An Attenuated Variant of the GDVII Strain of Theiler’s Virus Does Not Persist and Does Not Infect the White Matter of the Central Nervous System

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The DA strain of Theiler’s virus causes a persistent and demyelinating infection of the white matter of spinal cord, whereas the GDVII strain causes a fatal gray-matter encephalomyelitis. Studies with recombinant viruses showed that this difference in phenotype is controlled mainly by the capsid. However, conflicting results regarding the existence of determinants of persistence in the capsid of the GDVII strain have been published. Here we show that a GDVII virus whose neurovirulence has been attenuated by an insertion in the 5′ noncoding region does not persist in the central nervous systems of mice. Furthermore, this virus infects the gray matter efficiently, but not the white matter. These results confirm the absence of determinants of persistence in the GDVII capsid. They suggest that the DA capsid controls persistence by allowing the virus to infect cells in the white matter of the spinal cord.

Theiler’s virus is a murine picornavirus related to encephalomyocarditis virus and Mengo virus, two members of the Cardiovirus genus. Although all strains of Theiler’s virus are closely related, serologically and at the nucleic acid sequence level (17, 19, 20, 22), they can be divided into two groups based on the disease they cause after intracranial inoculation. Most strains, including the DA and BeAn strains, cause a biphasic disease of the central nervous system (CNS) (13). The first phase, or early disease, is an acute encephalomyelitis which occurs during the first days following inoculation. During this period, the virus infects mainly neurons in the gray matter of the brain and spinal cord. Most animals survive and enter the second phase of the disease, during which the virus persists in the white matter of the spinal cord, mainly in macrophages and oligodendrocytes (2, 15, 21). Viral persistence is associated with focal inflammatory lesions in which numerous demyelinated axons can be seen (6, 13). Persistence and late disease occur only in genetically susceptible mouse strains, such as the SJL/J strain (3). Resistant strains clear the infection at the end of the early phase of the disease, mainly through a vigorous, class I-restricted, cytotoxic T-lymphocyte response (7, 11). Accordingly, the nude mutation confers susceptibility on mice with an otherwise resistant background (26, 29). In contrast to the DA and BeAn strains, strain GDVII is highly neurovirulent. It replicates permissively in neurons during the early phase, killing its host in a matter of days (27). Based on the observation that the GDVII strain does not persist in the rare survivors (12), determinants of persistence have been mapped by recombining cDNA obtained from the DA (or BeAn) and GDVII strains. The results obtained by different groups are, on the whole, consistent and show that the capsids of the DA and BeAn strains contain the main determinants of persistence (1, 16). However, some studies with recombinant viruses suggested that determinants of persistence were also present on the capsid of the GDVII strain (8, 25), an observation which, if confirmed, would make the interpretation of the phenotype of recombinant viruses very difficult. The persistence of GDVII virus is difficult to assess directly since the virus kills the majority of mice within the first week postinoculation. Therefore, attenuated GDVII viruses are invaluable for investigation of the question. Variants in which attenuation is due to modifications in the cis-acting control elements of the 5′ noncoding region are particularly suitable for this purpose since the rest of the genome, including the region coding for the capsid proteins, consists of GDVII sequences (24). In this study we made use of mutant GD-43 (formerly GD/I.27-43) (23), which harbors an engineered 27-nucleotide-long insertion just upstream of the initiator codon. This insertion contains an AUG in a nonoptimal context (ua uAU/Gu), followed by a stop codon 3 nucleotides further down. While being attenuated for mice, GD-43 exhibits an almost wild-type phenotype in BHK-21 cells (23, 24).

We tested the ability of virus GD-43 to persist in the CNS of susceptible inbred SJL/J mice. Eighteen 3-week-old SJL/J mice were inoculated intracerebrally with 40 μl of phosphate-buffered saline containing 10⁵ PFU of GD-43 virus and sacrificed at days 6, 14, 22, and 45 postinoculation. The CNS was removed and studied for histopathology and for the presence of viral antigen, as described in a previous publication (2). In the four mice which were analyzed 6 days postinoculation, viral antigen and numerous foci of inflammation were found in the gray matter of the CNS. Most infected cells had the morphology of neurons (Fig. 1), as already reported for the early phase of infection with the DA or GDVII virus. A minority of infected cells could not be identified by morphological criteria, mainly because of cytopathic effect. Four mice were sacrificed 14 days postinoculation. A small number of infected cells were observed in the gray matter of the brain and spinal cord of one mouse, which was paralyzed, whereas no viral antigen was found in the other three mice. Five mice were sacrificed 22 days postinoculation, and another five were sacrificed 45 days
postinoculation. No viral antigen could be detected in the CNS of any of the 10 animals. However, some inflammation was observed in the white matter of the spinal cord, mainly in some of the mice sacrificed 22 days postinoculation.

We then compared the levels of viral RNA present in the CNS of GD-43- and DA-infected SJL/J mice, 45 days after inoculation. Two groups of SJL/J mice were inoculated with 10^5 PFU of GD-43 or DA virus and sacrificed 45 days postinoculation. Total brain and spinal cord RNA was extracted by the method of Chomczynski and Sacchi (5), and viral RNA was quantitated by dot blot hybridization as described elsewhere (4). Briefly, a series of fivefold dilutions of total RNA was dotted on a Hybond C+ membrane, starting with 10 μg of total RNA per dot, and hybridized with a radioactive probe consisting of nucleotides 5428 to 6944 of the GDVII genome labeled with 32P (5 × 10^6 dpm/μg) by random priming. No viral RNA could be detected in the spinal cords or the brains of the GD-43-infected mice. As expected, all the DA-infected mice had viral RNA, mainly in their spinal cords (Fig. 2). In conclusion, both the histological and dot blot analyses showed that virus GD-43 doesn’t persist in the CNS of susceptible SJL/J mice.

The DA and BeAn strains of Theiler’s virus infect the gray and white matter of the CNS successively. Our previous studies indicated that modifications of the DA capsid alter the tropism of the virus for the white matter (9). We hypothesized that only those strains able to infect white-matter cells may persist. However, because the strain is highly virulent, the tropism of the GDVII virus is difficult to study at late times postinoculation. On the other hand, the GD-43 virus, an attenuated Theiler’s virus with a GDVII capsid, offers the possibility of examining this point. To perform this study under stringent conditions, we used nude mice which, because they are immunocompromised, remain persistently infected even when inoculated with a non-persisting recombinant Theiler’s virus (9). Groups of 4-week-old BALB/c nu/nu mice were inoculated with 10^5 or 10^4 PFU of DA or GD-43 virus and sacrificed at various times after inoculation for histological analysis of the CNS. Most mice inoculated with 10^4 PFU of either virus presented with severe signs of encephalitis and were sacrificed between days 13 and 17 postinoculation. The majority of mice inoculated with 10^5 PFU survived longer and could be examined at 21 days postinoculation. Antigen-positive cells were found in the gray matter of the brain in both DA- and GD-43-infected mice. In the spinal cord, on the other hand, the pattern of infection depended on the virus (Fig. 3). Ten mice inoculated with virus DA were analyzed 13 or 15 days postinoculation, and five mice were analyzed 21 days postinoculation. At 13 to 15 days postinoculation, the number of infected cells in the white matter was similar to, or greater than, the number of infected cells in the gray matter. Figure 3A illustrates the presence of viral antigen in the white matter at this time. At 21 days postinoculation, a large number of virus-infected cells were found in the white matter and only a small number were found in the gray matter (Fig. 3B). Six mice inoculated with virus GD-43 were analyzed between days 13 and 17 postinoculation, and five mice were analyzed at day 21 postinoculation. In contrast to the findings with virus DA, only a few antigen-positive cells were found in the white matter and they were present in some mice only. Almost all infected cells were in the gray matter at both 13 to 15 (Fig. 3C) and 21 (Fig. 3D) days postinoculation.

<table>
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<tr>
<th>Virus</th>
<th>No. of infected cells/longitudinal section</th>
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<tr>
<td></td>
<td>Gray matter</td>
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<tr>
<td>DA</td>
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<tr>
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* BALB/c nu/nu mice were inoculated with 10^5 PFU of the DA or GD-43 virus and sacrificed 21 days later. For each mouse, two longitudinal sections of the entire spinal cord were processed for the detection of viral antigens. The sections were systematically scanned under a microscope, and infected cells in the gray and white matter were counted. The figures are the average for two sections.
Most of these cells could be identified as neurons by their morphology. The difference in localization of the GDVII and GD-43 viruses was documented further by counting the number of infected cells in the gray and white matter of the spinal cord 21 days postinoculation. Two longitudinal sections of the entire spinal cords of four BALB/c nu/nu mice inoculated with $10^2$ PFU of either virus were systematically scanned under a microscope, and viral antigen-positive cells in gray and white matter were counted (Table 1). On average, 93% of DA virus-infected cells were in the white matter and 95% of GD-43 virus-infected cells were in the gray matter.

In the present work, we show that an attenuated GDVII virus doesn’t persist in the CNS of SJL/J mice. Our findings are in complete agreement with those recently reported by Lipton et al. (14). Those authors attenuated the GDVII virus either by exchanging part of the 5’ noncoding region with that of the BeAn virus or by deleting part of the L gene. These attenuated viruses did not persist in SJL/J mice. Thus, it is now clear that the capsid of virus GDVII does not contain determinants that would lead to a persistent infection if the animals were to survive. Previous results suggesting that the capsids of both DA and GDVII viruses contain determinants of persistence (8, 25) might be explained by the difficulties encountered in interpreting the phenotype of recombinant viruses with hybrid capsids. More than a specific linear sequence of amino acids, persistence may require conformational determinants in a region made of the VP2 “puff” and the VP1 CD loop (1, 10, 28, 30). Our results indicate that these determinants might be absent from the native GDVII capsid.

The DA and BeAn viruses persist in macrophages and oligodendrocytes in the white matter of the spinal cord (2, 15, 21). Here we report that the nonpersistent GD-43 virus doesn’t efficiently infect the white matter of the spinal cord. These results, and our previous findings with recombinant viruses (9), suggest that the capsid may control persistence by determining the tropism of the virus for glial cells of the white matter of the spinal cord. The conformation of the capsid may enable the virus to interact with a specific receptor on these cells. Interestingly, recent results suggest that the persistent BeAn strain binds to the cell surface differently than the GDVII virus (28).

FIG. 3. Histological findings in BALB/c nu/nu mice. Viral capsid antigens were detected by immunocytochemistry, with a rabbit hyperimmune serum, in longitudinal sections of the spinal cord. (A) Mouse infected with DA virus and sacrificed 13 days postinoculation (magnification, ca. ×109). (B) Mouse infected with DA virus and sacrificed 21 days postinoculation (magnification, ca. ×68). Arrows in panels A and B point to foci of antigen-positive cells in the white matter. (C) Mouse infected with GD-43 virus and sacrificed 13 days postinoculation (magnification, ca. ×109). (D) Mouse infected with GD-43 virus and sacrificed 21 days postinoculation (magnification, ×125). Arrows in panels C and D point to infected cells in the gray matter. WM, white matter; GM, gray matter.
REFERENCES


