Attenuation of neurovirulence of Theiler’s murine encephalomyelitis virus strain GDVII is not sufficient to establish persistence in the central nervous system

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Virus recombinants constructed from Theiler’s murine encephalomyelitis virus (TMEV) strain GDVII, which causes a rapidly fatal encephalitis in mice, and the less virulent BeAn, which persists in the murine central nervous system (CNS) and causes inflammatory demyelination, and a GDVII mutant deleted of 46 of 76 leader protein amino acids were analysed for virus persistence in the CNS. The two recombinant and mutant viruses principally contain GDVII sequences including the nucleotides encoding the polyprotein and 3′ untranslated region. These viruses were found to replicate in the CNS of mice but they did not produce acute encephalitis or paralysis, i.e. they were attenuated in neurovirulence compared to the GDVII parent. More important, none of the viruses persisted in the mouse CNS nor caused chronic demyelination. Thus, attenuation of GDVII neurovirulence alone is not sufficient to establish TMEV persistence. This result is discussed in the context of a genomic determinant for persistence.

The Theiler’s murine encephalomyelitis viruses (TMEV), members of the genus Cardiovirus in the family Picornaviridae, can be divided into two groups based on their neurovirulence characteristics after intracerebral (i.c.) inoculation of mice. Highly virulent strains, such as GDVII virus, cause a rapidly fatal encephalitis in mice. The less virulent strains, such as BeAn and DA, are characterized by at least a 10⁴-fold reduction in the mean 50% lethal dose (LD₅₀) compared with the virulent group and by their ability to persist in the central nervous system (CNS). TMEV persistence which leads to immunopathological damage of myelin is mediated by major histocompatibility (MHC) class II-restricted Th1 lymphocytes directed at virus epitope(s) (Miller et al., 1990; Welsh et al., 1990; Lipton et al., 1997; Monteyne et al., 1997).

Previously, we reported that mice infected with a TMEV recombinant containing predominantly GDVII sequences, designated Chi 2, had focal areas of inflammatory, demyelinating lesions at 28 days post-infection (p.i.) in the absence of persisting virus, but the demyelinating lesions did not become larger thereafter (Lipton et al., 1991). We concluded that viruses from either TMEV neurovirulence group can produce demyelination but that the progression and chronicity of the demyelinating process requires virus persistence.

Recombinant TMEVs, constructed by exchanging corresponding genomic regions between the highly virulent GDVII and less virulent BeAn or DA cDNAs, have been used to map a determinant for virus persistence (and demyelination) to the P1 sequences encoding the capsid proteins (Calenoff et al., 1990; Fu et al., 1990; McAllister et al., 1990; Rodriguez et al., 1992). However, there are conflicting results regarding whether this determinant can include GDVII sequences (Fu et al., 1990; Rodriguez et al., 1992; Adami et al., 1997). Persistence of GDVII virus is difficult to assess directly because few or no infected animals survive at low inoculation doses, e.g. 1–10 LD₅₀. The results with Chi 2 suggest that GDVII sequences in the capsid are not sufficient to establish CNS persistence.

Here we report on the lack of CNS virus persistence in mice inoculated i.c. with two additional recombinants and a mutant TMEV containing predominantly GDVII sequences. Fig. 1 schematizes the parental and recombinant viruses used in this study. Construction of the cDNA clones and some properties of the derived viruses have already been described for BeAn and Chi VL(In668) (Lipton et al., 1991; Pritchard et al., 1992, 1993; Bandyopadhyay et al., 1993), whereas those of LAN46 and Chi 5L are described here. Chi 5L contains BeAn sequences between nucleotides 933 (KpnI restriction endonuclease site) and 1142 (SspI site) in a virus that is otherwise GDVII. Construction of Chi 5L was verified by restriction endonuclease

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The three viruses were somewhat growth compromised compared to BeAn virus, with mean virus titres in p.f.u./g tissue as follows: BeAn, $1 \times 10^6$ (n = 4); Chi 5L, $5.4 \times 10^5$ (n = 5); LAN46, $7.0 \times 10^4$ (n = 6); Chi VL(In668) $9 \times 10^4$ (n = 5). However, these CNS titres at day 6 p.i. were the same as those previously reported for Chi 2 (Lipton et al., 1991). In contrast to the results reported for Chi 2, early poliomyelitis was not seen after inoculation of mice with these recombinant and mutant viruses. In addition, none of the recombinant or mutant viruses produced persistent CNS infection as determined by standard plaque assay, nor did they produce clinical or pathological demyelinating disease (Table 1). PCR-amplification was also performed to confirm whether TMEV persisted in mice taken at later times p.i. (Fig. 2). Total RNA isolated from spinal cord was reverse transcribed (1-2 µg RNA in a 25 µl reaction) using Moloney murine leukemia virus reverse transcriptase in the presence of a primer (1.8 µM) corresponding to the complement of viral nucleotides 828–809 (reading 5'–3'). Two µl of each cDNA reaction was PCR-amplified in a 50 µl reaction using this negative-strand primer and a positive-strand primer equivalent to BeAn nucleotides 527–546. The sequences of the primers and the hybridization...
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Fig. 2. Autoradiogram of a Southern blot of PCR-amplified cDNA derived from total RNA isolated from BeAn-infected and uninfected mouse spinal cords. The PCRs for the BeAn-infected mouse contained 10-fold serial dilutions of the cDNA as indicated. The other PCRs contain undiluted cDNA from the reverse transcriptase reactions. The 40 PCR cycles consisted of 1 min denaturations at 94 °C, 2 min anneals at 58 °C, and 1 min extensions at 72 °C. Ten µl of each PCR reaction was electrophoresed on a 1·2% agarose gel and transferred to nitrocellulose. The chimera 5L-RT lane refers to the absence of reverse transcriptase in the corresponding cDNA reaction, and the two 5L lanes are for two difference mice. The 300 bp TMEV-specific band and a 400 bp mouse product, nonspecific for TMEV, are labelled.

An shown in Fig. 2, no TMEV RNA was found in Chi 5L-infected mice at 112 days p.i. using conditions that detected a TMEV-specific band at a 10⁻³ dilution of cDNA for a BeAn-infected mouse 212 days p.i. in addition to the Chi 5L data shown in Fig. 2 (a total of three mice were tested; one is not shown), negative results were obtained for undiluted cDNA reverse-transcribed from RNA from mice infected with Chi VL(In668) (three mice tested at 337 days p.i.), and with LΔN46 (one mouse tested 161 days p.i.) (not shown). A TMEV-specific PCR-amplified band from BeAn-infected mouse spinal cord RNA can be detected for at least 12 months p.i. (not shown).

Therefore, two TMEV recombinants and a leader protein mutant that largely contain GDVII sequences (Fig. 1) were attenuated in neurovirulence and did not persist or produce demyelination in the mouse CNS. Previously, we reported that Chi 2, which contains GDVII sequences encoding the polyprotein and 3’ untranslated region (UTR), did not persist; however, Chi 2-infected mice developed acute flaccid paralysis (poliomyelitis) and survived (Lipton et al., 1991). We have no explanation why Chi 5L-, Chi VL(In668)- or LΔN46-infected mice failed to develop acute polio. The lack of virus persistence with Chi 5L, Chi VL(In668) and LΔN46 supports the notion that TMEV persistence is required to produce the chronic demyelinating pathology (Chamorro et al., 1986). A continuous virus-antigen drive is apparently needed for chronic immunopathological destruction of myelin to occur in this infection. Miller et al. (1997) have recently shown that autoimmune responses to myelin epitopes arise as a late consequence of TMEV infection and ongoing myelin breakdown. Delayed-type hypersensitive responses to the immunodominant proteolipid protein (PLP) peptide 139–151 were detected at day 52 p.i., and responses to other neuroantigen epitopes developed at later times. This was explained on the basis of an immune response to newly exposed epitopes, ‘epitope spreading’, and suggested that at later times in this infection autoimmune responses enhance the myelin damage. It will now be important to determine whether similar autoimmune responses develop in mice infected with Chi 5L, Chi VL(In668) or LΔN46, where there is no initial myelin breakdown, as well as in Chi 2-infected mice where myelin breakdown takes place only before day 30 p.i. (Lipton et al., 1991).

With one exception a TMEV persistence determinant(s) has been mapped to the capsid of BeAn or DA (Tangy et al., 1991;
McAllister et al., 1990; Adami et al., 1997). The exception is a GDVII–DA virus recombinant, designated GD1B-2C/DAFL3, which is partially neurovirulent and persists in the CNS (Fu et al., 1990; Rodriguez et al., 1992). GD1B-2C/DAFL3 contains GDVII sequences in most of the capsid, i.e. in the carboxyl half of VP2 (amino acids 152–267) and in VP3 and VP1. We have assembled a series of TMEV recombinants in which BeAn sequences progressively replaced those of GDVII starting at the 5’ end of the capsid protein genes to more finely map a persistence determinant within the P1 (capsid) sequences (Adami et al., 1997). TMEV persistence was restored only when BeAn sequences extended from the leader to approximately half-way through VP1 (169 of 276 VP1 residues) in a recombinant designated Chi 40. Therefore, in contrast to the GDVII–DA recombinant GD1B-2C/DAFL3, the capsid of our GDVII–BeAn recombinant Chi 40 consisted mainly of BeAn sequences. This and other evidence (Jarrousse et al., 1994) suggests that persistence of TMEV recombinants may depend on the conformation of the capsid. The results of the present study suggest that the native conformation of the GDVII capsid found in Chi 5L, Chi VL(Inc68) or LΔN46 does not result in CNS persistence. Further work is necessary to clarify the role of conformation of the TMEV capsid in CNS persistence and determine the exact mechanism by which this may lead to persistence.

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References


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