 0.04% collagenase, 0.02% α-chymotrypsin, 0.03% DNase and 4% fetal calf serum. B cell preparations were enriched using discontinuous Ficoll gradients and purified by the methods of differential adhesion and treatment with cysteine-free culture medium [8]. Cells were plated into multiwell plates ([23] 16 mm wells/plate, Falcon Plastics, Cockeyville, Maryland, USA) and maintained with TCM 199 [Grand Island Biological Company (GIBCO), Grand Island, New York] containing 10% fetal calf serum (M. A. Bioproducts, Walkersville, Maryland, USA), glucose (16.5 mmol/l) and gentamicin (10 mg/l). Cultures were maintained at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. Since these primary cultures contained variable numbers of B cells, the amounts of insulin released differed somewhat from one experiment to the next. Insulin in the culture medium was determined by the method of Wright et al. [9] using I^{125} labelled porcine insulin (Cambridge Nuclear, Billerica, Massachusetts, USA) diluted with cold porcine insulin (provided by Dr. R. Chance, Eli Lilly, Indianapolis, Indiana, USA) and guinea pig anti-bovine serum (Linco Research, Manchester, Missouri, USA) with purified mouse insulin (Novo Research Institute, Copenhagen, Denmark) serving as a standard. Using this method, insulin concentrations as low as 50 mU/l can be detected.

Interferon Production and Assay

Confluent monolayers of mouse L 929 cells (> 10^7 cells/flask) were infected with 10^3 tissue culture infectious dose, 50% effective ($TCID_{50}$) of EMC virus. Two days later, the medium [Eagle's minimum essen-

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tial medium (MEM), GIBCO, containing 5% heat-inactivated (50° C, 30 min) chicken serum] and gentamycin (10 mg/l) was withdrawn and clarified by centrifugation at 500 × *g* for 30 min initially, followed by 144,000 × *g* for 60 min. The supernatant was dialyzed for 48 h at 4° C in Sorenson's buffer (pH 2) and against Hanks' balanced salt solution (GIBCO, pH 7.4) for an additional 48 h.

Assays of L cell interferon were carried out on monolayers of the same cell type in 35-mm plates. Aliquots of the test preparation were incubated with the cells for 24 h and 40–90 plaque-forming units of vesicular stomatitis virus (New Jersey strain) were used in the challenge. Monolayers were overlayered with a 1.5% agar solution containing Eagle's MEM and 5% calf serum. Plaques were counted 3 days after staining with neutral red. Titres (units) are expressed as the reciprocal of the dilution of the interferon preparation necessary to produce a 50% reduction in plaques corrected to international units (1 unit in our assay equals 1.2 units of NIH mouse reference interferon G-002-904-511 obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA).

Viruses

The derivation and properties of the M variant and the non-diabetogenic E [10], r^+ [11] and Mengo [12, 13] strains of EMC have been reported previously. The M variant was selected initially using a critical dilution technique. Mice were inoculated SC with a dilute preparation of virus that produced hyperglycaemia in fewer than half of the animals and virus was isolated from the heart tissue of diabetic mice 5 days later. This process was repeated 21 times. All four strains were passaged in L 929 cells for use in these studies. Endpoints were based on cytolysis in tube cultures of L cells and calculated with the Reed-Muench formula [14]. B cell cultures were infected with 10^3 plaque forming units of virus. Since the number of pancreatic B cells in primary cultures varied from experiment to experiment, it was not possible to determine accurately the multiplicity of infection.

Results

Interferon release by cultures infected with the different strains of EMC virus is summarized in Figure 1. In each of three experiments, the mouse heart suspension of the M variant induced substantially greater amounts of interferon than did the three other antigenically similar strains of EMC.

Attempts to demonstrate defective interfering particles in the culture medium of L cells infected with the mouse heart suspension of the M variant were unsuccessful. In these experiments, virus in 25 ml of tissue culture fluid was pelleted by centrifugation (144,000 × *g* for 60 min), washed with Hanks' balanced salt solution and resuspended in 5 ml of fresh medium. This concentrated suspension of virus proved cytolytic 24 h after inoculation into tube cultures of L cells. Thus, it seems likely that interference can be attributed largely, if not exclusively, to substances released into the culture medium during the course of infection.

Monolayers predominantly comprised of B cells from diabetes-'susceptible' CD-1 and 'resistant' C_{57} bl/6 mice were used 8 days after preparation. Since cultures maintained with glucose supplemented medium (16.5 mmol/l) secrete insulin into the culture medium for prolonged periods [8], a decrease in insulin release would indicate a loss of B cell viability. Fifty units of L cell interferon in 0.5 ml of medium were added to each culture well. The medium was withdrawn after 24 h and the monolayers infected with 10^3 $TCID_{50}$ of virus in 0.2 ml of medium. After incubation for 2 h at 37° C, the cultures were washed three times, and 0.5 ml of fresh medium was added. At 12-h intervals, thereafter, the medium was removed, frozen and stored (−20° C) for insulin and viral assays. Uninfected, interferon-treated

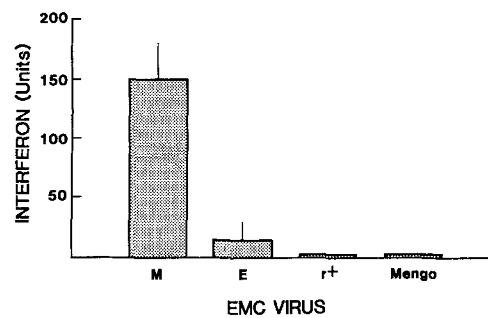

Fig. 1. Interferon release by mouse L-929 cells infected with 10^3 $TCID_{50}$ of the M, E, r^+ or Mengo variant of EMC virus. Each bar represents the mean ± SEM of three separate experiments

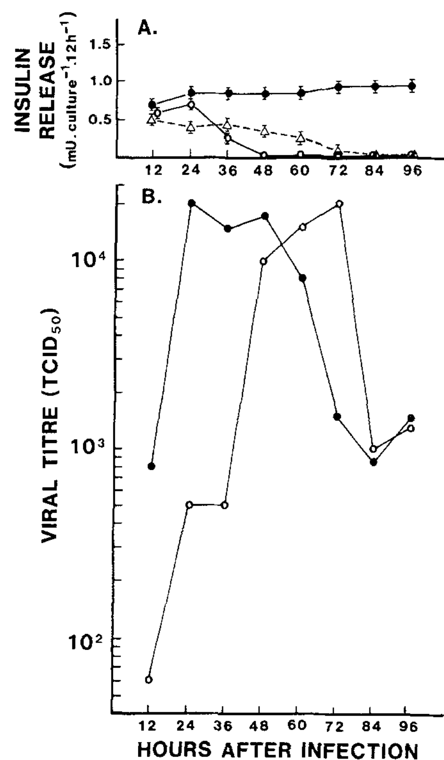

Fig. 2 A and B. Insulin and virus release in CD-1 pancreatic monolayer cultures after interferon treatment and inoculation with 10^3 $TCID_{50}$ of the M-variant. **A** Insulin release data are expressed as mean ± SEM of the concentration of insulin released by three separate cultures, determined at 12 h intervals after infection. ●——● -uninfected interferon; ○——○ -virus infected cultures; △——△ -virus infected interferon treated cultures. **B** Virus data are expressed as the geometric mean of the virus released by three separate cultures determined at 12 h intervals after infection. ●——● -virus infected cultures; ○——○ -virus infected interferon treated cultures

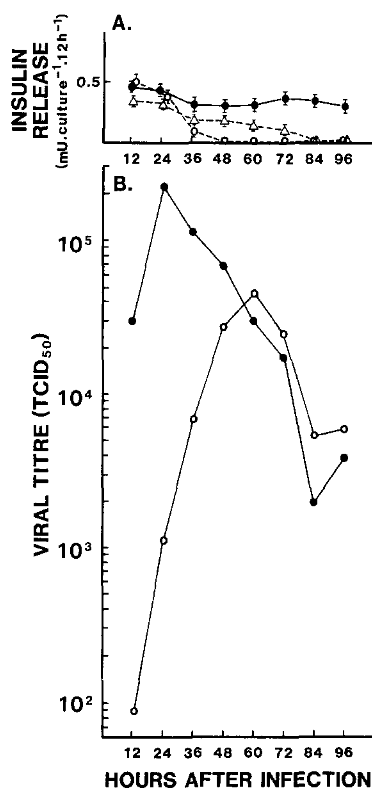

Fig. 3 A and B. Insulin and virus release in C_{57} bl/6 pancreatic monolayer cultures after interferon treatment and inoculation with 10^3 $TCID_{50}$ of the M-variant. **A** Insulin release data are expressed as mean ± SEM of the concentration of insulin released by four separate cultures determined at 12 h intervals after infection. ●——●-uninfected interferon treated cultures; ○——○-virus infected cultures; △——△-virus infected interferon treated cultures. **B** Virus data are expressed as the geometric mean of the virus released by four separate cultures determined at 12 h intervals. ●——●-virus infected cultures; ○——○-virus infected interferon treated cultures

cultures and infected cultures that had not been treated with interferon served as controls. The results of these studies are summarized in Figures 2 and 3.

Insulin release by cultures of B cells from the diabetes-'susceptible' CD-1 mice (Fig. 2 A) increased slightly during the 24 h after infection but decreased precipitously thereafter. By 48 h, microscopic examination showed that all cells were lysed, and insulin was not detected in the medium. Viral replication (Fig. 2 B) accompanied these changes. In contrast, insulin was recovered from the culture medium of interferon-treated, virus-infected cultures for 72 h, and the appearance of cytolysis was more protracted. As might be expected, viral production by these cultures was delayed although the interferon-treated CD-1 cells ultimately released infectious virus in amounts comparable to the untreated, infected controls. Interferon had no apparent effect on insulin production and release by uninfected B cells.

Similar results were obtained in studies using cultures of B cells prepared from C_{57} bl/6 mice which are diabetes-'resistant' (Fig. 3 A). However, in each experiment, the amount of infectious virus released by the interferon-treated cultures were reduced approximately five to tenfold in comparison with untreated cultures (Fig. 3 B).

Discussion

The M variant of EMC proved to be a highly effective inducer of interferon production and release by L cells (in contrast to other strains of EMC). The reason is obscure, but it is of interest in view of the unique pathogenic properties of this virus variant. Relatively small amounts of this interferon transiently protected B cells in culture. Under the artificial conditions of these experiments, the 'breakthrough' of the infection is not surprising since duration of protection afforded by interferon in vitro customarily is variable [15].

The results of experiments with B cells prepared from C_{57} bl/6 mice could explain the relative resistance of this strain to the diabetogenic effect of EMC. Although the islets in infected animals of this strain exhibit focal cytopathological changes and partially degranulate, injury to the islet tissue is more severe in mice of 'susceptible' strains [16]. Since virus production by C_{57} bl/6 B cells was suppressed by interferon in vitro, a similar effect in vivo might be responsible for the attenuated islet damage observed in C_{57} bl/6 animals.

It has recently been reported by Yoon et al. that the M variant contains at least two variants [17]. One of the variants isolated was diabetogenic and the other was not. We have also isolated variants in this laboratory that differ in their ability to affect islet tissue. The M variant virus preparation used in this study was purified by a critical dilution technique. While this does not preclude the possibility that this preparation was comprised of a mixture of viruses having different pathogenic potential, the inoculum used does produce hyperglycaemia in over 80% of CD-1 mice. Although C_{57} bl/6 mice do sustain a systemic viral infection, hyperglycaemia usually does not occur.

Our observations fail to account for the variability in the long-term outcome of the infection in 'susceptible' strains of animals. Compelling recent information suggests that an autoimmune response may be critical in a sustained attack on the islets after the subsidence of the infection [18, 19]. In addition to its anti-viral action, interferon could play an intermediary role in this process because of its capacity to serve as an activator of macrophages [20] and natural killer cells [21]. An infiltrate of macrophages in the insular tissue and phagocytosis of injured B cells commonly is observed in infected mice during early convalescence [22, 23]. Variations in immune injury to the islets might account for differences in the severity of the insular lesion.

Yoon, et al. [4] claim that viral receptors on the plasma membrane of islet B cells determine the outcome of infection with EMC virus. The data reported here and elsewhere strongly argue against this notion because

cells from both the 'susceptible' and 'resistant' strains are injured to a comparable extent by the virus in vitro and in vivo [8, 24]. An alternative hypothesis might be more plausible; namely, that genetically influenced host defence mechanisms determine the severity of EMC virus infection and the associated damage of B cells.

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