Immunofluorescence Studies on the Antigenic Interrelationships of the Hughes Virus Serogroup (Genus Nairovirus) and Identification of a New Strain

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SUMMARY

Titrations of hyperimmune antisera by indirect immunofluorescence using each virus of the Hughes serogroup (Hughes, Zirqa, Punta Salinas, Soldado and Farallon) demonstrated their individual antigenic identities. Furthermore, an antigenically related virus, designated Puffin Island (PI) virus, was shown both by indirect immunofluorescence and by neutralization in XTC cells to be distinguishable from the other viruses. These viruses readily established persistent infections in Vero cells after producing only moderate cytopathic effects. Treatment of persistently infected cultures with either fluorodeoxyuridine or bromodeoxyuridine made no significant difference to the percentage of immunofluorescent cells. Attempts to demonstrate haemagglutination by Zirqa virus were unsuccessful.

The Hughes serogroup are constituent viruses of the genus Nairovirus, family Bunyaviridae (Bishop et al., 1980; Casals & Tignor, 1980), comprising Hughes (HUG), Farallon (FAR), Zirqa (ZIR), Punta Salinas (PS) and Soldado (SOL) virus. They are all tick-borne viruses usually infecting Ornithodoros (Alectorobius) capensis which is a common ecto-parasite of nesting marine birds on many oceanic islands (Kohls et al., 1965). Humans bitten by infected ticks experienced pruritus persisting for several days (Converse et al., 1975). The morphology and morphogenesis of SOL virus have been examined by electron microscopy (Chastel et al., 1979) and ZIR virus was reported to haemagglutinate goose erythrocytes (Varma et al., 1973). With the exception of Yunker et al. (1979), there is little information specifically regarding either the precise antigenic relationships between these viruses or their replicative pathways during infections. Consequently, these and other properties are assumed on the basis of a slight antigenic relationship with Crimean-Congo haemorrhagic fever (CCHF) virus (Casals & Tignor, 1980). In this paper using indirect immunofluorescence we have examined the antigenic interrelationships of the recognized viruses within the Hughes serogroup and have included a new candidate, previously assumed to be SOL virus, for this group. Furthermore, we report our attempts to produce haemagglutination with Zirqa virus.

The viruses HUG, PS, SOL and FAR were kindly supplied by Dr J. Casals (Yale Arbovirus Research Unit, New Haven, Conn., U.S.A.). ZIR was isolated as reported by Varma et al. (1973). PI virus was isolated by Johnson et al. (1979) from a pool of argasid ticks [O. (A.) maritimus] and was previously referred to as SOL virus. Each virus was maintained as a 20% suspension of infected newborn mouse brains. Vero cells (Flow Laboratories) were subcultured in Leibovitz L15 medium (Gibco) with 5% foetal bovine serum, 10% tryptose–phosphate broth, penicillin and streptomycin (100 units/ml and 100 µg/ml respectively). Xenopus (XTC-2) cells (Pudney et al., 1973) were subcultured as above but incubated at 28°C. Hyperimmune guinea-pig antisera against each virus were prepared by intramuscular inoculation of infected mouse brain suspension mixed with an equal volume of complete adjuvant. This was repeated 2 weeks later, followed after a further 2 weeks by intraperitoneal inoculation without adjuvant. The sera were collected after a further 2 weeks for use in the experiments to be described. For indirect
Table 1. Antigenic cross-reactivity of Hughes viruses studied by indirect immunofluorescence

<table>
<thead>
<tr>
<th>Antibody</th>
<th>SOL</th>
<th>PI</th>
<th>PS</th>
<th>HUG</th>
<th>ZIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOL</td>
<td>160*</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>PI</td>
<td>&lt;10</td>
<td>160</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>PS</td>
<td>&lt;10</td>
<td>20</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HUG</td>
<td>40</td>
<td>80</td>
<td>320</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>ZIR</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>20</td>
<td>320</td>
</tr>
<tr>
<td>FAR</td>
<td>160</td>
<td>320</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
</tr>
</tbody>
</table>

* Titre of antibody expressed as reciprocal of highest dilution showing immunofluorescence.

Table 2. Neutralization of HUG, SOL and PI virus by plaque reduction in XTC cell cultures

<table>
<thead>
<tr>
<th>Antibody</th>
<th>HUG</th>
<th>SOL</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUG</td>
<td>&gt;3-0*</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>SOL</td>
<td>&lt;1</td>
<td>2.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>PI</td>
<td>2.0</td>
<td>&lt;1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* log10 Neutralization index estimated by comparison of plaque numbers obtained in control and antibody-treated samples.

immunofluorescence either infected or non-infected Vero cells on glass coverslips were washed in phosphate-buffered saline (PBS) and fixed in acetone at 4 °C. They were treated with guinea-pig antisera for 40 min at 37 °C, then washed with PBS, and fluorescein-conjugated anti-guinea-pig immunoglobulin (Nordic Immunological Laboratories) was added for 40 min at 37 °C. After further washing with PBS, coverslips were mounted in glycerol–saline (90%) and viewed with an incident light research fluorescence microscope (Vickers M17). For neutralization tests viruses were diluted in L15 medium containing 2% foetal calf serum and antibiotics. Equal volumes of virus dilution and a 1:10 dilution of hyperimmune guinea-pig antiserum of the appropriate type were mixed at 28 °C for 1 h. XTC-2 cells were added to each mixture and, after incubation for 3 h at 28 °C, L15 medium containing 2% foetal calf serum, 1.5% carboxymethylcellulose and antibiotics was added. The cultures were incubated for 5 days at 28 °C before staining with 0.1 naphthalene black prepared in 0.1 M-sodium acetate and 6% acetic acid. Neutralization was considered to have occurred if at least 90% of the plaques were inhibited.

Vero cells infected with the Hughes serogroup viruses were used in both homologous and heterologous immunofluorescence titrations of the guinea-pig hyperimmune antisera. Although Farallon virus was not included, antiserum against Farallon virus was included in the titrations. The results (Table 1) illustrate the relatively close antigenic relationships between all of these viruses. On the other hand, each strain of virus could also be distinguished from each other strain by virtue of the distinct patterns of cross-reactivity. PI virus was included in the titrations and, although originally described as SOL virus because of cross-reactivity with SOL in complement fixation tests, it appears to be a distinct strain of virus.

Homologous and heterologous neutralization tests were performed with each of the three viruses, PI, SOL and HUG, using XTC-2 cells exactly as described above. The one-way reactivity between SOL and PI was distinct, SOL antiserum neutralizing SOL but not PI or HUG, whereas PI antiserum caused neutralization only of PI and HUG virus. As expected, HUG antiserum neutralized all three viruses although neutralization titres were lower in the heterologous tests (Table 2).

During the course of this investigation, we noticed that at all times after infection in Vero cells it was possible to subculture the infected monolayers without losing cultures due to virus-induced cell death. We therefore maintained and subcultured each virus for 10 serial passages, each time monitoring the cells by immunofluorescence for the presence of virus-specific anti-
gens. With the exception of PI virus all of the strains established persistent infections in which on average 20 to 40% of the cells contained cytoplasmic viral antigen. With PI virus normally only approximately 1% of the cells contained virus-specific antigen after the second subculture. Each of the viruses produced only moderate cytopathic effect (c.p.e.), usually within the first 10 days, that disappeared soon after the third subculture (15 days). HUG and ZIR usually produced the most noticeable c.p.e. and the highest percentage of infected cells. In these cases we were able to freeze the persistently infected cell lines in liquid nitrogen and subsequently resuscitate the cultures. The others were not tested in this way. When 200 µg/ml of either BUdR or FUdR (Sigma) were incorporated in the maintenance medium of HUG- or ZIR-persistently infected cultures there was no obvious change in either the appearance of the cells within the next 48 h, or in the proportion of antigen-bearing cells.

It was previously reported (Varma et al., 1973) that ZIR virus produced haemagglutination of goose erythrocytes. In view of the importance of such a claim with respect to the classification of the Hughes viruses we decided to repeat this work in the hope that we could perform haemagglutination inhibition tests with each of the above antisera.

ZIR virus was inoculated intracerebrally into newborn TO mice and when at least 10% had died, usually on day 7, brains were removed from the surviving mice and stored at −70 °C until processed. Twenty percent brain suspensions of ZIR virus were prepared for the haemagglutination tests. Also, ZIR virus-infected Vero cells (input multiplicity 0-1) showing c.p.e. on day 5 post-infection were freeze-thawed three times in the supernatant medium. After centrifugation at 2000 g for 15 min, the virus was precipitated in polyethylene glycol (6%) and sodium chloride (0.4 M). After overnight incubation at 4 °C the precipitate containing the antigen was collected by centrifugation at 15,000 g for 30 min.

Haemagglutination titrations as described by Clarke & Casals (1958) were attempted with all of the above preparations using goose erythrocytes at pH 5.8 up to pH 7.2. The tests were repeated at 4 °C, 20 °C and 37 °C. No haemagglutination was demonstrated with any of the above preparations. Furthermore, in view of the report by Casals & Tignor (1974) that Nairoviruses produce haemagglutination at pH 7.0 to 7.6, the above samples were retested under these modified conditions. Haemagglutination was not detected with any of the preparations described above.

On the basis of serological cross-reactivity using haemagglutination inhibition and neutralization tests, the Hughes viruses are considered to be related to Crimean-Congo haemorrhagic fever virus (Casals & Tignor, 1980). None of the Hughes viruses is known to produce haemorrhagic symptoms in humans or birds. Severe pruritus, fever, headache and erythema have been reported however in humans bitten by ticks known to be infected with either SOL, PS or ZIR virus (Hoogstraal et al., 1970; Converse et al., 1975). There are very few detailed reports describing either their antigenic relationships or their mode of replication. Our knowledge of these viruses is based upon their known serological cross-reactivity by complement fixation, neutralization and indirect immunofluorescence (Converse et al., 1976; Yunker et al., 1979), the morphogenesis of SOL virus by electron microscopy (Chastel et al., 1979) and by analogy with other viruses of the genus Nairovirus (Bishop et al., 1980; Casals & Tignor 1980).

Several more characteristics of the Hughes viruses can now be included. By indirect immunofluorescence titrations with hyperimmune antisera against each of the representative viruses, they each show distinct antigenic characteristics as well as cross-reactivity. Furthermore, the degree of relationship of each virus with one another appears different from that demonstrated in complement fixation tests by Converse et al. (1976) and indirect immunofluorescence titrations reported by Yunker et al. (1979). This probably reflects the different methods of serum preparation and the different procedures for analysing the virus relationships. For example, we have found that many flaviviruses produce a different pattern of immunofluorescence when the infected cells are fixed with cold acetone (−20 °C) for 10 min as opposed to room temperature (20 °C) for 15 to 20 min (unpublished observations). Such differences might account for the apparent unrelatedness of both SOL and ZIR virus with the others as reported by Yunker et al. (1979). In our hands, and using indirect immunofluorescence, both SOL and ZIR virus were clearly closely related to most of the other recognized Hughes viruses.
In previous reports all viruses isolated from Puffin Island were found to be identical to SOL virus (Converse et al., 1976) by complement fixation tests whereas the PI virus reported here and isolated independently (Johnson et al., 1979) was sufficiently different by both neutralization and immunofluorescence tests from SOL and from all of the other Hughes viruses to justify its inclusion as a separate member of the Hughes serogroup. It would be of interest to compare PI virus with others isolated from areas near the United Kingdom to see whether or not they resemble PI virus rather than SOL virus.

All of the recognized Hughes viruses readily established persistent infections in Vero cells. Attempts to discover a cellular DNA dependence using BUdR and FUdR were unsuccessful although this failure could be due to the very slow rate of virus turnover in the cells. Finally, we totally failed to demonstrate a haemagglutination antigen in ZIR virus. ZIR virus does not therefore appear to be unique among the Hughes viruses in its ability to produce haemagglutinin.

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REFERENCES


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