

Tolerance to the Induction of Interferon by Vaccinia Virus in Germfree Mice¹

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Introduction

Interferon is produced in response to the treatment of animal cells with certain viruses. It is a cell-produced protein which inhibits multiplication but does not directly inactivate animal viruses (5). This characteristic represents an initial defense mechanism of the host against viral invasion. It is probable that intrinsic host resistance is determined by local tissue factors and foremost among these is the interferon mechanism. Antibody responses to many acute viral infections are often too little and too late to influence the outcome of viral diseases (5). Rabbits were rendered tolerant to an otherwise effective interferon-inducing dose of virus by pretreatment with either sindbis virus or with *E. coli* endotoxin (3). In our studies, germfree mice were made tolerant to the induction of interferon by vaccinia virus. Germ-free mice were used because of their lack of a bacterial flora which has been shown to influence interferon production (4). Vaccinia virus was selected because it is a poor inducer of interferon (2). This characteristic may exemplify an anti-interferon mechanism.

Materials and Methods

Cells. The L strain (clone MCN) of Earle's mouse fibroblast was carried in a nutrient medium (NM) containing 60% Scherer's and 30% 199 supplemented with 10% horse serum. The pH was adjusted to 7.2 with 5.0% sodium bicarbonate. Penicillin (100 units/ml) and streptomycin (0.1 mg/ml) were used.

Virus. Vaccinia virus WR strain (neurotropic) was maintained by passage in MCN cells. Dilutions of stock virus were adsorbed for 2 hr at 37°C on confluent sheets of 48 hr monolayers in screw-capped prescription bottles. Medium was added and bottles were reincubated for 24 hr. The vaccinia virus was harvested by the freeze-thaw technique. Fluid was clarified by centrifugation at 600 rpm for 10 min at 5°C. The clear viral suspension was vialled in 1 ml volumes and stored at -60°C. Vesicular stomatitis virus (VSV) used in the interferon assays was, also, propagated in MCN cells. The NM was, however, adjusted to pH 7.6. After a 45 min adsorption period, virus-infected cells were incubated at 37°C for 1 to 2 days. VSV was harvested as described for vaccinia virus.

Virus Titration. Vaccinia virus was assayed by the plaque method in MCN cells grown in falcon flasks (10⁶ cells/flask). Stock virus was

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serially diluted in NM. One tenth ml of each dilution was introduced and thoroughly spread over the surface of the monolayer. After a 2 hr adsorption period at 37°C, the inoculum was decanted. The cell sheet was washed 2 times with 1 ml of NM. Two ml of NM was added to the flask. Following incubation for 48 hr, medium was removed and 2 ml of a dye solution (5% crystal violet in 20% ethanol) was added. The dye remained in contact with the monolayer for 2 to 4 min. Distilled water was used to wash excess dye from the cell monolayer. The plaques were readily visible and easily counted.

VSV was titrated by formation of plaques on MCN cells. Stock virus preparations were serially diluted in NM and adsorbed onto MCN cells for 2 hr as described for vaccinia. Flasks were incubated for 48 hr with a mixture of 2 ml double strength NM plus 2 ml of 2% Difco Noble agar. Within 48 hr, 0.5 ml of neutral red (1/2000) was added and plaques were counted after 2 hours.

Interferon Assay. Whole blood was collected and allowed to stand for ½ hr at room temperature. Serum was separated from the clot and centrifuged at 2,000 rpm. for 15 minutes. The serum interferon preparation was acidified to pH 2, and dialysed overnight against KCl-HCl buffer. To insure removal of infectious particles, the serum was centrifuged at 40,000 rpm for 2 hr. The preparation was assayed by the 50% inhibition endpoint of plaque formation on MCN cells, utilizing VSV as the challenge virus. One ml of diluted interferon preparation was adsorbed on MCN cells for 5 hours at 37°C. The VSV was introduced and the assay was performed as described in the above section.

Results

TABLE 1
Inhibition of Interferon Induction with High Titers
of Vaccinia Virus.

Vaccinia Inoculum ¹ (PFU)	Interferon Titer (Reciprocal of Serum Dilution) ²	
	Group 1	Group 2
1.9 X 10 ⁹	11	—
1.9 X 10 ⁸	15	15
1.9 X 10 ⁷	48	40
1.9 X 10 ⁶	36	35
1.9 X 10 ⁵	<10	<10
1.9 X 10 ⁴	<10	<10
None	<10	<10

1. Animals sacrificed 7 hours post-inoculation.

2. Interferon titers represent the 50% plaque inhibition endpoint of vesicular stomatitis virus on MCN cells.

Table 1 shows the relationship between virus challenge dose and interferon production seven hours post-challenge. Results of two series

of experiments are shown. Germfree mice were challenged intravenously with 1.9×10^4 to 1.9×10^9 plaque forming units (PFU) of vaccinia virus. Maximum interferon induction occurred with a viral inoculation of 1.9×10^7 PFU. Challenges with either higher or lower viral concentrations yielded less interferon than this optimal dose.

Tolerance of mice to the induction of interferon by vaccinia after a prior challenge of vaccinia virus is demonstrated in Table 2. Mice were first inoculated intraperitoneally with vaccinia virus. The range of viral

TABLE 2
Tolerance to the Induction of Interferon by Prechallenge
with Vaccinia Virus

First Inoculation Vaccinia Virus ¹ (PFU)	Second Inoculation Vaccinia Virus ² (PFU)	Interferon Titer (Reciprocal of Serum Dilution) ³
7.6×10^8	1.52×10^7	<10
7.6×10^7	1.52×10^7	15
7.6×10^6	1.52×10^7	15
7.6×10^5	1.52×10^7	38
None	1.52×10^7	38
None	None	<10

1. Twelve-hour interval between inoculations.

2. Animals were sacrificed 7 hours after the second inoculation.

3. Interferon titers represent 50% plaque inhibition endpoint of vesicular stomatitis virus on MCN cells.

dosage was from 7.6×10^5 to 7.6×10^8 PFU. After 12 hours, all mice were again challenged with the optimum interferon producing dose (1.52×10^7 PFU). Seven hours after the second challenge, animals were sacrificed and serum samples collected. Mice which were prechallenged with vaccinia virus in excess of 7.6×10^5 PFU were rendered hyporeactive to an otherwise effective interferon-inducing dose of vaccinia. Both the time of induction, 7 hours, and the inducing dose of vaccinia virus, 10^7 PFU, were optimal for our system.

Discussion and Summary

Even under optimal conditions, vaccinia virus does not induce animals to produce high concentrations of interferon. In studying this phenomena, we observed that increasing dosages of vaccinia, above the optimal dose of 10^7 PFU, induced germfree mice to produce less interferon. It is possible that the higher doses of vaccinia are toxic to the interferon producing cells in the reticuloendothelial system. Thus, a hyporeactive or tolerant state was created. This phenomenon of hyporeactivity to vaccinia virus is augmented (Table 2) by a vaccinia virus challenge 12 hours prior to an otherwise effective interferon-inducing dose of vaccinia.

We hope that further investigations, using this assay system, will disclose the nature of this vaccinia tolerance. This problem is being considered from the point of view that the vaccinia virus is toxic to the cells of the reticuloendothelial system of the mice. Vaccinia toxicity to mouse leucocytes has been demonstrated by Glasgow and Habel (1, 2).

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