Induction of Interferon by Group C Arboviruses

Brief Report

By

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With 1 Figure
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Summary

Several group C arboviruses are able to induce interferon in chick embryo fibroblasts, primary human amnion and mouse L cells.

The group C arboviruses were first isolated in the Amazon region (3) and soon were found to be antigenically distinct from other arboviruses (2) presently belonging to the Bunyaviridae family (4).

Several group C arboviruses induce interference in chick embryo fibroblasts (CEF) as well as in primary human amniotic and mouse L-A9 cells against vaccinia, vesicular stomatitis (VSV) and Sindbis viruses (Golgher et al. in preparation). Marituba virus inactivated by ultraviolet light but not by heating at 56°C, was also able to induce interference in CEF. Interference was established 12 hours after treatment of CEF by this virus (Mezencio et al., submitted for publication).

These data suggested that the interference phenomenon observed may have been mediated by interferon. This suggestion now derives further support from the demonstration in this paper that group C arboviruses can indeed stimulate an antiviral substance in CEF with the characteristics of an interferon.

CEF were obtained according to Henle et al. (5). The cells were grown in medium 199, supplemented with 5 per cent sheep serum, sodium bicarbonate and antibiotics. For maintenance of the cells, the serum concentration was lowered to 1 per cent.

Oriboca (Be An 17), Murutucu (Be An 974), Caraparu (Be An 3994), Itaqui (Be An 12797), Apeu (Be An 848) and Nepuyo (Be An 10709) viruses were furnished by the American Type Culture Collection and Marituba (Be An 15) virus by Dr. Francisco P. Pinheiro, Belem, Brazil. VSV (Indiana strain) and Newcastle disease virus (NDV) (Victoria strain) were kindly supplied by Dr. Kurt Paucker,
Philadelphia, USA; Sindbis virus was donated by Dr. Norman B. Finter, Beckenham, Kent, England, and vaccinia virus was obtained from the smallpox vaccine prepared by the Department of Public Health in Massachusetts, USA. The viruses were grown in either Vero or HeLa cells, with the exception of NDV which was propagated in embryonated eggs. Group C arboviruses were titrated in tube cultures of Vero cells; other viruses by plaque assay in CEF.

Standard chick interferon was prepared in CEF exposed to NDV irradiated with a germicidal lamp (8) for 60 sec (optimal dosage). The fluids were collected 24 hours later and dialysed against pH 2 and subsequently against pH 7. Aliquots were stored at $-20^\circ$ C. The interferon activity of the chick interferon standard and of the fluids from cultures infected with group C arboviruses was titrated by adding serial dilutions to CEF monolayers which were infected 24 hours later with approximately 100 plaque forming units of Sindbis virus. The 50 per cent plaque reduction endpoint was determined. Materials from each experiment were titrated in the same batch of CEF (coefficient of variation 8 per cent) and the chick interferon standard was included with each titration. One interferon unit, as expressed in this paper, is equivalent to 0.29 reference units (research standard A 62/4).

Crystalline trypsin (lot 4620), soy-bean trypsin inhibitor (lot 9418) and actinomycin D (lot 4136) were purchased from Nutritional Biochemical Co., Cleveland, Ohio, USA.

Two bottles of CEF were drained and infected with 0.4 ml of each of the following viruses: Apeu, Caraparu, Itaqui, Marituba, Murutucu, Nepuyo and Oriboea, using the input multiplicity listed in Table 1. After two hours of adsorption at $37^\circ$ C, maintenance medium was added to a total volume of 6 ml and at 48 hours post-infection the fluids were collected, dialysed first against pH 2 buffer, then against pH 7 buffer, and titrated for interferon activity. Titers of a representative experiment are shown in Table 1. All the viruses were able to induce antiviral activity with the exception of Itaqui and Nepuyo viruses. However, in later experiments fluids from CEF cultures infected with Nepuyo virus also had low levels of antiviral activity. Since Itaqui virus caused interference in CEF with an efficiency comparable to that of Marituba virus (GOLHER et al., in preparation), this could be related to an undetectable amount of interferon, the production of a pro-interferon not liberated from the cells (9), or a type of interference not mediated by interferon (7).

Table 1. Induction of interferon in chick embryo fibroblasts by group C arboviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Multiplicity</th>
<th>Interferon units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apeu</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>Caraparu</td>
<td>0.02</td>
<td>16</td>
</tr>
<tr>
<td>Itaqui</td>
<td>0.02</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Marituba</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Murutucu</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>Nepuyo</td>
<td>17</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Oriboea</td>
<td>17</td>
<td>64</td>
</tr>
</tbody>
</table>

a Cells infected for 48 hours
b Input multiplicity of infection