THE NECESSITY FOR CELLULAR RNA AND PROTEIN SYNTHESIS FOR VIRAL INHIBITION RESULTING FROM INTERFERON

Royce Z. Lockart Jr.
Department of Microbiology, The University of Texas
Austin, Texas.

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While there is still no clear understanding of the mechanism by which interferon exerts its antiviral action, it has been well established that it acts at some stage of the virus-cell interaction subsequent to those of adsorption and penetration (Ho, 1961; Wagner, 1961; Grossberg and Holland, 1963, Lockart, Sreevalsan and Horn, 1962). Several lines of evidence have indicated that the replication of viral RNA is inhibited (Lockart, Sreevalsan and Horn, 1962; DeSomer, Prinzie, Denys and Schonne, 1962; Levy, Snellbaker and Baron, 1963). Recently, Taylor (1964) reported that virus inhibition failed to occur in cells treated with Actinomycin D and that the viral-inhibitory effects of interferon could be partially reversed by the addition of the drug. Because Actinomycin D acts by inhibiting transcription from DNA to RNA (Goldberg and Rabinovitz, 1962; Baltimore and Franklin, 1962; Reich, Franklin, Shatkin and Tatum, 1961), this suggests that virus inhibition is cell mediated. We will present data which confirm the findings of Taylor and indicate the importance of protein synthesis for the viral inhibitory activity to be mediated.

Interferon was prepared by infecting monolayer cultures of chick-embryo (CE) cells with a small plaque-forming variant of Western equine encephalomyelitis (WEE) virus. The interferon was precipitated from the medium by the addition of ammonium sulfate to 75 percent saturation and redissolved in 0.01 M phosphate buffer pH 7.0. Prior

to use, 2-3 ml of interferon solution in a 60 mm petri dish were exposed for 5 min. to an 8 watt GE germicidal lamp at a distance of 15 cm. Such treatment renders WEE virus incapable of interference but has no effect on the action of interferon. Interferon was diluted in twofold steps in Eagle's medium with 3 percent calf serum (EC) and two ml were added to CE monolayers. The greatest dilution capable of preventing the appearance of cytopathologic effects in the cultures when they were challenged with a large multiplicity of WEE virus 17-24 hours later was considered to contain one protective unit (PU) of interferon. Puromycin dihydrochloride was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. It was diluted in EC medium and used at a final concentration of 50 μg per ml. Actinomycin D was provided by Merk, Sharp and Dohme, Co., West Point, Penn. It too was diluted in EC medium and used at a final concentration of 5 μg per ml.

The incubation of monolayer cultures of CE cells (approx 1-2 million cells) for 2 hours at 37° with 30 PU of interferon followed by washing and challenge with 20 PFU per cell of WEE virus resulted in about a 90 percent reduction in the virus titers of fluids obtained 17-24 hours post-inoculation (PI). Incubation of the cultures for 1 hour at 37° with EC medium containing 5 μg per ml of actinomycin prior to incubation with interferon prevented virus inhibition from occurring. These results are shown in Table 1. They confirm those published by Taylor (1963) who used Semliki Forest virus.

Taylor also reported that viral inhibition resulting from more prolonged incubation with interferon was partially reversed by subsequent incubation with actinomycin. To see how rapidly actinomycin-resistant viral inhibition developed, the following experiment was performed. Monolayer cultures of CE cells were incubated for 1, 2, and 3 hours at 37° with 50 PU of interferon. Cultures to serve as controls were incubated with EC medium. At each time mentioned, two control and two interferon-treated cultures were removed, washed 3 times with warm buffered saline and challenged by the addition of 20 PFU per cell of WEE virus. Also, at each time, two cultures of each were washed, and incubated an hour at 37° with EC medium containing puromycin

TABLE 1

Inhibition of Interferon Action By Actinomycin D.

EXP. NO.	Preincubated with actinomycin (1 hr at 37°)	Incubated additionally with 30PU of interferon (2 hrs at 37°)	Virus titers (17-24 hrs PI)
1	-	-	1.8×10^8
	-	+	1.9×10^7
	+	-	1.8×10^8
	+	+	1.8×10^8
2	-	-	7.7×10^9
	-	+	2.3×10^8
	+	-	2.1×10^9
	+	+	2.2×10^9

and actinomycin. They were then washed an additional 3 times and challenged as above with WEE virus. Samples of fluid were removed 17-24 PI and titered. Results of two such experiments are plotted in Figure 1A. The solid lines indicate that virus inhibition increased with each hour of incubation with interferon. The dotted lines indicate that as a result of an additional hour of incubation with medium containing puromycin and actinomycin, much of the inhibition was lost. Despite the partial prevention of virus inhibition after the hour's incubation with puromycin and actinomycin, the amount of unreversible inhibition became greater with each hour of incubation with interferon. It should be pointed out that an additional hour of incubation, after the removal of interferon, with medium or medium containing puromycin, has never resulted in a loss of inhibition but frequently a slight gain. It can be concluded that a product whose production or action is not susceptible to actinomycin is necessary for viral inhibition to become stabilized. Protein is the most likely possibility.

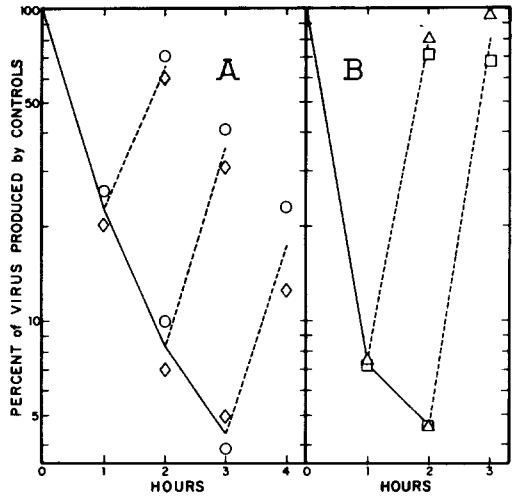

Fig. 1 Onset of viral inhibition resulting from interferon and its prevention (or reversal) with actinomycin D. Each point connected with solid lines indicates the viral yields obtained after that period of incubation of cultures with interferon prior to the removal of interferon by washing and challenge of the cultures with large multiplicities of WEE virus. The points to which the dotted lines lead indicate the yields obtained when replicate cultures were incubated an additional hour, after the removal of interferon, with medium containing actinomycin and puromycin. After the additional hour of incubation, the cultures were again washed and challenged with WEE virus. A. Cells were incubated with 50 PU of interferon diluted in EC medium. B. Cells were incubated for 30 min. with EC medium containing puromycin before the addition of 50 PU of interferon in EC medium containing puromycin.

Experiments similar to those just described were done except that the cultures were incubated for 30 min. at 37° with EC medium containing puromycin prior to the addition of interferon. Also, interferon was added in medium containing puromycin. Control cultures were incubated with EC medium containing puromycin but no interferon for identical periods. After 1 and 2 hours of initial incubation with interferon, the additional incubation, and challenge with virus was as already described. The results are shown in Figure 1B. As before, the solid lines indicate that viral inhibition increased with time even though puromycin was present during its uptake. Again, as

indicated by the dotted lines, much of the inhibition of virus production in those cultures incubated an additional hour with medium containing puromycin and actinomycin was prevented.

Two differences can be seen between the data plotted in Fig. 1A and that in Fig. 1B. When interferon uptake occurred in the presence of puromycin (1B) prior to incubation with actinomycin, less viral inhibition resistant to the action of actinomycin resulted. There was also a failure for an increased amount of actinomycin-resistant inhibition to occur with an increased period of incubation with interferon. These data point out the importance of protein synthesis as well as that of DNA-dependent RNA for the resulting viral inhibitory activity of interferon. Several explanations are possible. The new proteins may be required to stabilize other products or they may themselves be the actual inhibitors.

It appears that interferon acts as an inducer, causing sensitive cells to produce a new protein(s) which in some yet unexplained manner is necessary for the inhibition of virus reproduction to result. The production of the new protein must be of cellular origin and mediated through a DNA-dependent RNA. This would account for the actinomycin sensitivity of the inhibitory activity. Evidence reported by Levy, Snellbaker, and Baron, (1963) who found an increased uptake of label into RNA of normal chick-embryo cells incubated overnight with interferon also supports this idea. To explain the inhibition which resulted when interferon was added in the presence of puromycin and not incubated further with actinomycin, one might postulate the following. In the presence of puromycin and interferon, DNA-dependent RNA induced by the interferon was produced. Upon the removal of puromycin in order to infect the cells, sufficient protein was synthesized to permit inhibition of virus reproduction. When, however, after removal of interferon, an additional hour of incubation was carried out under conditions where both protein synthesis and further DNA-dependent RNA synthesis were inhibited, that RNA already present lost its activity and very little inhibition resulted.

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