Prolonged Infection of Interferon-Treated Cells by Vesicular Stomatitis Virus: Possible Role of Temperature-Sensitive Mutants and Interferon

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(Accepted 8 July 1977)

SUMMARY

Lγ cells were treated with 100 reference units/ml of mouse interferon and then infected with a wild-type vesicular stomatitis virus (VSV) at a multiplicity of 10 to 60 p.f.u./cell. Prolonged infection of cultures ensued, lasting from 14 to at least 60 days. Less than 1% of the cells produced infectious virus, but more than 10% produced detectable levels of VSV antigens. No small virion RNA forms (< 42S) characteristic of defective interfering (DI) virus particles were detected. The virus produced appeared to be temperature sensitive. There was decreased plaquing efficiency at 37 or 39 °C compared to 32 °C and termination of the chronic infection due to c.p.e. within a few days after shift to 32 °C. The cultures resisted superinfection with wild-type VSV or with a heterologous virus, encephalomyocarditis (EMC) virus. Treatment of cultures with rabbit anti-mouse interferon globulin resulted in a marked increase in virus titres and termination of the chronic infection. Prolonged VSV infection in this system may be related both to the emergence of temperature-sensitive mutants and to endogenous interferon production rather than to cyclical generation of DI particles.

INTRODUCTION

In cells chronically infected with vesicular stomatitis virus, there is evidence that persistence of the infection may be due to defective interfering (DI) virus particles (also referred to as T particles) and a number of workers (Holland & Villarreal, 1974; Palma & Huang, 1974; Holland, Villarreal & Breindl, 1976) have investigated the possible role of such forms in chronic virus infections in animal cells. However, in several earlier reports (Chany, 1961; Glasgow & Habel, 1962; Wagner et al. 1963; Rodriguez & Henlé, 1964) it was suggested, but not proven, that in some chronic virus infections endogenous production of interferon may be at least in part responsible for a persisting infection.

Recent reports have also suggested that persisting infections initiated with VSV might be due to the emergence of temperature sensitive (ts) mutants of VSV. These mutants act as conditionally defective particles that both interfere with and are rescued by the wild-type virus at non-permissive temperatures, i.e. 37 or 39 °C (Youngner et al. 1976; Youngner & Quaglia, 1976). In the present study, Lγ cells were treated with interferon and then infected with VSV at a high virus:cell multiplicity. Under these conditions chronic
infections, persisting for 2 to 3 weeks, or in several cases even longer, were established. The findings in these cultures suggested that the prolongation of the infection was more likely to be due to emergence of ts mutants and to endogenous interferon generated in the system than to cyclical production of DI particles.

METHODS

Viruses. The Indiana strain of VSV originally obtained from Mr C. Buckler (National Institute of Allergy and Infectious Diseases, Bethesda, Md.) was plaque-purified three times and passaged by us at low multiplicities in VERO cells. It was assayed as plaque-forming units (p.f.u.) in VERO cells, or as 50% tissue culture infectious doses (TCID$_{50}$) by c.p.e. in VERO or L$_Y$ cells in microtitre assay plates. EMC virus was assayed by c.p.e. in L$_Y$ cell monolayers.

Cells. L$_Y$ cells were originally obtained from J. Youngner (University of Pittsburgh, Pa.). They were grown in monolayers in microtitre wells in Eagle's minimum essential medium (MEM) with 10% foetal calf serum (GIBCO, Grand Island, N.Y.) and have proved to be highly sensitive to and excellent producers of mouse interferon (Youngner, Thacore & Kelly, 1972). VERO cells and baby hamster kidney cells (BHK 21) were obtained from the National Institutes of Health media section and were also grown in Eagle's MEM and 10% foetal calf serum.

Interferon. Mouse interferon was prepared and partially purified on an antibody affinity column (Ogburn, Berg & Paucker, 1973) in the laboratory of Dr K. Paucker (Pennsylvania College of Medicine, Philadelphia, Pa.). The specific activity of the preparations employed was at least $2 \times 10^7$ mouse reference units per mg of protein. Interferon was assayed by its ability to inhibit the replication of VSV by 0.5 log$_{10}$ in a single growth cycle in L$_Y$ cells. Titres are expressed in terms of the N.I.H. research reference standard of mouse interferon.

Fluorescent-antibody studies. Infected and uninfected monolayer cultures were gently trypsinized and cell smears were prepared on Teflon-coated well-slides and air-dried. They were immediately fixed in cold acetone for 10 min, allowed to dry, and reacted for 30 min at 37 °C with a drop of a 1:100 dilution of a rabbit antiserum (kindly donated by Dr J. Youngner) with a neutralizing antibody titre to VSV of 1:16000. After 30 min of incubation, the slides were washed three times in phosphate-buffered saline, and the cells were stained for 30 min at 37 °C with a 1:10 dilution of fluoresceinated goat anti-rabbit gamma globulin (Miles Laboratories, Inc., Elkhart, Ind.). At the end of the incubation period, the slides were again washed three times in phosphate-buffered saline, mounted in a 50% glycerol solution, and examined under ultraviolet light with a Zeiss Universal fluorescence microscope with an exciting filter no. BG-12 and a barrier filter no. 53 (Friedman & Costa, 1976).

Infectious centre assay. Infected L$_Y$ cells were washed, counted, diluted in tenfold steps and mixed with $5 \times 10^6$ VERO cells, enough to form a confluent monolayer in a 60 mm tissue culture dish. After the cells had attached (2 h) they were overlaid with Eagle's plaque medium and 0.9% agarose. Plaques were counted after 24 to 96 h.

Rabbit anti-mouse and anti-human interferon globulins. Anti-L cell interferon globulin was prepared by Dr K. Paucker in rabbits by inoculation of partially purified L cell interferon which had been produced in his laboratory. Pooled sera from a single animal were inactivated at 56 °C for 30 min. Globulin was precipitated twice by 50% saturated ammonium sulphate. The globulin was then sequentially adsorbed with sheep erythrocytes, insolubilized
Chronic infection in interferon-treated cells

Monolayers containing 2 to $4 \times 10^6$ L$_y$ cells were treated for 14 h with mouse interferon (100 units/ml). These cells and untreated control cultures were washed and then infected with VSV at a multiplicity of 30 to 60 p.f.u./cell. The virus had been produced in VERO cultures infected at a multiplicity of 0.01; it titred $2 \times 10^8$ p.f.u./ml.

At the multiplicity of infection used, all of the L$_y$ cells in both the untreated and interferon-treated cultures appeared to be infected with virus since the chance of any one cell escaping infection under these conditions is very small (estimated at far less than $10^{-8}$). In the control cultures obvious virus c.p.e. was present in all cells by 8 h after infection and by 24 to 36 h after infection, the cells were completely destroyed (Fig. 1). Little c.p.e. (less than 10% of the cells destroyed) was observed in the interferon-treated cultures, although interferon treatment has no effect on virus adsorption (Friedman, 1966). By 8 h after infection more than 90% of the cells in the culture were strongly positive for VSV antigen in a fluorescent...
antibody assay and by 24 h after infection, more than 99% of the cells were positive. Nevertheless, virus production was inhibited by more than 10000-fold (Fig. 1).

The cultures which had been treated with interferon were subcultured by splitting 1:5 at 24 h after infection and then at 4-day intervals. Medium was changed at 2-day intervals. At the indicated time periods after infection samples of culture fluids were frozen at −70 °C in 0.1 ml aliquots. Later they were assayed on microtitre dishes in tenfold serial dilutions. Virus production in these cultures persisted throughout the experiment. The cultures were carried for 22 days after infection at which time they were frozen for storage in liquid nitrogen.

The titres of virus produced from the cultures were at the level of 10¹ to 10³ p.f.u./0.1 ml throughout the study. In two other prolonged VSV infections similarly initiated in Lυ cells, higher virus titres (10³ to 10⁴ p.f.u./0.1 ml) were generally observed and virus c.p.e. was noted in both on the 14th day after infection. In other studies c.p.e. was seen in chronic VSV-producing cells after 24 days of culture; in yet another culture, virus production persisted for more than 60 days. Similar results were obtained in cultures infected at a virus to cell multiplicity of 0.1 to 10.

Virus RNA from interferon-treated, persistently infected cultures

Several species of VSV RNA may be isolated from culture fluids of infected cells. When cultures are infected by virus pools containing high multiplicities of DI particles, virus RNA is formed in a range of different sizes which are all smaller than the normal RNA of the virion (42S). Virus pools containing only infectious virus particles characteristically display only a 42S RNA.

To optimize and amplify any existing DI particles in a population of VSV resulting from a prolonged infection, BHK cells, which are excellent DI particle producers (Perrault & Holland, 1972), were infected with undiluted samples from days 1, 3 and 5 of chronic infection of Lυ cells. No virion RNA smaller than 42S could be identified. Similar experiments on undiluted samples from days 3 and 5 also showed no indication of virion RNA of less than 42S. Intracellular species of virus RNA produced during prolonged infections with VSV were also studied. Even though reduced amounts of virus RNA were present in interferon-treated cells during prolonged infection, qualitatively the same species of virus RNA were present as were seen in acute VSV infections (J. M. Ramseur and R. M. Friedman, unpublished data).

The results suggested that in this system, production of significant quantities of DI particles was not required to maintain a chronic infection; although DI particles might be present, other workers (Youngner et al. 1976) and we ourselves have found it extremely difficult to generate significant amounts of DI particles in Lυ cells even under conditions of repeated infections with high titres of VSV.

Emergence of ts mutants of VSV during prolonged infections of interferon-treated cultures

Persistent infection with VSV with a high ratio of DI particles to infectious virus can be established from wild-type virus or ts mutants of VSV (Holland & Villarreal, 1974; Youngner et al. 1976). In one case, the prolongation of the infection initiated by wild-type VSV appeared to depend on the spontaneous emergence of ts mutants of VSV during incubation at 37 °C. These mutants interfered with the growth of the wild-type virus, persisted, and became the dominant type of virus present (Youngner et al. 1976; Youngner & Quagliana, 1976). We found that if interferon-treated cultures in which prolonged infection with VSV at 37 °C was established, were shifted to 32 °C, there was c.p.e. and a more
Chronic infection in interferon-treated cells

Table 1. Temperature shift studies in interferon-pre-treated cultures infected with VSV at 10 p.f.u./cell

<table>
<thead>
<tr>
<th>Interferon pre-treatment (units/ml)*</th>
<th>Day of shift from 37 to 32 or 39 °C</th>
<th>Measured at day</th>
<th>Virus titre (p.f.u./0·1 ml) at</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>1</td>
<td>8·0 × 10³†</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>6‡</td>
<td>4·4 × 10³</td>
</tr>
<tr>
<td></td>
<td>7‡</td>
<td>6·8 × 10³†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8‡</td>
<td>7·6 × 10³†</td>
<td></td>
</tr>
</tbody>
</table>

* 18 h at 37 °C.
† 100 % virus-induced c.p.e. observed at this time.
‡ Cells were subcultured at days 1 and 5.

Table 2. Evidence for emergence of temperature-sensitive mutants during prolonged infection of Lut cells with VSV at 37 °C

<table>
<thead>
<tr>
<th>Interferon pre-treatment (units/ml)*</th>
<th>Time after infection with VSV (days)†</th>
<th>Virus titre (p.f.u./0·1 ml × 10⁻⁷) at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>32 °C 39 °C</td>
</tr>
<tr>
<td>100</td>
<td>1‡</td>
<td>8000 8000</td>
</tr>
<tr>
<td></td>
<td>1§</td>
<td>150 100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4 3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>&lt; 1 &lt; 1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>800 150</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>1·6 0·4</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>40 2·6</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>120 5·0</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>40 0·6</td>
</tr>
</tbody>
</table>

* 18 h at 37 °C.
† Cells were infected at a multiplicity of 10 p.f.u./cell.
‡ After 24 h there was complete c.p.e. Virus samples at this time were assayed at 32 or 39 °C.
§ Culture fluids were harvested at indicated times after infection and assayed for infectious virus.

Table 3. Inability to establish prolonged infection in interferon-treated Lut cells infected with VSV and incubated at 32 °C

<table>
<thead>
<tr>
<th>Interferon treatment (units/ml)</th>
<th>Day after VSV infection</th>
<th>Titre of virus assayed at 32 °C (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>1·7 × 10⁷</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>1·8 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4·0 × 10³</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6·0 × 10³</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4·1 × 10⁵</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1·2 × 10⁶*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6·2 × 10⁶*</td>
</tr>
</tbody>
</table>

* 100 % virus-induced c.p.e. observed at this time.

than 100-fold rise in virus titres within 48 h (Table 1). Cultures left at 37 °C or shifted to 39 °C remained chronically infected. The wild-type virus employed in this study grew to high titres within 24 h after infection at all three temperatures, but its growth at 39 °C was slightly lower than at 32 or 37 °C (Table 1). VSV often appears spontaneously to have an increased plaquing efficiency at 32 °C compared to 37 or 39 °C but with increasing...
culture time, this temperature-sensitive effect became more dominant (Table 2), a finding which suggested the emergence of temperature-sensitive mutants. The low yields of virus recovered on day 5 were probably due to the residual effects of interferon treatment on day 0 (Fig. 2a) and to the subculture at day 4.

Furthermore, we have been unable to initiate a state of prolonged infection in L cells which were treated with 100 units/ml of interferon for 16 h at 37 °C, then infected with VSV and incubated at 32 °C (Table 3). This result and the results seen in the experiment summarized in Table 1 were possibly due to c.p.e. by ts mutants after the temperature downshift (Youngner et al. 1976; Youngner & Quagliana, 1976). The exact nature of the temperature-sensitive mutation which emerged under these conditions is currently under investigation by selectively cloning the virus from the chronic infection.
Chronic infection in interferon-treated cells

![Graph showing virus titres after infection of interferon-treated or chronically VSV-infected Lγ cells with 1000 p.f.u. of EMC virus.](image)

**Fig. 3.** Virus titres after infection of interferon-treated or chronically VSV-infected Lγ cells with 1000 p.f.u. of EMC virus. ▲—▲, Cultures pre-treated with interferon, and infected with 30 p.f.u. of VSV per cell at day 0; ○—○, cultures pre-treated with interferon, but not infected at day 0 (these cultures were passaged on days 1, 5, and 9 after infection).

**Resistance of VSV producer cultures to superinfection with homologous or heterologous viruses**

Lγ cells which chronically produced VSV (Fig. 1) must have been resistant to VSV infection, since they were not destroyed by their endogenously produced virus. This point was demonstrated by experiments in which 10⁸ chronically infected Lγ cells were co-cultivated with 2 × 10⁶ VERO cells, or uninfected Lγ cells. These mixed-cell cultures showed almost complete virus c.p.e. in less than 14 h.

In order to confirm that the cultures producing VSV in a prolonged infection (Fig. 1) were also resistant to VSV, they were superinfected with 10⁵ p.f.u. of VSV per culture (Fig. 2). When normal Lγ cell controls with the same passage history as the Lγ cells with a prolonged VSV infection were infected at various times with 10⁶ p.f.u. of VSV, complete c.p.e. and virus growth to 10⁶ to 10⁷ p.f.u./0.1 ml were regularly noted within 48 h after infection (Fig. 2c). Other cultures were treated with interferon at the same time as the VSV producer cells but were not immediately infected with a high multiplicity of VSV; these were also infected with 10⁵ p.f.u. of VSV at various times during their passage (Fig. 2b). There was inhibition of virus growth in the original cultures and early in the first passage (Fig. 2b, points at P₀ and P₁). Later in the first passage and in all subsequent passages, however, normal virus growth to 10⁶ to 10⁷ p.f.u./0.1 ml was consistently observed. Therefore, after about 5 days in these cultures there was no residual inhibitory effect of the interferon treatment on day 0.

Quite different results were obtained in Lγ cells with prolonged VSV infection (Fig. 2a), cultures similar to those which chronically produced 10⁴ to 10⁵ p.f.u. of VSV per 0.1 ml (Fig. 1). When superinfected with 10⁵ p.f.u. of VSV there was no increase in virus yield; compared to control cultures (Fig. 2b, c) there was a marked inhibition of VSV production throughout the entire course of the chronic infection (Fig. 2a). These findings of inhibition
of homologous virus replication were consistent with an inhibition of VSV production due to DI particles (Holland & Villarreal, 1974), or to ts mutants of VSV (Youngner et al. 1976; Youngner & Quagliana, 1976). However, they were also consistent with a non-specific antiviral activity. In contrast, results obtained in cultures superinfected with heterologous EMC virus were quite inconsistent with the hypothesis that the resistance to super-infection was completely due to either DI particles or ts mutants (Holland & Villarreal, 1974; Youngner et al. 1976; Youngner & Quagliana, 1976). In cultures infected with 10³ p.f.u. of EMC virus per culture on day 11 or 15 after interferon treatment and primary VSV infection, there was a marked inhibition of EMC yields as compared with results in cultures treated with interferon on day 0, but not infected with virus until day 11 or 15 (Fig. 3).

Evidence suggestive of a chronic interferon effect

One characteristic of an inhibition of virus growth due to low levels of interferon is that only partial inhibition of virus functions is observed. In Semliki Forest virus infected cells, concentrations of interferon which markedly inhibited yields of infectious virus had a much less inhibitory effect on intracellular levels of single-stranded virus RNA and almost no effect on production of virus replicative forms (Mécs et al. 1967). Similarly, in the chronic VSV-producing cultures in Fig. 1, fluorescent-antibody studies at day 8, during the second passage, indicated that 12% of the cells were positive for virus antigen. Repeated studies during the course of this experiment and others have indicated a similar percentage of cells positive for virus antigen. When the same cells at day 8 (Fig. 1) were also tested for infectious centre formation, only 0.9% scored as positive. Thus, at least ten times more cells were producing virus antigen than produced infectious virus.

In addition very low concentrations of interferon have sometimes been found in the tissue culture fluids from chronic VSV-producing cells. On day 2, there were 6 units of interferon/ml and on day 15, 5 units/ml, but no measurable concentrations of interferon were detected on days 4 and 8. It is important to note that in L₂ cells, concentrations of interferon as low as 2 units/ml completely inhibited c.p.e. due to VSV infection at a multiplicity of 10 p.f.u./cell (data not shown).

A role for interferon in the maintenance of chronic infection in these cultures also seemed possible from the results of experiments employing rabbit anti-mouse interferon globulin. When this globulin was added to cultures with prolonged VSV infections, virus titres rose

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**Table 4. Stimulation of virus growth in prolonged VSV infection in L₂ cultures treated with rabbit anti-mouse interferon globulin**

<table>
<thead>
<tr>
<th>Time after addition of anti-interferon globulin (h)</th>
<th>Virus titre (p.f.u./0.1 ml x 10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-mouse interferon globulin (1:500)</td>
</tr>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>48</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>72</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>96</td>
<td>40</td>
</tr>
</tbody>
</table>

* L₂ cell cultures were treated with 100 units/ml of mouse interferon and, after 18 h, infected at a multiplicity of 100 p.f.u./cell with VSV. Prolonged infection ensued. After 5 days, 4 ml of anti-mouse interferon globulin or anti-human interferon globulin was added to some of the cultures at a final dilution of 1 to 500 or 1 to 250.
† 100% virus-induced c.p.e. observed at this time.
Table 5. Virus growth in L₀Y cells infected with Sindbis virus at 37 °C with or without interferon treatment

<table>
<thead>
<tr>
<th>Interferon treatment (units/ml)</th>
<th>Days after infection</th>
<th>Virus titre (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m.o.i. = 100</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>5 x 10⁶</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>1 x 10³</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

significantly and the cultures were destroyed (Table 4). The anti-mouse interferon globulin used had no significant effect on wild-type VSV infection in normal L cells. Also, rabbit anti-human foreskin fibroblast interferon globulin (enough to neutralize 240 units of human interferon) or purified rabbit IgG added to culture media for several days did not stimulate virus titres in VSV chronic infection and cell death did not occur (Table 4).

** Attempt to establish a prolonged infection with Sindbis virus in interferon-treated L₀Y cells

When interferon-treated L₀Y cells were infected with Sindbis virus at multiplicities of infection (m.o.i.) of 100 or 10 p.f.u./cell, there was a significant inhibition of virus production and of virus-induced c.p.e. (Table 5). High titres of interferon (50 to 300 units/ml) were produced and virus titres dropped off rapidly over the 6 days following infection until little or no virus could be recovered from the cultures. No virus-induced c.p.e. was ever detected in the subsequent passages of these cells.

**DISCUSSION**

The evidence in this paper suggests that the prolonged VSV infection seen in interferon-treated L₀Y cells is probably due both to emergence of ts mutants of VSV and to endogenous interferon production rather than to the effects of DI particles. No small species of VSV RNA could be isolated from culture fluids of the producer L₀Y cells on days 1, 3, or 5 after infection by passage of virus in BHK cells. Also, electron microscopy studies of negatively stained VSV samples from interferon-treated cells showed no evidence of significant quantities of DI particles (J. M. Ramseur and R. M. Friedman, unpublished data). On the other hand, the VSV from the cultures with prolonged infection contained ts mutants. Also, the production of infectious virus was more markedly inhibited than was the production of virus antigens. There was marked resistance to both homologous and heterologous virus infection, results which were more consistent with an interferon effect than with an inhibition due to DI forms or to ts mutants. In the case of cells infected with a high concentration of DI forms there was no resistance to superinfection with EMC (Holland & Villarreal 1974). Resistance induced by ts mutants also appears to be homologous (Youngner et al. 1976; Youngner & Quagliana, 1976). An exception to the latter was resistance to pseudorabies virus in cells persistently infected with VSV (Youngner et al. 1976; Youngner & Quagliana, 1976). In addition, low levels of interferon could be isolated from the culture fluids of the chronic VSV-producing cells; and also, rabbit anti-mouse interferon globulin stimulated virus growth. At present, we are attempting to see whether substances which inhibit
interferon action, such as cholera toxin or thyrotropin (Friedman & Kohn, 1976), will rapidly raise virus titres in cultures with prolonged VSV infections.

The prolonged VSV infection seen in interferon-treated L_Y cells may mimic some natural chronic virus infections because high titres of interferon could be generated in the stage of acute infection. This interferon might inhibit the growth of the infecting virus and thus initiate reciprocal cycles of virus and interferon production. A few tissue culture systems which seem to conform to this model have been reported (Chany, 1961; Glasgow & Habel, 1962) in addition to the L_Y cell-VSV system discussed in this paper.

The genesis of the crises which terminated most of the reported experiments by causing complete c.p.e. is not understood. In other systems it has been suggested that the c.p.e. was due to destruction of interferon after trypsinization of the cell monolayers (Glasgow & Habel, 1962; Wagner et al. 1963) or possibly to emergence of viruses which were not ts mutants (Youngner et al. 1976; Youngner & Quagliana, 1976). These are probably not the correct explanations, because the antiviral activity of interferon persists long enough for cultures to generate more endogenous interferon after depletion by trypsin treatment, and interference by ts mutants seemed to be very effective in controlling VSV growth (Youngner et al. 1976; Youngner & Quagliana, 1976). If the prolonged VSV infection was partially related to endogenous interferon production, then among possible causes for the crises leading to c.p.e. were: (1) induction of a refractory state of the cells to the antiviral activity of the endogenously produced interferon (Friedman, 1966); (2) loss of the ability of the cultures to produce interferon (Chany & Vignal, 1970); or, (3) a slow build up of titres of wild-type virus high enough to overcome the protective effect of low concentrations of interferon.

Since both ts mutants and endogenous production of interferon seemed to play roles in maintaining prolonged VSV infections of L_Y cultures, it is interesting to speculate on a possible interrelationship between these factors. While VSV is a poor inducer of interferon, it is possible that its ts mutants might be better inducers. The emergence of these ts mutants could inhibit endogenous infection directly by an interference mechanism which would tend to inhibit the emergence of wild-type viruses (Youngner et al. 1976; Youngner & Quagliana, 1976). The interferon generated would tend both to moderate the infection by the ts mutant and to slow up the emergence of rapidly growing viruses. Thus the two factors may work in concert to maintain the chronic infection. It would be important to know, however, whether chronic interferon production favours the emergence of ts mutants or whether ts mutants tend to be better inducers of interferon. These problems are under study. It is also, of course, possible that endogenous interferon production and emergence of ts mutants are not directly related.

In one important respect the present study differs from previous studies of chronic virus infection in which the chronic state may have been due to interferon (Chany, 1961; Glasgow & Habel, 1962). In the present system exogenous interferon treatment was used to initiate prolonged infection. Since the capacity of cells to form interferon can be changed by pre-treating them with interferon (Friedman, 1966), the prolonged infection may have been induced by production of interferon early in infection due to this priming effect.

Under the conditions employed in this study, it was consistently possible to initiate prolonged infections with VSV, a finding which may be of both theoretical and practical interest. By using similar methods we have been attempting to set up prolonged infections by a number of viruses in interferon-sensitive cells with an awareness of the delicate balance between virus production and endogenous interferon generation. In contrast to the VSV-L_Y chronic infection, Sindbis virus infection of interferon-treated L_Y cells gave rise to high
titres of endogenous interferon. This appeared to cause the complete elimination of Sindbis virus infection from the cultures, rather than to give rise to a chronic infection. Similar results were obtained in herpes simplex virus infection of L- cells (Friedman & Costa, 1976).

On the practical side, it would be important to learn whether repeated treatment of patients with high levels of interferon might convert an acute virus disease into a chronic infection.

After this work had been completed, Nishiyama (1977) reported generally similar conclusions in a study of L cells persistently infected with VSV.

We wish to thank F. Mottram and C. Yee for their technical assistance and Dr J. Costa for help in carrying out fluorescent antibody studies.

Much of this material was presented at the 77th annual meeting of the American Society of Microbiology (abstracts of the annual meeting of the ASM, S342, p. 336, 1977).

This work was performed by J.M.R. as a partial fulfilment of requirement for a Ph.D. in Genetics at George Washington University.

REFERENCES


(Received 6 April 1977)