An Explanation for Increased Interferon Production At Low Multiplicities of Infection (34995)

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In several systems of virus-cell interactions, the multiplicity of infection seems to play a significant role in determining the amount of interferon synthesized by the cells. Optimal interferon yields are obtained in some systems when the multiplicity is relatively low (1–5); higher multiplicities seem to inhibit interferon production in such systems. An attempt was made to explain the phenomenon by demonstrating that at low multiplicities more than one cycle of virus replication is necessary for prolonged and increased interferon production.

Materials and Methods. Media and cell cultures. Chick embryo cell cultures were prepared as previously described (6). Growth medium for the establishment of chick cell cultures consisted of Gey's balanced salt solution (BSS) with 5% calf serum, 0.1% lactalbumin hydrolysate, 0.1% proteose peptone, and 0.06% sodium bicarbonate. Maintenance medium used for assays with vaccinia virus consisted of BSS with 0.11% sodium bicarbonate, 0.1% proteose peptone, 0.1% yeast extract, and 0.1% lactalbumin hydrolysate. An agar overlay medium consisting of 0.5% Ionagar in maintenance medium with 5% calf serum was used for assays employing Semliki Forest virus.

Viruses. Vaccinia virus, strain NY 914, was grown on the chorioallantois of 11-day-old chick embryos. Semliki Forest virus was grown by inoculating newborn mice intracerebrally with about 1×10^4 PFU and harvesting the brains when the mice became moribund 36–48 hr later. Vaccinia virus and interferon were assayed by the methods described by Lindenmann and Gifford (7). One PDD_{50} unit (50% plaque-depressing dose) in our assay system is equal to 1.4 units of the International Research Reference Standard.

Antiserum. Semliki Forest virus rabbit antiserum was a gift from Dr. Stephen T. Toy, Department of Preventive Medicine, Case Western Reserve University. This antiserum inactivated 99% of the virus in 1 hr at 37° when employed at a final dilution of 1:20.

Results and Discussion. Interferon production at different multiplicities of infection. The effect of multiplicity of infection of Semliki Forest virus on interferon production in chick embryo cell cultures was studied. Figure 1 shows the 24-hr yields of interferon in chick embryo cell cultures infected with dif-

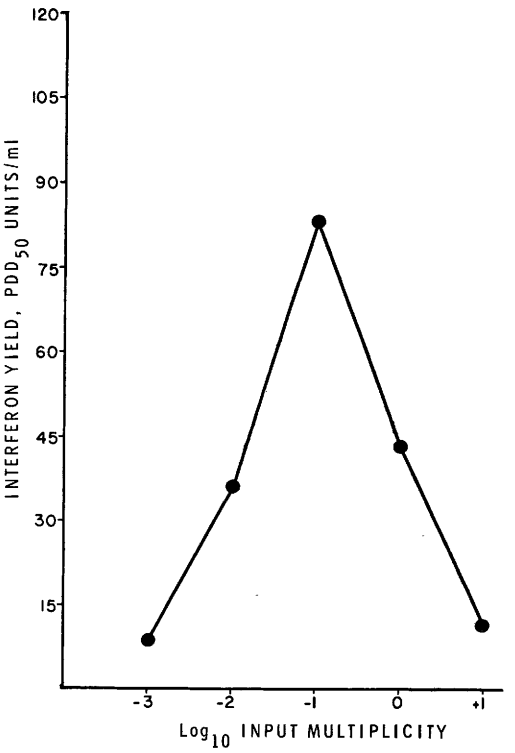

FIG. 1. Interferon yields at 24 hr in chick embryo cell cultures infected with various input multiplicities of Semliki Forest virus.

TABLE I. The Effect of Two Multiplicities of Virus on Interferon Production.

Exp. no.	Multiplicity of infection (PFU/cell)	Interferon yield, PDD_{50} units/ml: Hours after infection 8	12	24	% Inhibition at 10 PFU[a]
1	0.1	21.9	36	78.4	
	10	23.6	25.2	24.8	68.4
2	0.1	12.1	37.2	86.6	
	10	14.3	16.0	16.2	81.2
3	0.1	9.7	33.2	65.6	
	10	9.2	10.1	9.5	85.5

[a] Percent inhibition compared to yield of interferon in cell cultures infected at a multiplicity of 0.1 at 24 hr after infection.

ferent multiplicities of Semliki Forest virus. The highest yield of interferon was obtained when an input multiplicity of about 0.1 PFU/cell was employed. These results are in agreement with Toy and Gifford (2). When the input multiplicity was increased to 1 or greater, the 24-hr interferon yields were much reduced. The mechanism of the multiplicity effect was studied since it might provide insight into conditions for maximal interferon production.

Interferon production as a function of time after infection. To further delineate the difference in interferon yield as a function of multiplicity of infection, the production of interferon in cell cultures at various times after infection was studied with two different multiplicities. The results of three separate experiments are shown in Table I. Essentially the entire yield of interferon was obtained by 8 hr with cultures infected at 10 PFU/cell. When a multiplicity of 0.1 PFU/cell was employed, there was a significant increase in interferon production beyond 12 hr after infection. These data indicated that the infection of all the cells with active virus (*i.e.*, at a multiplicity of 10), resulted in an earlier termination of interferon synthesis. The reason for the prolongation of interferon synthesis at low multiplicity was then considered.

Interferon production in the presence of immune serum. Several possibilities were considered in attempts to explain the multiplicity effect. For example, the higher yields of interferon at lower multiplicities might be due to: (a) induction of interferon by "inactive" virus particles in the virus preparation which may not inhibit host macromolecular synthesis, (b) effect of different multiplicities of active virus on the time of shutting off of host macromolecular synthesis, or (c) several cycles of virus replication resulting in induction of increasing numbers of cells. Each of these possibilities might result in alteration in amount of interferon synthesized. For brevity, however, we shall consider only the experiments which seem to explain the phenomenon. When chick embryo cell cultures are infected at a multiplicity of 0.1, a maximum of 10% of the cells are initially infected. Since infected cells apparently produce interferon for a maximum of 8 hr, the continuous synthesis of interferon by cultures infected at low multiplicities may well be due to the subsequent infection of the remaining cells by the progeny virus. To test this possibility, the kinetics of interferon production in chick cells infected at 0.1 PFU/cell, with or without the addition of antiserum or Semliki Forest virus, was studied. The progeny virus produced by cells initially infected would be neutralized by the immune serum present and would thus be unable to infect the remaining cells. The cell monolayers were exposed to virus at room temperature for 1 hr. Cell cultures were then washed once with BSS, supplied with 2 ml of maintenance

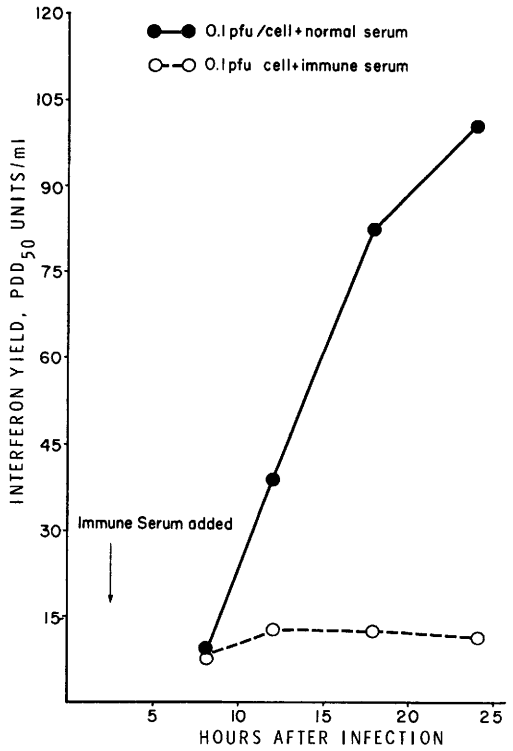

FIG. 2. Kinetics of interferon production in presence of immune serum prepared against Semliki Forest virus. Details have been described in the text.

medium, and incubated at 37°. After 2.5 hr of incubation, 0.1 ml of immune serum, or normal rabbit serum, was added. The results are shown in Fig. 2. In cell cultures containing normal serum, the production of interferon was similar to that described in Table I. However, in the presence of immune serum, the total yield of interferon was obtained within 12 hr of infection. The prolongation of interferon synthesis by cultures infected at low multiplicities is thus apparently due to the infection of the remaining cells in the culture by the progeny virus.

An intriguing question is why the total quantity of interferon synthesized is greater in cell cultures infected at the lower multiplicity than with the higher multiplicity. The reason for the increased yield is not obvious since the same number of cells were employed in both cases and eventually all of the cells were infected and would contribute to the total yield. At the low multiplicity, more than one cycle of virus replication was required for infection of all the cells which would result in a prolonged synthesis but it would not explain the increased yield. However, it has been reported (8–9) that treatment of cells with interferon before the addition of virus enhances the subsequent production of interferon. Thus, at lower multiplicities of infection, interferon produced in the first cycle might enhance the production of interferon in cells infected during subsequent cycles of viral replication, and would thus result in higher total yields of interferon. Unfortunately, the mechanism of this phenomenon of interferon enhancement of additional interferon production is as yet unclear.

Summary. Relatively low multiplicities of Semliki Forest virus induce optimal yields of interferon in chick embryo cell cultures. High multiplicities apparently inhibit interferon synthesis; however, a different mechanism is shown to be responsible. The increase yield of interferon at low multiplicities is shown to be associated with a prolongation of interferon synthesis due to multiple cycles of virus replication. The possibility of interferon induced in the first cycle of replication having a priming effect on interferon production in subsequent cycles is suggested.

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