Variants of the HR Strain of Sindbis Virus Lethal for Mice

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SUMMARY

We have selected two similar variants of Sindbis HR virus which are lethal for mice. Consecutive brain to brain passage series were conducted in suckling and weanling mice. Specific anti-Sindbis HR neutralization tests and protection tests demonstrate that these viruses are derived from the parent Sindbis HR.

The HR strain of Sindbis virus was selected by Burge & Pfefferkorn (1966) from a standard Sindbis virus stock about a decade ago. This strain and temperature-sensitive (ts) mutants derived from it have been the strains of choice for molecular studies of Sindbis virus and often for the togavirus Group A in general (Pfefferkorn & Burge, 1967). Although these Sindbis strains have been well characterized on a molecular basis, they have only recently been used in studies of viral pathogenesis and immunity (Peck, Brown & Wust, 1975; Schluter, Bellomy & Brown, 1974; Schluter & Brown, 1974). In an effort to extend the potential usefulness of the molecular knowledge of these strains to future studies on pathogenesis and immunity among alphaviruses by us and others, attempts were made to select a variant of HR that was lethal for mice. A variant of Sindbis virus (AR86) lethal for mice has been reported (Weinbren, Kokernot & Smithburn, 1956) and made available to us by Dr B. M. McIntosh (of the South African Institute for Medical Research) through Dr J. Casals at Yale University. No molecular studies of the variant or its parent have been described and no ts mutants have been reported to our knowledge.

Undiluted (10³⁵-² p.f.u./0.05 ml) or 1:10 diluted HR rarely kills mice by the intracerebral (i.c.) route and not at all by the intraperitoneal (i.p.) route of injection; however we have found that HR multiplies and induces homologous protection against the AR86 lethal variant and the variants reported in this note, and cross-protection against Semliki Forest virus (Peck et al. 1975). Two separate mouse passage series were begun simultaneously. One was by consecutive brain to brain passage in 1- to 3-day old Ha/ICR mice (10 passages) and the other was by brain passage in 4- to 5-week old Ha/ICR mice (14 passages). Ten consecutive passages of HR in chick embryo (CE) cell culture or in chick embryos (by the yolk sac route) did not result in an increased lethality for mice. The stock of HR used to initiate passage was a plaque purified CE cell culture supernatant fluid which contained about 10³⁵-² p.f.u./ml. The low speed supernatant fluid from a pooled 10% (w/v) mouse brain suspension in Medium 199 or Difco Heart Infusion Broth was used in each passage. Only those suspensions which contained peak p.f.u. of virus (as determined after assay of those suspensions made 1, 2 and 3 days after injection) were chosen for the next passage. The following additional procedures have been described previously: the preparation of CE cells, plaquing of virus, LD₅₀ assays in mice and embryonated eggs (Hammon, 1969; Schluter et al. 1974); plaque and mouse neutralization tests (Brown et al. 1969); mouse immunization and protection tests (Brown & Officer, 1975).

In both passage series, peak titres increased erratically until about the 6th passage in suckling mice and the 12th passage in weanling mice. The suckling mouse brain passage 10 (HR-SMB-10) and the weanling mouse brain passage 14 (HR-WMB-14) were the stock...
Table 1. Mouse lethality of HR variants*

<table>
<thead>
<tr>
<th>Virus</th>
<th>CE cell cultures (p.f.u./ml)</th>
<th>Chick embryos (LD₅₀/ml)</th>
<th>Mice (LD₅₀/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>y.s. route</td>
<td>All. route</td>
</tr>
<tr>
<td>HR</td>
<td>10⁶-1</td>
<td>10⁶-4</td>
<td>10⁶-8</td>
</tr>
<tr>
<td>HR-SMB-10</td>
<td>10⁹-1</td>
<td>10⁹-4</td>
<td>10⁷-6</td>
</tr>
<tr>
<td>HR-WMB-14</td>
<td>10⁹-1</td>
<td>10⁹-4</td>
<td>10⁷-6</td>
</tr>
</tbody>
</table>

* Tenfold dilutions of virus in Medium 199 were inoculated on to primary chick embryo fibroblasts, into 8-day old embryonated chicken eggs by the yolk sac (y.s.) route and 10-day old eggs by the allantoic (All.) route, into weanling mice by the intracerebral (i.c.) and intraperitoneal (i.p.) routes. Plaques on chick embryo cell cultures were counted on day 3 post infection by neutral red agar overlay. The embryonated eggs were examined daily for viability for a period of 3 days post infection and the mice were examined daily for 2 weeks.

Table 2. Specific neutralization of HR-SMB-10*

<table>
<thead>
<tr>
<th>Loss in p.f.u. or LD₅₀ titre (log₁₀/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In CE cell cultures</td>
</tr>
<tr>
<td>Normal ascites fluid†</td>
</tr>
<tr>
<td>Anti-HR ascites fluid</td>
</tr>
<tr>
<td>Anti-SF ascites fluid</td>
</tr>
</tbody>
</table>

* 0.1 ml of a 1/100 dilution of stock virus suspensions was mixed with 0.9 ml of a 1/5 dilution of the various ascites fluids and incubated for 45 min at 37 °C. Tenfold dilutions of the mixture were assayed in CE cells (p.f.u.) and in 4-week old mice by the i.c. route.

† Used as control – but titre after incubation did not differ significantly from the stock virus titre.
‡ Not done.

The results of Table 1 show that the two mouse adapted strains of virus, as opposed to parent HR, were highly virulent by the i.c. route and moderately virulent by the i.p. route; the virulence of the strains were not significantly different from HR for embryonated eggs by either the yolk sac or allantoic routes of injection. The results in Table 2 show that HR-SMB-10 virus is a variant of Sindbis and not a contaminating virus acquired during passage, because it was neutralized by anti-HR ascites fluid from HR immunized mice made several years ago, but not by normal ascites fluid nor anti-Semliki Forest virus ascites fluid. This was confirmed in immunization and protection experiments. Four to five-week old mice were immunized with living HR (10⁶-2 p.f.u. or 10⁵-2 p.f.u./dose) by the i.c. route and were challenged 33 days later with 50 LD₅₀ (~ 500 p.f.u.) of HR-SMB-10. The results showed complete protection in the immunized mice (8/8 and 6/6) and no protection in the immunized controls (0/10). Similar results were obtained after HR-WMB-14 virus challenge following immunization of 4- to 5-week old mice with one injection of 10⁷ p.f.u. of living HR by the i.p. route. Furthermore, HR-immunized mice were similarly protected against the mouse lethal variant of AR86.

Thus, we report the selection of two apparently similar variants of Sindbis HR which are lethal for mice by the i.c. and i.p. routes of injection. It is our belief that such virulent strains will be valuable in relating the pathogenesis and immunity to the molecular biology of Group A togaviruses.
We appreciate the excellent technical assistance of Mrs Virginia Derrick. L. R. B. is a Public Health Service predoctoral trainee (TO 1-A100435) of the National Institute of Allergy and Infectious Diseases.

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REFERENCES


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