Effect of Tacaribe Virus Infection on Host Cell Protein and Nucleic Acid Synthesis

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SUMMARY

Tacaribe virus stocks were prepared which induced definite lytic responses in Vero cells infected at multiplicities giving synchronous infection. Under these conditions, the first signs of cytopathic effect (c.p.e.) appeared at about 30 h post-infection and cell lysis occurred after 40 h. Before the onset of cytopathic changes, the virus induced inhibition of host cell protein, DNA and RNA (primarily rRNA) synthesis. These were designated c.p.e. (+) virus stocks. The effect of virus on host cell macromolecular synthesis and development of c.p.e. were not related to the virus isolate, but to the conditions under which the virus was produced. Thus, from a single virus clone, working stocks were derived which could or could not induce inhibition of host cell functions and c.p.e. development. The virus stocks that did not induce inhibition are defined as c.p.e. (−). Analysis of [3H]leucine-labelled proteins from Vero cells infected with either the c.p.e. (+) or the c.p.e. (−) virus stocks revealed synthesis of two virus-specific polypeptides migrating with mobilities corresponding to mol. wt. 68000 and 79000. These are presumed to correspond, respectively, to the nucleoprotein and to the minor polypeptide p79. In cells infected with the c.p.e. (+) virus stock, the virus-specific polypeptides were synthesized at times when there was a drastic inhibition of host cell protein synthesis. The yield of infectious progeny during the first 24 h of infection is similar in Vero cells infected with either the c.p.e. (+) or the c.p.e. (−) virus stocks. The proportion of defective interfering particles was much higher in the c.p.e. (−) than in the c.p.e. (+) virus stocks. The results presented here are the first demonstration that an arenavirus affects the biosynthetic machinery of the host cell.

INTRODUCTION

The arenaviruses are a group of enveloped viruses with genetic information encoded in two segments of single-stranded RNA. The virions contain, in addition, rRNA of cellular origin. Three members of this group, Junin, Machupo and Lassa viruses, cause serious diseases in man. Tacaribe virus, another member of this group, is antigenically related to Junin virus but is not pathogenic for human beings.

Arenaviruses infect a variety of cell lines in culture. They characteristically produce persistent infections in the host cell and the cytopathic effect (c.p.e.) is, in most instances, absent or only slightly apparent (Pedersen, 1979; Rawls & Leung, 1979). These properties of arenaviruses probably account for the generally accepted view that they do not disrupt vital cellular functions such as protein synthesis (Saleh et al., 1979; Buchmeier et al., 1978; Gimenez et al., 1983). It has been reported, however, that standard (plaque-forming) arenaviruses readily kill cells and that cells are spared through defective interfering (DI) particle intervention (Dutko & Pfau, 1978). If this were the case, standard arenaviruses should be able to affect vital biochemical mechanisms and this would ultimately lead to cell death. Every arenavirus preparation appears to be a mixture of standard and DI particles. The ratio varies according to the virus strain and depends on the conditions under which the virus has been produced. These are related among other
factors to the cell line used for virus growth, the m.o.i., and the time elapsing between infection of the cells and the harvesting of the supernatant fluids (Martínez Peralta et al., 1981; Dutko & Pfau, 1978; Pedersen, 1979).

On the basis of these observations we considered that it should be possible to obtain arenavirus preparations with a ratio of standard viruses to DI particles that would induce lysis upon infection of susceptible cells at multiplicities at which the culture should be synchronously infected. This would permit study of the mechanism whereby infection by an arenavirus eventually leads to cell death. To this end, we prepared Tacaribe virus stocks that induce definite lytic responses in Vero cells, even when infected at high multiplicities, and examined the effect of virus infection on host cell macromolecular synthesis. The results indicated that Tacaribe virus is able to affect host cell protein, DNA and RNA synthesis.

**METHODS**

**Cells.** BHK21 (Clone 13) cell monolayers were grown in MEM (Glasgow modification) containing 5% calf serum. Vero cells were grown in MEM (Earle's salts) with 5% horse serum. The two cell lines were from the American Type Culture Collection. Cells were shown to be free of mycoplasma by the method of Schneider et al. (1974).

**Virus.** Tacaribe virus strain T.RVL.II 573 (from C. J. Pfau, Rensselaer Polytechnic Institute, Troy, N.Y., U.S.A.) was kindly provided by M. Weissembacher (Instituto de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina), who performed five mouse brain passages. Unless otherwise indicated virus stocks were prepared as follows. Virus was plaque-cloned in Vero cells, and a plaque plug was used to infect approximately 10⁶ BHK21 cells. The supernatant fluid was collected on the fourth day after infection and used to produce a secondary infection in BHK21 cells at a m.o.i. of 0.05 or less. The culture medium, with titres of approximately 10⁵ p.f.u./ml, was collected on the third day after infection and clarified by centrifugation at 3000 g for 20 min. Some 30 ml of the clarified supernatant was layered on top of 8 ml of a solution containing 20 mM-Tris-HCl pH 7.20, 2 mM-EDTA, 30% (w/v) glycerol and virions were sedimented by centrifugation for 75 min at 45000 r.p.m. in the 55-2 Ti rotor of a L8-55 Beckman ultracentrifuge. The pelleted virions were resuspended in 300 to 600 μl of MEM containing 20% horse serum and 10% glycerol and stored at -70 °C. Virus stocks had titres of 10⁶ to 10⁷ p.f.u./ml. The virus preparations were identified as Tacaribe virus by neutralization with specific antiserum prepared in rabbits against the original Tacaribe virus stock from C. J. Pfau. Analysis by polyacrylamide gel electrophoresis of the RNAs from virus stocks labelled for 3 days with [3H]uridine revealed the large (L; 32S) and small (S; 25S) viral RNAs (Vezza et al., 1978) and 28S and 18S rRNAs. The ratio of the amount of radioactivity in the L and S viral RNAs lay between 1.3 and 1.6. The proportion of rRNA varied in the different virus stocks.

Supernatant fluids from uninfected BHK21 cells processed in parallel in the same way as the infected fluids were used for mock-infection of cells.

**Infection and mock infection of cells.** Exponentially growing cultures were used for infection. Virus dilutions of 0.2 ml were inoculated and allowed 60 min for adsorption at 37 °C before the monolayers were overlaid with 2 ml of culture medium. For mock infection, the supernatant stocks prepared from uninfected BHK21 cells were diluted and inoculated in the same way as the virus stocks. There was no difference between mock-infected and untreated cells as regards growth characteristics and macromolecular biosynthesis, thus discounting the presence of toxic substances in the supernatant fluids of BHK21 cells.

**Assay of infectivity.** Infectious virus was titrated by counting plaques on Vero cell monolayers as described by Mifune et al. (1971).

**Measurement of DI particles.** DI particles were assayed by their ability to form foci of protected Vero cells as described by Popescu et al. (1976).

**Measurement of protein, DNA and RNA synthesis.** Infected or mock-infected cell monolayers in 35 mm plastic Petri dishes were labelled at the times indicated for 1 h with either [3H]leucine (2 μCi/ml), [3H]thymidine (1 μCi/ml) or [3H]uridine (1 μCi/ml) in the appropriate culture medium. When required, actinomycin D (AMD) was added (15 μg/ml) 15 min before isotope addition. At the end of the labelling period, the medium was aspirated and the cell monolayers were washed three times with cold phosphate-buffered saline (PBS). The cells were detached by scraping in the presence of PBS, transferred to a centrifuge tube, adjusted to 10%, trichloroacetic acid (TCA) and kept at 4 °C for 30 min. Acid-precipitable material was pelleted at 1600 g for 10 min. An aliquot of the supernatant solution was withdrawn for measurement of acid-soluble radioactivity. The TCA-precipitable pellets were washed twice with cold 5% TCA. The final pellets were dissolved in 0.2 ml of Protosol (New England Nuclear) and counted in a toluene-based scintillation fluid. Each determination was performed in triplicate. Triplicate dishes of unlabelled infected or mock-infected cell monolayers were washed with PBS as described above and used for DNA determination, by the fluorometric assay described by Erwin et al. (1980). The TCA-soluble and -insoluble radioactivities were normalized for the DNA content of the cultures. The TCA-soluble...
radioactivity did not decrease at any time post-infection, indicating that the uptake of the label was unimpaired (results not shown).

**Labelling and analysis of cell and viral proteins by gel electrophoresis and fluorography.** Mock-infected or infected Vero cells were labelled with 100 μCi/ml of [3H]leucine in a medium containing one-tenth the normal amount of leucine. The cells were labelled for 2 h, at the times indicated. At the end of the incorporation period the monolayers were washed three times with PBS, the cells were detached by scraping in the presence of PBS, and transferred to a tube. Aliquots of the cell suspension containing about 2 x 10⁶ cells were pelleted at 800 g for 5 min and the pellet was resuspended in 50 μl of electrophoresis sample buffer (62.5 mM-Tris-HCl pH 6.8, 10% glycerol, 1% SDS, 1% 2-mercaptoethanol). For immunoprecipitation of viral proteins, about 2 x 10⁷ cells were pelleted as before, and the pellet was resuspended in 200 μl of RIPA buffer (50 mM-Tris–HCl pH 7.2, 150 mM-NaCl, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100) containing 1 mM-PMSE and 1 mM-TLCK and cellular debris was removed by centrifugation at 12000 g for 10 min. The supernatants were mixed with 10 μl of undiluted rabbit anti-Tacaribe virus serum and were incubated for 3 h at 37 °C followed by 12 h at 4 °C. The immunocomplexes were subsequently precipitated by incubation for 1 h at 4 °C with 100 μl of a 10% suspension of heat-killed formalin-fixed *Staphylococcus aureus* (Cowan I strain) (Kessler, 1975). The precipitates were pelleted at 12000 g for 10 min and the pellets were washed twice with 500 μl of RIPA buffer. Antigen–antibody complexes were eluted by resuspension in 50 μl of electrophoresis sample buffer and heating at 100 °C for 10 min.

Proteins were analysed by electrophoresis on 8 to 14% acrylamide–SDS slab gels prepared according to Laemmli (1970). Samples dissolved in the electrophoresis sample buffer were heated at 100 °C for 5 min. Electrophoresis was performed at 80 V for 14 h in a buffer containing 50 mM-Tris, 0.38 M-glycine, 0.1% SDS. Following electrophoresis the gels were prepared for fluorography by the method of Bonner & Laskey (1974) and exposed to preflashed Kodak XK-1 medical X-ray films at −70 °C.

**Assay of RNA polymerases (RNA nucleotidyltransferase, EC 2.7.7.6) in permeabilized cells.** Infected or mock-infected Vero cell monolayers were washed twice with cold PBS and permeabilized with the addition of a solution containing 0.34 M-m sucrose, 0.036 M-HEPES pH 7.3, 5 mM-MgCl₂, 0.012 M-KCl, 2 mM-dithiothreitol, 0.5 mM-PMSE and 0.001% Triton N-101 (2 ml of this solution for about 10⁶ cells). The cells were then detached by scraping, transferred to a tube and pelleted by centrifugation (1000 g, 5 min). The permeabilized cells were resuspended in a solution containing 0.34 M-m sucrose, 0.03 M-Tris–HCl pH 7.9, 5 mM-MgCl₂, 2 mM-dithiothreitol, 0.5 mM-PMSE; aliquots were taken for the assay of RNA polymerases and for DNA determination. DNA-dependent RNA polymerases were assayed by measuring the incorporation of [3H]UTP (1500 to 2000 Ci/mol) into RNA as previously described (Iapalucci-Espinoza et al., 1977) with the following differences: the reaction mixture contained, in addition, MnCl₂ at a final concentration of 1:5 mM and the incubation was for 10 min at 30 °C. Assays were performed in triplicate, in the absence or in the presence of 15 μg of α-amanitin/ml. When 200 μg of α-amanitin/ml was added, there was no detectable additional inhibition, indicating that in our experimental conditions the α-amanitin-insensitive activity corresponds to RNA polymerase I (Weil & Blatti, 1976). The RNA polymerase II activity was estimated as the difference between the total activity and that of RNA polymerase I.

**Materials.** Media and culture sera were from Gibco; culture flasks and Petri dishes from NUNC; [5,6-3H]uridine 5-triphosphate; [6-3H]thymidine, [5,6-3H]uridine and [1-4,5,3(n)-3H]leucine were purchased from New England Nuclear. The α-amanitin was kindly provided by Professor Th. Wieland of the Max Planck Institute, Heidelberg, F.R.G. AMD was a gift from Merck, Sharp and Dohme Argentina.

**RESULTS**

Effect of Tacaribe virus infection on nucleic acid and protein synthesis in Vero cells

When Vero cells are infected with virus stocks prepared as indicated in Methods at multiplicities at which the culture should be synchronously infected, the first signs of c.p.e. appeared at about 30 h post-infection as rounding and detachment of the cells. This is followed about 10 h later, by a definite lytic response of the cell monolayer. These virus preparations are defined as c.p.e. (+). The studies that follow were performed within the first 24 h after infection, before the onset of cytopathic changes.

In the experiment depicted in Fig. 1, Vero cells were infected with Tacaribe virus at different multiplicities and at the times indicated, cells were pulse-labelled with [3H]thymidine. A decrease in the rate of incorporation of the radioactive precursor in the infected cells was observed; this decrease was proportional to the m.o.i. The maximal effect was observed at multiplicities at which the cell culture should be synchronously infected. Autoradiographic analysis of cells pulse-labelled with [3H]thymidine showed that 24 h after infection with about 10 p.f.u./cell, less than 3% of the cell nuclei were labelled, while in the uninfected cells, 40 to 60% of the nuclei were labelled, a value corresponding to that of a non-synchronous growing culture.
Fig. 1. Effect of Tacaribe virus infection on incorporation of \([^{3}H]\)thymidine into DNA in Vero cells. Cells were either mock-infected or infected with Tacaribe virus at the following m.o.i.: 0.1 (□), 1 (○), 6 (△), 60 (●). At the times indicated, cells were pulse-labelled with \([^{3}H]\)thymidine and the trichloroacetic acid-precipitable radioactivity was determined and corrected for the DNA content of the cultures as indicated in Methods.

Fig. 2. Effect of Tacaribe virus infection on RNA synthesis in Vero cells. Cells were mock-infected or infected at a m.o.i. of 6. At the times indicated the cultures were either pulse-labelled with \([^{3}H]\)uridine and the acid-precipitable radioactivity was estimated (○), or the cells were permeabilized and the activity of RNA polymerases I (△) and II (□) was estimated as indicated in Methods. In the mock-infected cells the activity of RNA polymerases I and II were, respectively, 89 and 105 pmol UMP incorporated per mg of DNA. The broken line represents the incorporation of \([^{3}H]\)uridine into infected cells in the presence of AMD as the percentage of that in mock-infected cells without AMD.

Fig. 3. Effect of Tacaribe virus infection on the incorporation of \([^{3}H]\)leucine in Vero cells. Cells were mock-infected or infected at a m.o.i. of about 6. At the times indicated, cells were pulse-labelled with \([^{3}H]\)leucine and the acid-precipitable radioactivity was determined as indicated in Methods. △, ○, □. Infections performed with virus stocks prepared as described in Methods from independently cloned viruses. ■, Incorporation of \([^{3}H]\)leucine in cells infected with a virus stock prepared as follows: virus from one of the stocks that inhibits protein synthesis (the one shown as □) were used to infect BHK21 cells at a m.o.i. of 7. The supernatant fluid was collected on the fifth day after infection and the viruses were purified as indicated in Methods.
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(result not shown). This result, together with the finding that the uptake of $[^3H]thymidine$ is not affected in the infected cells (results not shown), indicates that the decreased incorporation after virus infection reflects inhibition of host cell DNA synthesis.

The results in Fig. 2 show that in Vero cells infected with Tacaribe virus the incorporation of $[^3H]thymidine$ decreased as compared to that in the mock-infected controls. It can also be seen that the AMD-resistant incorporation in infected cells is less than 3\% of that observed in mock-infected cells not exposed to the inhibitor. This low level of incorporation reflects the amount of viral RNA synthesized, since treatment of the cells with AMD does not reduce the yield of infective virus (R. López, unpublished observations).

The decreased incorporation of $[^3H]thymidine$ after virus infection might reflect a decline in either transcription or processing, or an increased degradation of RNA. To determine whether transcription is affected, we examined the activity of the RNA polymerases in permeabilized cells. In addition, this procedure circumvents the problem of differences in the specific radioactivity of the nucleoside triphosphate pools in infected and uninfected cells. The results indicate (Fig. 2) that RNA polymerase I activity in Vero cells permeabilized at different times after virus infection declined in parallel with the incorporation of $[^3H]thymidine$ into RNA in the intact cells. RNA polymerase II activity was only slightly affected. It is concluded, therefore, that the reduced incorporation of radioactive thymidine in virus-infected cells reflects primarily inhibition of rRNA transcription.

The results in Fig. 3 show that infection of Vero cells with Tacaribe virus stocks prepared as indicated in Methods affects the incorporation of $[^3H]leucine$ into proteins. It can also be seen that the effect of the virus is not related to the virus isolate used for infection, since virus stocks prepared from virus cloned in independent experiments gave similar effects. It should be pointed out that the uptake of the labelled leucine remains unaffected at all times after infection (results not shown). Thus, the decreased incorporation of the amino acid is indicative of the inhibition of host cell protein synthesis.

An examination of the conditions used for the preparation of the working stocks showed that these are critical for the outcome of the virus infection in Vero cells. For instance, in the experiment shown in Fig. 3, a c.p.e. (+) virus stock was subjected to a further passage in BHK21 cells at high multiplicity and the resulting virus preparation was unable to induce inhibition of host cell protein synthesis. This virus stock did not induce c.p.e. in Vero cells infected at multiplicities at which all cells in the culture should be infected. At an input multiplicity \(\leq 0.05\), however, signs of c.p.e. appeared at about day 5 post-infection, but the cell monolayer recovered afterwards. [These virus preparations are defined as c.p.e. (−).] Nevertheless, different working stocks exhibited a spectrum of abilities to inhibit Vero cell macromolecular synthesis and to induce c.p.e. depending on the conditions under which the viruses had been produced. With increasing numbers of passages in BHK21 cells, higher m.o.i. of passage, and delayed harvesting of supernatants fluids, the virus produced showed decreasing abilities to induce inhibition of host cell macromolecular synthesis and c.p.e. A strong correlation between the last two parameters was always observed (results not shown).

We would like to point out that we were unable to induce c.p.e. and to inhibit host cell macromolecular synthesis in BHK21 cells and in BALB/c/3T3 cells infected with Tacaribe virus stocks that are c.p.e. (+) for Vero cells. At most, a transient inhibition of host protein synthesis followed by a rapid recovery was observed (results not shown).

Viral protein synthesis and yield of infectious virus in Vero cells infected with the c.p.e. (+) or c.p.e. (−) virus stocks

The effect of infecting Vero cells with either the c.p.e. (+) or c.p.e. (−) stocks of Tacaribe virus on the relative rates of cellular and viral protein synthesis was examined. Cells were mock-infected or infected at an input multiplicity of about 2 p.f.u./cell with either stock and at various times after infection the cultures were pulse-labelled with $[^3H]leucine$ and the labelled polypeptides were analysed by SDS–polyacrylamide gel electrophoresis and fluorography (Fig. 4). The results show that total protein synthesis in Vero cells infected with the c.p.e. (−) virus stock proceeded at about the same rate at 8 and 24 h. In contrast, in the cells infected with the
Fig. 4. Polyacrylamide gel electrophoresis of proteins synthesized in mock-infected and in Tacaribe virus-infected Vero cells. Cells were mock-infected (lanes 1 and 7) or infected at a m.o.i. of about 2 with either a c.p.e. (−) stock (lanes 2, 3, 8 and 9) or a c.p.e. (+) stock (lanes 4, 5, 10, 11 and 12). The c.p.e. (+) virus stock was prepared as described in Methods. The c.p.e. (−) stock was prepared as follows. The c.p.e. (+) virus stock was used to infect BHK21 cells at a m.o.i. about 5. The supernatant fluids were collected on the fourth day post-infection and a further passage in BHK21 cells at a m.o.i. about 5 was performed. The culture medium was collected on the fourth day after infection and the viruses were purified as indicated in Methods. Cells were labelled as indicated in Methods at the following times after infection: 6 h (lanes 2, 4, 8 and 10), 13 h (lanes 5 and 11), and 24 h (lanes 3, 6, 9 and 12). At this last time the mock-infected cells were also labelled. At the end of the incorporation period the cultures were processed as indicated in Methods. Lanes 1 to 6 are fluorograms of the proteins immunoprecipitated with anti-Tacaribe virus antiserum. Lanes 7 to 12 correspond to the analysis of total cellular proteins. Lane V shows the [3H]leucine-labelled proteins of purified Tacaribe virus. The position of marker proteins (low mol. wt. calibration kit, Pharmacia) are indicated in the right. Films were exposed for 2 days.

c.p.e. (+) virus stock there was a drastic inhibition of synthesis of most proteins at 24 h with the exception of two polypeptides (mol. wt. 68 000 and 79 000). These polypeptides did not appear in the mock-infected cells and are synthesized in cells infected with either the c.p.e. (+) or the c.p.e. (−) virus stock. The polypeptide band of mol. wt. 68 000 migrates at the same mobility as the viral nucleoprotein (N) and immunoprecipitates with anti-Tacaribe virus serum. The
polypeptide of mol. wt. 79000 (p79; Gard et al., 1977) detected in the immunoprecipitates when the gels were exposed for longer periods than those shown in Fig. 4. Under our conditions of cell labelling, we were unable to find a protein with mobility similar to that of the viral glycoprotein even after overexposure of the gels. It is noteworthy that at 8 h similar amounts of viral N protein were synthesized in cells infected with either the c.p.e. (+) or the c.p.e. (−) virus stocks whereas at 24 h, when there was a clear inhibition of host cell protein synthesis in the cells infected with the c.p.e. (+) virus stock, the rate of synthesis of the N protein in the latter was higher than that corresponding to cells infected with the c.p.e. (−) virus stock.

The pattern of viral polypeptide synthesis shown in Fig. 4 is similar to that reported by Gimenez et al. (1983) in Tacaribe virus-infected Vero and BHK21 cells labelled with [35S]methionine. However, we did not detect the minor protein of mol. wt. 105 000 suggested by these authors to be of viral origin.

The results in Table 1 compare the yield of infectious virus in the supernatant fluids of Vero cells infected at similar multiplicities with either the c.p.e. (+) or the c.p.e. (−) stocks of Tacaribe virus. It can be seen that the yields of infectious progeny were very similar, differing by less than twofold, at times when the differential effect on host cell macromolecular synthesis was manifested (see Fig. 3 and 4).

### Possible role of DI particles in the outcome of virus infection in Vero cells

Two observations suggested that the proportion of DI particles in our virus preparations played a role in determining both the degree of inhibition of host cell macromolecular synthesis and c.p.e. development. In the first place, these two properties of a given virus stock were related to the conditions used for virus preparation, i.e. increasing numbers of passages in BHK21 cells, higher m.o.i. of the passage, and delayed harvesting of the supernatant fluids [conditions known to favour the genesis of DI particles (Pedersen, 1979; Rawls & Leung, 1979; Martinez Peralta et al., 1981)] led to a decrease in the capability for inducing inhibition of host cell macromolecular synthesis and c.p.e. In the second place, virus preparations that were c.p.e. (+) for Vero cells did not affect host cell macromolecular synthesis nor induce c.p.e. in BHK21 cells, a cell line which apparently favours the production of DI viruses (Dutko & Pfau, 1978). The amounts of DI particles in our c.p.e. (+) and c.p.e. (−) virus stocks were measured, therefore, by a procedure which allowed counting of individual DI particles by their ability to form foci of Vero cells protected against the cytolytic activity of infectious virus (Popescu et al., 1976).

It was found that the ratio of DI particles to infective virus in a c.p.e. (+) stock is of the order of $10^{-4}$ while that in a c.p.e. (−) virus stock varied between 0.2 and 20 depending on the conditions used for preparation of the stock (Table 2). Furthermore, it was found (Table 2) that a
Table 2. Relationship between the input of DI particles at infection and protein synthesis inhibition in Vero cells

<table>
<thead>
<tr>
<th>DI particles/cell*</th>
<th>[%H]Leucine incorporation † (% of mock infected)</th>
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<tbody>
<tr>
<td>0.001 ‡</td>
<td>26</td>
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<tr>
<td>0.06§</td>
<td>34</td>
</tr>
<tr>
<td>0.13§</td>
<td>37</td>
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<tr>
<td>0.25§</td>
<td>53</td>
</tr>
<tr>
<td>0.63∥</td>
<td>86</td>
</tr>
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</table>

* Indicates the amount of DI particles/cell at infection.
† At 20 h, infected and mock-infected cells were pulse-labelled with [%H]leucine and the acid-precipitable radioactivity was measured as indicated in Methods.
‡ Vero cells were infected at a m.o.i. of 3 with a c.p.e. (+) stock prepared as indicated in Methods. The stock contained $1 \times 10^8$ p.f.u./ml and $5 \times 10^4$ DI particles/ml.
§ Cells were infected with mixtures of the c.p.e. (+) and c.p.e. (−) stocks. In each case the m.o.i. was 3.
∥ Cells were infected at a m.o.i. of 3 with a c.p.e. (−) stock prepared as indicated in the legend to Fig. 4. The stock contained $3 \times 10^7$ p.f.u./ml and $6.8 \times 10^0$ DI particles/ml.

c.p.e. (−) virus stock protects Vero cells from the inhibition of protein synthesis induced by a c.p.e. (+) stock. There is an inverse relationship between the input of DI particles at infection and the degree of protein synthesis inhibition.

DISCUSSION

The results in this study demonstrate that infection of Vero cells with Tacaribe virus inhibits host cell protein, DNA and RNA (primarily rRNA) synthesis. This is, to our knowledge, the first demonstration that an arenavirus affects the biosynthetic machinery of the host cell.

The virus-induced inhibition of host cell macromolecular synthesis was found to be associated with c.p.e. development. A strong correlation between these parameters was observed in Vero cells infected with virus stocks obtained under different conditions, in infections performed with mixtures of c.p.e. (+) and c.p.e. (−) virus stock and, also, in the response to infection of different cell lines. This correlation is not surprising, since it is known that in virus-mediated cytopathology the biochemical lesions usually include the inhibition of host cell macromolecular synthesis (Shatkin, 1983).

Studies performed with Pichinde and lymphocytic choriomeningitis viruses support the notion that standard arenaviruses are able to kill cells and that DI particles play a role in masking their cell-killing potential (Dutko & Pfau, 1978). Our results suggest that standard Tacaribe virus particles have in addition the ability to inhibit host cell macromolecular synthesis and that DI particles counteract this effect. This could be achieved either by inhibiting standard particle production or more directly by interfering with the mechanism whereby standard viruses affect the biosynthetic machinery of the cell. With regard to this last possibility, it is worth mentioning that there is no appreciable difference between the yields of infectious viruses from Vero cells infected with the c.p.e. (+) or the c.p.e. (−) virus stocks at times when the differential effect of either stock on protein synthesis is observed (compare Table 1 with Fig. 3 and 4).

The results in the present report are at variance with the usual view that arenaviruses do not affect host cell macromolecular synthesis. It has to be pointed out that most studies dealing with intracellular events in arenavirus infections were performed in BHK21 cells (Buchmeier et al., 1978; Saleh et al., 1979; Buchmeier & Oldstone, 1979; Harnish et al., 1981; Dimock et al., 1982). In this cell line we did not find inhibition of host cell macromolecular synthesis, regardless of the virus preparation used for infection. As far as we know, there is only one report on Vero cells infected with Tacaribe virus, in which no inhibition of host cell protein synthesis was observed (Gimenez et al., 1983). The virus preparation used in this study was apparently obtained under conditions different from our c.p.e. (+) virus stocks and, as shown in the present report, this is critical for the outcome of the virus infection.
We are now performing a detailed analysis of the virus-specific proteins and RNAs synthesized in Vero cells infected with either the c.p.e. (+) or the c.p.e. (−) Tacaribe virus stocks, as a first step towards the understanding of the molecular events that lead to the inhibition of host cell macromolecular synthesis in arenavirus-infected cells.

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