Comparison of Interferon Induction by Active and Inactive Semliki Forest Virus

By R. M. GOORHA AND G. E. GIFFORD

Department of Immunology and Medical Microbiology, College of Medicine, University of Florida, Gainesville, Florida 32601

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SUMMARY

Semliki Forest virus, inactivated by incubation at 37°, induced considerably more interferon than did the live virus. Infection by live virus produced an earlier termination of interferon synthesis (at 8 hr) which correlated with a profound inhibition of cellular RNA synthesis. Heat-inactivated Semliki Forest virus did not inhibit cellular RNA synthesis until much later and permitted continuation of interferon synthesis for an additional 4 hr, thereby increasing the yield of interferon by about four- to sixfold.

INTRODUCTION

Inactivation of a virus abolishes its ability to induce interferon. Often, however, suitably inactivated viruses are good inducers of interferon and sometimes even better than the untreated virus (Isaacs & Lindenmann, 1957; Burke & Isaacs, 1958; Ho & Enders, 1959; Henderson & Taylor, 1961; Ho & Breinig, 1962; Gifford & Hellar, 1963; Lockart, 1963; Ho, 1964). In several systems, such as Newcastle disease and fowl plague viruses in chick embryo cells, significant amounts of interferon can only be induced by inactivated viruses (Ho, 1964; Gandhi & Burke, 1970). Our previous results indicated that heat-inactivated Semliki Forest virus induced considerably more interferon than live virus in chick embryo cells (Goorha & Gifford, 1970). This paper represents an attempt to explain the difference in the interferon-inducing abilities of live and inactivated Semliki Forest virus.

METHODS

Cells. Primary cultures from 10- to 11-day-old, eviscerated and decapitated chick embryos were prepared according to the method of Lindenmann & Gifford (1963a). Confluent cell monolayers were usually formed within 2 days and contained approximately $4 \times 10^6$ cells on an 18 cm.$^2$ surface.

Virus. Stock preparations of Semliki Forest virus (SFV), a group A arbovirus, consisted of infected newborn mouse brain as a 10% suspension in maintenance medium. SFV was assayed on chick embryo cell monolayers employing an overlay consisting of 1% methyl cellulose (1500 centipoise) in Eagle's MEM with 10% calf serum. Vaccinia virus was grown on the chorioallantois of 11-day-old chick embryos and assayed as previously described (Lindenmann & Gifford, 1963b).

Media. The growth medium consisted of Gey's balanced-salt solution (BSS) with 0.1% lactalbumin hydrolysate, 0.1% proteose peptone, and 5% calf serum. The maintenance medium consisted of BSS with 0.1% lactalbumin hydrolysate, 0.1% proteose peptone, and
0.1% yeast extract. A modified maintenance medium, in which 2.5% calf serum replaced the yeast extract, was usually employed for the determination of RNA synthesis.

**RNA synthesis.** Chick embryo cell monolayers were exposed to Semliki Forest virus at a multiplicity of 10 p.f.u./cell, or to an equivalent amount of the heat-inactivated virus, in 0.2 ml. volume with or without 4 μg. of actinomycin D. This amount of actinomycin D inhibited about 95% of cellular RNA synthesis within 1 hr. The virus was allowed to adsorb for 60 min. at room temperature and the cultures were then washed twice with 2 ml. of modified maintenance medium. Each cell culture was supplied with 2 ml. of the same medium and incubated at 37°. At various time intervals thereafter, 0.3 ml. of medium containing 30 μc of [3H]uridine and 37.5 μmoles each of unlabelled thymidine and deoxycytidine, was added to each culture and incubated for 45 min. at 37°. Incorporation of [3H]uridine was stopped by adding 0.1 ml. of unlabelled uridine (5 mmoles) to the cultures and immersing them in an ice bath. The medium was drained from the cultures and cell monolayers were extracted four times with ice cold, 5% perchloric acid (PCA) during a 30 min. period. The RNA was then hydrolyzed in 2 ml. of 5% PCA for 30 min. at 80°. An 0.2 ml. portion of this RNA hydrolysate was used for determination of [3H]uridine incorporation in a Packard 'Tri-Carb' liquid scintillation spectrometer.

**Interferon.** Supernatant fluids from cultures were collected for interferon assays and heated at 65° for 30 min. to destroy the infectivity and interferon inducing ability of the residual virus. Vaccinia virus and interferon were assayed by the methods described by Lindenmann & Gifford (1963b). One PDD50 unit in the present study is equal to 1.4 units of the International Research Reference Standard.

**Reagents.** [3H]uridine (7 c/mM or 28.1 c/mM) was purchased from New England Nuclear Corporation, Boston, Mass., Actinomycin D was a gift from Merck Sharpe, and Dohme, Rahway, New Jersey.

### RESULTS

**Heat inactivation of Semliki Forest virus**

Interferon-inducing ability of Semliki Forest virus is lost when the virus is heated at high temperatures which rapidly inactivate the virus. For example, when we heated SFV at 54° for 10 min., both the infectivity and the interferon inducing ability of the virus were lost. When infectivity was inactivated at 37°, however, the virus was still able to induce interferon (Goorha & Gifford, 1970) suggesting that the inactivation at the two temperatures is due to different mechanisms. In the current study SFV was inactivated by incubating the stock virus suspension in sealed glass ampoules at 37° for various periods of time, and the residual infectious virus was then measured. Fig. 1 shows a representative study of the exponential decay of SFV at 37°. One log. (90%) decrease in infectivity titre occurred per 3.2 ± 0.2 hr of incubation. The inactivated virus employed in the subsequent studies has been incubated at 37° for 24 hr, a treatment which reduced the titre from about 10⁶ p.f.u./ml to less than 5 p.f.u./ml.

**Interferon production with live or heat-inactivated Semliki Forest virus**

Chick embryo cell cultures were exposed to live Semliki Forest virus at a multiplicity of 10 p.f.u./cell or to an equivalent amount of inactivated virus. As shown in Table 1, considerably more interferon was consistently produced by cell cultures exposed to inactivated virus. A comparative study of the kinetics of interferon production in cell cultures exposed to the two virus preparations was then made. Fig. 2 shows that interferon synthesis was nearly completed by 8 hr following infection with live virus, but that interferon synthesis continued
for about 12 hr in cells exposed to an equivalent amount of inactivated virus. The maximal yield of interferon with the inactivated virus was about four times greater than that induced by the live virus. The increased yield of interferon obtained with inactive virus may be explained by the synthesis of interferon for 4 hr longer than that obtained with live virus.

Fig. 1. Exponential decay of infectivity of Semliki Forest virus at 37°. Samples of virus were removed at various times during incubation and assayed for residual virus.

Table 1. Interferon induction by live or heat-inactivated Semliki Forest virus

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Virus preparation</th>
<th>Interferon yield PDD50 units/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Live*</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Inactivated†</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>Live</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Inactivated</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>Live</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Inactivated</td>
<td>68</td>
</tr>
</tbody>
</table>

* Multiplicity of 10 p.f.u./cell.
† Virus was inactivated at 37° for 24 hr and equivalent amount of the preparation was employed as for the live virus-infected cultures.
RNA synthesis in the induced cells

Interferon induction by viruses probably represents a derepression event in the induced cells (Burke, 1966). Continued host RNA and protein synthesis would thus be required for optimal yields of interferon. It was considered possible that differences in interferon yields induced by live or heat-inactivated Semliki Forest virus may be related to some differential effect on host macromolecular synthesis. Therefore, the effect of live or heat-inactivated virus on RNA synthesis of the induced chick embryo cells was studied. Fig. 3 shows RNA synthesis in chick embryo cells infected with a multiplicity of 10 p.f.u./cell of live virus with or without actinomycin D. An increase in total RNA synthesis was observed which reached a peak at about 5 hr after infection. In individual experiments, this increase varied between 40 and 80% of the control uninfected cells. This early increase was followed by a rapid decline in total RNA synthesis in the infected cells, and by 12 hr following infection was approximately 14% of that of the control cells. Actinomycin D-resistant RNA synthesis (presumably of virus origin) increased until 8 hr following infection, and declined thereafter. Cellular RNA synthesis, plotted as the difference between the total RNA and actinomycin D-resistant RNA synthesis at each point, showed a pronounced inhibition beginning about 5 hr following the infection. From 8 hr onward, practically all the RNA synthesized was of virus origin (actinomycin D-resistant).

Fig. 4 shows a correlation of virus RNA synthesis with the production of infectious progeny. Virus maturation occurred between 5 and 10 hr and was essentially completed by 10 hr. Virus-specific RNA was first detected at about 3 hr and reached its maximum rate of synthesis at approximately 8 hr. There was a lag period of about 2 hr between the synthesis of virus-specific RNA and the appearance of infectious progeny virus. Thus, interferon
Table 2. Effect of heat-inactivated virus on \(^{[3}H\)uridine uptake of chick embryo cell cultures

<table>
<thead>
<tr>
<th>Time after exposure to inactivated virus (hr)</th>
<th>(^{[3}H)uridine uptake*</th>
<th>(^{[3}H)uridine uptake of controls</th>
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<tr>
<td>3</td>
<td>3258</td>
<td>3347</td>
</tr>
<tr>
<td>6</td>
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<td>12</td>
<td>2886</td>
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<td>2705</td>
<td>2322</td>
</tr>
<tr>
<td>18</td>
<td>2593</td>
<td>1794</td>
</tr>
</tbody>
</table>

* \(^{[3}H\)uridine, 20 \(\mu\)c, was added to each culture at the indicated time and incubated for 45 min. at 37°. Cultures were then extracted with cold PCA and the RNA was hydrolyzed with PCA at 80°. The counts/min. are averages of duplicate cultures, each counted twice for 10 min.

Fig. 3. RNA synthesis in chick embryo cell cultures infected with Semliki Forest virus at a multiplicity of 10 p.f.u./cell with or without actinomycin D. Actinomycin D-sensitive cellular RNA was plotted as the difference between total RNA synthesis and actinomycin-resistant RNA synthesis. ●●●●, no actinomycin; ○—○, actinomycin-resistant; □—□, actinomycin-sensitive RNA.
production ceased at a time when progeny virus synthesis was maximal and cellular RNA synthesis was severely inhibited. The cessation of interferon synthesis is most probably due to cell death as a consequence of virus infection.

Table 2 shows RNA synthesis in chick embryo cells exposed to heat-inactivated Semliki Forest virus at a multiplicity equivalent to 10 p.f.u./cell of the live virus. Cellular RNA synthesis was not inhibited by inactivated virus for at least 12 hr. At 15 hr, cellular RNA synthesis was 86% of the controls and decreased at 69% at 18 hr after exposure to the inactivated virus. We have previously shown that virus-specific RNA synthesis could not be detected in cells exposed to inactivated virus up to at least 12 hr after exposure (Goorha & Gifford, 1970). Thus, it seems that prolonged synthesis and a higher yield of interferon are obtained when the RNA synthesis of the induced cells is not suppressed.
Interferon induction by Semliki Forest virus

DISCUSSION

Interferon production in cells induced by viruses is intimately associated with cellular RNA synthesis (Heller, 1963; Wagner, 1964). The work presented here is consistent with the hypothesis that viruses which rapidly shut off host RNA synthesis are poor inducers of interferon and that viruses which do not alter host RNA synthesis are generally good inducers (Aurelian & Roizman, 1965; Wagner & Huang, 1966). Aurelian & Roizman (1965) found that infection of dog kidney cells with herpes virus at a multiplicity of 100 p.f.u./cell caused rapid inhibition of cellular RNA synthesis and no interferon was produced. However, at a multiplicity of 12 p.f.u./cell, inhibition of cellular RNA synthesis was delayed and interferon was produced. Wagner & Huang (1966) found that Newcastle disease virus-induced interferon synthesis in Krebs-2 cells was terminated when cells were superinfected with vesicular stomatitis virus (VSV). Since VSV-infected cells exhibited a profound and almost immediate decline in the rate of cellular RNA synthesis, it was suggested that VSV prevented interferon synthesis by inhibiting RNA synthesis. Recently, Gandhi & Burke, (1970) reported that Newcastle disease and fowl plague viruses inhibited protein synthesis in chick embryo cells and did not induce interferon, while irradiated NDV and fowl plague viruses had little effect on cellular protein synthesis and did induce interferon.

In the present study, live Semliki Forest virus at a multiplicity of 10 p.f.u./cell caused marked inhibition of cellular RNA synthesis and induced small amounts of interferon, while heat-inactivated virus in equivalent amounts did not inhibit cellular RNA synthesis and induced much higher yields of interferon. The small amount of interferon induced by live Semliki Forest virus may be due to the fact that cellular RNA synthesis continued during the initial stages of infection and presumably allowed the formation and translation of interferon specific messenger RNA. The early cessation of interferon synthesis in cells infected with live virus is most probably due to cell death since production of RNA, infectious virus and interferon all stopped abruptly at about the same time. The continued synthesis of interferon for an additional 4 hr may explain the increased yield of interferon with inactive virus as compared with that obtained with live virus. The termination of interferon synthesis at 12 hr may be related to the stability of interferon-specific messenger RNA. Wagner & Huang (1965) reported that interferon-specific messenger RNA was stable for 10 hr in Krebs-2 cells. Similarly, Burke (1966) reported that interferon synthesis induced by Semliki Forest virus was completed in 10 hr in chick embryo cells under restrictive conditions when virus replication did not take place. Unfortunately, the mechanism for control of the synthesis of interferon specific messenger RNA synthesis is not known but may be due to the formation of another protein which inhibits further interferon synthesis (R. M. Friedman, personal communication, 1970).

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REFERENCES


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