Cross-Neutralization Study of Seven California Group (Bunyaviridae) Strains in Homoiothermous (PS) and Poikilothermous (XTC-2) Vertebrate Cells

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SUMMARY

Antigenic relationships among seven California group strains were studied by a plaque-reduction neutralization test (PRNT). Cross-reactions occurred in most cases but three subgroups were noted: (1) the major serogroup contained the viruses of California encephalitis, LaCrosse, Snowshoe Hare and Trahyña (including the Lumbo strain) whereas (2) Jamestown Canyon and (3) Trivittatus viruses were distinct. There was no significant difference between the PRNT results in mammalian (PS) cells incubated at 37 °C and amphibian (XTC-2) cells incubated at 28 °C. Trivittatus virus failed to produce plaques in XTC-2 cells.

INTRODUCTION

The establishment of the XTC-2 cell line from the toad Xenopus laevis by Pudney et al. (1973) and the susceptibility of these cells to infection by many arboviruses (Leake et al. 1977) has made it possible to compare the cross-neutralization activity of sera produced against members of the California (CAL) group viruses, both at 28 °C in XTC-2 cells and at 37 °C in PS cells.

The antigenic relationships among CAL group viruses have been studied primarily by complement-fixation (Casals, 1962; Whitman & Shope, 1962; Hammon & Sather, 1966; Sather & Hammon, 1967; Thompson et al. 1972; Sprance & Shope, 1977), immunodiffusion in gels (Murphy & Coleman, 1967; Calisher & Maness, 1970; Papadopoulos et al. 1970; Wellings et al. 1970) or immunoelectrophoresis (Wellings et al. 1971). Neutralization comparisons of these viruses have previously been performed in mice (Whitman & Shope, 1962; Sather & Hammon, 1967) or in mammalian (BHK-21, Vero) cells, usually based on c.p.e. (Thompson et al. 1972; Brummer-Korvenkontio et al. 1973; Issel, 1973). Seawright et al. (1974) employed a PRNT, using LaCrosse and Snowshoe Hare viruses and anti-LaCrosse immune serum. Recently, Lindsey et al. (1976) studied the cross-reactions among ten CAL group viruses and their antisera by PRNT on mammalian cells.

METHODS

Cells. XTC-2 cells were kindly supplied by Dr C. J. Leake, and PS cells (stable pig-kidney cells, derived by Inoue & Ogura, 1962) by Dr J. S. Porterfield. Both cell lines were

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Table I. Virus strains

<table>
<thead>
<tr>
<th>Virus</th>
<th>Abbreviation</th>
<th>Strain</th>
<th>Passage level in suckling mouse brains</th>
<th>Infectivity of stock virus as suckling mouse i.c. log LD$_{50}$ per ml of 10% brain suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>California encephalitis</td>
<td>CE</td>
<td>BFS 283</td>
<td>14</td>
<td>8.9</td>
</tr>
<tr>
<td>La Crosse</td>
<td>LAC</td>
<td>5663</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>Snowshoe Hare</td>
<td>SSH</td>
<td>Original</td>
<td>25</td>
<td>8.7</td>
</tr>
<tr>
<td>Lumbo</td>
<td>LUM</td>
<td>AR 1881</td>
<td>6</td>
<td>8.2</td>
</tr>
<tr>
<td>Tahyña</td>
<td>TAH</td>
<td>92 (Bárdós)</td>
<td>20</td>
<td>8.4</td>
</tr>
<tr>
<td>Jamestown Canyon</td>
<td>JC</td>
<td>GIV-2235</td>
<td>7</td>
<td>7.5</td>
</tr>
<tr>
<td>Trivittatus</td>
<td>TVT</td>
<td>7348</td>
<td>4</td>
<td>6.6</td>
</tr>
</tbody>
</table>

maintained in L-15 medium (Leibovitz, 1963) supplemented with 10% (v/v) tryptose phosphate broth, 10% (v/v) heat-inactivated (56 °C for 30 min) foetal calf serum (FCS) and antibiotics (‘complete medium’) following the procedure of Madrid & Porterfield (1969). ‘Test medium’, used in the PRNT, contained a lower concentration (2%) of FCS.

Viruses. Table 1 shows a list of the viruses used. All the strains were passaged intracerebrally (i.c.) in suckling mice and stored after low-speed centrifugation as 10% infected brain suspensions in phosphate buffered saline (PBS) at −80 °C. For the PRNT, the virus stocks were diluted in test medium to obtain concentrations of approx. 1250 to 2500 p.f.u./ml, i.e. about five times more than were subsequently used in the test, and 0.5 ml portions were stored at −80 °C.

Antisera. The same virus stocks were used for both PRNT and the preparation of specific immune sera. Adult female T.O. strain mice (mean weight 20 g) were inoculated intraperitoneally (i.p.) once with 0.25 ml of the stock viruses. The blood sera were collected 11 days later, and stored at −20 °C. Control serum against 10% normal suckling mouse brain was prepared in a similar way. All sera were heat-inactivated at 56 °C for 30 min before testing.

Plaque-reduction neutralization test. The PRNT procedure was basically the constant virus–twofold serum dilution test described by Madrid & Porterfield (1969) adapted to the microtechnique of Chanas et al. (1976). The unit volume (drop) of the test was 39 μl (s.d. ± 3.6 μl). One drop of test virus containing approx. 15 p.f.u. was added to one drop of serum dilution in each well. After 60 min at 28 °C a drop of suspension containing 25,000 (XTC) or 30,000 (PS) cells was added to each test well. After a further 3.5 h at 28 °C (XTC) or 37 °C (PS), two drops of overlay medium containing 1.5% carboxymethylcellulose in test medium were introduced in each well. Each microplate contained a single virus serotype to be tested against antisera to all of the listed viruses; controls on each plate included mouse serum against normal suckling mouse brain, a test dose of virus, and cells without virus. The microplates were maintained at 28 °C (XTC) or 37 °C (PS) in sealed plastic bags and stained with naphthalene black solution four days later. The serum neutralization titres are expressed as the dilution that reduced the plaque count by 50%.

For evaluation of the cross-neutralization patterns among the viruses, the serum titres were transformed into $-\log_2$ values, and all sera were then arbitrarily standardized to have the homologous titre of 8 ($-\log_2$ of 1:256). If, for example, an anti-A serum had a titre against A virus 1:64, the $-\log_2$ titres of that anti-A serum against all viruses were increased by 2 ($8 - 6 = 2$). Average bilateral titres for each pair of viruses were then computed as the arithmetic means of the standardized $-\log_2$ titres of both (i) virus A against anti-B virus
serum and (2) virus B against anti-A virus serum. The matrix of these mean reciprocal standardized titres was analysed by the average linkage clustering procedure (Sneath & Sokal, 1973).

RESULTS

Distinct plaques were produced in both cell lines by all viruses except TVT virus which failed to produce plaques in XTC-2 cells; CE virus plaqued poorly in XTC-2 cells. The plaques were larger in PS than in XTC cells (about 1·2 to 1·4 mm against 0·7 to 0·8 mm) but they were clearer in the latter.
Table 4. Cross-plaque reduction neutralization tests*

<table>
<thead>
<tr>
<th>Virus</th>
<th>CE</th>
<th>LAC</th>
<th>SSH</th>
<th>LUM</th>
<th>TAH</th>
<th>JC</th>
<th>TVT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>8,8</td>
<td>6,6</td>
<td>5,5</td>
<td>6,6</td>
<td>4,3</td>
<td>5,4</td>
<td>(4)</td>
</tr>
<tr>
<td>LAC</td>
<td>8,8</td>
<td>6,5</td>
<td>5,7</td>
<td>4,5</td>
<td>3,4</td>
<td>5,2</td>
<td>(2)</td>
</tr>
<tr>
<td>SSH</td>
<td>8,8</td>
<td>5,7</td>
<td>5,7</td>
<td>4,5</td>
<td>3,4</td>
<td>5,2</td>
<td>(2)</td>
</tr>
<tr>
<td>LUM</td>
<td>8,8</td>
<td>7,7</td>
<td>7,7</td>
<td>4,4</td>
<td>4,5</td>
<td>4,5</td>
<td>(4)</td>
</tr>
<tr>
<td>TAH</td>
<td>8,8</td>
<td>5,4</td>
<td>5,4</td>
<td>4,5</td>
<td>4,5</td>
<td>4,5</td>
<td>(3)</td>
</tr>
<tr>
<td>JC</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>(8)</td>
</tr>
<tr>
<td>TVT</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>8,8</td>
<td>(8)</td>
</tr>
</tbody>
</table>

*Results are expressed as $-\log_2$ of mean standardized bilateral titres: first figure for PS cells, second for XTC-2 cells.

† Only the titres of anti-TVT sera against the other viruses are given since no plaques were produced by TVT virus in XTC-2 cells.

![Dendrogram](image)

Fig. 1. Dendrogram of the relationships in neutralization among the strains compared (average linkage clustering); PS and XTC-2 cells, average. Upper axis: $-\log_2$ standardized bilateral titres. Lower axis: $-\log_2$ standardized bilateral titres.

Table 2 and Table 3 show the results of cross-PRNT in both cell lines. The hypothetical homologous titres of TVT in XTC-2 cells has been estimated to be $1:128$, twice as high as in PS cells since the homologous titres in XTC cells were on average about twice as high as in PS cells. The average bilateral standardized titres are presented in Table 4. There were no marked differences between the PRNT data at 37 °C in PS cells and at 28 °C in XTC-2 cells. After calculating the average titres for each pair of viruses, using the mean bilateral value found in (a) PS cells and (b) XTC-2 cells (from Table 4), the average linkage clustering yielded the results that are illustrated in the dendrogram of Fig. 1. Choosing a $-\log_2$ level of 5 (titre $1:32$), which means an eightfold lower average titre compared to the homologous titres (8), three clusters of the viruses can be noted in the dendrogram. The major subgroup contains CE, LAC, SSH, TAH and LUM, whereas JC and TVT viruses appear to be separate, each having antigenic characteristics sufficiently different (eightfold on average in PRNT) from the other viruses. In the first subgroup, antigenically very close pairs of serotypes are LAC with SSH, and LUM with TAH, the differences between their average homologous and heterologous titres being only about twofold. SSH and TAH seem to be strains of LAC and LUM serotypes, respectively, deprived of some antigenic component(s). This might be deduced from the finding that anti-SSH and anti-TAH sera do not neutralize LAC and LUM viruses, respectively, to such an extent as anti-LAC and anti-LUM sera neutralize SSH and TAH viruses, respectively. That particular antigenic relationship between LAC and SSH serotypes has also been noted by Sather & Hammon (1967) and between LUM and TAH serotypes by Kunz et al. (1964) and Sather & Hammon (1967).
DISCUSSION

No significant differences in the neutralization results obtained were observed between the two cell systems used at different incubation temperatures. However, PS cells are preferred since plaques were shown by all the virus strains tested (although the plaques may not have been as clear as in XTC cells in some cases).

The use of single-dose immune sera produced by early bleeding of the animals has been shown to yield the most specific results with closely related arbovirus (including CAL group viruses) antigens (Casals, 1967; Murphy & Coleman, 1967; Wellings et al. 1970, 1971; Sprance & Shope, 1977). Nevertheless, our serological tests suggested in both cell systems that the viruses used were antigenically very close, with the exception of TVT and JC viruses. These results confirm those of previous workers (Hammon & Sather, 1966; Murphy & Coleman, 1967; Sather & Hammon, 1967; Calisher & Maness, 1970; Papadopoulos et al. 1970; Wellings et al. 1971; Lindsey et al. 1976). It has been suggested that there is a marked antigenic instability within the CAL group of viruses possibly due to laboratory passage level, previous passage in vertebrate hosts and/or invertebrate vectors, or geographic distribution (Hammon & Sather, 1966; Murphy & Coleman, 1967; Calisher & Maness, 1970; Issel et al. 1975; Lindsey et al. 1976).

The CAL group includes at present 11 serotypes (Porterfield et al. 1975/6) of which we had no opportunity to study Bocas (BOC), Inkoo (INK), Jerry Slough (JS), Keystone (KEY), Melao (MEL) and San Angelo (SA). INK is considered to be closely related to TAH serotype (Brummer-Korvenkontio et al. 1973). JS to JC (Calisher & Maness, 1970) and SA to strains of CE, SSH and LAC (Hammon & Sather, 1966; Sather & Hammon, 1967; Calisher & Maness, 1970; Lindsey et al. 1976). KEY is a distinctly separate virus (Murphy & Coleman, 1967; Calisher & Maness, 1970; Wellings et al. 1971; Lindsey et al. 1976). MEL has been described either as being similar to the CE subgroup (Whitman & Shope, 1962; Murphy & Coleman, 1967; Wellings et al. 1971) or by others as distinct (Hammon & Sather, 1966; Sather & Hammon, 1967; Calisher & Maness, 1970; Lindsey et al. 1976). BOC has now been shown to be a coronavirus, probably mouse hepatitis virus (R. E. Shope, personal communication). Thus, the CAL group of viruses seems to comprise several antigenic subgroups: (1) CE, LAC, SSH, SA, LUM, TAH, INK; (2) KEY, Waycross strain of KEY serotype, and intermediate serotypes JC, JS approaching the first subgroup; (3) TVT; (4) MEL (separate?). Further studies should be performed to clarify the taxonomic position of several serotypes.

SSH and LAC viruses, which are at present given separate status in the International Catalogue of Arboviruses (Berge, 1975; Karabatsos, 1978), exhibited similar close relationships to each other, as did the prototype and LUM strains of TAH to each other, although LUM is considered to be a strain of TAH. The recent work of Clewley et al. (1977) comparing SSH and LAC has established differences in the RNA species of the strains. The wide geographic separation and the antigenic similarity between TAH and LUM strains suggests that it would be worthwhile examining the relationship of these strains more closely.

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REFERENCES


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