Interfering Particles from a Culture Persistently Infected with Parana Virus

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SUMMARY

The existence of defective interfering (DI) particles has been well documented for a number of virus systems (Huang & Baltimore, 1970). Such particles are currently of great interest since their ability to cause homologous interference has been implicated as a possible regulatory factor in the establishment of chronic or persistent virus disease (Huang & Baltimore, 1970). We have recently reported that about 50 cell generations after infection of BHK 21/13S cells with either lymphocytic choriomeningitis virus (LCMV) or Parana virus, infective virus could no longer be detected in either the cells or the culture medium (Staneck et al. 1972). However, LCMV-infected cultures continued to produce particles with an interference activity which closely resembled DI virus (Welsh, O'Connell & Pfau, 1972). The data presented here indicate that Parana, another arenavirus (Rowe et al. 1970), can initiate the nearly exclusive synthesis of DI virus under the same conditions.

Welsh et al. (1972) found that LCM-DI virus could cause homotypic and heterotypic but not heterologous interference in L cells. The same type of result was found in BHK 21/13S cells exposed to Parana DI virus (Table 1). BHK 21/13S confluent monolayers (1 to 2 × 10^7 cells/Falcon flask no. 3012) were treated at 37°C with 1 ml of cell-free medium from a BHK 21/13S suspension culture either uninfected or persistently infected with Parana virus. The inocula were removed 1 h later. The monolayers were washed and then exposed to 1 ml of either standard Parana virus (input multiplicity 0.05 p.f.u./cell), LCMV strain CA1371 (0.09 p.f.u./cell) or VSV (0.05 p.f.u./cell). After 1 h at 37°C the inocula were again removed, the monolayers were washed, and the medium was replaced in each flask to the original 5 ml vol. The medium from each monolayer was removed 24 h post-infection and assayed in duplicate for p.f.u. (Pulkkinen & Pfau, 1970). The Parana virus yield from cells pre-treated with Parana DI virus was 33% of that obtained from untreated cells while the LCMV yield from these pre-treated cells was 53% of the controls. The vesicular stomatitis virus (VSV) yield from both pre-treated and non-treated cells was virtually identical. This reduction in homotypic challenge virus yield was consistently about 20% greater than that observed after heterotypic challenge following treatment of monolayers with Parana DI virus. The absolute degree of inhibition observed in any one experiment varied with the DI virus stock used as well as with the input multiplicity of the challenge virus. The standard error in these titrations was no greater than 5%. In the LCM-DI virus system, the homotypic interference with synthesis of infective virus was seen as an inhibition in infective centre formation when measured immediately after removal of the challenge virus inoculum (Welsh et al. 1972). The results presented in Table 2 show that cultures treated with DI and standard Parana virus synthesized fewer p.f.u. after 24 h than cultures treated with standard virus alone. This inhibition was approximately comparable to the decrease in infective centres at that time. The protocol used for these experiments was the same as that described for the results presented in Table 1 except that: the Parana DI virus was concentrated 20-fold by the...
Table 1. Virus synthesis in cells pre-treated with Parana DI preparations

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Total extracellular p.f.u. yield (24 h after infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI-treated</td>
</tr>
<tr>
<td>Parana</td>
<td>$1.2 \times 10^6$</td>
</tr>
<tr>
<td>LCMV</td>
<td>$4.7 \times 10^5$</td>
</tr>
<tr>
<td>VSV</td>
<td>$5.5 \times 10^5$</td>
</tr>
</tbody>
</table>

Table 2. Infective centre and p.f.u. yields from cells treated with Parana DI virus

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Total infective centre yield</th>
<th>Total extracellular p.f.u. yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI-treated</td>
<td>Control</td>
</tr>
<tr>
<td>Parana*</td>
<td>$4.0 \times 10^5$</td>
<td>$2.0 \times 10^7$</td>
</tr>
<tr>
<td>Parana†</td>
<td>$2.2 \times 10^6$</td>
<td>$2.0 \times 10^7$</td>
</tr>
<tr>
<td>LCMV*</td>
<td>$1.9 \times 10^6$</td>
<td>$3.7 \times 10^6$</td>
</tr>
</tbody>
</table>

* Pre-treatment with DI virus (1 ml) 1 h before challenge.
† Simultaneous treatment with DI virus (1 ml) and challenge virus (1 ml).

differential sedimentation conditions (Welsh et al. 1972) used for standard Parana virus; the challenge Parana and LCM virus input multiplicities were 0.5 and 0.1 p.f.u./cell, respectively; and the monolayers 24 h post-challenge were dispersed with trypsin and assayed for infective centres (Welsh et al. 1971; Welsh & Pfau, 1972). Treatment of cells with Parana DI virus prior to the addition of challenge virus was found to be more effective in infective centre and p.f.u. inhibition (98% and 99%, respectively) than simultaneous infection with both types (89% and 98%, respectively), again similar to the LCMV-DI system (Welsh et al. 1972). The Parana DI virus led to interference with LCMV synthesis, with nearly as much inhibition of infective centre as p.f.u. yield (43% and 47% reduction, respectively). The above observations were found consistently, although, as noted for the data presented in Table 1, the absolute amounts of inhibition varied from one experiment to the next depending on the DI virus stock used and the input multiplicity of the challenge virus. Thus, the Parana DI virus inhibition of p.f.u. yield seems to be the direct result of a reduction in the number of cells producing sufficient standard virus to score as infective centres, rather than the result of inefficient synthesis of virus by normal numbers of infective centres.

Welsh et al. (1972) found that maximal inhibition of L cell infective centres required early interaction between LCM-DI virus and the challenge standard virus. Similar results were obtained in the present system. For the Parana virus experiments, 8 BHK monolayers were prepared and four of these were exposed to DI virus as outlined in Table 1. After 1 h, a pair of monolayers (with and without DI virus treatment) were challenged with standard Parana virus. The inocula were removed 1 h later and the replacement medium (Pulkkinen & Pfau, 1970) contained 2% instead of the standard 10% foetal calf serum (necessary to decrease the cell division rate and preserve the integrity of the monolayers over the long time periods necessary for this experiment). The 24 h extracellular p.f.u. (standard virus) yield was 85% lower in the DI-treated monolayer than in the control. The other six monolayers received media containing 2% foetal calf serum and were infected with standard virus, in pairs, at 12, 24 and 48 h after removal of the DI virus. As the interval between DI virus treatment and standard virus challenge increased, the difference in p.f.u. yield between
normal and DI-treated monolayers decreased. Thus, with a 12 h interval between DI virus treatment and standard virus challenge, the p.f.u. yield 24 h after challenge was 66% lower than that from the monolayer never pre-exposed to DI virus. Similarly, if the challenge with standard virus was delayed 24 and 48 h after DI virus treatment, the p.f.u. yields were reduced by only 21% and 16%, respectively, of the control monolayers.

Welsh et al. (1972) found that the interfering activity of LCM-DI virus was much more resistant to u.v. inactivation than the standard plaque forming virus, and did not markedly change in u.v. sensitivity when diluted. They suggested that the DI virus might have a lower nucleic acid content than standard virus. Similar results were obtained with Parana virus. Using the identical procedure for u.v. inactivation as previously described (Welsh et al. 1972), 30-fold concentrated stocks of standard Parana virus and Parana DI virus were irradiated for 15 and 60 s. After 15 s, the standard virus titre dropped from $2.6 \times 10^7$ p.f.u./ml to $7.0 \times 10^5$ p.f.u./ml. An additional 45 s of irradiation decreased the titre to $2.6 \times 10^4$ p.f.u./ml. The ability of standard, unirradiated virus to initiate ineffective centres was 16%. Pre-treatment of a BHK monolayer with unirradiated DI virus lowered the number of infective centres formed after challenge with this standard virus to 0.85%. After 15 s of irradiation, the ability of the DI virus to lower infective centre formation was not altered. Even after the DI virus was irradiated for 60 s, the number of infective centres formed by the challenge virus rose only to 1.65%.

Various types of experiments suggested that LCM-DI virus was not the heat-inactivated (37 °C) breakdown product of the standard plaque forming virus (Welsh, 1972). A culture persistently infected with Parana virus was diluted from 3 to $4 \times 10^6$ cells/ml to $5 \times 10^4$ cells/ml and the medium assayed for infective virus every 8 h for 72 h. It was assumed that before heat-inactivated virus could appear in the culture medium, a burst of synthesis of infectious virus should occur which would be detectable in the standard assay procedure. At no time, however, even with undiluted samples, were p.f.u. detected. Although all Parana DI virus preparations used in these experiments were uniformly devoid of detectable p.f.u., they would initiate p.f.u. synthesis in normal cells within 36 h after infection (Staneck et al. 1972). This suggested that the Parana DI preparations may contain low levels of standard virus which, given a new environment of uninfected cells, could replicate sufficiently to reach detectable levels. The presence of such low levels of standard virus particles is consistent with the theory that the standard virus is required for DI virus replication (Huang & Baltimore, 1970).

Thus, the Parana and LCM-DI virus systems appear to be similar and share many of the properties of the DI virus as defined in other systems (Huang & Baltimore, 1970). The Parana DI virus did not form plaques, initiated homotypic and heterotypic interference, and was more resistant to u.v. inactivation than standard virus. Defective interfering particles have also been found for Pichinde virus (E. A. Wright & C. J. Pfau, unpublished observations). The dramatic fluctuations in p.f.u. titre observed in early stages of establishing persistent infections with LCM, Parana and Pichinde viruses( Lehmann-Grube, Slenczka & Tees, 1969; Staneck et al. 1972; Wright & Pfau, 1973), with DI virus synthesis eventually predominating, have also been noted in Junin virus-infected mouse fibroblasts (Martinez Segovia, Holstein & Grazzioli, 1967). Since Junin and Pichinde are also arenaviruses (Rowe et al. 1970), the ability to synthesize DI virus readily in vitro may be a common characteristic of all the viruses of this group.
Short communications

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REFERENCES


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