Enhanced or Inhibited Plaque Formation of Superinfecting Viruses in Yaba Virus-infected Cells

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Tsuchiya, Tagaya & Tsuruhara (1969) reported that Yaba virus caused overt cytological changes in a cynomolgus monkey kidney cell line, JINET (Tsuchiya, Takayama & Tagaya, 1969). Although markedly altered in morphology, the virus-infected JINET cells could be maintained in culture for periods up to 2 to 3 weeks. We have now examined the susceptibility of these cells to superinfection with other viruses and found that with some viruses plaque numbers were larger and more numerous in cells infected with Yaba virus than in uninfected counterparts. With other viruses plaque production was completely suppressed in Yaba-infected cells. We describe here the results of comparative plaque assay of various viruses in uninfected JINET cells (J(N)) and JINET cells infected with Yaba virus (J(Y)).

JINET cells were grown as previously described (Tsuchiya, Takayama & Tagaya, 1969), and maintained in superinfection experiments in Earle's salt solution containing 0.5% lactalbumine hydrolysate and 0.15% sodium bicarbonate. Yaba virus was propagated and titrated in JINET cells as described by Tsuchiya, Tagaya & Tsuruhara (1969). The strain names and propagation substrates of the superinfecting viruses are listed in Table I. Pre-infection with Yaba virus was performed by inoculating 1 ml. of Yaba virus (10^6 TCID 50/ ml.) into 2-oz prescription bottle cultures of JINET cells (2 × 10^6 cells/bottle) containing 4 ml. of fresh maintenance medium. Uninfected control cultures received 5 ml. of the fresh medium only. The treated cultures were incubated at 36° for 3 days. Comparative plaque assay of superinfecting viruses was then performed as follows. The medium was removed from cell monolayers and the virus, at various concentration in maintenance medium, was added in 0.2 ml. amounts to each of 2 to 5 bottle cultures of J(Y) and J(N). The inoculum was allowed to adsorb for 2 to 3 hr at 36° with occasional rocking of the cultures and then overlaid with 5 ml. of medium 199 containing 1% Noble agar (Difco), 0.225% sodium bicarbonate, 2% bovine serum, and 0.002% neutral red. The plaques were counted 3 to 14 days after inoculation with superinfecting viruses.

The results of the comparative assay of superinfecting viruses in the two types of cells are summarized in Table I. With MAHONEY, MEF 1, SAUKETT and LEON 12b strains of poliovirus, echovirus 7, vesicular stomatitis virus and herpes simplex virus plaque numbers and size were enhanced in J(Y). The fact that the plaque number of MEF 1 strain of poliovirus was enhanced by only 6.2 times under conditions where with other poliovirus strains they were enhanced by 39.5 to 61.7 times suggests that the degree of enhancement may depend on the strain or type of superinfecting virus. The plaques of an unclassified simian virus SV28 were enhanced only in number. Sindbis virus and Japanese encephalitis virus (JEV) produced plaques in J(Y) but not in J(N). A simian myxovirus SV5 formed as many plaques in J(Y) as in J(N), although the plaque size was diminished in the former cells. In contrast, plaque formation by vaccinia and cowpox viruses, a simian adenovirus SV15 and a simian papovavirus SV40 was completely suppressed in J(Y). The mechanism of this inhibition of plaque formation is not known but it is probably not mediated by interferon because Yaba virus does not itself induce interferon in JINET cells (unpublished observation). Moreover, viruses known to be highly sensitive to interferon were enhanced in J(Y).
The enhancing component present in Yaba virus preparations was neutralized by a specific Yaba virus antiserum, inactivated by heating at 60°C for 12 min., and sedimented by centrifugation at 30,000 rev./min. for 2 hr. The enhancing component may therefore be the Yaba virus particle itself. In this respect, it is quite different from ‘enhancer’ described by Kato, Okada & Ota (1965), ‘blocker’ by Isaacs & Rotem (1966) and ‘stimulon’ by Chany and Brailovsky (1967).

Table 1. Comparative plaque titration of various viruses in Yaba virus-infected and uninfected JINET cells

<table>
<thead>
<tr>
<th>Virus type</th>
<th>Strain</th>
<th>Propagated in</th>
<th>Plaque no.*</th>
<th>Plaque size †</th>
<th>Ratio J(Y)/J(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>J(Y)</td>
<td>J(N)</td>
<td>J(Y)</td>
</tr>
<tr>
<td>Polio I</td>
<td>MAHONEY</td>
<td>pMK</td>
<td>852-0</td>
<td>138</td>
<td>150</td>
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<tr>
<td>2</td>
<td>MEF I</td>
<td>JINET</td>
<td>232-5</td>
<td>37-8</td>
<td>10-8</td>
</tr>
<tr>
<td>3</td>
<td>SAUKETT</td>
<td>JINET</td>
<td>716-0</td>
<td>16-2</td>
<td>15-0</td>
</tr>
<tr>
<td>3</td>
<td>LEON 124</td>
<td>JINET</td>
<td>850-0</td>
<td>21-5</td>
<td>12-3</td>
</tr>
<tr>
<td>Echo</td>
<td>WALLACE</td>
<td>pMK</td>
<td>43-0</td>
<td>5-8</td>
<td>7-0</td>
</tr>
<tr>
<td>SV287</td>
<td>9182</td>
<td>JINET</td>
<td>46-6</td>
<td>28-3</td>
<td>1-0</td>
</tr>
<tr>
<td>Sindbis</td>
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<td>270-0</td>
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</tr>
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<td>JEV</td>
<td>NAKAYAMA</td>
<td>Mouse brain</td>
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<td>None</td>
<td>0-9</td>
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<tr>
<td>VSV</td>
<td>NEW JERSEY</td>
<td>JINET</td>
<td>227-0</td>
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<td>3-5</td>
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<tr>
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<td>76-3</td>
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<tr>
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<td>HF</td>
<td>HeLa</td>
<td>233-4</td>
<td>4-4</td>
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</tr>
<tr>
<td>SV15</td>
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<td>JINET</td>
<td>None</td>
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<td>SV40</td>
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<td>Vaccinia</td>
<td>DIE</td>
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<tr>
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<td>3615-0</td>
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</tr>
</tbody>
</table>

Abbreviations: J(Y) = Yaba virus-infected JINET cells; J(N) = uninfected JINET cells; pMK = primary monkey kidney cells; CAM = chorioallantoic membrane; JEV = Japanese encephalitis virus; VSV = vesicular stomatitis virus; HSV = Herpes simplex virus.

* Plaque number represents the average of 2 to 5 cultures and is expressed in terms of the number obtained by inoculating the same dilution to both types of cells.
† Plaque size indicates the average diameter of 10 plaques measured at random and is expressed in mm.

For the conditions of preinfection of cell cultures with Yaba virus, see the text.

We have also demonstrated (Tsuchiya & Tagaya, to be published) that JEV which induces interferon production in J(N) fails to do so in J(Y) and that the plaque number of SAUKETT virus in J(Y) is not affected by pretreatment of the cells with exogenous monkey interferon (titre of 1024 when assayed in JINET cells against challenge by SAUKETT virus). These facts may explain, at least in part, the mechanism of the enhancing phenomenon described above. It seems unlikely, however, that the enhancement is always explicable on the basis of the inhibitory effect of Yaba virus on the production and action of interferon in JINET cells because SAUKETT virus which does not produce appreciable amounts of interferon in J(N) (to be published) was nevertheless greatly enhanced in J(Y).

Further characterization of the phenomenon will be made in the future.

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REFERENCES


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