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Monoclonal Antibodies to the Matrix (E1) Glycoprotein of Mouse Hepatitis Virus Protect Mice from Encephalitis

JOHN O. FLEMING,*,†,[1] RICHARD A. SHUBIN,* MARK A. SUSSMAN,† NANCY CASTEEL,[2] AND STEPHEN A. STOHLMAN*,†

*Departments of *Neurology and †Microbiology, University of Southern California School of Medicine, Los Angeles, California 90033*

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Monoclonal antibodies to the matrix or E1 glycoprotein of mouse hepatitis virus (MHV) were tested for their ability to protect mice from a normally lethal challenge of MHV-4. Four antibodies were tested, and two of these, J.1.3 and J.3.9, were protective. Protection did not correlate with virus neutralization *in vitro*, antibody isotype, recognition of a unique E1 antigenic site, or dependence on complement *in vivo*. Survival from acute encephalitis was followed by subacute demyelination, as has been shown with protection mediated by neutralizing monoclonal antibodies against the major glycoprotein, E2. These results demonstrate that antibodies which are specific for a viral matrix protein are able to alter the course of disease.

Mouse hepatitis viruses (MHV) are useful agents for studying experimental infections of the gastrointestinal and central nervous systems (*1*). MHV are members of the Coronavirus group of animal viruses. The virion is composed of three structural proteins, the major glycoprotein (E2 or peplomer), a minor glycoprotein (E1 or matrix), and a nucleocapsid protein (N) (*2*). In order to better understand the pathogenesis and immune response induced by these viruses, attempts have been made to alter the course of infection by administering monoclonal antibodies specific for viral structural proteins. Buchmeier and colleagues showed that some, but not all, neutralizing anti-E2 monoclonal antibodies protected mice from challenge with the neurotropic strain MHV-4 (JHMV) (*3*). Interestingly, mice protected from acute encephalitis by anti-E2 monoclonal antibodies subsequently developed demyelination. Wege *et al.* (*4*) demonstrated that anti-E2 monoclonal antibodies protected suckling rats from MHV-4; these investigators also found that only anti-E2 monoclonal antibodies that both neutralized virus and inhibited cell fusion were protective. Nakanaga *et al.* (*5*) examined monoclonal antibodies reactive with MHV-2 and showed that both anti-E2 and anti-N monoclonal antibodies protected mice from lethal MHV-2 induced hepatitis. Most recently, Lecomte *et al.* (*6*) have reported that an anti-N monoclonal antibody which neutralizes MHV-3 *in vitro* in the presence of complement protects mice from lethal MHV-3 challenge.

In view of the demonstrations that anti-E2 and anti-N monoclonal antibodies protected rodents infected with MHV, we sought to determine whether monoclonal antibodies specific for the third structural protein, the E1 or matrix glycoprotein, could alter disease. Four monoclonal antibodies directed against the E1 glycoprotein of MHV-4 were evaluated for their ability to neutralize virus *in vitro* and protect mice from a normally fatal viral challenge (Table 1). Unless stated otherwise, experiments used 6-week-old male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) (seronegative for MHV) which were given 100 μg of purified monoclonal antibody intraperitoneally (ip) on the same day as intracerebral (ic) inoculation of 2000 plaque-forming units (PFU) of MHV-4, strain JHMV DS (*7*). Talbot and Buchmeier (*8*) have recently shown that administration of murine monoclonal antibodies ip results in significant, persistent plasma antibody titers in mice. In the absence of passively administered antibody, challenge with 2000 PFU ic of MHV-4, strain JHMV DS, led to a fatal encephalitis within 10–12 days in 26 of 29 mice.

Monoclonal antibodies J.1.3 and J.3.9 were found to protect mice from lethal ic challenge with MHV-4 (Table 1). However, monoclonal antibodies J.2.7 and J.3.11 consistently failed to protect mice, even when the amount of antibody was increased to 200 μg per recipient (data not shown). Protection did not correlate with IgG subclass, as IgG2a antibodies were both protective (J.1.3 and J.3.9) and nonprotective (J.3.11). Also, protection did not correlate with virus neutralization *in vitro* in the presence of complement, since protective monoclonal antibodies J.1.3 and J.3.9 were neutralizing and nonneutralizing *in vitro*, respectively (Table 1). This result was unexpected, since, with the exception of two monoclonal antibodies reported by Nakanaga *et al.* (*5*), all previously described anti-MHV monoclonal antibodies which protect *in vivo* neutralize virus *in vitro*.

[1] To whom requests for reprints should be addressed.

[2] Present address: Department of Microbiology, Chicago College of Osteopathic Medicine, Chicago, IL 60615.

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TABLE 1

MONOCLONAL ANTIBODIES SPECIFIC FOR THE E1 GLYCOPROTEIN

Monoclonal antibody[a]	Isotype[b]	Relative avidity[c]	Virus neutralization[d]		Protection (alive/total)[e]
			No complement	Complement	
None	—	—	—	—	3/29
J.1.3	IgG2a	1.0	$<5 \times 10^2$	4×10^5	16/19
J.2.7	IgG3	0.42	$<5 \times 10^2$	$<5 \times 10^2$	0/4
J.3.9	IgG2a	8.9	$<5 \times 10^2$	$<5 \times 10^2$	9/12
J.3.11	IgG2a	0.05	$<5 \times 10^2$	$<5 \times 10^2$	0/4

[a] Monoclonal antibodies were derived as previously described and shown to be specific for the MHV E1 molecule by radioimmunoprecipitation (*39*).

[b] Isotype was determined by enzyme linked immunosorbent assay (ELISA) (Mono Ab-Id EIA, Zymed Laboratories, So. San Francisco, CA). All light chains were of κ type. The isotypic characterizations by ELISA were found to be at variance with that previously reported, when double immunodiffusion was employed (*39*). This difference probably reflects the increased accuracy of the ELISA.

[c] Relative binding of monoclonal antibodies to MHV-4 was determined by ELISA and quantified by the multiplicity of normal activity method (*40*). Antibody concentration was determined as previously described (*40*). Relative avidity was expressed as the quotient of relative binding ÷ concentration. Relative avidities were normalized to J.1.3.

[d] Approximately 100 PFU of MHV-4 were incubated with serial dilutions of monoclonal antibodies for 30 min at 37°. A subsequent incubation with or without complement (rabbit serum, Accurate Biochemical, Hamilton, Ontario, Canada, diluted 1:40) was carried out at 37° for 30 min. Virus was assayed as previously described (*9*). The reciprocal of the dilution of antibody which inhibited 50% of MHV-4 was taken as the neutralizing titer.

[e] C57BL/6 male mice, 6 weeks old, were simultaneously given 2000 PFU of virus ic and 100 μg of monoclonal antibody ip. The antibodies were produced under serum-free conditions, purified by Sepharose–protein A chromatography, and quantified by ELISA as previously described (*40*).

In order to determine if protective antibodies were associated with a unique topographic site on the E1 molecule, we performed competitive binding studies with biotinylated antibodies, using previously described methods (*9*). In this regard, Talbot *et al.* (*10*) have shown that the MHV-4 E1 has at least two major antigenic sites. Talbot *et al.* (*11*) measured antibodies against one of these sites on E1 and found that significant amounts of antibody were produced during experimental infection. In preliminary studies, it was found that J.1.3 and J.3.9 recognize different antigenic sites on E1. Furthermore, nonprotective antibodies J.2.7 and J.3.11 appear to bind to a site which overlaps the site recognized by J.1.3 (data not shown). Thus, it seems unlikely that protection is associated with binding to a single critical determinant on E1. Although we did find a correlation between protection and the high relative avidities of monoclonal antibodies J.1.3 and J.3.9 (Table 1), additional testing with a larger panel of anti-E1 monoclonal antibodies will be required to evaluate the significance of this observation.

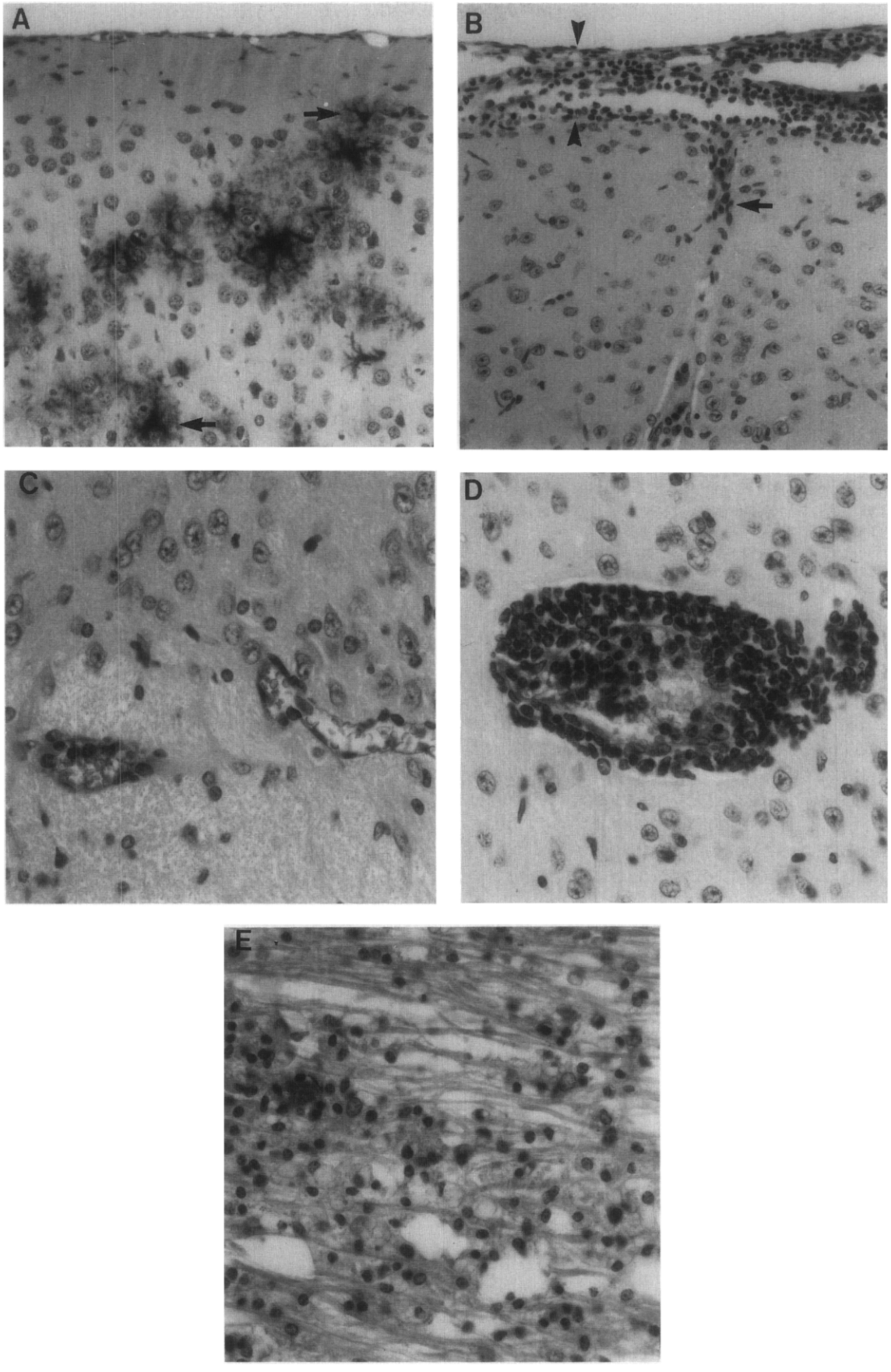
These initial findings were extended by more detailed studies *in vivo*. By administering graded amounts of purified J.1.3, we found that the minimal protective dose was approximately 40 μg. This result is similar to that of Boere *et al.* (*12*) who found that 10 μg of purified nonneutralizing monoclonal antibody against Semliki Forest virus was required for protection. By contrast, Boere *et al.* found that the minimal amount of protective neutralizing monoclonal antibody was only 0.1 μg (*12*). Other (*13–15*), but not all (*16*), studies have also shown that nonneutralizing antiviral monoclonal antibodies are much less effective than neutralizing monoclonal antibodies, based on the amount of antibody needed for protection. To date the minimum amount of protective monoclonal antibodies directed against the N or

TABLE 2

BRAIN VIRUS TITERS AFTER PASSIVE ANTIBODY PROTECTION[a]

Monoclonal antibody	5 days postinoculation		10 days postinoculation	
	PFU/g	*n*	PFU/g	*n*
None	6.5 ± 0.4	7	3.8 ± 1.0	7
J.1.3	4.6 ± 0.8	5	3.7 ± 0.7	6
J.2.7	5.5 ± 0.7	2	4.4 ± 0.5	4
J.3.9	6.1 ± 0.5	3	4.3 ± 0.5	4
J.3.11	6.2 ± 1.1	4	4.3 ± 0.5	2

[a] Male 6-week-old C57BL/6 mice were given 2000 PFU of MHV-4 ic and 100 μg of the indicated monoclonal antibodies ip on Day 0. On Days 5 and 10 after inoculation, the number (*n*) of mice indicated were sacrificed, and viral titers of brain homogenates were determined by limiting dilution assay on DBT or L2 cells as previously described (*9*). For each group the titer is expressed as the mean ± one standard deviation in PFU virus per gram of tissue.


A
B
C
D
E

E2 MHV protein has not been determined in absolute units.

To evaluate the effect of delayed treatment with protective antibody, 100 μg of J.1.3 was given to groups of five mice at different times relative to viral challenge (Day 0). Antibody protected all mice at Day +1 and Day +2 postinoculation. At Day +3 only one of five mice survived, and at Day +4, no mice survived. Since MHV replication (*9*) and early mononuclear cell inflammation (*17*) are present in the brains of mice at 2 days p.i., these results indicate that monoclonal antibodies are not simply prophylactic or preventative, as they will promote recovery in mice after encephalitis has been initiated.

Since the *in vitro* studies indicated that one of the anti-E1 monoclonal antibodies, J.1.3, neutralized virus in the presence of complement (Table 1), we sought to determine what role complement might play *in vivo* during protection mediated by passive administration of antibody. This issue was studied by two approaches. First, antibody-mediated protection was studied in B10.D2/nSnJ mice, which have normal complement, and in B10.D2/oSnJ mice, which are deficient in the C5 component of complement (Jackson Laboratory) (*18*). Monoclonal antibody J.1.3 (100 μg/mouse) was given ip on the same day that 4000 PFU of MHV-4 was given ic to groups of eight mice that were 6 weeks of age. Both normal and complement-deficient mice tested by this protocol survived the infection. By contrast, only two of eight B10.D2/nSnJ and one of eight B10.D2/oSnJ mice survived without antibody protection.

In a second experiment, cobra venom factor (CVF) (Diamedix, Miami, FL) (*19*) was used to deplete 6-week old C57BL/6 mice of complement component C3 prior to ic challenge with 2000 PFU of virus. Mice were given 1 unit of CVF intravenously. Serum complement was measured by complement-dependent lysis of ^{51}Cr-labeled rabbit red blood cells coated with goat antibody to rabbit erythrocytes (Organon Teknika-Cappel, Malvern, PA). All mice treated with CVF were found to be depleted of C3. Four groups of four mice each were studied: (1) virus only, (2) virus plus CVF, (3) virus plus J.1.3, and (4) virus plus J.1.3 and CVF. All mice receiving virus alone (group 1) and all mice receiving virus plus CVF (group 2) died by Day 12 postinoculation. On the other hand, all mice given 100 μg of J.1.3 on Day 0 survived, either without (group 3) or with (group 4) CVF depletion of complement. Thus, mice either genetically deficient in complement or pharmacologically depleted of complement were nevertheless protected by an anti-E1 monoclonal antibody from a normally lethal MHV-4 infection. These results suggest that complement does not play an essential role *in vivo* during protection conferred by anti-E1 monoclonal antibody.

In order to monitor virus replication *in vivo,* brain virus titers during passive protection were determined at 5 and 10 days postinoculation (Table 2). Protection by antibody J.1.3 was associated with a modest decrease in brain viral titer at Day 5 after infection, similar to that noted after protection by anti-E2 monoclonal antibodies (*3*). By contrast, mice protected by monoclonal antibody J.3.9 had no decrease in viral titer relative to that of unprotected control animals. These data indicate that survival does not necessarily depend on inhibition of net viral replication. They further raise the possibility that antibodies may protect mice by altering qualitative characteristics of infection, such as the cellular distribution of virus or the severity of viral cytopathogenicity.

To explore these possibilities, histological studies of the nervous system of unprotected and protected mice were undertaken. Several general neuropathological patterns were consistently noted. In unprotected mice, viral antigen was readily demonstrable in neurons of the cerebral cortex and hippocampus 5 days after inoculation (Fig. 1A). Inflammation in meninges, brain parenchyma, and perivascular spaces (Fig. 1C) was usually minimal to moderate. By contrast, mice protected with antibody J.1.3 showed much less involvement of neurons (Fig. 1B), although inflammatory infiltrates in meninges, brain parenchyma, and perivascular spaces were often severe (Fig. 1D). Subacutely, at Day +30, protected mice showed rarefaction and intense inflammation in white matter, typical of MHV-4-induced demyelination (*9*) (Fig. 1E).

FIG. 1. Immunoperoxidase staining of C57BL/6 mouse tissues after infection with MHV-4. A monoclonal antibody against MHV-4 N protein was used to demonstrate viral antigen by previously described methods (*9*). (A) Cerebral cortex 5 days after ic inoculation of 2000 PFU of MHV-4. Note that many cells, including neurons (arrows), contain viral antigen. Original magnification ×250. (B) Cerebral cortex at 5 days after inoculation with 2000 PFU of MHV-4 ic and protection by 100 μg of monoclonal antibody J.1.3 given ip on the day of viral inoculation. Note marked inflammation in meninges (arrowheads) and perivascular (Virchow-Robin) spaces (arrow). In other sections, viral antigen was rarely noted in scattered neurons (data not shown). Original magnification ×250. (C) Representative blood vessels from cerebral cortex of the animal shown in (A). The perivascular infiltrate is minimal. Original magnification ×400. (D) Representative blood vessel from the cerebral cortex of animal shown in (B). Note intensive inflammation surrounding the vessel. Original magnification ×400. (E) Spinal cord 30 days after protection by monoclonal antibody J.1.3 under conditions noted in (B). Note intense inflammation and rarefaction in this longitudinal section of white matter. Original magnification ×400. Demyelination was confirmed by luxal fast blue staining (data not shown).

These studies demonstrate that administration of monoclonal antibodies specific for the E1 protein of MHV protect mice from lethal viral challenge. The mechanism of this protection, however, remains uncertain. The evidence we have gathered indicates that anti-E1 monoclonal antibodies did not protect by virtue of a particular antibody isotype or by means of recognition of a specific topographic site on E1. Neither *in vitro* neutralization nor reduction in viral replication *in vivo* appeared to be an essential characteristic of protective antibodies; similarly, complement activity *in vivo* also did not appear to be necessary for protection. On the other hand, histologic studies revealed distinctive findings which may indicate how anti-E1 monoclonal antibodies protect mice from MHV-4 challenge. We found that protected mice had increased parenchymal and perivascular cellular infiltration when compared to unprotected controls, similar to results reported by Nakanaga *et al.* (*5*). This suggests that passive anti-MHV antibody administration enhances cell-mediated immunity. One mechanism by which antibody may enhance cell-mediated immunity to viruses involves binding to Fc receptors on antigen presenting cells or to specific receptors on T cells. Experimentally, effector functions of T cells have been shown to be increased by these means after administration of antibodies to hepatitis B virus (*20*).

Another significant histological finding in the animals which had been protected by anti-E1 monoclonal antibodies was that neurons were relatively spared by MHV-4. This observation is consistent with that of Buchmeier and colleagues (*3*), who found that mice treated with anti-E2 monoclonal antibodies and challenged with MHV-4 showed little involvement of neurons. These mice also survived to show subacute demyelination. By contrast, mice that did not receive anti-E2 antibodies had an acute, fatal necrotizing encephalomyelitis with neuronophagia. These studies parallel those with temperature-sensitive (*21, 22*), plaque morphology (*7, 23*) and antigenic (*9, 24–26*) variants of MHV-4 which show increased propensity for demyelination. Thus, it appears that any variable which allows animals to survive the initial MHV-4 encephalitis, perhaps by attenuating viral cytopathogenicity for neurons, permits subacute demyelination to be manifest. Similarly, Harty *et al.* (*27*) demonstrated that nonneutralizing antibodies to lactate dehydrogenase-elevating virus protected mice from motor neuron disease, although the level of viral replication was unchanged. In these studies, antibody appeared to block the infection of neurons, while having no effect on the infection of nonneuronal central nervous system cells. Taken together, these findings are consistent with the observation that neurons are usually the critical targets of highly neurovirulent viruses. (*28*). Nonneutralizing monoclonal antibodies have also been shown to protect animals against other neurotropic viruses, including Sindbis (*29–31*), Semliki Forest (*12, 32*), Western equine encephalitis (*33*), Venezuelan equine encephalitis (*13*), St. Louis encephalitis (*14*), herpes simplex (*34*), vesicular stomatitis (*15*), and yellow fever viruses (*16, 35*).

Our findings contrast with prior studies using monovalent (*36*) or monoclonal antibodies (*3*) which concluded that anti-E1 antibodies do not protect against MHV challenge. The differences between our study and previous reports may reflect the antibodies tested, the viruses used for challenge, or other experimental variables. To our knowledge, our results are the first demonstration that antibodies directed against a viral matrix protein will protect mice from viral challenge. Thus, immune responses to a matrix or minor glycoprotein, which is almost entirely embedded in the virion envelope (*37, 38*) and might not be expected to be highly immunogenic (*36*), may nevertheless influence the outcome of disease. These observations may have significant implications for the design of vaccines and the understanding of anti-viral immunity.

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