Isolation and Characterization of Two Plaque Size Variants of Theiler's Murine Encephalomyelitis Virus (DA Strain)

By E. L. OLESZAK, J. L. LEIBOWITZ and M. RODRIGUEZ

1Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston, 6431 Fannin, Houston, Texas 77030 and 2Departments of Neurology and Immunology, Mayo Medical School, 200 First Street S.W., Rochester, Minnesota 55905, U.S.A.

(Accepted 25 May 1988)

SUMMARY

We have isolated two plaque size variants of Theiler's murine encephalomyelitis virus (TMEV) strain DA. One variant, TMEV-CL (CL), produced large plaques, while the other, TMEV-Ds (Ds), produced small plaques in L-2 cells. The Ds variant yielded a lower titre in BHK cells and had a significantly slower growth rate when compared to CL and DA. In contrast, Ds replicated to a higher titre in the central nervous system (CNS) of infected mice than the large plaque counterpart and DA. Furthermore, the Ds (but not CL) variant was temperature-sensitive, replicating 130- to 500-fold more at 37 °C than at 39 °C. Although Ds, CL and DA were able to establish persistent CNS infections in mice, only the Ds variant and DA induced demyelinating disease in SJL/J mice. Therefore persistence of TMEV in the CNS is not sufficient to produce demyelinating disease. These two variants of the DA strain of TMEV will be useful for study of the viral genetic elements important in the mechanism of virus-induced demyelination.

Theiler's murine encephalomyelitis virus (TMEV) is a picornavirus which produces a unique biphasic central nervous system (CNS) disease in the mouse, its natural host (Lipton, 1975; Theiler, 1937). The early (acute) phase of infection is characterized by replication of virus in CNS grey matter, causing disease resembling poliomyelitis (Brahic et al., 1981; Dal Canto & Lipton, 1982; Lipton, 1980). Several weeks later surviving animals develop the second phase of the disease (late demyelinating disease) (Dal Canto & Lipton, 1975; Lipton, 1975; Rodriguez et al., 1983a). Persistent TMEV infection in mice is considered to be an excellent model for the study of multiple sclerosis (Lipton et al., 1986; Rodriguez et al., 1987). There are two subgroups of TMEV which differ in their biological activity. The first group is represented by GDVII and is highly virulent, results in acute disease, produces large plaques in vitro and does not persist (Lipton, 1980). The second group is represented by the DA strain. It is less virulent, produces small plaques in vitro and causes relapsing demyelinating disease (Daniels et al., 1952; Lehrich et al., 1976; Lipton, 1980). This has suggested that the ability of various strains of TMEV to persist is dependent on plaque size (Lipton, 1980).

The parental DA strain of TMEV used in our laboratory produces a wide spectrum of plaque morphologies when assayed on L-2 cells (Fig. 1 a). The origin and growth of this strain have been described (Daniels et al., 1952; Rodriguez et al., 1983a, b). We selected two of the largest (B1, C1) plaques and three of the smallest (A5, D5, E5) and clonally isolated them by two cycles of plaque-to-plaque purification. Small stocks of these cloned viruses were then grown in BHK-21 cells and characterized further. Representatives of each plaque size morphology, clones D5 and C1 are shown in Fig. 1. Clone D5 produces plaques of 0.75 ± 0.13 mm in diameter while CL produces plaques 1.51 ± 0.16 mm in diameter on L-2 cells (Fig. 1b and c, respectively).
In order to determine whether either of the plaque variants was attenuated with respect to its ability to produce CNS disease, we challenged 4- to 6-week-old SJL/J mice (Jackson Laboratories, Bar Harbor, Me., U.S.A.) intracerebrally with $2 \times 10^5$ p.f.u. Ds, CL or the parental DA virus. As shown in Fig. 2, on day 4 all three viruses grew to a similar titre in the CNS ($3.7 \times 10^4$ to $6.47 \times 10^4$ p.f.u./g tissue). After day 4 there was a gradual decrease in CNS virus titre. At day 45 (the latest time tested in this experiment) the residual virus content was approximately the same for parental DA and CL viruses ($1 \times 10^2$ p.f.u./g). In contrast, clone Ds replicated to a higher titre throughout the course of infection. At day 45 the amount of Ds virus in the CNS was approximately 10-fold higher than that of the parental virus or clone CL. No mice infected with any of these viruses showed evidence of clinical or histopathological signs of acute neuronal disease. These two variants of TMEV were genetically stable, since similar differences in plaque size were observed when virus was re-isolated from the CNS of mice infected with either clone Ds (0.62 ± 0.15 mm diam.) or clone CL (1.51 ± 0.24 mm diam.) (not shown).

However, these viruses differed in the histopathology they induced in infected mice. The parental DA virus induced prominent demyelination in the spinal cord (Fig. 3a) in association with inflammatory infiltrates as has been described previously (Rodriguez et al., 1983a). All SJL/J mice infected with the small plaque variants (As, Ds or Es) also showed the characteristic pathological abnormalities induced by this virus (Fig. 3b). However, in general the extent of
Fig. 3. (a) Intensive inflammation surrounding blood vessels in the spinal cord of an SJL/J mouse infected with wild-type DA virus (45 days p.i.). Inflammatory cells are infiltrating the substance of the white matter and are intimately associated with demyelinated axons. (b) Primary demyelination (arrow) in an animal infected with small plaque variant A₅ virus isolated from DA. Similar lesions were seen with D₅ and E₅ viruses. Araldite-embedded sections stained with 1% toluidine blue. Bar markers represent 25 μm.

Table 1. Pathological abnormalities in the spinal cords of SJL/J mice infected with plaque-purified small plaque and large plaque variants of DA virus 45 days after infection

<table>
<thead>
<tr>
<th>Virus</th>
<th>Glycol methacrylate</th>
<th>Araldite</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Meningeal inflammation</td>
<td>White matter inflammation</td>
</tr>
<tr>
<td>A₅</td>
<td>30.3 ± 9.0†</td>
<td>33.1 ± 9.0</td>
</tr>
<tr>
<td>B₅</td>
<td>1.3 ± 0.7</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>C₅</td>
<td>1.3 ± 1.3</td>
<td>1.6 ± 1.6</td>
</tr>
<tr>
<td>D₅</td>
<td>49.6 ± 5.1</td>
<td>49.2 ± 3.5</td>
</tr>
<tr>
<td>E₅</td>
<td>51.6 ± 6.1</td>
<td>49.6 ± 2.2</td>
</tr>
<tr>
<td>Wild-type</td>
<td>21.8 ± 6.5</td>
<td>23.0 ± 7.0</td>
</tr>
</tbody>
</table>

* Preparation of tissue for light and electron microscopy and pathological scoring was used as previously described (Rodriguez et al., 1986). A score of 100 represents the presence of inflammation or demyelination in every quadrant of every spinal cord section examined in one mouse.
† Data are presented as mean ± S.E.M. (five mice in each group).

Pathological abnormalities was more extensive with the small plaque variant compared to parental DA (Table 1). In contrast, mice infected with the large plaque variants (B₅ or C₅) showed minimal inflammation and demyelination in the spinal cord at 45 days even though these viruses grew to high titres in the CNS (Fig. 4a, b). Three of five mice infected with B₅ and four of five mice infected with C₅ showed no demyelinating lesions in a detailed analysis of
Fig. 4. (a) Cross-section of the spinal cord from a mouse infected with large plaque variant CL virus isolated from DA. There are no inflammatory infiltrates or demyelinating lesions. The vast majority of spinal cord sections (277/282, 98%) from mice infected with clone CL virus showed no pathological abnormalities. (b) Perivascular inflammatory cells surrounding a blood vessel in the meninges of a mouse infected with clone CL virus. Note that these inflammatory cells are not infiltrating the substance of the white matter, and there is no demyelination. Araldite-embedded sections stained with 1% toluidine blue. Bar markers represent 100 μm.

Fig. 5. Kinetics of growth of DA (○), Ds (■) and CL (□) variants of TMEV in BHK cells infected at an m.o.i. of 0.35. Aliquots of supernatants were withdrawn at 2 h intervals and virus titres were determined by plaque assay on L-2 cells.

sections of 25 blocks from each spinal cord. The other mice showed small areas of perivascular inflammation usually unassociated with demyelination (Fig. 4b). The extent of demyelination or inflammation was 15 to 20 times less in large plaque variant infection than in small plaque variant infection (Table 1).

We examined the ability of CL and Ds to form plaques at different temperatures. As shown in Table 2, the relative efficiencies of plating at 33.5 °C, 37 °C and 39 °C for DA virus and clone CL were quite similar. The titres were generally similar at all three temperatures (Table 2) although
Table 2. Titration of DA, Ds and CL variants of TMEV at different temperatures*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Temperature (°C)</th>
<th>Expt. 1 (p.f.u./ml)</th>
<th>Expt. 2 (p.f.u./ml)</th>
</tr>
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<tbody>
<tr>
<td>DA</td>
<td>37</td>
<td>7.5 × 10^8</td>
<td>3.5 × 10^8</td>
</tr>
<tr>
<td></td>
<td>33.5</td>
<td>4.5 × 10^8</td>
<td>1.5 × 10^8</td>
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<tr>
<td></td>
<td>39</td>
<td>1.5 × 10^8</td>
<td>2.5 × 10^8</td>
</tr>
<tr>
<td>CL</td>
<td>37</td>
<td>4 × 10^8</td>
<td>3 × 10^8</td>
</tr>
<tr>
<td></td>
<td>33.5</td>
<td>2.5 × 10^8</td>
<td>3.5 × 10^8</td>
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<tr>
<td></td>
<td>39</td>
<td>1 × 10^8</td>
<td>3 × 10^8</td>
</tr>
<tr>
<td>Ds</td>
<td>37</td>
<td>2.5 × 10^7</td>
<td>6.5 × 10^6</td>
</tr>
<tr>
<td></td>
<td>33.5</td>
<td>1.5 × 10^7</td>
<td>5 × 10^6</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>5 × 10^4</td>
<td>5 × 10^4</td>
</tr>
</tbody>
</table>

* The infected BHK cells were incubated for 3 days and stained with crystal violet.

in some experiments the titre of these two viruses was somewhat lower when assayed at 39 °C compared to titres obtained at lower temperatures. In contrast, Ds formed 130- to 500-fold more plaques at 37 °C than at 39 °C, but formed plaques with equal efficiency at 33.5 °C and 37 °C. Therefore clone Ds represents a natural temperature-sensitive mutant of TMEV.

The growth kinetics of DA, clones Ds and CL in BHK cells infected at identical m.o.i. (0.35) are presented in Fig. 5. Exponential virus growth began at 8 h post-infection (p.i.) for all three viruses. DA and clone CL exhibited a similar pattern of growth, reaching a maximum titre (1 × 10^7 p.f.u./ml) about 14 h p.i. In contrast, the replication and/or spread of clone Ds was significantly slower (Fig. 5), although by 27 h p.i. clone Ds reached a maximum yield (5 × 10^6 p.f.u./ml) similar to those of DA and clone CL.

A relationship between virus plaque size and virulence has been postulated for a variety of animal viruses including picornaviruses (Colter et al., 1964; Takemori et al., 1957; Takemoto & Habel, 1959; Takemoto, 1966; Vogt et al., 1957). Lipton (1980) studied in detail the association between the disease induced by TMEV strains and their plaque morphology. The large plaque strains, represented by GDVII and FA viruses, were highly virulent, primarily replicated in neurons, produced an acute, fatal polioencephalitis which primarily affected the grey matter, and did not persist in the CNS of mice surviving the acute infection. In contrast, five strains forming small plaques in tissue culture (DA, WW, TO4, Yale and BeAn8386) produced minimal acute neuronal infection, but produced a persistent infection of the white matter resulting in demyelination (Brahic et al., 1981; Dal Canto & Lipton, 1975, 1982; Lehrich et al., 1976; Lipton, 1975). It should be pointed out that plaques produced by the CL plaque variant are ‘large’ (1-5 mm in size) only when compared to the TO subgroup of TMEV. When compared to the 5 mm or greater plaques produced by the GDVII subgroup of TMEV, they are clearly small plaque viruses.

The histopathology induced by the plaque variants we have identified correlates with their ability to persist in the animal. Initially, both viruses replicated to the same extent in the CNS of infected mice, reaching titres of about 10^4 p.f.u./g of CNS tissue at 4 days p.i. However, the small plaque (demyelinating) virus Ds persisted in the CNS to a much greater extent than the non-demyelinating CL variant, maintaining titres more than 10-fold higher than those present in CL-infected mice. Since CL virus replicates as well as DA parental virus but does not induce disease, it seems unlikely that the level of viral replication in the brain is the only factor responsible for demyelination. In contrast to their behaviour in vivo, in tissue culture the small plaque virus Ds in general grew less well than the large plaque virus CL, reaching lower final titres and replicating at a slower rate. In addition, Ds was temperature-sensitive in cell culture, having a plating efficiency at 39 °C at least 100-fold less than that observed at 33.5 °C or 37 °C. The large plaque CL virus is not temperature-sensitive.

The molecular basis for the different biological behaviour of the Ds and CL variants of DA virus is unknown at this time. Nitayaphan et al. (1985) have postulated a crucial role for a trypsin-sensitive site on the TMEV VP1 protein for the differences observed in the
Short communication

neurotropism and virulence of the GDVII and TO-like strains of TMEV. Although the GDVII and TO-like viruses are closely related it is clear that there are enough differences in their sequences to make identifying the biologically important differences difficult. A molecular analysis of the sequence differences between the D₅ and C₉ variants of the DA virus should simplify matters somewhat. Since these viruses arose from the same parental stock they should differ in their sequence at only a small number of sites. In addition, D₅ and C₉ fail to produce lethal neural disease in contrast to GDVII. This will allow a more direct analysis of the genetic differences between D₅ and C₉ which contribute to demyelination without the added complication of neuronal disease. A correlation of these sequence differences with their differing abilities to persist and induce demyelination should give us a clue to at least some of the viral genetic elements which are important for the induction of this disease.

M.R. is the recipient of Teacher Investigator Award NS 00849 from the NIH and is supported by the National Multiple Sclerosis Society (RG-1878) and the Searle Foundation.

REFERENCES


(Received 11 March 1988)