Restricted Virus Replication in the Spinal Cords of Nude Mice Infected with a Theiler's Virus Variant

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The Daniels strain of Theiler's murine encephalomyelitis produces a chronic disease which is an animal model for human demyelinating disorders. Previously, we selected a neutralization-resistant virus variant producing an altered and diminished central nervous system disease in immunocompetent mice which was evident during the later stage of infection (after 4 weeks) (A. Zurbriggen and R. S. Fujinami, *J. Virol.* 63:1505–1513, 1989). The exact epitope determining neurovirulence was precisely mapped to a capsid protein, VP-1, and represents a neutralizing region (A. Zurbriggen, J. M. Hogle, and R. S. Fujinami, *J. Exp. Med.* 170:2037–2049, 1989). Here, we present experiments with immunoincompetent animals to determine viral replication, spread, and targeting to the central nervous system in the absence of detectable antibodies or functional T cells. Nude mice were infected orally, and the virus was monitored by plaque assay, immunohistochemistry, and in situ hybridization. Early during the infection (1 week), the variant virus induced an acute disease comparable to that induced by the wild-type virus in these nude mice. Alterations in tropism in the central nervous system were not apparent when wild-type parental Daniels strain virus was compared with the variant virus. Moreover, variant virus replicated in tissue culture (BHK-21 cells) to similarly high titers in a time course identical to that of the wild-type virus (A. Zurbriggen and R. S. Fujinami, *J. Virol.* 63:1505–1513, 1989). However, replication of the variant virus versus the wild-type virus within the spinal cord of athymic nude mice infected per os was substantially restricted by 6 weeks postinfection. Therefore, the reduced neurovirulence in the later stage (6 weeks) of the disease is most likely due to a diminished growth rate or spread of the variant virus in the central nervous system rather than to marked differences in viral tropism.

Theiler's murine encephalomyelitis virus (TMEV), a positive single-stranded RNA virus, is a naturally occurring murine picornavirus which upon oral (per os) infection of young adult mice leads to an asymptomatic enteric infection. However, after intracerebral (i.c.) infection with the Daniels (DA) strain (10) of TMEV in susceptible mice (17), a consistent biphasic disease of the central nervous system (CNS) was observed (18). The acute disease resembles poliomyelitis. Most immunocompetent SJL/J mice survive the acute poliomyelitis without having severe clinical symptoms and progress to a chronic, demyelinating disease observed in the CNS (7, 19).

The mechanisms leading to TMEV-induced demyelination are still under investigation. Different reports favor various hypotheses, for example, immune-mediated mechanisms leading to demyelination (20, 27). Other observations suggest mechanisms in which TMEV plays a central role in demyelination (3, 6, 21, 29, 30). Various reports consider a combination of immune-mediated and virus-induced mechanisms (2, 34).

By using combined immunohistochemistry and in situ hybridization techniques, virus antigen within macrophages, astrocytes, and oligodendrocytes in the CNS of infected immunocompetent animals has been observed (2). Of these infected cells, 25 to 40% were identified as oligodendrocytes (2). Infection of oligodendrocytes, the myelin-producing cells, could support a direct virus-induced demyelinating mechanism (by affecting myelin metabolism) as well as an immune-mediated mechanism (through humoral or cellular

immune responses against TMEV antigens in oligodendrocytes) (2). In studies involving immunoincompetent nude mice, CNS tissue also contained infected cells which were identified as oligodendrocytes (30).

Recent observations have shown that antigenically distinct mutants from various neurotropic viruses can be selected with neutralizing monoclonal antibodies (12, 23, 33). Some of the selected virus variants were markedly less neurovirulent, inducing infection that was attenuated compared with infection with the wild-type virus (9, 13, 14, 31).

Previously, we described an altered and diminished disease pattern in immunocompetent SJL/J mice after i.c. infection with a neutralization-resistant DA virus variant, H7A6-2 (37). This variant virus was selected with a neutralizing monoclonal antibody to TMEV capsid protein VP-1. After 3 weeks postinfection (p.i.), mice infected with the variant virus had much less CNS inflammation. In addition, the number of viral antigen-containing cells in the spinal cords of variant virus-infected mice was markedly decreased compared with the number in the spinal cords from the parental DA virus-infected mice.

To further characterize the variant virus, enzyme-linked immunoassays, neutralization assays, and direct RNA sequencing were performed (38). A site was proposed as an important determinant for persistence. The variant virus contained one single point mutation in VP-1, leading to an amino acid replacement within a neutralizing epitope. This epitope is located in an exposed loop on the surface of the virus (38). No changes in the 5' noncoding region as well as an adjacent region from VP-2 were observed.

To cause the modified pathogenesis, the variant virus may be cleared faster by the immune system than the wild-type virus is, or variant-virus replication could be restricted in the

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TABLE 1. Enumeration of infectious virus in the serum of nude mice^a

Time p.i.	Mouse no. ^b	PFU/ml of serum ^c
3 days	1	1.4×10^3
	All others	—
1 wk	5	2.0×10^2
	All others	—
2 wk	2	1.5×10^2
	4	5.1×10^1
	All others	—
3 wk	1	2.0×10^2
	2	4.0×10^2
	3	—
	4	—
	5	1.0×10^2
4 wk	1	1.0×10^2
	2	1.0×10^2
	3	1.5×10^2
	4	3.0×10^2
	5	3.0×10^2
6 wk	1	2.0×10^2
	2	2.0×10^2
	3	1.5×10^2
	4	—
	5	1.5×10^2

^a Assays were performed as described by Zurbriggen and Fujinami (37) and Zurbriggen et al. (38).

^b Five mice per group were infected per os with DA virus.

^c —, Falling below the limit of detection of this plaque assay (50 PFU of virus per ml).

CNS in the presence or absence of an antiviral immune response compared with wild-type virus replication.

A means to study the involvement of the immune system is the use of nude mice. Nude mice infected with the DA strain of TMEV have rising titers of infectious virus in the CNS and fail to produce anti-TMEV antibodies or sensitized virus-specific T lymphocytes (29, 30). Moreover, nude mice can develop a demyelinating disease after i.c. infection with DA TMEV. Infection of oligodendrocytes and neurons and demyelinated foci in the CNS of TMEV-infected nude mice have been documented by immunohistochemical and ultrastructural means (29, 30).

In this study, we infected athymic nude mice with the wild-type DA virus and the less neurovirulent variant virus H7A6-2 to determine the influence of the immune system on infection. Nude mice were infected per os rather than the i.c. route, since infection of enteroviruses per os is more likely to mimic the naturally acquired infection. Virus titers and histopathological appearances of viral antigen and RNA in the CNS of variant virus versus those of wild-type virus in nude mice were analyzed. It was found that the variant virus, H7A6-2, was able to induce an acute disease similar to that of the parental DA virus. However, during the later stage of disease, after 4 weeks of the infection, variant-virus replication in the CNS was more limited than that of the wild-type virus. These results suggest that the late diminished disease is due to a restricted growth or spread of the variant virus, H7A6-2, within the CNS.

Infectivity assay. In a pilot experiment, sera from nude mice infected per os with DA virus (five mice per group)

were screened for infectious virus (Table 1). Only one of five mice contained detectable infectious virus in the serum at 3 days and at 1 week p.i. At 2 weeks p.i., infectious virus was isolated from serum specimens of two mice and at 3 weeks p.i. was isolated from serum specimens of three mice. By 4 weeks p.i., all mice had approximately 2×10^2 PFU/ml in serum (Table 1). Four of five animals contained infectious virus in their sera at 6 weeks p.i. The titers of infectious virus in these mice ranged from 200 to 300 PFU/ml of serum (Table 1), with the exception of a single mouse at 3 days p.i.

CNS tissues from five mice per group were examined for infectious virus at 1 and 4 weeks p.i. This was at a time when virus was first detected and a time when all animals were viremic, respectively. A viral plaque assay was performed on BHK-21 cell monolayers with brain and spinal cord homogenates from wild-type-virus- or variant-virus-infected nude mice 1 and 4 weeks p.i. The results of this plaque assay are summarized in Table 2.

At 1 week p.i., no differences in the amount of infectious virus in the CNS from wild-type-virus- and variant-virus-infected nude mice were observed (Table 2). Wild-type-virus-infected nude mice contained about 7.7×10^3 PFU/g of brain tissue, and variant-virus-infected nude mice had approximately 4.8×10^3 PFU/g of brain tissue. In the spinal cords of wild-type-virus-infected animals, an average of 4.7×10^4 PFU/g of tissue was detected, and from spinal cords of variant-virus-infected nude mice, 6.1×10^4 PFU of infectious virus per g of tissue was detected. In both groups (wild-type-virus- and variant-virus-infected animals), the spinal cords contained 6 to 10 times more infectious virus than the brain tissues (Table 2). Since the amount of virus in the CNS was higher than that in sera, virus titers cannot be attributed to contaminating blood and therefore represent replication.

At 4 weeks p.i., a difference in the amounts of infectious wild-type virus and variant virus isolated from brain and spinal cord tissue homogenates was observed (Table 2). Brain tissue from wild-type-virus-infected nude mice (5.2×10^5 PFU/g) contained 10 times more infectious virus than variant-virus-infected tissue (5.3×10^4 PFU/g). The amount of infectious virus isolated from variant-virus-infected spinal cord tissue (1.8×10^6 PFU/g) was four times less than that from wild-type-virus-infected spinal cord homogenates (8.3×10^6 PFU/g). Similar to spinal cords at the 1 week p.i. time point, spinal cords at the 4 week p.i. time point contained 16 times more infectious wild-type virus and 34 times more variant virus than were found in brain tissue (Table 2).

The titers of wild-type virus and variant virus progressively increased in brain and spinal cord. The amount of wild-type virus in brain and spinal cord was six times more than the amount of variant virus (Table 2). The number of PFU per gram of brain tissue in wild-type-virus-infected nude mice increased from 7.7×10^3 at 1 week p.i. to 5.2×10^5 at 4 weeks p.i. In spinal cord tissue of wild-type-virus-infected mice, titers increased from 4.7×10^4 at 1 week p.i. to 8.3×10^6 at 4 weeks p.i. Both parental DA virus and the virus variant replicated more efficiently or to higher titers in the spinal cord than in the brain (Table 2).

Variant virus H7A6-2 and wild-type DA virus were also examined for their plaque morphologies. No apparent difference in size, shape, or turbidity of plaques produced by either virus strain was observed. Variant virus and the parental DA virus before and after injection into mice produced similar plaque sizes in BHK-21 cells.

Immunohistochemistry. The distribution of viral antigen in the CNS was determined by immunohistochemistry with a

TABLE 2. Comparisons of infectious virus isolated from CNS tissue of athymic nude mice infected with wild-type virus and variant virus H7A6-2^a

Time p.i. (wk)	Mouse no. ^b	PFU of:			
		DA per g of		H7A6-2 per g of	
		Brain	Spinal cord	Brain	Spinal cord
1	1	1.8×10^4	4.6×10^3	1.1×10^4	3.7×10^4
	2	3.9×10^3	2.5×10^4	5.1×10^2	5.6×10^4
	3	1.0×10^3	1.5×10^5	2.5×10^2	3.0×10^4
	4	6.8×10^3	5.1×10^4	3.1×10^3	1.1×10^5
	5	8.8×10^3	5.1×10^3	9.2×10^3	7.4×10^4
	Avg	7.7×10^3	4.7×10^4	4.8×10^3	6.1×10^4
4	1	1.9×10^5	2.5×10^5	5.7×10^3	7.3×10^5
	2	3.9×10^5	3.7×10^6	2.2×10^3	1.5×10^6
	3	2.5×10^5	9.4×10^6	7.3×10^4	1.1×10^6
	4	8.9×10^4	1.1×10^7	9.8×10^3	2.2×10^6
	5	1.7×10^6	1.7×10^7	1.7×10^5	3.5×10^6
	Avg	5.2×10^5	8.3×10^6	5.3×10^4	1.8×10^6

^a Assays were performed as described by Zurbriggen and Fujinami (37) and Zurbriggen et al. (38).

^b Five mice per time point were infected per os.

rabbit anti-DA virus hyperimmune serum. Sagittal brain sections and longitudinal and cross-sectional spinal cord sections were examined at 1, 4, and 6 weeks p.i. In addition, the number of variant-virus antigen-positive cells in brain and spinal cord were compared with the number of wild-type-virus antigen-positive cells on a scale from 0 to ++++ (37) (Tables 3 and 4).

One week after infection with virus, no difference in the number or distribution of antigen-positive cells in the brain or spinal cord was observed for wild-type-virus- and variant-virus-infected nude mice. In both variant-virus-infected and parental DA virus-infected nude mice, viral antigen was found in three of five animals. Single infected cells were distributed throughout brain and spinal cord in similar amounts (Tables 3 and 4). These data are consistent with those presented in Table 2, where at 1 week p.i. comparable amounts of infectious virus could be demonstrated in variant-virus- and wild-type-virus-infected mice.

At 4 weeks after infection, single infected glial and neuronal cells were present in the brain stems of all five wild-type-virus-infected nude mice (Table 3). Two of these five mice had two small clusters of infected cells as well. The number of virus-infected cells in H7A6-2-infected animals was somewhat lower than in wild-type DA-infected mice,

TABLE 3. Comparison of the number of antigen-positive cells in the brain on a scale from 0 to ++++^a

Virus strain	Rating (no. of mice with viral antigen/total no. of mice) at time p.i.:		
	1 wk	4 wk	6 wk
Wild-type DA	+ (3/5)	+ to ++ (5/5)	+ to ++ (4/4)
H7A6-2	+ (3/5)	+ (3/5)	+ (4/4)

^a Nude mice (five mice per group) were infected per os with wild-type DA virus or variant virus H7A6-2. Immunohistochemistry was performed as described by Zurbriggen and Fujinami (37).

TABLE 4. Comparison of the number of antigen-positive cells in the spinal cord on a scale from 0 to ++++^a

Virus strain	Tissue source ^b	Rating (no. of mice with viral antigen/total no. of mice) at time p.i.:		
		1 wk	4 wk	6 wk
Wild-type DA	GM	+ (3/5)	+ (5/5)	+ (4/4)
	WM	+ (3/5)	++ to +++ (5/5)	++++ (4/4)
H7A6-2	GM	+ (2/5)	+ (5/5)	+ (3/4)
	WM	+ (3/5)	+ to ++ (5/5)	+ to ++ (4/4)

^a Nude mice (five mice per group) were infected per os with wild-type DA virus or variant virus H7A6-2. Immunohistochemistry was performed as described by Zurbriggen and Fujinami (37).

^b GM, Gray matter; WM, white matter.

and in two mice no viral antigen was detected in the brain. There were no observable differences in cell tropism.

Differences in the numbers of virus-infected cells occurred primarily within the spinal cord at 4 weeks p.i. (Table 4). Mice infected with variant virus H7A6-2 had mild involvement of ventral root entry zones in the white matter and infection of anterior horn cells in the gray matter. In contrast, nude mice infected with wild-type virus had a more widespread distribution of viral antigen-positive cells throughout the white matter of the spinal cord. In addition, more anterior horn cells in the gray matter of the spinal cord were positive for viral antigen. Thus, by 4 weeks p.i. two conclusions could be made. First, the white matter in the spinal cords of infected mice had more antigen-positive cells than gray matter did, and second, the white matter from wild-type-virus-infected mice had more antigen-positive cells than white matter of variant-virus-infected mice. The number of infected cells somewhat parallels the data obtained with the plaque assay (Table 2).

By 6 weeks after per os infection, the distribution of virus-infected cells within brain and spinal cord was essentially the same in wild-type DA virus-infected and variant-virus-infected nude mice, although the numbers of infected cells in the white matter of the spinal cord markedly differed. In contrast, only minor differences in the number of infected brain cells were observed for parental DA virus- and variant-virus-infected mice (Table 3). Infected cells were exclusively located within the brain stem in small clusters or as single cells. In contrast, obvious differences in the number of viral antigen-positive cells in the spinal cord were found for wild-type and variant virus-infected nude mice at this time point (Table 4). Wild-type-virus-infected mice manifested massive virus replication and spread within the white matter of the spinal cord. Throughout the length of the spinal cord, most areas of the white matter contained wild-type virus (Fig. 1a), while single infected cells were present in the gray matter of these animals. However, nude mice infected with variant virus had only mild involvement of the white and gray matter within the spinal cord (Fig. 1b). Virus antigen or infected cells were limited in amount and in numbers compared with the high numbers of wild-type virus-infected cells. Animals infected with variant virus had much lower numbers of infected cells in spinal cord at 6 weeks p.i. than did mice infected with wild-type virus (Table 4).

In situ hybridization. Overall, the in situ hybridization data correlated well with the results obtained by immunohistochemistry. A probe encoding the DA structural protein VP-1 and part of VP-3 was used to detect the presence of viral nucleic acids in sections; in parallel, immunohistochemistry

FIG. 1. (a) Longitudinal spinal cord section (overview) from a nude mouse 6 weeks after oral infection with wild-type DA virus. Viral antigens are widely spread throughout the white matter (see references 37 and 38 for the method). (Antibody to TMEV, ABC method; magnification, $\times 150$). (b) Single spinal cord cells infected with variant virus H7A6-2 (6 weeks p.i.). (Antibody to TMEV, ABC method; magnification, $\times 480$).

was also used. This probe was highly specific for TMEV and has been previously characterized (35). At 1 week p.i., only single CNS cells containing viral nucleic acids were observed scattered throughout the brain and spinal cord. The number of in situ hybridization-positive cells was similar to the number of viral antigen-containing cells. No obvious differences in the number and distribution of TMEV-containing cells between wild-type-virus- and variant-virus-infected nude mice were detected at 1 week p.i.

At 4 weeks p.i., differences between wild-type-virus RNA- and variant-virus RNA-containing cells in the spinal cord were observed; differences were also observed with immunohistochemistry for viral antigen. Spinal cords of wild-type-virus-infected nude mice contained more in situ hybridization-positive cells in the gray matter. This was true of the white matter in spinal cords from wild-type-virus-infected mice versus variant H7A6-2 virus-infected animals.

At 6 weeks p.i., wild-type-virus-infected nude mice had much higher numbers of cells containing viral RNA in the spinal cord white matter (Fig. 2a and b) than variant virus-infected mice (Fig. 2c and d). However, the differences in the amount of wild-type and variant viral RNA observed within the brain stem were only minor.

No variations regarding cell tropism between the two virus strains were noticed by using in situ hybridization. Most of the viral RNA detected with in situ hybridization was

localized within the white matter of the spinal cord (Fig. 2a and b), as observed with immunohistochemistry.

Previously, we found that a neutralization-resistant variant virus, H7A6-2, was markedly less neurovirulent in immunocompetent SJL/J mice than the parental wild-type DA virus (37). The exact nature by which the variant virus or host alters the disease was the focus of the present study. Several hypotheses concerning the mechanism have been discussed in previous reports (37, 38). These include an altered cell tropism for the variant virus, resulting in a diminished disease. This hypothesis was not supported by previous studies using immunocompetent SJL/J mice, in which no differences in cell tropism were noted (37). During the early stage of the disease, no variations in virus distribution in immunocompetent SJL/J mice infected with wild-type DA virus or H7A6-2 variant virus were demonstrated by using immunohistochemistry or in situ hybridization. Moreover, by 2 to 4 weeks p.i., variant virus was cleared from the CNS (37). In the present study, we found no differences in cell tropism within brain and spinal cord for wild-type-DA virus- and variant-virus H7A6-2-infected nude mice. This, too, suggests that no change in viral cell tropism between the wild-type DA and variant viruses has occurred, allowing for different patterns of disease in the CNS. Furthermore, the route of infection did not affect or alter virus distribution within the CNS. Similar regions in the brain

FIG. 2. (a) Longitudinal section of a spinal cord from a nude mouse infected with wild-type DA virus (6 weeks p.i.). In situ hybridization was with a cDNA probe complementary to VP-1 and part of VP-3 of the DA strain (35). Viral RNA has a location similar to that of viral antigen in Fig. 1a. Magnification, $\times 100$. (b) Higher magnification of a wild-type-virus-infected spinal cord 6 weeks p.i. (In situ hybridization; magnification, $\times 150$.) (c) Single neuron in the spinal cord (6 weeks p.i.) containing variant H7A6-2 virus RNA. The grains are localized within the cytoplasm of the cell. (In situ hybridization; magnification, $\times 480$.) (d) Infected cells with variant-virus RNA (spinal cord section at 6 weeks p.i.). (In situ hybridization; magnification, $\times 480$.)

stem and in the spinal cord infected after various routes of virus inoculation have been described (22, 30, 35, 36).

In contrast to our observations with TMEV (described above), a modified cell tropism of variant viruses has been suggested by several groups to be responsible for an altered neurovirulence (9, 25, 28). For example, Dalziel and colleagues selected neutralization-resistant murine coronaviruses with a reduced neurovirulence (9). A sparing of neuronal infection by the variant coronavirus was proposed to be the explanation of why a chronic demyelinating disorder, rather than the acute fatal disease usually induced by the wild-type virus, resulted, thus suggesting an altered viral tropism (9). Another explanation is that their coronavirus is an attenuated variant, since the coronavirus titers of variant-virus-infected mice at all tested time points were much lower than the wild-type titers (9), thereby allowing more mice to survive the initial infection and develop a demyelinating disease. In support of this, the few surviving animals infected with the wild-type murine coronavirus which did not die developed a demyelinating disease (9). These data suggest that the altered disease observed in the surviving variant-virus-infected mice most likely is due to a restricted virus replication rather than to a change in cell tropism.

To eliminate a possible influence of the immune system on viral cell tropism and/or virus replication, we infected athymic nude mice (barring natural killer cells). In addition, we chose the oral route of infection in these studies, since most nude mice infected via the i.c. route with either virus strain die within 2 to 3 weeks p.i. (22, 30, 36) and since an oral infection represents the natural route of infection. Immunocompetent mice (in contrast to nude mice) require the i.c. route of infection to produce a reproducible CNS disease. No viral RNA or antigen in the brains and spinal cords of SJL/J mice infected in our laboratory per os could be demonstrated (data not shown). However, in nude mice (unlike the situation in immunocompetent mice), the route of inoculation did not affect virus distribution within the CNS but did delay the time course of the disease development (22).

Our results from the plaque assay, immunohistochemistry, and in situ hybridization demonstrated that wild-type virus as well as variant virus could spread to the CNS in the absence of a functional immune system. Both viruses, DA as well as the variant virus, infected the CNS probably via the blood from the gut, as suggested in Table 1. Virus spread to and within the CNS of infected nude mice by viremia has been proposed in a preceding study (36). There TMEV-infected cells associated with blood vessels were demonstrated by in situ hybridization in the brains and spinal cords of nude mice.

In our studies, immunohistochemistry as well as in situ hybridization results demonstrate major differences in the ability of the two TMEV virus strains to replicate within the CNS late in infection. At 6 weeks p.i., much more wild-type DA virus than variant virus H7A6-2 was found in the spinal cord. Even though wild-type DA virus infected larger numbers of cells than variant virus, both had a predilection for white matter in the spinal cord. Most of the spinal cord white matter in wild-type-DA virus-infected nude mice contained viral antigen and viral RNA at this time. In contrast, the number of variant-virus-infected cells was somewhat restricted even in the absence of a functional antiviral immune response. The differences in the number of infected cells within immunocompetent SJL/J mice infected with wild-type virus or variant virus were at this time point more obvious (37), probably because of the addition of an antiviral immune

response further restricting spread and growth in the CNS. Thus far, direct comparisons cannot be made between SJL/J mice infected via the i.c. route and athymic nude mice inoculated per os. Immunologic depletion experiments in the SJL/J mouse will address this issue.

Diminished virus replication within the CNS does not necessarily reflect a defect of variant-virus attachment to the CNS cells or a deficiency in infecting CNS cells. Variant virus H7A6-2 produced an acute disease similar to that produced by the wild-type DA virus in immunocompetent (37) and immunoincompetent mice. The degree of the acute disease was defined by the amount of infectious virus isolated from CNS tissue and by the number of infected cells in the CNS. The values of infectious virus, determined with plaque assays, as well as the number of infected cells, evaluated by immunohistochemistry and by in situ hybridization, during the early stage (1 week p.i.) of the disease were comparable for wild-type-virus- and variant-virus-infected nude mice. Similar results were observed by Roos and coworkers in the acute disease produced by DA escape mutants with a mutation at the carboxyl end of VP-1 (28). Nevertheless, the overall replication of the variant virus is not defective. Variant virus and wild-type DA virus replicate in BHK-21 cells to comparable high titers in a similar time courses (37). However, this growth rate in vitro does not necessarily reflect virus replication in the CNS of infected animals. For example, small-plaque DA variants were reported to produce lower titers and have a slower growth rate in BHK-21 cells than large-plaque variants or the parental DA viruses (26). In contrast, in the mouse CNS, small-plaque variants reached higher values of infectious virus than large-plaque DA variants or the DA virus strain (26). However, in another related study, poliovirus Mahoney and Sabin 1 attenuation in vivo paralleled a diminished cytopathic effect of the virus in vitro (15). Furthermore, neutralization-resistant variants mutated in the antigenic site 1 of the mouse-adapted poliovirus Lansing type 2 had a reduced ability to replicate in the mouse CNS (16). Moreover, poliovirus type 2 is avirulent in primates, yet it grows as well as virulent virus in tissue culture (24). Thus, limited virus replication and/or spread may be specific to CNS cells.

To examine the observed restriction of variant-virus replication in the CNS, we are currently investigating the growth kinetics of wild-type versus variant virus in various CNS-derived mouse cell lines and newborn-mouse brain cell cultures. This approach has been successful for studying poliovirus neurovirulence with human neuroblastoma cell lines (1). In these studies, Sabin attenuated poliovirus had a limited growth rate in neuroblastoma cells in vitro. Restricted virus replication may be a natural mechanism for wild-type TMEV in developing a persistent infection. Synthesis of the TMEV capsid proteins has been reported to be restricted in mouse CNS cells compared with synthesis in BHK-21 cells (4). Replication in the CNS of the variant H7A6-2 virus versus wild-type virus in mice may be too limited to allow the establishment of a persistent infection and leads to successful elimination of variant virus by the host.

The inability of variant virus H7A6-2 to replicate to the same high titers as wild-type DA virus does in the spinal cord at the late stage of the disease may be due to a defect in virus production after cell attachment and penetration. Mutations within capsid protein VP-1 of poliovirus type 2 affect uncoating of the virus (11). In addition, attenuated poliovirus type 3 mutants retain a translation deficiency compared with the neurovirulent strains (32). However, these poliovirus vari-

ants were mutated within the 5' noncoding regions, in contrast to the observed alteration of amino acid 101 in VP-1 of variant virus H7A6-2 (38). One way of evaluating translation is comparing the number of mRNA-positive cells to the number of antigen-positive cells. In our study, we could not demonstrate a marked translation deficiency. CNS tissue contained similar numbers of variant-virus RNA- and antigen-positive cells. A further possibility is a restriction of virus replication at the level of RNA transcription. TMEV persistence has been suggested to be due to an inhibition at the level of minus-strand RNA synthesis (5).

This study also demonstrated a predilection of both virus strains for the spinal cord white matter. Most of the viral antigen as well as viral RNA was detected within the white matter of the spinal cords (Fig. 1a and 2a). During the entire observation period, more virus was found in spinal cord than in brain. Yet nude mice infected by the i.c. route show in the early phase of the disease higher titers of infectious virus in the brain (36). This most likely reflects the initial injection site rather than a preferential replication site. In nude mice infected i.c., spinal cord values from later time points during the infection surpassed those in brain (36). Similarly, SJL/J mice infected via the i.c. route had at the early stage of the disease more virus in the brain than in the spinal cord (36, 37). However, thereafter TMEV persists mainly in the white matter of the spinal cord (8, 21, 37).

In this report, we describe infection of nude mice per os with a wild-type DA virus and a neutralization-resistant virus variant. Wild-type virus as well as variant virus infects the CNS in the absence of an intact antiviral immune response and produces a similar acute disease. Subsequently, in the chronic disease, variant-virus H7A6-2 replication in the CNS of nude mice is limited. Reduced ability of variant virus H7A6-2 to replicate or spread was not observed in tissue culture (BHK-21 cells) but was confined to the mouse CNS.

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