Pseudotypes of Vesicular Stomatitis Virus and Pichinde Virus

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SUMMARY

Super-infection of Pichinde virus-infected cells with vesicular stomatitis virus (VSV) resulted in the production of pseudotype virus which was not neutralized by antiserum to VSV but which was neutralized by antiserum to Pichinde virus. Analysis of pseudotype virus production in relation to the kinetics of replication of Pichinde virus demonstrated that pseudotype virus production occurred when super-infection with VSV was initiated 8 h or more after infecting the cells with Pichinde virus. The quantities of pseudotype virus produced correlated with the quantities of Pichinde virus antigen detected on the surface of the cells both during acute infection and in cells chronically infected with Pichinde virus. The observations indicate that pseudotype of VSV and Pichinde virus are readily formed and that the formation of pseudotype virus may be used to examine the Pichinde virus antigens expressed on the surface of infected cells.

INTRODUCTION

Cells infected with vesicular stomatitis virus (VSV) and some other enveloped viruses produce particles which contain the genome of one virus and an envelope containing antigens of the other virus (Choppin & Compans, 1970; Zavada, 1972; Huang et al. 1974). The VSV pseudotype particles represent a useful tool to study certain aspects of virus expression, especially in cells infected with members of the retrovirus group. The Arenaviruses, like the retroviruses, produce minimal perturbation of host cell macromolecular synthesis and chronically infected cultures are readily established. The viruses are enveloped, and mature by budding at the plasma membrane (Dalton et al. 1968; Murphy et al. 1970). Thus, a study was undertaken to produce VSV pseudotype virus in cells infected with Pichinde virus—a member of the Arenavirus group. In this paper we report the production of pseudotype virus which possesses the genome of VSV and the coat of Pichinde virus.

METHODS

Cell cultures. The established cell lines BHK21 and Vero were grown in Eagle's minimal essential medium (MEM) containing 0.75 g/l NaHCO₃, 10 mM-HEPES buffer, antibiotics (100 units/ml of penicillin and 100 µg/ml of streptomycin) and 10% foetal bovine serum.

Virus and virus assays. The An 3739 strain of Pichinde virus (Trapido & Sanmartin, 1971) was used. Virus stocks were prepared in BHK21 cells and the virus was assayed by plaque formation in Vero cells (Mifune et al. 1971). The HR-LT strain of the Indiana serotype of
VSV (Nakai & Howatson, 1968) was obtained from Dr L. Prevec, Department of Biology, McMaster University, Hamilton, Ontario. Virus stocks were prepared in BHK21 cells. The virus was assayed by plaque formation in confluent monolayers of BHK21 cells as described elsewhere (Kang & Prevec, 1969). Under the conditions of assay for VSV, Pichinde virus did not form plaques.

Antisera. Antiserum to VSV was prepared in rabbits and was kindly supplied by Dr L. Prevec. The method of production of the antiserum has been described previously (Kang & Prevec, 1969).

Antiserum to Pichinde virus was raised in LVG/Lak hamsters (Charles River/Lakeview, N.J.). Animals 6 weeks of age or older were injected intraperitoneally with 2.5 × 10⁴ p.f.u. of virus and the animals were exsanguinated by cardiac puncture 5 weeks later. Sera were assayed for antiviral antibodies by complement-fixation test and high-titred sera were pooled. Rabbit anti-hamster IgG was obtained from Dr J. Gauldie (Department of Pathology, McMaster University).

Assays for Pichinde virus antigen. Virus antigens located in the cytoplasm or on the surface of infected cells were detected by direct immunofluorescence. Immunoglobulins from anti-Pichinde virus serum were separated and conjugated with fluorescein isothiocyanate; the specificity of the reagent has been described previously (Rawls et al. 1976). For the detection of antigens on the surface of infected cells, the monolayers were dispersed with trypsin. After washing in phosphate buffered saline (PBS) containing 2% foetal bovine serum, 1 × 10⁵ cells were incubated for 30 min at 37 °C with the conjugated IgG, washed three times and the pellet of cells which resulted from the final centrifugation was re-suspended in two drops of buffered glycerol. The cells were then spotted on a slide, covered with a coverslip and examined on a fluorescent microscope. For detection of antigen in the cytoplasm, the cells were seeded on to coverslips and incubated at 37 °C until the cells spread. The cells were then fixed with acetone and stained (Rawls et al. 1976).

Antigens on the surface of the cells were also quantified by binding of ¹²⁵I-labelled immune hamster IgG. The labelling was performed using chloramine T and free ¹²⁵I was separated from bound ¹²⁵I by column chromatography. The binding assay was carried out using 1 × 10⁶ monodispersed cells and 50 μl of the iodinated IgG preparations as described previously (Rawls et al. 1976). The binding was expressed as ct/min/10⁶ cells.

Infectious centre assay. Monolayers of cultures to be assayed for infected cells were mono-dispersed with trypsin. The cells were washed twice with cold growth medium, re-suspended in medium and counted in a haemocytometer. The cells were diluted to 100, 200 and 500 cells per 0.1 ml vol. This amount was pipetted on to monolayers of Vero cells grown in 60 mm plastic Petri dishes. Each sample was assayed in triplicate. The cultures were incubated at 37 °C for 1 h and then carefully overlaid with medium containing 1% agar. The cultures were incubated at 37 °C and a second agar overlay containing neutral red was added after 4 days. Plaques were enumerated after an additional 24 to 48 h of incubation.

RESULTS

Pseudotype of VSV and Pichinde virus

Cells co-infected with Pichinde virus and VSV were found to yield pseudotype virus. This was demonstrated by infecting BHK21 cells with about 10 p.f.u./cell of Pichinde virus and then after 24 h of incubation super-infecting the cells with 5 p.f.u./cell of VSV. After an additional 12 h of incubation, the yield of VSV was assayed before and after treatment with antisera to VSV. The results of a representative experiment are shown in Table 1.
Pseudotypes of VSV and Pichinde

Table 1. *Yield of VSV from BHK21 cells which had or had not been infected with Pichinde virus*

<table>
<thead>
<tr>
<th>Cell culture system</th>
<th>Titre of VSV (p.f.u./ml)*</th>
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<tbody>
<tr>
<td>No serum treatment</td>
<td>Treated with antiserum</td>
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<tr>
<td>Normal BHK21</td>
<td></td>
</tr>
<tr>
<td>Pichinde virus infected BHK21†</td>
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<td></td>
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</table>

* The cultures were harvested after 12 h of incubation and assayed on monolayers of BHK21 cells (Pichinde virus does not produce plaques under the conditions of the assay for VSV).
† Equal vol. of virus and a 1:5 dilution of antiserum to VSV were mixed, incubated at 37 °C for 30 min and then assayed for surviving virus.
‡ BHK21 monolayers were infected with 10 p.f.u./cell of Pichinde virus and incubated 24 h before infection with 5 p.f.u./cell of VSV.

Table 2. *Reduction of virus infectivity by antiserum to Pichinde virus*

<table>
<thead>
<tr>
<th>Virus preparation</th>
<th>Titres of surviving virus after treatment with:*</th>
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<tbody>
<tr>
<td></td>
<td>Diluent</td>
</tr>
<tr>
<td>Pichinde virus</td>
<td>1.6 × 10^5</td>
</tr>
<tr>
<td>Pseudotype† virus</td>
<td>4.2 × 10^4</td>
</tr>
<tr>
<td>VSV</td>
<td>7.2 × 10^5</td>
</tr>
</tbody>
</table>

* To 0.1 ml of virus preparation was added 0.1 ml of diluent, antiserum to Pichinde virus or normal hamster serum; the latter two were diluted 1:5. The reaction mixtures were incubated at 37 °C for 60 min. Where appropriate, 0.1 ml of rabbit antiserum to hamster IgG was added and the mixtures were incubated for an additional 60 min. Diluent was then added to give a final vol. of 0.5 ml and the mixtures were centrifuged at 5000 g for 30 min. The supernatant fluids were removed and assayed for virus as indicated in the Methods.
† Pseudotype virus produced as indicated in footnote of Table 1.

The yields of VSV from Pichinde virus infected and normal BHK21 cells were similar indicating that infection of cells with Pichinde virus did not appreciably interfere with the replication of VSV. The antiserum to VSV reduced the titre of virus grown in normal cells to 1.9 × 10^5 p.f.u./ml; however, 1.7 × 10^5 p.f.u./ml of virus replicated in Pichinde virus-infected cells survived treatment with VSV antiserum. The presence of over 3 logs more surviving virus in harvest from Pichinde virus-infected cells when compared to harvest from normal cells suggest the presence of pseudotype virus.

The data shown in Table 2 indicate that the pseudotype virus was antigenically related to Pichinde virus. As repeatedly observed in the past, Pichinde virus was not readily neutralized by convalescent serum obtained from hamsters infected with the virus. The pseudotype was also not neutralized by the convalescent serum. The infectivity of Pichinde virus could be reduced by the addition of anti-hamster IgG to the mixture of hamster anti-Pichinde serum and virus. The addition of anti-hamster IgG also abolished the infectivity of the pseudotype virus. This reduction in infectivity appeared to be specific since the infectivity of VSV was not altered by the treatments and the use of normal hamster serum instead of antiserum to Pichinde virus did not significantly reduce the titres of pseudotype virus.
Kinetics of appearance of Pichinde virus antigens (○—○) and pseudotype virus (●—●) at different times after BHK21 cells were infected with 10 p.f.u./cell of Pichinde virus and incubated at 37 °C. At the intervals indicated, cells from one set of cultures were assayed for binding of anti-Pichinde virus 125I IgG and cells in another set of cultures were infected with 5 p.f.u. of VSV. The cultures super-infected with VSV were harvested 12 h after infection and the fractions of virus not neutralized by antiserum to VSV were plotted as p.f.u./ml.

The eclipse period of Pichinde virus is about 6 to 8 h, after which progeny virus is formed by budding at the plasma membrane of the cell. The kinetics of appearance of pseudotype virus in relation to the replication of Pichinde virus was examined. The results are illustrated in Fig. 1. Pichinde virus antigen, as detected by 125I–labelled antiviral IgG, appeared between 6 and 8 h after infection and increased thereafter. Cells infected with Pichinde virus and incubated for 0 to 6 h before super-infection with VSV yielded $1.5 \times 10^3$ to $3.6 \times 10^3$ p.f.u./ml of non-neutralizable virus when harvested 12 h later. Infection with VSV after 8 h or more of incubation of the Pichinde virus-infected cells resulted in substantially greater titres of non-neutralizable virus. These observations indicate that pseudotype virus production parallels the appearance of Pichinde virus antigen on the surface of the cells.

The kinetics of the production of pseudotype virus in relation to super-infection with VSV is shown in Fig. 2. Cells infected with Pichinde virus and incubated for 24 h were expressing virus antigens at the cell surface when super-infected with VSV. Pseudotype virus could be detected in the culture medium 2 to 4 h after infection and in all samples collected thereafter. Maximum production of VSV and pseudotype virus was observed 6 to 8 h after infection and pseudotype virus titres paralleled the VSV titres. The amount of Pichinde virus antigen detected on the surface of the cells was not noticeably altered by the production of VSV.
Fig. 2. Kinetics of the production of pseudotype virus (●) and VSV (▲) in cells expressing antigens of Pichinde virus on the cell surface (○). BHK21 cells were infected with 10 p.f.u./cell of Pichinde virus. After 24 h of incubation at 37 °C, the cells were super-infected with VSV at 5 p.f.u./cell. The yield of VSV and non-neutralizable VSV (pseudotype virus) produced during the time intervals shown as well as the amount of Pichinde virus antigen present at the end of the time interval were determined. To evaluate virus yields, medium from the cultures was aspirated, the cultures were washed twice with warm medium and then warm medium was added. Cultures were then harvested after the appropriate period of incubation. Control values not plotted included 125I anti-Pichinde virus IgG binding to uninfected BHK21 cells - 3 × 10³ ct/min/10⁶ cells, BHK21 cells infected with VSV only - 4.4 × 10⁴ ct/min/10⁶ cells; BHK21 cells infected with Pichinde virus only and incubated 6, 12 and 24 h after mock VSV infection - 25.1 × 10⁶, 37.1 × 10⁶ and 18.9 × 10⁶ ct/min/10⁶ cells, respectively.

Pseudotype virus production as a function of multiplicity of infection of Pichinde virus

Interference of virus replication with high multiplicities of infection has been observed with several arenaviruses. Lower yields of infectious Pichinde virus and complement fixing antigen were observed when Vero cells were infected at high multiplicity than at low multiplicity (Carter, 1972). A similar phenomenon was not found when BHK21 cells were used. The production of pseudotype virus was, therefore, examined in Vero and BHK21 cells infected at different multiplicities of Pichinde virus. The results presented in Table 3 show that pseudotype virus production in BHK21 cells increased from 1.2 × 10⁴ to 1.3 × 10⁵ p.f.u./cell at inputs of 0.01 to 1 p.f.u./cell of Pichinde virus. Similar yields were obtained at inputs of 10 to 100 p.f.u./cell. In Vero cells, a similar increase in pseudotype virus yield was also observed at inputs of 0.01 to 1 p.f.u./cell of Pichinde virus; however, at higher multiplicities both pseudotype virus and infectious virus yields were lower. Thus, the high multiplicity interference of Pichinde virus yield observed in Vero cells was also reflected in pseudotype virus yields.

Pseudotype virus production in chronically infected cultures

Members of the arenavirus group characteristically produce chronic infection in vitro (Rowe et al. 1970). In the chronically infected cultures, there is a disproportionately lower yield of infectious virus than antigen containing cells when compared to cultures acutely infected (Lehmann-Grube et al. 1969; Staneck et al. 1972; Cole et al. 1973; Hotchin et al. 1973).
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Table 3. Influence of multiplicity of infection of Pichinde virus in BHK21 and Vero cells on pseudotype virus production

<table>
<thead>
<tr>
<th>Multiplicity of infection (p.f.u./cell)*</th>
<th>Vero</th>
<th>BHK21</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pichinde virus</td>
<td>Pseudotype</td>
</tr>
<tr>
<td>0.01</td>
<td>2.5 x 10^6</td>
<td>3.2 x 10^5†</td>
</tr>
<tr>
<td>0.1</td>
<td>1.5 x 10^6</td>
<td>1.8 x 10^5†</td>
</tr>
<tr>
<td>1.0</td>
<td>2.9 x 10^6</td>
<td>1.1 x 10^5</td>
</tr>
<tr>
<td>10</td>
<td>1.6 x 10^6</td>
<td>5.3 x 10^5</td>
</tr>
<tr>
<td>100</td>
<td>1.5 x 10^6</td>
<td>1.0 x 10^5</td>
</tr>
</tbody>
</table>

* Vero cells or BHK21 cells were infected with Pichinde virus at the multiplicity indicated. The cultures were incubated for 24 h at 37 °C at which time the tissue culture fluid was harvested and assayed for infectious Pichinde virus. At the time of harvest of culture fluid the monolayers were infected with 5 p.f.u./cell of VSV and after an additional 12 h of incubation the cultures were harvested and assayed for pseudotype particles.

† Titres of VSV yields not neutralized by antiserum to VSV. The total yields of VSV from all cultures were similar and ranged from 4 to 7 x 10^5 p.f.u./ml.

Table 4. Pseudotype virus production in chronically infected cultures

<table>
<thead>
<tr>
<th>Conditions of BHK21 cells culture</th>
<th>Titre of Pichinde virus (p.f.u./ml)</th>
<th>Pseudotype virus (p.f.u./ml)</th>
<th>Infectious centres</th>
<th>Immunofluorescence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>—</td>
<td>3.5 x 10^5</td>
<td>0</td>
<td>27 (4+)†</td>
</tr>
<tr>
<td>Infected* (24 h)</td>
<td>7.6 x 10^6</td>
<td>8.5 x 10^4</td>
<td>32</td>
<td>27 (4+)†</td>
</tr>
<tr>
<td>Infected 4th passage</td>
<td>4.0 x 10^6</td>
<td>3.0 x 10^5</td>
<td>12</td>
<td>13 (2+)</td>
</tr>
<tr>
<td>Infected 11th passage</td>
<td>3.8 x 10^6</td>
<td>2.1 x 10^5</td>
<td>11</td>
<td>17 (2+)</td>
</tr>
<tr>
<td>Infected 21st passage</td>
<td>6.8 x 10^6</td>
<td>1.9 x 10^5</td>
<td>7</td>
<td>11 (2+)</td>
</tr>
</tbody>
</table>

* BHK21 cells were infected with about 1 p.f.u./cell and one set of cultures were assayed for Pichinde virus in the culture fluid, the percentage of cells infected, antigen expression on the cells and pseudotype virus production after 24 h of incubation. Another set of cultures were subdivided by trypsinization at 3 to 4 day intervals and these cultures were assayed for the same parameters at the indicated passage levels.

† The numbers in parentheses indicates the intensity of fluorescence with 4+ being maximal.

DISCUSSION

The presence of two unrelated replicating enveloped viruses may give rise to particles which contain the genome of one virus and surface antigens of the other virus. In the yields
Pseudotypes of VSV and Pichinde from the cells infected with both Pichinde virus and VSV we found particles which were not neutralized by antiserum to VSV but which upon assay formed plaques characteristic of VSV. Furthermore, the infectivity of these particles could be reduced with antiserum to Pichinde virus under conditions which resulted in the reduction of infectivity of this virus. These findings suggest that pseudotype particles containing the genome of VSV and the surface antigens of Pichinde virus were formed.

The kinetics of the appearance of Pichinde virus antigens on the surface of cells infected with Pichinde virus and the ability of the cells to produce increased titres of non-neutralizable virus upon super-infection with VSV supports the contention that pseudotype particles were formed (Fig. 1). Antigens of Pichinde virus were detected between 6 and 8 h after infection with this virus and it is at this time that progeny virus appears in the culture fluid (Mifune et al. 1971). After 8 h of incubation, the titres of non-neutralizable VSV correlated directly with the quantities of Pichinde virus antigen detected on the surface of the cells, suggesting that the amount of Pichinde virus antigens present on the cells at the time of infection influences the quantity of pseudotype virus produced. This is of interest since in these experiments 12 h lapsed between super-infection with VSV and harvest for pseudotype virus assay. The apparent absence of pseudotype virus production in cultures incubated 0 to 6 h after infection with Pichinde virus suggests that Pichinde virus antigen synthesis is stopped by super-infection with VSV as has been demonstrated for murine leukaemia virus (Witte & Baltimore, 1977). However, in cultures incubated for 24 h after infection with Pichinde virus there was no evidence of depletion of Pichinde virus antigens at the surface of cells after super-infection with VSV. These apparent contradictory observations could be accounted for by an excess pool of Pichinde virus antigens on the surface of the cells at the time of super-infection with VSV. Alternatively, VSV may inhibit Pichinde virus antigen synthesis early in the replicative cycle but not later in the cycle. Further studies will be required to clarify whether Pichinde virus antigens are in excess or are continually synthesized in the presence of VSV replication.

Not surprisingly, the production of pseudotype virus was found to correlate with the expression of Pichinde virus antigens. In addition to the experiments in which the kinetics of Pichinde virus and pseudotype virus were examined, this was found to be true in chronically infected cultures and under conditions where interference associated with high multiplicities of infection was observed. At multiplicities of 10 and 100 p.f.u./cell, lower yields of Pichinde virus and pseudotype virus were observed in Vero cells than at 1 p.f.u./cell. This interference has been attributed to defective interfering particles present in virus stocks (Welsh & Pfau, 1972). For lymphocytic choriomeningitis virus, defective interfering particles inhibited the expression of both intracytoplasmic and cell surface antigens of standard virus (Welsh & Oldstone, 1977). The synthesis of Pichinde virus antigens detected by immunofluorescence staining of fixed cells and by complement fixation was found to be reduced at high multiplicities of infection (Carter, 1972). Thus reduced pseudotype virus production correlates with reduced antigen expression.

Intracytoplasmic and cell surface antigens of Pichinde virus were found to increase together during the initial phases of replication of Pichinde virus. After the peak yield of virus was reached, a marked decrease in virus production was observed. A decrease in cell surface antigens was associated with the decrease in virus production but the percentage of cells containing intracytoplasmic antigen was not appreciably decreased (Buchmeier, 1976). Similar patterns of expression of these antigens and virus yields have been observed in cultures during acute infections with lymphocytic choriomeningitis virus (Hotchin et al. 1975; Welsh & Oldstone, 1977). Subculturing of cultures infected with lymphocytic
choriomeningitis virus gave rise to chronically infected cultures in which there appeared to be a disproportionate expression of intracytoplasmic and cell surface antigens (Cole et al. 1973; Hotchin et al. 1975). We found evidence of Pichinde virus antigen synthesis in 7 to 17% of cells in chronically infected BHK21 cultures. The amounts of antigens and the yields of infectious virus per infected cell were reduced in the chronically infected cultures as compared to acutely infected cells. Despite the reduced amounts of antigen, pseudotype virus was produced in the chronically infected cultures. In these cultures, the yields of pseudotype virus were roughly proportional to the amounts of antigens expressed on the surface of the cells as quantified by binding of immune globulin. The observations suggest that pseudotype virus formation is an indirect measure of the expression of Pichinde virus antigens at the surface of the infected cell.

REFERENCES


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