Strain-dependent Virulence Characteristics of Bluetongue Virus Serotype 11

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SUMMARY
Two strains of bluetongue virus (BTV) serotype 11, UC-2 and UC-8, were identified by the electrophoretic migration pattern of their genomic RNA segments on polyacrylamide gel electrophoresis. Significant differences in virulence of these two viruses could be demonstrated by subcutaneous inoculation of newborn mice. No signs of disease were observed in mice infected with UC-2. Mice infected with UC-8 died of a severe necrotizing encephalitis, which resembled lesions in bovine and ovine foetuses infected with BTV.

Bluetongue virus (BTV) is the prototype virus of the genus Orbivirus, which belongs to the double-stranded RNA family Reoviridae. Twenty-three serotypes of BTV have been identified to date (Knudson & Shope, 1985). This classification, based on serology, does not reflect expression of clinical disease (Della Porta et al., 1982). Comparisons of the virulence of different BTV isolates have been limited to serotypes. However, there is evidence that BTV virulence is not serotype-dependent, e.g. BTV serotype 2 is the most frequent cause of bluetongue disease in sheep in South Africa, whereas U.S. strains of this serotype cause only inapparent or clinically mild infections (Barber & Collisson, 1983).

Mice have been used as the model for studying the pathogenesis of BTV-induced cerebral malformation of ovine foetuses (Narayan & Johnson, 1972; Richards & Cordy, 1967). In addition, various aspects of the immunology involved with BTV infection have been investigated in mice (Jeggo & Wardley, 1982; Letchworth & Appleton, 1983). The objectives of the present study were therefore to select genetically different strains of one serotype (BTV-11) and identify virulence differences using the mouse as a laboratory animal model.

Two strains of BTV-11 were used and designated UC-2 and UC-8. UC-2 was isolated from a calf and UC-8 from a deer by inoculation of blood into 11-day-old embryonated chicken eggs (Foster & Luedke, 1968; Goldsmit & Barzilai, 1968). The isolated virus was passaged twice in Vero cells, plaque-cloned twice in Vero cells (five additional passages), and a virus stock prepared by amplifying the virus titre on BHK-21 cells. The supernatant from BHK-21 cells was diluted 1:1 with buffered lactose peptone, divided into aliquots and stored at -70 °C. The virus was titrated by a plaque method (Stott et al., 1978) on Vero cells before and after mouse inoculation to check for any loss of infectivity. The serotype was determined by the plaque inhibition (disc) method as described previously (Stott et al., 1978).

The selection of the two virus strains used in this study was based on distinct differences in their genomic RNA profile as determined by polyacrylamide gel electrophoresis (PAGE). Briefly, BHK-21 cells were grown in 24-well plates, infected with the virus and harvested when c.p.e. was 100%. Cells were then pelleted in an Eppendorf centrifuge and resuspended in 100 μl 0.1 M-sodium acetate buffer pH 5 with 1% SDS. Double-stranded RNA was extracted with 100 μl of a 3:2 phenol:chloroform suspension (Squire et al., 1983).

Electrophoresis was done on a 10% discontinuous SDS–Tris–glycine-buffered polyacrylamide gel at 20 mA for 16 h (Laemmli, 1970). The gels were silver-stained according to the
Fig. 1. Area of malacia in the mouse cerebral cortex 12 days after inoculation with BTV-11 strain UC-8. Bar marker represents 100 μm.

Fig. 2. Focal necrosis with mineralization (lower left) and non-suppurative meningitis in the mouse cerebral cortex 19 days after inoculation with BTV-11 strain UC-8. Bar marker represents 100 μm.

Fig. 3. Electrophoretic migration patterns of genomic dsRNA from BTV-11 strains (a) UC-2, (b) UC-2 with UC-8 and (c) UC-8. Numbers indicate genome segments.
Table 1. Results of infecting newborn mice with two strains of BTV-11 at a dose of $10^3$ p.f.u./mouse

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>UC-2</th>
<th>UC-8</th>
<th>Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. inoculated</td>
<td>29</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Dead mice</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>% Mortality</td>
<td>0</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Virus isolation</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Brain lesions</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

procedures of Sammons et al. (1981) modified for BTV by Squire et al. (1983). Following plaque-cloning, serotype and genomic profiles of all isolates were reconfirmed by plaque inhibition and PAGE, respectively. The experiments were carried out in newborn BALB/c mice. Pregnant mice were obtained from Charles River Breeding Laboratories (Wilmington, Mass., U.S.A.) and their progeny inoculated within 24 h of birth.

A preliminary study, estimating the effect of the dose, used a high dose inoculum of $5 \times 10^5$ p.f.u./animal and a low dose inoculum of $5 \times 10^2$ p.f.u./animal. Two litters of mice were inoculated subcutaneously with each virus strain.

In the principal experiment, mice were inoculated with $10^3$ p.f.u. of virus diluted in phosphate-buffered saline by the subcutaneous route. Twenty-nine mice were inoculated with UC-2 and 25 with UC-8. Fourteen mice injected with diluent served as negative controls. Mice were checked daily for 20 days after inoculation. Dead animals were collected for virus isolation from the brain. Additionally, three dead mice from each group were used for virus isolation from pooled visceral organs and the eviscerated body. Surviving mice were killed at the end of the experiment and their brains collected for virus isolation. The organs were homogenized in MEM, sonicated and cellular debris was removed by centrifugation at 700 g for 15 min. Virus isolation and titration on Vero cells was done in 96-well plates. Titres were determined according to the method of Reed & Muench (1938).

Lesions in the brains of mice inoculated with the two virus strains were evaluated in a separate experiment. Newborn BALB/c mice were inoculated subcutaneously and killed when signs of disease became obvious. They were dissected and their organs fixed in 6% buffered formalin. Coronary sections of brain were embedded in paraffin, cut on a microtome and stained with haematoxylin and eosin.

The RNA profiles of the two virus strains showed the most obvious differences in the electrophoretic mobilities of genome segment 5 (Fig. 3). Minor differences were observed in genome segments 1, 9 and 10.

In the preliminary study, when the mice were inoculated with the high dose of $5 \times 10^5$ p.f.u./animal, UC-8 killed all six inoculated mice. One out of eight mice inoculated with UC-2 died at this dose. When mice were inoculated with the low dose of $5 \times 10^2$ p.f.u./animal seven out of ten mice inoculated with UC-8 died. All 11 mice inoculated with UC-2 survived. The results obtained from the principal experiment are summarized in Table 1. The differences in mortality between the groups inoculated with UC-2 and UC-8 were statistically significant ($\alpha = 0.001$). Mice died 7 to 16 days after inoculation with UC-8, with the average incubation period being 12.55 days.

Virus recovered from the brains of dead mice had titres ranging from $1.1 \times 10^8$ to greater than $1.4 \times 10^{11}$ TCID$_{50}$/g of brain. Twenty days after inoculation, virus could still be recovered from the brains of three survivors inoculated with UC-8, but not from the 29 mice inoculated with the avirulent strain UC-2. Virus was re-isolated from one of three eviscerated bodies of mice which had been inoculated with UC-8.

Serotype and genomic RNA electrophoretic patterns of re-isolated viruses were indistinguishable from the virus used for inoculation. The virus titre did not change in any of the three virus strains during storage.

A necrotizing encephalitis and non-suppurative meningitis were found in the mice infected with the virulent strain, UC-8 (Fig. 1, 2).
This is the first report which demonstrates differences between BTV strains for virulence in mice. This difference in virulence was demonstrated between two strains of BTV-11. UC-8 was demonstrated to be neurovirulent in newborn mice, whereas UC-2 was avirulent. The virulent virus reached the brain after subcutaneous injection, replicated to high titre, and produced lesions similar in nature to those observed in foetal lambs and foetal calves after inoculation with the vaccine strain of BTV-10 and the U.S. prototype of BTV-10 (McLachlan & Osburn, 1983; Osburn et al., 1971). Therefore, the mouse is a potential model for studying differences in the pathogenesis of virulent and avirulent strains of BTV infection in foetal ruminants. The difference in virulence is not the result of cell culture history. The virulent and the avirulent strains have been isolated in the same way and have the same number of passages on the same cell lines.

The analysis of the genome by PAGE was used to select different strains. However, this technique does not permit identification of genetic characteristics responsible for virulence. Analysis of oligonucleotide fingerprints of each of the genome segments of BTVs has shown that substantial sequence divergence is not restricted to segments with different electrophoretic mobilities (Gorman, 1983).

The virus was consistently re-isolated from the brain of affected animals, indicating that the ability to cause neural lesions in newborn mice is a characteristic of the virulent strain. The neural lesions in the neonatal mouse are similar to those observed in foetal ruminants (McLachlan & Osburn, 1983; Narayan & Johnson, 1972; Osburn et al., 1971; Richards & Cordy, 1967). The laboratory animal used herein may provide a tool for distinguishing virulent and avirulent strains of BTV. However, further work needs to be done to determine how valid the mouse model is in reflecting infections with virulent or avirulent strains of BTV in foetal ruminants.

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REFERENCES


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