The Influence of the Host Cell on the Inhibition of Virus Protein Synthesis in Cells Doubly Infected with Vesicular Stomatitis Virus and Mengovirus

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SUMMARY

The ability of mengovirus to inhibit the synthesis of vesicular stomatitis virus (VSV) proteins and of VSV to inhibit the synthesis of mengovirus proteins during double infection in three different cell lines was investigated. Although cellular protein synthesis was inhibited after infection of cells by each virus, the ability of one virus to decrease translation of the mRNA species of the co-infecting virus varied with the cell type. Superinfection of mengovirus-infected L-929 cells by VSV resulted in essentially no inhibition in the synthesis of either mengovirus or VSV proteins. In HeLa cells and CHO cells the synthesis of both VSV and mengovirus proteins was inhibited under conditions of simultaneous or sequential infection. The inhibition of VSV protein synthesis after infection of HeLa cells by mengovirus was not a result of a modification or inactivation of virus mRNAs. When extracted from doubly infected cells, the VSV mRNAs manifested normal biological activity, as determined by their ability to stimulate the synthesis of VSV proteins in a micrococcal nuclease-treated cell-free system from L cells.

The interference or non-interference of one virus by another in different cell lines was also measured by quantifying the number of infectious particles produced in each cell line. The results were similar to those reported above for protein synthesis inhibition. These experiments suggest that the interference of mengovirus with VSV mRNA translation in HeLa cells is not necessarily reflective of the mechanism by which mengovirus inhibits cellular protein synthesis. Also, the host cell appears to influence the extent or nature of the interference of one virus by the other.

INTRODUCTION

The inhibition of cellular protein synthesis by picornaviruses has been studied extensively, but the precise mechanisms that allow discrimination between cellular and virus mRNA in translation have not been elucidated (Lucas-Lenard, 1979). The block in cellular mRNA translation seems to occur at the level of initiation of protein synthesis (Leibowitz & Penman, 1971).

When VSV-infected HeLa cells are superinfected with poliovirus, the translation of VSV mRNAs is inhibited (Doyle & Holland, 1972; Ehrenfeld & Lund, 1977). The kinetics of poliovirus-induced inhibition of cellular and VSV protein synthesis are the same, suggesting that they take place by the same mechanism (Ehrenfeld & Lund, 1977). Thus, the inhibition of VSV mRNA translation by poliovirus has been considered to be a model
system for studying cellular protein synthesis inhibition by viruses (Ehrenfeld & Lund, 1977; Rose et al. 1978). Recent studies suggest that the inhibition results from an inactivation of an initiation factor necessary for VSV (Rose et al. 1978) and cellular mRNA (Helentjaris & Ehrenfeld, 1978) translation. Helentjaris & Ehrenfeld (1978) have shown that crude preparations of initiation factors from poliovirus-infected HeLa cells support the translation of poliovirus but not of cellular mRNA. Subsequently, Rose et al. (1978) reported that the inhibition of VSV mRNA translation in extracts from poliovirus-infected HeLa cells is reversed by the addition of initiation factor eIF4B from rabbit reticulocytes. The active component is probably a 24,000 mol. wt. protein that co-purifies with eIF4B (Sonenberg et al. 1978) and interacts with the 5'-cap structure on mRNA (Shafritz et al. 1976; Sonenberg et al. 1979). It has been suggested that poliovirus mRNA, lacking a cap structure (Hewlett et al. 1976), can bypass cap-dependent initiation (Rose et al. 1978). Thus, if virus infection resulted in the inactivation of the initiation factor necessary for the binding of capped mRNAs to ribosomes, poliovirus mRNA translation would not be affected by it.

In our laboratory we have been studying the mechanism by which mengovirus inhibits L-929 cell protein synthesis. To simplify the identification of the inhibited proteins after infection, we also turned to the model system discussed above, except that mengovirus and L cells were used in place of poliovirus and HeLa cells. Surprisingly, when L cells were co-infected with VSV and mengovirus, cellular protein synthesis was inhibited, but not VSV protein synthesis. When HeLa cells and CHO cells were co-infected with mengovirus and VSV, cellular, VSV and mengovirus protein synthesis was reduced. These results suggest that the mechanism by which VSV or mengovirus interfere with each other is not necessarily the mechanism by which VSV or mengovirus inhibit cellular protein synthesis. The host cell appears to influence the nature of the interference.

METHODS

Cells. Monolayers of mouse L-929 cells (1 x 10⁷ cells/plate) were cultivated in Eagle's minimum essential medium (MEM) supplemented with 6% foetal bovine serum. Monolayers of HeLa cells (3 x 10⁶ cells/plate) were cultivated in Dulbecco's modified Eagle's medium (DME) supplemented with 5% calf serum. Confluent monolayers (5 x 10⁶ cells/plate) of Chinese hamster ovary (CHO) cells which require proline for growth were cultivated in DME supplemented with 5% foetal bovine serum and non-essential amino acids. In each case 60 mm (Falcon Plastics) dishes were used.

Viruses. VSV, HR-C, a heat-resistant strain of the Indiana serotype, was grown on mouse L cell monolayers. Confluent monolayers were infected at a multiplicity of infection (m.o.i.) of 10⁻⁸ p.f.u./cell. The virus stock was harvested after 24 h at 36°C. Cell debris was removed by low speed (1000 g) centrifugation and the virus was pelleted at 30000 rev/min for 90 min in a Beckman 60 Ti rotor. The virus pellet was resuspended in 1/100 original cell supernatant volume of buffer containing 10 mM-tris-HCl, pH 7.4, 100 mM-NaCl and 1 mM-EDTA and stored at -70°C. The yield of virus was approx. 800 to 1000 p.f.u. per mouse L cell.

Mengovirus (small plaque variant) was grown in mouse L cells. Confluent monolayers were infected at an m.o.i. of 10. Virus stock was harvested after 24 h at 36°C by freezing and thawing. Cell debris was removed by low speed centrifugation at 1000 g and the supernatant was made 6% in polyethylene glycol 6000 (adjusted to pH 7.5 with 100 mM-tris-HCl). The solution was stirred overnight in the cold and the precipitate was collected by centrifuging at 15000 g for 5 min at 4°C. The pellet containing the virus was resuspended in 1/100 the original cell supernatant volume of phosphate-buffered saline (PBS) and stored at -70°C.
**Virus interference in different cell lines**

**Plaque assay of viruses.** Both VSV and mengovirus were plaqued on L-929 cells. Appropriate 10-fold dilutions of the virus sample were made in PBS and 0.2 ml was added per 60 mm plate of confluent cells in duplicate. Virus was adsorbed for 30 min at 36 °C with periodic agitation of the plates to ensure good distribution of the viruses. A 2 ml overlay containing MEM, 3% foetal bovine serum and 0.8% agarose was added to each plate at the end of the adsorption period. After 24 to 36 h at 36 °C, 1 ml of a 0.1% neutral red solution in 0.9% NaCl was added to plates and incubated for 1 h at 36 °C. Plaques were then counted. For virus samples containing both viruses, plaque assays were performed as described except that two sets of two plates were used for each dilution. The overlay for one set of plates was prepared as described above. For the second set, rabbit anti-VSV antibody (kindly supplied by Dr P. I. Marcus) was added to the agarose overlay. This addition caused a 4 log reduction in the plaquing of VSV and had no effect on the plaquing of mengovirus. The titre for mengovirus was determined directly while the titre for VSV was determined by the difference between total plaques without antibody and the mengovirus plaques.

**Infection of cells with virus and in vivo labelling of protein.** For single infection or co-infection, cells were washed with warm PBS and infected at the indicated m.o.i. at 0 time with 0.2 ml of virus diluted in PBS. Following adsorption at 37 °C for 30 min, the monolayers were overlaid with 2 ml warm MEM + 5% serum and incubated at 37 °C. For superinfection by VSV, the monolayers, previously infected with mengovirus, were washed with warm PBS at 1.5 h post-infection (p.i.) and superinfected at the indicated m.o.i. with 0.2 ml VSV in PBS. After 30 min of adsorption at 37 °C, 2 ml MEM + 5% serum were added to each plate. In order to label proteins, infected and uninfected cell monolayers were washed with warm PBS at 5 h p.i. and overlaid with 1 ml MEM lacking methionine, plus 5%, dialysed serum and 10 μCi/ml 35S-methionine (at 600 to 1300 Ci/mmol; Amersham). The cells were incubated for 30 min at 37 °C, after which the medium was removed and 0.5 ml of sodium dodecyl sulphate (SDS) lysing buffer (10% SDS, 1% β-mercaptoethanol, 5 mM-urea) was added. Lysates were collected and dialysed overnight against SDS sample buffer containing 0.1% SDS, 0.1% β-mercaptoethanol and 2.5 mM-tris-glycine, pH 8.2.

**SDS-polyacrylamide gel electrophoresis.** Lysate samples in SDS sample buffer were boiled for 10 min, cooled and approx. 150 μg protein was loaded on to polyacrylamide slab gels which were prepared according to the method of Laemmli (1970). After electrophoresis (25 mA for approx. 3 h), gels were dried and exposed to Kodak X-Omat R X-ray film for 24 to 48 h before film development. Autoradiograms were scanned using a Joyce-Loebl densitometer and relative amounts of individual virus proteins were determined by measuring peak areas of densitometer tracings.

**Extraction and analysis of RNAs from infected cells.** In single infections, cell monolayers on 100 mm plates were infected at an m.o.i. of 10 with mengovirus or VSV and were treated with 5 μg/ml actinomycin D. In double infections, VSV was added 1.5 h after infection with mengovirus. In each case at 5.5 h after the initial infection, plates were washed twice with PBS and lysed with 4 ml RSBK-Triton lysing buffer (0.1% Triton X-100, 0.01 M-KCl, 0.01 M-tris-HCl, pH 7.4, 1.5 mM-magnesium acetate). Nuclei were pelleted by centrifuging at 1200 g for 5 min, washed with 1 ml RSBK containing 1% Nonidet P 40 (Shell Oil Co.) and 0.5% deoxycholate, and re-centrifuged (Penman, 1966). The two supernatants were combined and extracted three times with 45% re-distilled phenol, 5% metacresol, 0.05% β-hydroxyquinolinol, 49.5% chloroform and 0.5% isooamyl-alcohol. The resulting aqueous layer was adjusted to 0.24 M-ammonium acetate, pH 5, and was precipitated with 2 vol. of ethanol.
Fig. 1. Densitometer tracings of autoradiograms of \(^{35}\text{S}\)-methionine-labelled proteins from infected and uninoculated L-929 cells. Cells were infected with mengovirus at the m.o.i. indicated in the figure and then 1.5 h later with VSV as indicated. At 5 h.p.i. with mengovirus, cells were labelled for 30 min as described. During coinfection the m.o.i. of the viruses were as indicated in the figure. The labelled proteins were analysed by gel electrophoresis as described in Methods. (a) L-929 cells alone; (b) infected with VSV (m.o.i. 10); (c) VSV (m.o.i. 50); (d) mengovirus (m.o.i. 10); (e) mengovirus (m.o.i. 50); (f) coinfection with mengovirus (m.o.i. 10) and VSV (m.o.i. 10); (g) superinfection, first with mengovirus (m.o.i. 10) then with VSV (m.o.i. 10); (h) superinfection with mengovirus (m.o.i. 10) then VSV (m.o.i. 50); (i) superinfection with mengovirus (m.o.i. 50) then VSV (m.o.i. 10); (j) superinfection with mengovirus (m.o.i. 50) then VSV (m.o.i. 50).
For translation of RNA in vitro, S-30 extracts from uninfected L-929 cells were prepared essentially as described by Rose et al. (1978). Cells (7 × 10⁶) were pelleted and resuspended in 2 vol. of a buffer containing 10 mM-KCl, 1·3 mM-Mg(OAc)₂, 0·5 mM-dithiothreitol and 10 mM-HEPES-KOH, pH 7·4, and allowed to swell for 10 min on ice. Cells were lysed by 20 strokes with a Dounce homogenizer, and 0·2 vol. of five times concentrated incubation buffer, consisting of 150 mM-HEPES-KOH, pH 7·4, 15 mM-Mg(OAc)₂, 400 mM-KCl and 5 mM-dithiothreitol, was added. The supernatant from a 30000 g, 20 min centrifugation, was incubated for 45 min at 37 °C with 0·1 vol. of ten times concentrated energy mix (10 mM-ATP, 2 mM-GTP, 250 mM-creatine phosphate, 2 mg/ml creatine kinase) and dialysed for 2 to 4 h against 100 vol. of dialysis buffer (10 mM-HEPES-KOH, pH 7·4, 90 mM-KCl, 1·5 mM-Mg(OAc)₂, 7 mM-β-mercaptoethanol). Extracts were treated with 10 μg of micrococcal nuclease (Boehringer-Mannheim Biochemicals, Indianapolis, Ind., U.S.A.) per ml of extract for 20 min at 20 °C as described by Pelham & Jackson (1976). Samples of extract were stored at -70 °C.

In vitro translation reaction mixtures (50 μl) contained: 10 μl of extract (0·2 A²₈₀ units), 1 mM-ATP, 0·2 mM-GTP, 25 mM-creatine phosphate, 0·2 mg/ml creatine kinase, 80 mM-KCl, 30 mM-HEPES-KOH, pH 7·4, 3 mM-Mg(OAc)₂, 0·38 μM each of 19 amino acids, 1 mM-dithiothreitol, 2 μCi ³⁵S-methionine (600 to 1300 Ci/mmol, New England Nuclear Corp.) and 3 to 7 μg RNA (from the extraction described above). Reactions were incubated at 32 °C for 1 h and 1 vol. of twice concentrated SDS sample buffer (Laemmli, 1970) was added to stop the reaction. Samples were prepared for electrophoresis as described above. Gels were prepared for fluorography according to the method of Bonner & Laskey (1974).

RESULTS

Virus protein synthesis in L cells simultaneously infected with mengovirus and VSV or sequentially infected first with mengovirus and then with VSV

The object of this experiment was to determine the feasibility of using the inhibition of synthesis of VSV proteins by mengovirus as a prototype of cellular protein synthesis inhibition by mengovirus in L cells. To do this it was necessary to determine whether mengovirus, like poliovirus, inhibits VSV-directed protein synthesis in doubly infected L cells. In these experiments L cells were infected with mengovirus and VSV simultaneously, or were first infected with mengovirus and then with VSV 1·5 h later. This latter order was used for maximum detection of all virus proteins, since substantial mengovirus protein synthesis is not seen until 5 to 6 h p.i., whereas VSV protein synthesis occurs at least 1 h earlier. At 5 h after the initial infection, the cells were labelled for 30 min with ³⁵S-methionine as described in Methods. Afterwards, the cells were lysed and the proteins that were synthesized at this time after infection were identified by polyacrylamide gel electrophoresis.

Fig. 1 (a to e) shows the pattern of protein synthesis in L-929 cells alone, and in cells infected with either VSV or mengovirus at m.o.i. of 10 or 50. The results of infecting L cells first with mengovirus and then with VSV are shown in Fig. 1 (g to j). As can be seen, the synthesis of both VSV and mengovirus proteins occurred without apparent interference of one virus by another. By infectious centre assay it was determined that the efficiency of infection of L cells by mengovirus was 100% (Colby et al. 1974). Thus, it cannot be argued that only one half of the L cells was infected with mengovirus and the other half by VSV. When L cells were infected simultaneously with mengovirus and VSV, translation of both VSV and mengovirus mRNAs was inhibited (Fig. 1, panel f). This inhibition probably does not result from competition at the level of attachment, since the
Table 1. Effect of double infection on production of VSV proteins in different cell lines

<table>
<thead>
<tr>
<th>% VSV proteins synthesized*</th>
<th>HeLa</th>
<th>CHO</th>
<th>L-929</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mengovirus (m.o.i.) ... VSV (m.o.i.)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10†</td>
<td>31 ± 10</td>
<td>64 ± 8</td>
<td>100 ± 2</td>
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<td>50†</td>
<td>17 ± 3</td>
<td>95 ± 11</td>
<td>104 ± 2</td>
</tr>
<tr>
<td>50‡</td>
<td>12 ± 14</td>
<td>31 ± 6</td>
<td>nt</td>
</tr>
</tbody>
</table>

* 100% = the amount of VSV proteins (L, G, N/NS and M) as measured from densitometer tracings from single infections. Numbers are an average from two gels for L-929 and CHO cells, and an average of three gels for HeLa cells.
† Superinfection.
‡ Simultaneous infection.
§ Not tested.

Table 2. Effect of double infection on the production of mengovirus proteins in different cell types

<table>
<thead>
<tr>
<th>% Mengovirus proteins synthesized*</th>
<th>HeLa</th>
<th>CHO</th>
<th>L-929</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mengovirus (m.o.i.) ... VSV (m.o.i.)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10†</td>
<td>58 ± 14</td>
<td>63 ± 7</td>
<td>82 ± 10</td>
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<tr>
<td>50†</td>
<td>42 ± 3</td>
<td>68 ± 10</td>
<td>95 ± 13</td>
</tr>
<tr>
<td>50‡</td>
<td>22 ± 18</td>
<td>31 ± 6</td>
<td>nt</td>
</tr>
</tbody>
</table>

* 100% = the amount of mengovirus proteins (A, B, C and D) as measured from densitometer tracings from single infections. Numbers are an average from two gels for L-929 and CHO cells, and an average of three gels for HeLa cells.
† Superinfection.
‡ Simultaneous infection.
§ Not tested.

number of unattached infectious VSV or mengovirus particles recovered from the extracellular fluid after a 30 min adsorption period was only slightly greater (10 to 15%) in simultaneous infection than in single infection (data not shown).

For a relative quantification of these data, only densitometer tracings which allowed us to accurately measure total peak areas were used. The per cent reduction was calculated from peaks in which there was no overlap of mengovirus and VSV proteins. Mengovirus proteins A, B, C and E were used for these calculations as well as all of the VSV proteins. As shown in Table 1 the amount of VSV proteins synthesized was not changed in L cells which were first infected with mengovirus and then with VSV. As shown in Table 2 shows that the amount of mengovirus proteins synthesized under these same conditions was also hardly affected.

When L cells were infected simultaneously with mengovirus and VSV, synthesis of both VSV and mengovirus proteins was inhibited to the extents shown in Tables 1 and 2.
Virus interference in different cell lines

VSV and mengovirus protein synthesis in HeLa cells

The results of the experiments described above were quite different from those in which HeLa cells were doubly infected with VSV and poliovirus. Since mengovirus is a picornavirus-like poliovirus, we thought that the difference might lie in the cell line used in these studies rather than in the virus. The above experiments were therefore repeated except that HeLa cells were used in place of L cells. Fig. 2(a to e) shows the amount of protein synthesis in control (panel a) cells, cells infected with VSV singly at a multiplicity of 10 or 50 (panels b and c) and cells infected with mengovirus singly at an m.o.i. of 10 or 50 (panels d and e). The results of simultaneous or sequential infection with VSV and mengovirus are shown in Fig. 2(f to j). As can be seen, there was a drastic reduction in synthesis of VSV proteins under all the conditions shown. This is similar to the results observed in HeLa cells infected simultaneously with poliovirus and VSV (Doyle & Holland, 1972). However, whereas in the latter case the synthesis of poliovirus proteins was unaffected under these conditions, the synthesis of mengovirus proteins was inhibited also, but to a lesser extent, except when VSV was present at an m.o.i. of 50, in which case mengovirus protein synthesis was extensively inhibited.

These data were quantified as described above for L cells doubly infected with mengovirus and VSV and the results are shown in Tables 1 and 2.

VSV and mengovirus protein synthesis in CHO cells

Studies similar to those discussed above were also carried out in CHO cells. As observed in HeLa cells, the ability of CHO cells infected first with mengovirus and then with VSV to sustain both mengovirus and VSV-directed protein synthesis was reduced (Fig. 3, compare panels b to e with g to j). However, the level of inhibition of VSV-directed protein synthesis was considerably less in CHO cells than in HeLa cells. Furthermore, simultaneous infection of CHO cells by VSV and mengovirus resulted in little inhibition of VSV-directed protein synthesis but a substantial inhibition of mengovirus-directed protein synthesis (Fig. 3f).

The measurements of all the data from densitometer tracings are demonstrated in Tables 1 and 2. Although both mengovirus and VSV-directed protein synthesis were inhibited, it is clear that the level of inhibition in CHO cells was much less than that seen in HeLa cells.

Inhibition of cellular protein synthesis by mengovirus or VSV

Since the ability of mengovirus and VSV to interfere with one another might simply reflect the ability of each virus to inhibit cellular protein synthesis, the effect of infection of L cells, HeLa cells and CHO cells on total cellular protein synthesis was examined. Cells were infected with VSV or mengovirus and, at the times specified in Fig. 4, aliquots were removed and incubated with ^35S-methionine for 30 min. The incorporation of the ^35S-methionine into hot acid-insoluble material was determined and the products analysed by polyacrylamide gel electrophoresis.

As shown in Fig. 4 cellular protein synthesis was inhibited to the same extent and at comparable rates in HeLa and CHO cells after infection with mengovirus. The effect of VSV infection on cellular protein synthesis was also comparable in HeLa and CHO cells. In L cells the rate and extent of inhibition after infection by either mengovirus or VSV seemed to be less than in either HeLa or CHO cells. An examination of the products synthesized at late times (see gels in Fig. 4) revealed that the inhibition of cellular protein synthesis was occurring to the same extent as in HeLa and CHO cells, except that there were more VSV and mengovirus products made in L cells, thus masking the inhibition in acid-insoluble tests.
Fig. 2. Densitometer tracings of autoradiograms of 35S-methionine-labelled proteins from infected and uninfected HeLa cells. HeLa cells were infected with mengovirus and VSV as described in the legend to Fig. 1 and Methods. The labelled proteins were analysed by gel electrophoresis as described in Methods. (a) HeLa cells alone; (b) infected with VSV (m.o.i. 10); (c) VSV (m.o.i. 50); (d) mengovirus (m.o.i. 10); (e) mengovirus (m.o.i. 50); (f) co-infection with mengovirus (m.o.i. 50) and VSV (m.o.i. 10); (g) superinfection with mengovirus (m.o.i. 10) then VSV (m.o.i. 10); (h) superinfection with mengovirus (m.o.i. 10) then VSV (m.o.i. 50); (i) superinfection with mengovirus (m.o.i. 50) then VSV (m.o.i. 10); (j) superinfection with mengovirus (m.o.i. 50) then VSV (m.o.i. 50).
Fig. 3. Densitometer tracings of autoradiograms of $^{35}$S-methionine-labelled proteins from infected and uninfected CHO cells. CHO cells were infected with mengovirus and VSV as described in the legend to Fig. 1 and Methods. The labelled proteins were analysed by gel electrophoresis as described in Methods. (a) CHO cells alone; (b) infected with VSV (m.o.i. 10); (c) VSV (m.o.i. 50); (d) mengovirus (m.o.i. 10); (e) mengovirus (m.o.i. 50); (f) co-infection with mengovirus (m.o.i. 50) and VSV (m.o.i. 10); (g) superinfection with mengovirus (m.o.i. 10) then VSV (m.o.i. 10); (h) superinfection with mengovirus (m.o.i. 10) then VSV (m.o.i. 50); (i) superinfection with mengovirus (m.o.i. 50) then VSV (m.o.i. 50); (j) superinfection with mengovirus (m.o.i. 50) then VSV (m.o.i. 50).
Fig. 4. Analysis of proteins synthesized in HeLa, CHO and L-929 cells after infection with (a) mengovirus or (b) VSV. Cells were infected at 0 time with mengovirus or VSV at an m.o.i. of 50. At the indicated times cells were labelled with $^{35}$S-methionine for 30 min. Samples at each time point were analysed for trichloroacetic acid-precipitable counts and by gel electrophoresis and autoradiography as described in Methods.

**Virus mRNAs in single and double infections**

Although Ehrenfeld & Lund (1977) showed that poliovirus inhibition of VSV was not due to a reduction in the quality of VSV mRNAs, mengovirus might be acting by modifying the VSV mRNAs. To test this possibility, RNAs from HeLa cells infected with one or both viruses were analysed for their translation activity in cell-free extracts made from uninfected L-929 cells. As shown in Fig. 5, VSV mRNAs do function in this cell-free translation system in spite of the fact that they are translated very poorly in HeLa cells after mengovirus infection. Although the presence of an L cell protein whose mRNA is resistant to micrococcal nuclease partially obscures the N and NS proteins of VSV, the M protein is translated from RNA extracted from singly or doubly infected HeLa cells. This protein was not synthesized using RNAs extracted from uninfected HeLa cells (lane c) or from RNAs extracted from mengovirus-infected HeLa cells (lane d). The absence of detectable mengovirus proteins in lanes (d) and (e) probably results from the lack of cleavage of the synthesized precursor proteins in the L cell extract. Most of the time only the ‘A’ precursor is made in these extracts.
Fig. 5. SDS-polyacrylamide gel analysis of proteins synthesized in micrococcal nuclease-treated L cell extracts from mRNAs extracted from singly or doubly infected HeLa cells. Incubation conditions and extraction of RNAs were as described in Methods. (a) Mengovirus protein marker; (b) extract with no RNA; (c) extract with 7 μg RNA from uninfected cells; (d) extract with 7 μg RNA from mengovirus–infected cells; (e) extract with 7 μg RNA from doubly infected cells; (f) extract with 7 μg RNA from VSV-infected cells; (g) VSV protein marker.
### Table 3. Effect of double infection on the yield of infectious VSV in different cell types

<table>
<thead>
<tr>
<th>VSV (p.f.u./cell)*</th>
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<th>L-929</th>
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<tr>
<td></td>
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<tr>
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</tr>
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<td>50‡</td>
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* The p.f.u./cell was determined in L-929 cells as described in Methods.
† Superinfection.
‡ Simultaneous infection.
§ Not tested.

### Table 4. Effect of double infection on the yield of infectious mengovirus in different cell types

<table>
<thead>
<tr>
<th>Mengovirus (p.f.u./cell)*</th>
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<td>120±32</td>
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* The p.f.u./cell was determined in L-929 cells as described in Methods.
† Superinfection.
‡ Simultaneous infection.
Yields of p.f.u. of mengovirus and VSV during double infection

We also determined whether or not the presence or absence of inhibition of virus protein synthesis was paralleled by effects on the yields of infectious VSV and mengovirus during double infection. The yield of mengovirus during double infections was determined using antiserum against VSV, and the VSV titre was determined by the difference between total plaques without antibody and the mengovirus plaques.

First, the yield of each virus alone was determined in the different cell types. As shown in Table 3, the yield of infectious VSV was approximately the same order of magnitude in all three cell types. Similarly, the yield of mengovirus, as shown in Table 4, was approximately the same order of magnitude in all cell types. In terms of infectious units per cell, VSV and mengovirus were approximately equal in any one cell type, suggesting that neither virus had any a priori replicative advantage over the other.

During simultaneous infection, as shown in Tables 3 and 4, the yields of infectious VSV and mengovirus were reduced between 4- and 18-fold in nearly all the different cell types when the m.o.i. was 10 or 50 for each virus. In HeLa and CHO cells, superinfection by VSV resulted in a 3- to 20-fold inhibition of both VSV and mengovirus infectivity. In L cells the infectivity of neither virus was reduced by more than twofold. In most of the cases examined, there seemed to be a relationship between virus protein synthesis inhibition and reduction of infectivity.

DISCUSSION

In our laboratory we have been studying the mechanism by which mengovirus and VSV inhibit cellular protein synthesis in L cells. We supposed that since mengovirus is a picornavirus like poliovirus, it would interfere with VSV protein synthesis and replication in L cells in a manner analogous to the action of poliovirus in HeLa cells. However, as documented in this report, this is not the case. Both mengovirus and VSV protein synthesis can take place simultaneously in L cells (Fig. 1) without extensive interference of one virus by the other, provided that the viruses are not added simultaneously. The yields of infectious virus are also not inhibited severely under these conditions.

It should be noted that in the experiments by Doyle & Holland (1972), HeLa cells were first infected with VSV and then superinfected with poliovirus, or were infected with both viruses simultaneously. In each case, the poliovirus interfered with the growth of VSV. We found that if cells were either infected first with VSV and then superinfected with mengovirus (unpublished results) or first infected with mengovirus and superinfected with VSV, VSV protein synthesis was not inhibited in L-929 cells but was inhibited in HeLa and CHO cells. Quantification of the mengovirus proteins was poor with the former protocol since substantial mengovirus protein synthesis is not seen until 5 to 6 h p.i., whereas VSV protein synthesis occurs at least 1 h earlier. Therefore, the latter protocol was used which did not influence the results obtained for VSV protein synthesis but allowed for the accurate quantification of mengovirus protein synthesis.

In cells infected simultaneously the inhibition does not appear to take place at the level of attachment since the number of unattached infectious particles recovered from extracellular fluid at 30 min after simultaneous infection was only 5% higher for VSV and 15% higher for mengovirus than the amount recovered after single infection (unpublished data).

The fact that the translation of both VSV and mengovirus RNAs takes place simultaneously in L cells is probably not due to the use of mengovirus in place of poliovirus. As shown in Fig. 2 mengovirus, like poliovirus, does restrict VSV protein synthesis and replication in HeLa cells without reducing the template activity (Fig. 5) of VSV mRNAs.
However, in contrast to studies using poliovirus, the synthesis of mengovirus proteins is also inhibited, although to a much lesser extent than VSV protein synthesis.

These results do not arise from an overwhelming amount of growth of one virus over the other. The yields of infectious VSV and mengovirus were approximately the same in L cells and HeLa cells (Tables 3 and 4), yet the synthesis of both VSV and mengovirus proteins was unaffected in L cells, while in HeLa cells translation of both virus mRNAs was inhibited. The yield of infectious VSV was also equal to the yield of mengovirus in CHO cells and, as in the case of HeLa cells, the synthesis of both VSV and mengovirus proteins was inhibited.

These results also do not arise from differences in the inhibition of cellular protein synthesis by each virus. A significant difference in rates or extent might have indicated an overall resistance of a particular cell type to inhibition by the virus. However, the rates and extents of inhibition by mengovirus in each cell type were comparable, suggesting that the mechanism of inhibition of cellular protein synthesis in HeLa cells is not necessarily the same as the inhibition of VSV protein synthesis. It is to be noted that mengovirus inhibited cellular protein synthesis slower than VSV (see insert to Fig. 4).

Mengovirus RNA, like poliovirus RNA, is uncapped (Nomoto et al. 1976; Perez-Bercoff & Gander, 1978). If mengovirus, like poliovirus, inhibits cellular protein synthesis by inactivating the ‘cap’ binding protein (Sonenberg et al. 1979), then one would expect that VSV mRNA translation would be restricted in any cell line that has been infected with mengovirus, since VSV mRNAs are capped (Shatkin, 1976). Therefore, the fact that mengovirus replication does not interfere with VSV mRNA translation and VSV growth in L cells is surprising. This lack of effect could result from the quantity of the cap binding protein in L cells. It is possible that cap binding protein is present in such excess in L cells that infection by mengovirus does not inactivate it totally. This explanation, however, is not satisfactory since it would not explain why there would be sufficient cap binding protein for VSV protein synthesis in L cells, but not enough for cellular protein synthesis, which is inhibited, albeit slowly. It is also possible that the mechanism of interference of VSV mRNA translation by mengovirus in HeLa cells is not necessarily reflective of the mechanism by which mengovirus turns off cellular protein synthesis. The former process could well be influenced by the cell line in which the co-infection is taking place. Studies are underway in our laboratory to test these possibilities.

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REFERENCES


Virus interference in different cell lines


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