Temporal Relationship between Virus and Interferon Biosynthesis in L Cells Infected with Newcastle Disease Virus[1] (34194)

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Several studies have revealed that avirulent cell–virus interactions result in the production of more interferon than virulent cell–virus interactions (1, 2). These observations suggest that interferon may be the agent responsible for the abortive nature of the cell–virus interaction. However, several investigations of arboviruses grown in chick embryo fibroblasts (3–5) and in mouse L cells (5, 6) showed that interferon is not synthesized until relatively late in the viral replication cycle. These studies raise the question whether interferon is actually responsible for the avirulent or abortive nature of the cell–virus interaction. The present study involves an investigation of the temporal relationship between the cellular synthesis of interferon and the production of Newcastle disease virus in L cells. The role of interferon in the abortive replication of the virus is assessed.

Materials and Methods. The cultivation of cells and the preparation of virus stocks has been described (7). In all experiments L cell and chick embryo fibroblast (CEF) monolayers, grown in 2-oz French square bottles (A. H. Thomas Co., Philadelphia) or 30-ml plastic Falcon tissue culture flasks (B-D Laboratories, Los Angeles) were infected with Newcastle disease virus (NDV) at a multiplicity of 20–40 pfu/cell (plaque-forming units per cell). Following a 60-min adsorption period at 37° the monolayers were washed 3 times with Hanks' balanced salt solution (BSS) (8) and fed 4 or 5 ml of double strength Eagle's medium as previously described (7). At various times after infection the quantity of extracellular and intracellular interferon, virus and viral hemagglutinin was determined. The appearance of these components extracellularly was measured by removing and assaying an aliquot of medium. The intracellular content was determined by thoroughly washing the infected monolayers, removing the cells by 2 freeze–thaw cycles and disrupting the cells by sonication with the Branson sonifier (model S-65) at 20 kcps for 15 sec. Intracellular and extracellular NDV titers were determined by plaque assay on CEF cultures.

Viral hemagglutinin was determined by mixing dilutions of medium or sonicated cell fractions with an equal volume of 1% (v/v) suspension of chick erythrocytes as described by Wagner (5).

Intracellular and extracellular interferon preparations were adjusted to pH 2 with concentrated hydrochloric acid and incubated at 4° for 18–24 hr to inactivate the NDV. The samples were then neutralized with 10 *N* sodium hydroxide, diluted in growth medium (7) and 4 ml of each dilution was incubated on L cell monolayers for 24 hr. These cultures were subsequently challenged with 50–100 plaque-forming units of mengo virus, overlayed with agar containing medium, oxygenated and incubated at 37° for 24–36 hr, at which time plaques were counted. Interferon titers are expressed as the reciprocal of the dilution which resulted in a 50% decrement in plaque formation, relative to untreated controls.

Results. The replication of NDV (Roakin strain) in L and CEF cells was compared by measuring the extracellular infectious virus

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TABLE I. NDV and Viral Hemagglutinin Production in Primary Chick Embryo and L Cells.[a]

Cell line	Virus yield (pfu/cell)	Hemagglutinin titer
Chick embryo	64	320
	74	320
L cell	1.4	<8
	4.2	16
	1.9	8

[a] Cell monolayers were infected with a multiplicity of infection of 30. Virus and hemagglutinin were titered 24 hr after infection as described in "Materials and Methods."

and viral hemagglutinin at 24 hr after infection. The yield in L cells was only 2–6% of that obtained in CEF (Table I), demonstrating the abortive or inefficient nature of this cell–virus interaction. In addition, the abortive infectious cycle of NDV replication in L cells was reflected in the low quantities of viral hemagglutinin liberated during the 24-hr incubation period. In a subsequent series of experiments, infected L cell monolayers were tested for the possibility that viral hemagglutinin remained associated with the cell membranes or in the cytoplasm and was not released into the culture medium. However, only trace amounts of cell associated viral hemagglutinin were ever detected in NDV infected L cells.

The low yield of NDV per cell observed in the previous experiment may not represent actual viral replication, but may reflect the elution of viral inoculum from infected cells. These possibilities were tested by measuring NDV yields in actinomycin D-pretreated cells. The extracellular virus yield in cells treated with 0.1–10μg/ml actinomycin D was only 11% of the yield in untreated cells. This indicates the apparent increase in virus titers seen in the previous experiments actually represented viral replication. The kinetics of NDV synthesis in L cells was measured by infecting approximately 6×10^6 cells with NDV at a multiplicity of infection of 30 and titering extracellular and intracellular virus at various times after infection. The extracellular virus titer began to increase at approximately 5–6 hr after infection and reached a maximum at 24 hr (Fig. 1). In contrast to the liberation of extracellular virus, the intracellular concentration was maximal within 7 hr after infection and subsequently decreased as the virus was released.

In order to assess the role of interferon on the abortive cycle of viral replication, the intracellular and extracellular interferon concentration was determined. When L cells were infected with NDV and the medium was assayed for interferon at various intervals, none could be detected until approximately 7–8 hr postinfection. The titer of interferon in the medium did not significantly rise until 12 hr and reached a miximum concentration at 24 hr after infection (Fig. 2). Although viral replication was well advanced by 7 hr, no intracellular interferon could be detected by this time (Table II). Even after 7 hr only small amounts of interferon were observed in these cells. These results suggest that interferon is not produced in appreciable quantities until 7 hr after infection and that the release of interferon following its synthesis is very rapid.

Discussion. In the present study, the infec-

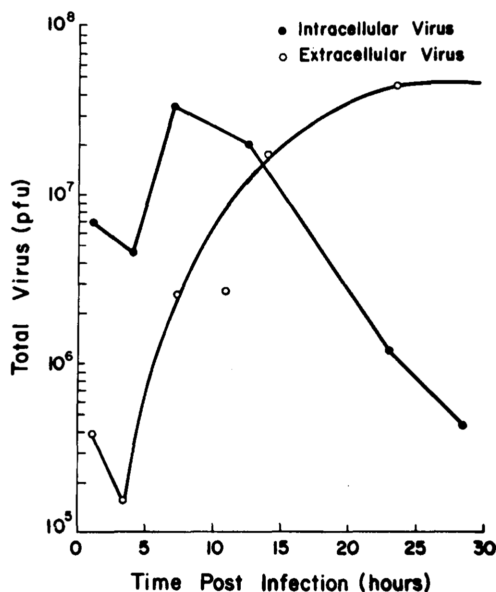

FIG. 1. Kinetics of NDV production in L cells; aliquots of medium and sonicated L cells were titered for NDV by plaque formation on CEF cells as described in "Materials and Methods"; (●), intracellular virus; (○) extracellular virus.

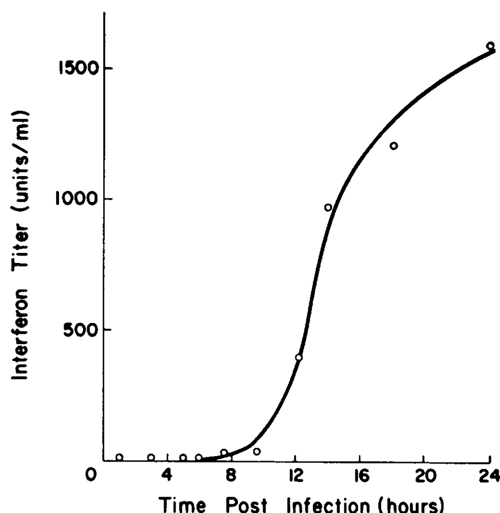

FIG. 2. Kinetics of the appearance of interferon in culture medium; aliquots of medium were assayed for interferon activity at different times post infection as described in "Materials and Methods."

tion of L cells by the Roakin strain of NDV resulted in an abortive or inefficient cycle of virus replication. These results concur with previous studies in which the Beaudette (9), Victoria (10), and Italien strains (11) of NDV were found to inefficiently replicate in L cells. However, in the studies of Wilcox (9) and Reda *et al.* (11), the production of large amounts of viral hemagglutinin was reported. Their results suggest that despite low yields of mature and infectious virus, the inefficient nature of viral replication was probably independent of viral directed protein synthesis. In contrast to these previous studies, the data presented in this report reveal that only trace amounts of viral hemagglutinin were produced in L cells infected with the Roakin strain of NDV. These results indicate a significant difference in the Roakin strain in that its interaction with L cells not only leads to inefficient virus production but that the synthesis of viral directed proteins is considerably reduced.

TABLE II. Intracellular Interferon following Infection of L Cell Monolayers with NDV.[a]

Time post-infection (hr)	Interferon titer	
	Expt. 1	Expt. 2
1	<25	—
3	<25	—
5	—	<20
6	<25	<20
7	—	<20
8	—	80
9	—	80
10	—	80
12	25	—

[a] At the appropriate times the cell monolayers were carefully washed and the cells disrupted as described in the text. Interferon titers are expressed as the reciprocal of the dilution providing 50% plaque reduction.

Although the NDV-L cell system has been extensively used by investigators to study several facets of interferon induction and synthesis, the role of interferon in the abortive cycle of infection has not been directly studied. In other host–virus systems, the temporal relationship between virus replication and the appearance of interferon indicated the synthesis of interferon lagged virus production (3–6). Wagner (5) indicated that an inherent problem in assessing the role of interferon on the outcome of viral infection resides with the technical problem of detecting small quantities of the viral inhibitor. We attempted to minimize this problem by employing a system where relatively large amounts of interferon are produced. Consequently, it was possible to determine with a degree of precision the time after infection when interferon is initially observed. By directly comparing the data in Figs. 1 and 2 it can be seen that interferon is initially observed at 7–8 hr after infection, at a time when the intracellular virus titer is already maximal. In addition, intracellular interferon is not observed before 7 hr postinfection and the maximum titers are found at 9–10 hr (Table II). The time of interferon synthesis is similar to that reported by Cantell and Paucker (12). This report also provides direct evidence in support of the notion of Younger and Scott (13) that the inefficient nature of NDV replication in L cells is due to an incompatibility in the cell–virus system and is not a result of "feed back" by endogenous interferon.

Summary. The temporal relationship between virus production and interferon biosynthesis was studied in L cells infected with NDV (Roakin strain). The results indicate that this strain of NDV inefficiently replicates progeny virus and that viral directed protein synthesis (hemagglutinin) is significantly depressed in L cells. These latter findings which are in contradistinction to the observations of others who used the Beaudette and Italien strains of NDV, reflect a primary difference in the characteristics of NDV strains. Evidence is also presented which indicates interferon appears very late in the replication cycle of the virus. These data substantiate the notion that interferon is not responsible for the abortive nature of viral replication.

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