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Pathogenicity of Antigenic Variants of Murine Coronavirus JHM Selected with Monoclonal Antibodies

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To analyze the pathogenesis of the neurotropic murine coronavirus JHMV, we used monoclonal antibodies to the E2 viral glycoprotein to select antigenic variant viruses. Monoclonal antibodies J.7.2 and J.2.2 were shown to bind to topographically distinct regions of the E2 molecule, and the variants selected with the two antibodies demonstrated very different disease pictures in mice. Variants selected with J.7.2 were, like the parental virus, highly virulent and caused an acute encephalitic illness. By contrast, J.2.2-selected variants predominantly caused a subacute paralytic disease clinically and extensive demyelination histologically. Antigenic differences among the variants and parental virus were readily demonstrable with anti-E2 monoclonal antibodies. However, no differences between the viruses could be shown in binding studies with monoclonal antibodies directed against either E1 or N, the other two JHMV structural proteins. Since only J.2.2 selected demyelinating variants with reduced neurovirulence, it is likely that this monoclonal antibody recognizes a subregion of the E2 molecule that is particularly important in JHMV pathogenesis.

JHM virus (JHMV) (MHV-4) is a highly neurotropic member of the murine coronavirus or mouse hepatitis virus family. It has been extensively studied in animal models of human neurological diseases (15, 26, 32, 40). JHMV infects all central nervous system (CNS) cell types, including oligodendrocytes, and causes encephalitis and primary demyelination in mice and rats. In some studies, a relapsing-remitting demyelinating disease, reminiscent of multiple sclerosis, has been noted (39). Under other conditions, a fulminant acute encephalitis has been found (14, 40). The outcome of infection in a given experiment depends on the viral strain, dose, and route of inoculation, as well as the age and strain of the animals (5, 38).

Murine coronaviruses have three structural proteins (25). The E1 glycoprotein has a molecular weight of 25,000 and probably serves as a matrix protein. The N nucleocapsid protein has a molecular weight of 60,000 and is intimately associated with viral RNA (30). The E2 glycoprotein, which is a 180,000-molecular-weight heterodimer, forms the projecting peplomers of the virus, and its functions are thought to include attachment to cells, induction of cell-to-cell fusion, and elicitation of neutralizing antibodies (3, 7, 25, 34). Competitive binding studies with monoclonal antibodies in several laboratories have identified at least six major antigenic sites on JHMV E2 (36, 37; Fleming, unpublished observations).

The functions ascribed above to the E2 protein suggest that it may play a particularly significant role in JHMV infections. We evaluated this hypothesis by using two anti-E2 monoclonal antibodies to isolate antigenic variants of JHMV. It has been shown in several virus systems that antigenic variant viruses, selected for resistance to neutralization by monoclonal antibodies, usually arise by single-point mutations (6, 16–18, 24). These viruses represent preexisting, spontaneous variants which occur at a frequency of approximately 10^{-4} to 10^{-5} in most RNA virus stocks (22). Treatment with an excess of a monoclonal antibody efficiently neutralizes the parental or wild-type virions and allows selection of resistant variants. Antigenic variants have proven ideal for studies of the genetics, antigenicity, and pathogenesis of many viruses (23), including several that are neurotropic (4, 9, 19, 24, 27).

In this study, we report that JHMV antigenic variants selected with different monoclonal antibodies cause different disease pictures in mice. Two monoclonal antibodies binding to topographically distinct sites on the E2 protein were used to select variants. Variant viruses selected with monoclonal antibody J.7.2 did not significantly differ from the parental strain employed, which principally causes an acute, fatal encephalitis. In contrast, another monoclonal antibody, J.2.2, consistently selected avirulent variant viruses. Mice infected with these variants survived the acute phase of infection and subsequently developed a paralytic, demyelinating disease. Thus, these observations suggest that subregions of the E2 molecule are essential determinants of pathogenesis in JHMV infections.

MATERIALS AND METHODS

Viruses, cells, and animals. The isolation and properties of the JHMV-DL strain, from which the antigenic variants were derived, have been previously described (29). Viruses were propagated and plaque assayed on DBT cells, a continuous murine brain tumor line, as previously described (30).

Six-week-old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) were used in all experiments. Representative animals were tested for anti-mouse coronavirus antibody by an enzyme-linked immunoabsorbent assay (ELISA) before use; all were negative. Mice were held for 48 to 72 h after arrival and then injected with the stated doses of virus intracerebrally (i.c.) in a 30-μl volume.

Antibodies. Hyperimmune and monoclonal antibodies to JHMV-DL used in these studies were developed in this

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laboratory (8). Monoclonal antibodies were used in the form of tissue culture supernatant, containing approximately 1 to 5 μg of specific antibody per ml. Negative control monoclonal antibody 7-16.17 (anti-H-2I^p) was a gift from Jeffrey Frelinger, University of North Carolina, Chapel Hill. (11).

Antibody binding assays. Topographical relationships among the binding sites of monoclonal antibodies to E2 were determined by competitive ELISAs, using a modification of the method of Wege et al. (37). Briefly, hybridomas were grown under serum-free conditions, and immunoglobulin was isolated by affinity chromatography with protein A-Sepharose (Pharmacia Diagnostics, Piscataway, N.J.). The purity of the antibodies, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (33), was greater than 95%. Antibody concentration was determined by the Bradford method (Bio-Rad Laboratories, Richmond, Calif.), and the antibodies were biotinylated (13), using biotin-x-NHS reagent (Calbiochem-Behring, La Jolla, Calif.).

The antigen in the competitive ELISA was JHMV-DL, in the form of clarified supernatant derived from DBT cells infected under serum-free conditions. Approximately 10^3 PFU of JHMV per well were adsorbed overnight on polystyrene microtiter plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.) at 4°C in 0.08% sodium azide. The plates were flicked, allowed to dry for 1 h, and postcoated with ELISA medium (0.1% bovine serum albumin and 0.2% Tween 20 in phosphate-buffered saline, pH 7.2) for 1 h. The postcoating and all subsequent steps were done at room temperature. Competing, unlabeled monoclonal antibody was first incubated on the plates for 2 h. In the second incubation, 10 ng of biotinylated monoclonal antibody per well was added and incubated for an additional 2 h. The competing antibody was not removed, and the plates were not washed between the two incubations. The remainder of the procedure and the criteria for competition were as described by Wege et al. (37), except that streptavidin-horseradish peroxidase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the conjugate.

Quantitative determinations of the binding of monoclonal antibodies to parental and variant JHMV were performed by radioimmunoassay (8). Relative binding of antibodies to the different viruses was evaluated by the criteria previously described (7).

Selection of antigenic variant viruses. Antigenic variants were selected by a modification of the procedure of Wiktor and Koprowski (41). Anti-JHMV monoclonal antibodies were used to select sets of antigenic variant viruses that escaped neutralization. In all incubations, monoclonal antibody was used at a final concentration sufficient to reduce the titer of JHMV-DL by approximately 4 $\log_{10}$ units. Antibody and virus were incubated for 45 min at 37°C. In each selection experiment, approximately 10^6 PFU of JHMV-DL were incubated with an anti-JHMV monoclonal antibody. The viruses surviving neutralization were isolated by plating at limiting dilution on DBT cells. Isolated plaques were picked, incubated with the selecting monoclonal antibody, and then propagated on DBT cells. Resistance to neutralization by the selecting monoclonal antibody was assessed by plaque assaying virus isolates after parallel incubations with either the selecting monoclonal antibody or the negative control monoclonal antibody, which is specific for H-2I^p (11). If the titers of the two assays were within 1 $\log_{10}$ unit of each other, the isolate was considered to be resistant to neutralization. This criterion was used throughout to distinguish antigenic variants and revertant viruses. Neutralization-resistant isolates were plaque purified two additional times after incubation with the monoclonal antibody and by plating at limiting dilution on DBT cells. Before each use, a portion of each stock of variant virus was tested to assure that reversion had not occurred.

Pathogenesis. Virus present in the tissues of infected mice was assayed by a modification of the protocol of Stohlman and Weiner (32). Briefly, tissues were removed aseptically, homogenized in Dulbecco phosphate-buffered saline (5%, wt/vol), clarified by centrifugation at 200 × *g* for 7 min, and assayed in duplicate on L2 cells in 24-well microtiter plates. Plates were observed for 72 h for the presence of viral cytopathic effect. In cases where mice were infected with antigenic variant viruses, the phenotype of recovered virus was ascertained by differential neutralization with anti-E2 and negative control monoclonal antibodies.

Initially, tissues were fixed by immersion in 10% formaldehyde in phosphate-buffered saline and then embedded in paraffin. Sections were stained with hematoxylin and eosin for routine histology, luxol fast blue for myelin, Bodian silver for axons, as well as with phosphotungstic acid hematoxylin for glial fibers. For immunohistochemistry, sections of paraffin-embedded tissue were stained by an avidin-biotin immunoperoxidase procedure (Vectastain; Vector Laboratories, Burlingame, Calif.), using monoclonal antibodies to the JHMV N protein (7) as primary antibodies.

For ultrastructural studies, mice were perfused with half-strength Karnovsky fixative by the intracardiac route. Tissues were immersed in the same fixative, postfixed in 2% OsO_4, enblock-stained in 1% uranyl acetate, and dehydrated in a graded series of ethanol solutions. Tissues were then embedded in Poly/Bed 812 (Polysciences, Warrington, Pa.) and 1-μm sections were cut and stained with toluidine blue. These sections were examined by light microscopy, and target areas were identified for electron microscopy. The blocks were then retrimmed, and silver sections were cut on a Sorvall MT 6000 ultramicrotome (Du Pont Co., Wilmington, Del.) and mounted on 180 honeycomb mesh grids (Ted Pella, Inc., Tustin, Calif.). The grids were examined and photographed on an AEI transmission electron microscope.

RESULTS

Selection of antigenic variant viruses. Selection of antigenic variants of JHMV-DL was done with two JHMV-specific monoclonal antibodies, designated J.7.2 and J.2.2 (7). We have previously shown that these antibodies bind to the major viral glycoprotein, E2 (7). Both antibodies neutralize viral infectivity in the absence of complement. To further characterize the binding sites of these antibodies on E2, J.7.2 and J.2.2 were labeled with biotin, and competitive ELISA studies were performed. Figure 1A shows that unbiotinylated J.7.2 effectively competed with biotinylated J.7.2, as indicated by the marked decrease in optical density when the unlabeled homologous competititor exceeded 1.0 ng. By contrast, the heterologous antibody J.2.2 did not significantly diminish the optical density associated with biotinylated J.7.2, even when as much as 10,000 ng of heterologous unlabeled competing antibody was added. Figure 1B shows the reciprocal experiment in which 10 ng of biotinylated J.2.2 was subjected to competition with graded amounts of unlabeled homologous and heterologous antibodies. As in experiment 1, only homologous antibody competed with the biotinylated antibody. Since the two monoclonal antibodies do not compete with each other during binding to E2, it may be concluded that they recognize topographically distinct antigenic domains of this molecule (10, 28, 42).

Viruses resistant to neutralization by either monoclonal

antibody J.7.2 or J.2.2 were isolated and cloned as described above. A total of 11 isolates selected with J.7.2 were obtained and designated 7.2-V-1 to 7.2-V-11. Similarly, a total of 15 isolates resistant to J.2.2 were selected and designated 2.2-V-1 to 2.2-V-15.

Antigenic properties of variant viruses. The antigenic properties of JHMV-DL and representative selected variants were compared in quantitative neutralizations with the following antibodies: (i) negative control monoclonal antibody 7-16.17 (anti-H-2I^p), (ii) anti-JHMV mouse serum, and (iii) JHMV-specific, anti-E2 monoclonal antibodies J.7.2 and J.2.2. JHMV-DL and variants 7.2-V-1 and 2.2-V-1 all were efficiently neutralized by anti-JHMV mouse serum (Table 1). Also, the monoclonal antibodies used in the selection procedures reduced the JHMV-DL stock approximately 4 $\log_{10}$ units. By contrast, both variants 7.2-V-1 and 2.2-V-1 were resistant to neutralization with their respective selecting monoclonal antibodies. Thus, the neutralizations with anti-JHMV serum show the variant viruses share common antigenic determinants with parental virus. On the other hand, the results of neutralizations with anti-E2 monoclonal antibodies also indicate that the variant viruses have some determinants that are antigenically distinct from those of the parental virus.

An additional finding was the resistance of variant 2.2-V-1 to neutralization by J.7.2. This result was unexpected, since J.7.2 and J.2.2 recognize different antigenic domains of E2 (see above). Increasing the concentration of neutralizing antibodies consistently demonstrated variant 2.2-V-1 was completely resistant to neutralization with its selecting antibody, J.2.2; however, it was only partially resistant to neutralization with the nonselecting antibody, J.7.2 (data not shown).

Quantitative antibody binding studies were used to search for antigenic differences in the other viral structural proteins. Using a panel of 19 anti-N and 6 anti-E1 antibodies (7; Fleming, unpublished observations), no differences were found between the binding of these monoclonal antibodies to variants 7.2-V-1 or 2.2-V-1 and their binding to parental virus (data not shown).

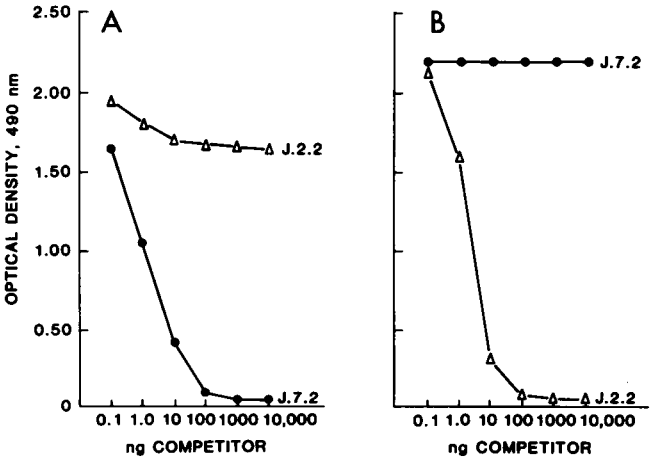

FIG. 1. Competitive ELISAs between biotinylated monoclonal antibody and unlabeled competitor monoclonal antibodies. In the assays, a constant amount (10 ng) of biotinylated antibody was tested with various amounts of competing antibody. (A) Competition of biotinylated J.7.2 with unlabeled, homologous J.7.2 (●), and unlabeled J.2.2 (△). (B) Reciprocal competition experiment with biotinylated J.2.2.

TABLE 1. Comparative neutralizations of JHMV-DL and variant viruses by different antibodies

Antibody[b]	Specificity	Virus neutralization index[a]		
		JHMV-DL	7.2-V-1	2.2-V-1
Anti-JHMV	Mouse hepatitis virus	5.0	4.9	5.3
J.7.2	E2	3.8	0.0	0.8
J.2.2	E2	4.1	4.0	0.0

[a] Determined by subtracting the $\log_{10}$ PFU of virus per milliliter mixed with neutralizing antibody from the $\log_{10}$ PFU of virus per milliliter mixed with negative control antibody 7-16.17.

[b] Used as hybridoma supernatants and diluted 1:10; the hyperimmune serum was diluted 1:100.

Growth of antigenic variant viruses in vitro. Since the E2 glycoprotein may be responsible for virus attachment to the target cells and for cell-to-cell fusion (3, 7, 25, 34), we examined the abilities of JHMV-DL, 7.2-V-1, and 2.2-V-1 to cause fusion of DBT cells. No differences were found. Also, variant viruses were examined for their ability to form plaques at 32, 37, and 39°C. No significant differences were found, indicating that the viruses are not temperature sensitive. Finally, in one-step studies of viral growth on DBT cells, there was essentially no difference in rate of growth or in maximal yield among the three viruses. These data provide evidence against a general defect in the growth characteristics of the antigenic variant viruses.

Pathogenesis. As a first step in characterizing the diseases caused by the JHMV antigenic variant viruses, their virulence was compared with that of parental virus. Various amounts of each virus were inoculated i.c. into groups of 10 mice. The 50% lethal dose (LD_{50}) at day 7 postinoculation (p.i.) for parental JHMV-DL virus was 4 PFU, and the LD_{50} for 7.2-V-1 was 25 PFU. By contrast, the LD_{50} for 2.2-V-1 was approximately 10,000 PFU. To examine whether other variants selected with the same monoclonal antibodies have similar virulence, three additional variants from each set were inoculated into groups of five mice i.c. at 1,000 PFU per mouse. When variants selected with J.7.2 were tested, all mice died, a result identical to that obtained with the prototype variant 7.2-V-1. By contrast, the three additional variants selected wtih J.2.2 showed the pattern of relative avirulence manifested by 2.2-V-1 and did not cause death at 1,000 PFU i.c. These data indicate that antigenic variants selected with J.2.2 were, in general, markedly less virulent than variants selected with J.7.2.

In subsequent experiments, the patterns of clinical disease during infection were monitored. Groups of mice injected i.c. with various amounts of parental virus or antigenic variants were observed daily. By day 3 or 4 p.i., the mice injected with JHMV-DL or four variants selected with J.7.2 showed signs of generalized encephalitis such as immobility, hunched posture, myoclonus, or convulsions. All these mice deteriorated rapidly and died by day 7. This encephalitic illness was seen at all doses of virus sufficient to infect the mice. Histological examination showed inflammation which involved all CNS regions (Fig. 2A and B), as has been demonstrated previously with wild-type JHMV (40). Although the entire CNS was involved, the predominant lesions were in gray matter, where neuronophagia and frank necrosis were noted.

In contrast, the disease produced by four variants selected

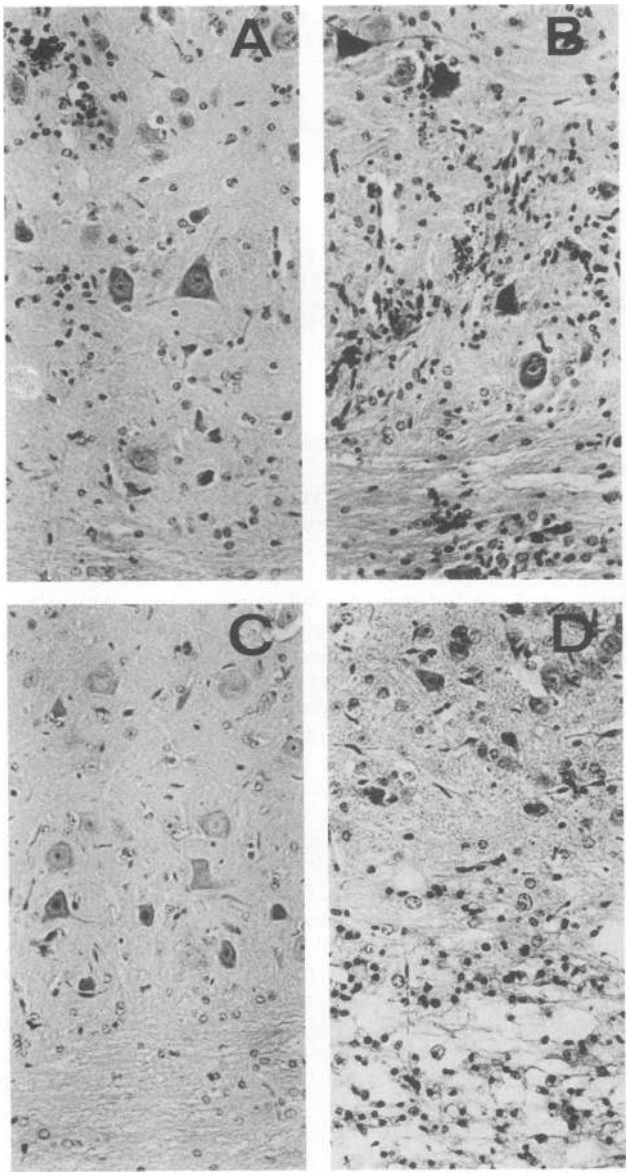

FIG. 2. Infected mouse spinal cords embedded in paraffin and stained with hematoxylin and eosin. In each illustration, a longitudinal section of spinal cord demonstrating the juction between gray and white matter is shown, with gray matter at the top of the figure and white matter at the bottom. (A) Infection with JHMV-DL, day 4 p.i. A small cell inflammatory infiltrate was seen, most prominently in gray matter. Magnification, ×100. (B) Infection with variant 7.2-V-1, day 4 p.i., showing extensive inflammatory cell infiltrate in both gray and white matter. Inflammation was most prominent in the gray matter, where foci of possible neuronophagia were noted. Magnification, ×100. (C) Infection with variant 2.2-V-1, day 4 p.i. Minimal scattered inflammatory cells were seen in gray and white matter. The section was otherwise normal. Magnification, ×100. (D) Infection with variant 2.2-V-1, day 19 p.i. Marked inflammation, loss of myelin, and rarefaction were seen in the white matter. Note the normal appearance of gray matter, where neurons appeared uninvolved. Magnification, ×100.

by monoclonal antibody J.2.2 differed markedly from the fulminant encephalitic illness observed with the parental strain or variants selected with J.7.2. Mice receiving 1,000 PFU or less i.c. of the J.2.2-selected variants were completely asymptomatic until day 8 or 9 p.i., when mild hindleg paralysis was noted in approximately 70% of the animals. Although the severity of paralysis increased between days 12 and 14 p.i., the majority of mice remained alert and did not show signs of generalized CNS dysfunction, such as lethargy or convulsions. There was variable recovery from this paralysis during the ensuing 2 to 3 months. Remarkably, severe paralysis could be induced by very low doses of virus (from 1 to 10 PFU i.c.). When the mice were given 100,000 PFU i.c., most mice died; interestingly, death occurred between days 12 and 14 p.i., in contrast to the early deaths caused by J.7.2-selected variants or parental virus. On histological investigation, mice acutely infected with 1,000 PFU of 2.2-V-1 i.c. showed only minimal inflammatory infiltrates in gray and white matter (Fig. 2C). By day 19 p.i., however, extensive demyelination and rarefaction were found in the white matter, while gray matter appeared normal (Fig. 2D). Mice were also given 10,000 or 100,000 PFU of 2.2-V-1 i.c. and examined at day 7 p.i., when both encephalitic and paralytic signs were first becoming apparent. These animals showed extensive cellular infiltrates in both gray and white matter and perivascularly; interestingly, neurons did not appear directly involved in these mice. Although only spinal cord pathology is illustrated in Fig. 2, similar morphological changes were found in brains of these mice. Histologic examination of the liver did not reveal abnormalities in any mouse.

To determine the distribution of viral antigen, immunohistochemical studies were done with a mixture of monoclonal antibodies with specificity for the JHMV N protein. During acute infection with JHMV-DL and variant 7.2-V-1, viral antigen was found in the cytoplasm of cells in both gray and white matter (data not shown); specifically, antigen was found in cells that had the obvious morphological features of neurons. During the early stages of infection with variant 2.2-V-1, viral antigen could be detected in both gray and white matter, although the staining was most intense in the white matter. Unequivocal staining of neurons was not demonstrable. As noted, mice infected with JHMV-DL or 7.2-V-1 did not survive the period of acute encephalitis. On the other hand, mice infected with 2.2-V-1 did survive the acute phase of the illness, and at day 19 p.i. low levels of viral antigen were demonstrable in the white matter of two of eight mice examined. No antigen was found in the neurons of these mice. Viral antigen could not be demonstrated in mice after day 19 p.i.

The nature of the white-matter lesions in mice infected with 1,000 PFU of 2.2-V-1 was studied further. Epon-embedded sections taken at day 21 p.i. (Fig. 3A and B) showed myelin loss, axon survival, inflammatory cells, and occasional extracellular vacuoles; also, active macrophages were noted next to naked axons and in perivascular loci. Mice studied at day 30 p.i. with luxol fast blue and silver staining also showed active lesions with primary demyelination. In addition, one mouse examined at 12 months p.i. showed loss of myelin, axon preservation, and gliosis. Apart from mild perivascular inflammation, the lesions in this mouse did not appear to be active.

Virus replication in mice. In view of the very different disease patterns exhibited by the two sets of JHMV variants, we sought to recover and characterize infectious virus from the brains of mice inoculated with either JHMV-DL, variant 7.2-V-1, or variant 2.2-V-1. Groups of mice were inoculated with 1,000 PFU of each virus i.c. and sacrificed at intervals for study. The kinetics of virus replication in the brains of these mice is shown in Fig. 4. Maximal virus titers were

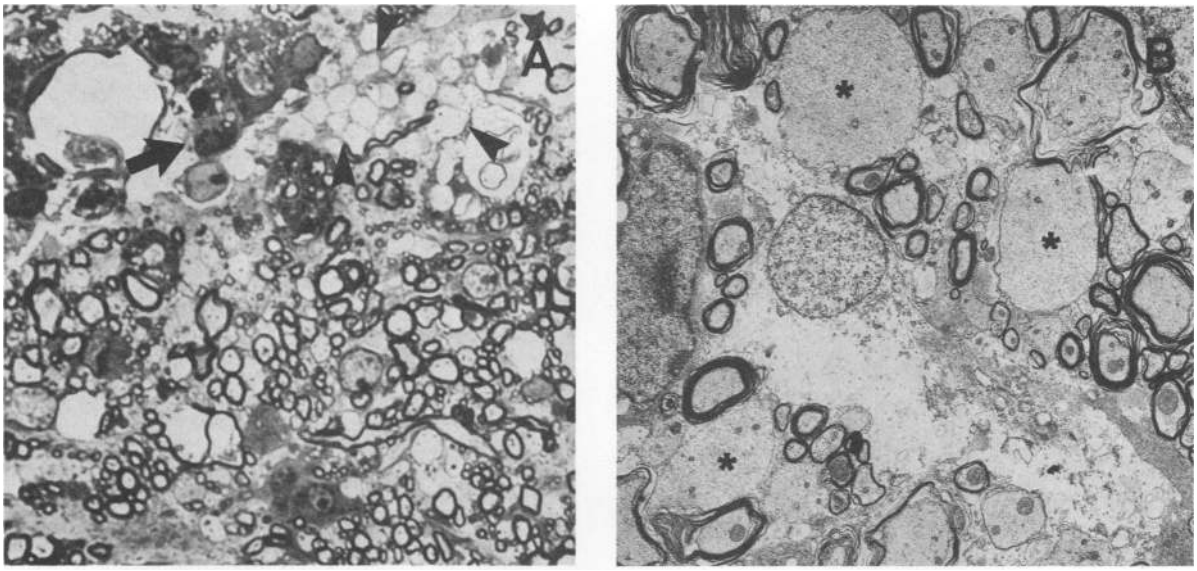

FIG. 3. Epon-embedded sections of spinal cord from a mouse given 1,000 PFU of variant 2.2-V-1, day 21 p.i. (A) Thick section stained with toluidine blue. Naked, demyelinated axons (arrowheads) and a perivascular infiltrate of inflammatory and phagocytic cells (arrow) were observed. Magnification, ×400. (B) Electron microscopy. Representative demyelinated axons are indicated by asterisks. Magnification, ×4,000.

reached between days 3 and 5 p.i. The maximum virus yields from mice given JHMV-DL and 7.2-V-1 were $10^{6.7}$ and $10^{5.8}$ PFU/g of brain, respectively, while the maximum yield from mice given 2.2-V-1 was $10^{5.3}$ PFU/g of brain. All mice given JHMV-DL were dead by day 5 p.i., and all mice injected with 7.2-V-1 were dead by day 7 p.i. The titer of recovered virus from mice injected with 2.2-V-1 was slightly lower than that of the other two viruses and gradually fell until day 19 p.i., after which infectious virus was not detectable in brain homogenates. All mice used in the study were tested for the presence of infectious virus in homogenates of liver and spleen; none was found in any mouse.

To determine whether in vivo replication of variant 2.2-V-1 had selected for revertants, we tested virus recovered from mice infected with 1,000 PFU of 2.2-V-1 i.c. by neutralization with polyclonal mouse anti-JHMV serum and monoclonal antibody J.2.2. Virus recovered from brain homogenates at day 5 p.i. was tested after one passage in vitro to increase the titer. These viruses were neutralized by the polyvalent antibody but not by J.2.2, indicating that at the time of maximum in vivo replication, the majority of the viral population had retained the phenotype of the input variant virus. Similar studies with variant 7.2-V-1 recovered from mice at day 6 p.i. also showed that the phenotype of the original imput virus was preserved.

DISCUSSION

We report here on the pathogenic properties of two sets of antigenic variants of the murine coronavirus JHMV. These variants were selected by neutralization of parental stock virus with monoclonal antibodies that recognize separate sites on the E2 viral glycoprotein. One group of variants was selected for resistance to monoclonal antibody J.7.2. In mice inoculated with these variants, encephalitic signs were prominent clinically, and marked gray matter lesions were present histologically. These findings are consistent with prior observations that suggest that the lethality of JHMV depends upon the extent of involvement of neurons and gray matter (14, 38).

The set of antigenic variant viruses selected by monoclonal antibody J.2.2 showed a very different pathogenesis for mice. These variants had a marked reduction in virulence and predominantly caused a paralytic disease, in which severe primary demyelination was noted. On the basis of the data presented above, it seems unlikely that a general defect in replication would account for the diminished neurovirulence of these variants. For example, the maximum yield of the prototype variant 2.2-V-1 was only $10^{0.5}$ PFU/g of brain lower than that of the highly virulent variant 7.2-V-1. In addition to decreased lethality, the group of J.2.2-selected variants consistently demonstrated pronounced demyelination, which resulted in obvious paralysis.

The sets of variants could be distinguished from each other and from parental virus on the basis of demonstrable antigenic variations of the E2 protein. However, no differences were shown in antigenic comparisons of the other viral structural proteins, E1 and N. The frequency with which neutralization-resistant JHMV variants were isolated was between 10^{-4} to 10^{-5}, which is consistent with the frequency of antigenic variants of other RNA viruses that have been proven to arise by single-point mutations (6, 16–18, 24). This observation suggests that the JHMV antigenic variants represent individual point mutations. Direct evidence for point mutations in the E2 gene must, of course, await RNA sequencing of the relevant JHMV strains. Sequence determinations would be most interesting in the case of variant 2.2-V-1, which is partially resistant to neutralization with J.7.2, the monoclonal antibody not employed in its selection. This finding suggests that the mutation selected by treatment with monoclonal antibody J.2.2, in addition to altering recognition by J.2.2, also results in conformational changes that secondarily affect interaction of the variant with monoclonal antibody J.7.2. Similar anomalies have been noted with other, independently selected, antigenic variants of JHMV (R. G. Dalziel, P. W. Lampert, P. J. Talbot, and M. J. Buchmeier, J. Virol., in press) and antigenic variants of influenza virus (21).

The finding that antigenic variants of JHMV with putative

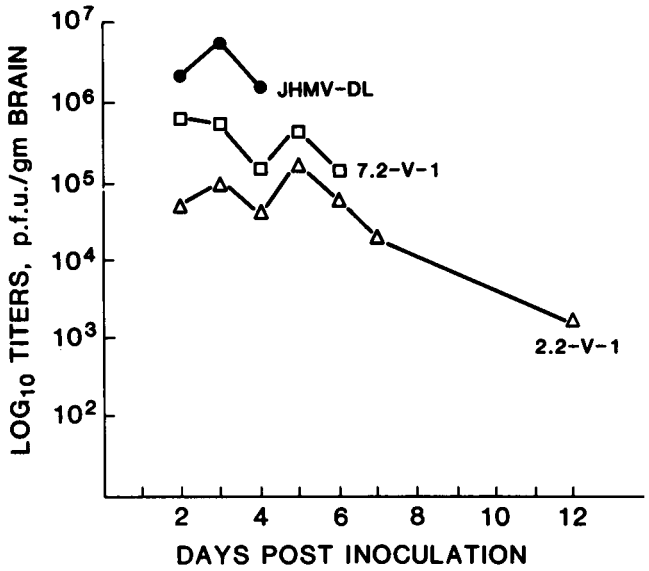

FIG. 4. Viral replication in the brains of mice injected i.c. with 1,000 PFU of JHMV-DL (●), antigenic variant virus 7.2-V-1 (□), or antigenic variant virus 2.2-V-1 (△). Each time point represents the mean titer from a group of three or six mice.

point mutations in the E2 protein cause very different diseases implies that this glycoprotein is a major determinant of pathogenesis. In this regard, it has been shown that the adoptive transfer of monoclonal antibodies specific for the E2 protein protects mice injected with parental virus from acute encephalitis (2; Fleming, unpublished observations). These animals survive and have extensive demyelination histologically. Buchmeier et al. (2) have suggested that the transferred anti-E2 monoclonal antibodies may alter local CNS environments in such a way as to favor infection of oligodendroglial cells and diminish viral entry into neurons. In other investigations, persistent in vitro infections of JHMV have yielded a variety of strains that exhibit changes in E2 and have altered neuropathogenicity (1, 12, 20). In vivo studies of JHMV infection of rats have shown that 5 to 7 days after JHMV infection, specific, stable alterations in E2 are evident (35). Taken together, these observations clearly implicate E2 in the phenomena of neurovirulence, persistence, and demyelination associated with JHMV infections. On the other hand, it is probable that JHMV pathogenesis ultimately will be shown to depend on several major variables, including JHMV proteins besides E2 and key host factors.

Finally, it should be noted that monoclonal antibody J.2.2, but not monoclonal antibody J.7.2, selected antigenic variant viruses with reduced neurovirulence. Thus, it would appear that J.2.2 is involved with a site on E2 whose integrity, i.e., parental conformation, is essential for the production of acute, fatal encephalitis. It is not known whether other sites on E2 play analogous, critical roles during paralytic, demyelinating disease. The availability of strains such as 2.2-V-1 may be useful in studies of this issue.

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