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Protective Effect of Monoclonal Antibodies on Lethal Mouse Hepatitis Virus Infection in Mice

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Neutralizing and nonneutralizing monoclonal antibodies to the peplomer glycoprotein and nucleocapsid protein of a mouse hepatitis virus (MHV), MHV-NuU, protected mice against lethal MHV-2 challenge. Histopathologically, livers of mice receiving protective antibodies showed some focal necrotic lesions with remarkable cellular infiltration instead of fulminant hepatitis caused by MHV-2.

Mouse hepatitis virus (MHV) is a member of the coronavirus group producing both acute and chronic diseases in various species of animals (15, 17, 25). Most MHV strains have been shown to have the nucleocapsid protein (NP) and the minor (E1) and major (E2) envelope glycoproteins (18, 19). E1 is probably of the viral matrix, whereas E2 forms the peplomers that have important roles in attachment to host cells (18, 19).

We described previously the production of monoclonal antibodies (MAbs) to low-virulence MHV-NuU, an isolate from a wasted nude mouse (7), and their specificity for viral polypeptides (10). Those MAbs were shown to be cross-reactive in vitro with other strains of MHV including the highly hepatitogenic strain MHV-2 (6, 11, 12).

This paper describes protective effects of MAbs against E2 as well as NP of MHV-NuU in highly virulent MHV-2 infection in mice.

VN activity of MAbs and passive mouse protection. Ascitic fluids containing the MAbs used in this study were prepared as described previously (10). They were examined for virus-neutralizing (VN) activity against MHV-2 and MHV-NuU by 50% plaque reduction assay with DBT cells as described elsewhere (20). In some experiments, samples were assayed for VN activity in the presence of fresh guinea pig complement (1:25) and anti-mouse immunoglobulin G (IgG) rabbit IgG (1:100) (Cooper Biomedical, Inc., West Chester, Pa.). MAbs 23-2:C2 and 14-1:C7 (Table 1), both recognizing E2, showed VN activity against MHV-2 as well as MHV-NuU. In contrast, no VN activity against MHV-2 was shown with three other MAbs, 7-2:A2 recognizing E2 and 10-1:D2 and 23-1:G8 both recognizing NP, as already reported for VN activity against MHV-NuU (10). The presence of guinea pig complement with or without anti-mouse IgG antiserum had no effect.

Five-week-old female BALB/c mice purchased from a commercial breeder (Charles River Japan, Atsugi, Japan) received intraperitoneal injections of 0.1 ml of ascitic fluid containing each MAb, and 24 h later they were challenged intravenously with 30 PFU of MHV-2, which killed infected mice within 6 days. Animals were observed for mortality for 10 days postchallenge. MAb titers were not reduced during at least 11 days in unchallenged mice.

MAb 23-2:C2, which had high VN activity, exhibited a remarkable protective effect (Table 1). MAb-treated mice showed no clinical signs during 10 days postchallenge. Similar protective effects were also observed with non-neutralizing MAbs 7-2:A2 and 10-1:D2, which recognize E2 and NP, respectively, whereas some treated mice showed clinical signs at 4 to 7 days postchallenge. Also, 80% of mice treated with MAb 14-1:C7, having a lower VN titer, survived for 10 days postchallenge. On the other hand, no protective effect was observed with MAb 23-1:G8 recognizing NP. The

TABLE 1. VN and passive protection in mice by Mabs

Antibody			Viral polypeptide specificity[a]	VN titer[b] to MHV-NuU	VN titer[b] to MHV-2		Mortality by challenge[c]
Group	Clone	Isotype			Without anti-mouse IgG and complement	With anti-mouse IgG and complement	
A	23-2:C2	IgG1	E2	1:8,000	1:20,000	1:20,000	0/17[d]
B	14-1:C7	IgG2a	E2	1:100	1:100	1:100	2/10
C	7-2:A2	IgG2a	E2	1:<5	1:<5	1:<5	0/10
D	10-1:D2	IgG2a	NP	1:<5	1:<5	1:<5	0/19
E	23-1:G8	IgG2b	NP	1:<5	1:<5	1:<5	15/15
Control[e]				1:<5	1:<5	1:<5	59/59

[a] Determined by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis with infected DBT cell extracts.
[b] Using DBT cells.
[c] An antibody sample (1:1) was given intraperitoneally, and 30 PFU of MHV-2 was inoculated intravenously 24 h later.
[d] Number dead/number tested on day 10 after virus challenge.
[e] Ascitic fluid from BALB/c mice injected with parental myeloma cells.

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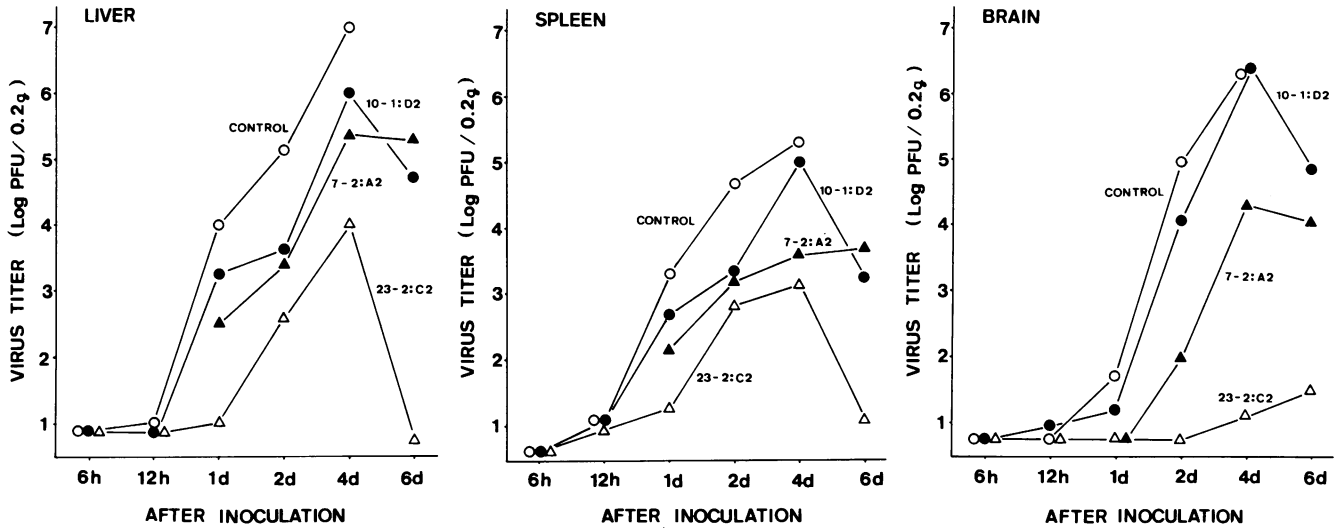

FIG. 1. Viral growth in livers, spleens, and brains of mice treated with MAb 23-2:C2 (△), 10-1:D2 (●), or 7-2:A2 (▲) and untreated controls (○). Ascitic fluid with or without MAb was given i.p. 24 h prior to i.v. challenge with 30 PFU of MHV-2. Each point represents the mean titer of three mice.

most protective MAb, 23-2:C2, was also effective against 30,000 PFU of MHV-2 challenge or at a dilution of 1:100.

The protective effect was lower when MAb administration followed the virus challenge, whereas some effect was seen with MAbs 10-1:D2, 23-2:C2, and 7-2:A2 given as late as 24 or 48 h following virus inoculation. With MAb 23-1:G8, no protection was shown after administration at any time before or after virus challenge.

When athymic nude mice were used instead of normal ones, time to death was longer in those animals having received protective MAbs, though all animals died within 14 days postchallenge. The effect of these protective MAbs was of passive immunity, because antibody was more effective when given prior to challenge infection and nude mice lacking T cells also could be protected to survive longer.

Effects of protective MAbs on viral growth in tissues and liver lesions in mice. By the same protocol as in the passive mouse protection test, mice were killed at intervals and tissue samples were removed. Virus titers were determined with DBT cells as previously described (4, 5). Liver samples were fixed with 10% phosphate-buffered Formalin and embedded in paraffin. Sections (3 μm) were stained with hematoxylin and eosin. On day 4 postchallenge (Fig. 1), more than 10^7 PFU of virus per 0.2 g was detected in livers of control mice that received ascitic fluid without MAbs. Those that had been treated with MAbs 23-2:C2, 7-2:A2, or 10-1:D2, showed peak virus titers of 10^4 to 10^6 PFU/0.2 g on day 4 postchallenge, and no virus was detectable on day 6 postchallenge in those that had received MAb 23-2:C2. Similar effects of MAbs on viral growth were seen in spleen and brain tissues (Fig. 1). Especially MAb 23-2:C2-treated mice showed only low virus titers in brain tissue, suggesting that spread of the virus to the brain was prevented by neutralizing MAb 23-2:C2 much better than by nonneutralizing MAbs 7-2:A2 and 10-1:D2.

Mice having received no MAb had severe liver lesions with poor infiltration of neutrophils and mononuclear cells on day 4 postchallenge (Table 2; Fig. 2, 3, 4). In contrast, mice treated with MAbs 23-2:C2 or 7-2:A2 had only a few necrotized lesions with considerable infiltration of mononuclear cells on day 4 postchallenge. Mice which had received MAb 10-1:D2, showed as many hepatic lesions as control

TABLE 2. Histopathological changes in the livers of MAb-treated mice after MHV-2 infection[a]

Days postinfection	MAb	No. of mice tested	No. of lesions[b]			Severity of liver changes[c]			
						Medium lesion		Large lesion	
			Small	Medium	Large	Necrosis	Cell infiltration	Necrosis	Cell infiltration
2	23-2:C2	4	0.3–1	0.3–1	0	+	++	−	−
	7-2:A2	4	0.3–1	<0.3	<0.3	+	++~+++	±	+++
	10-1:D2	4	1–3	0.3–1	0	+	+	−	−
	Control[d]	3	1–3	<0.3	0	+	±	−	−
4	23-2:C2	4	0.3–1	0.3–1	1–3	+	+~++	+	++
	7-2:A2	4	0.3–1	1–3	0.3–1	+	++	+	++
	10-1:D2	4	3–7	3–7	1–3	+~++	+~++	+~++	+~++
	Control	3	>7	>7	3–7	++~+++	±	+++	±

[a] Mice were given 0.1 ml of MAb 24 h before intravenous challenge with 30 PFU of MHV-2.
[b] Mean number of lesions per microscopic field at ×10 magnification by counting 10 to 28 fields.
[c] Intensity of necrosis or cell infiltration in the lesion: − = none, ± = slight, + = mild, ++ = moderate, +++ = severe.
[d] Ascitic fluid from BALB/c mice injected with parental myeloma cells.

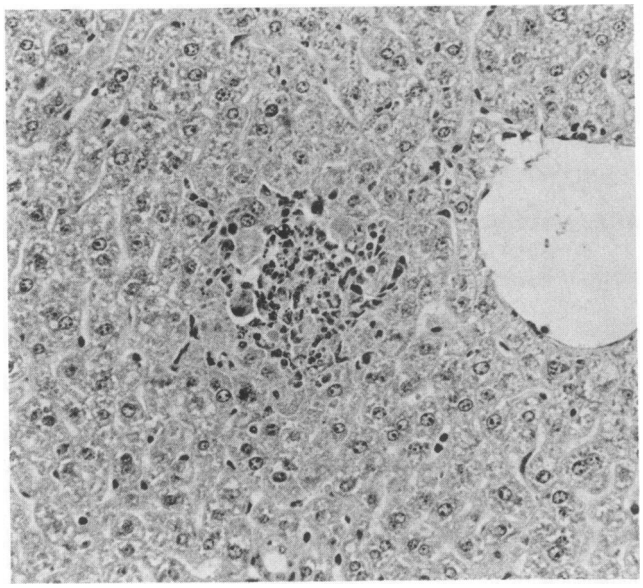

FIG. 2. Focal hepatic necrosis with inflammatory cells and cellular debris in the liver of an MAb 23-2:C2-treated and infected mouse on day 2 postinfection. Hematoxylin and eosin stain; magnification, ×350.

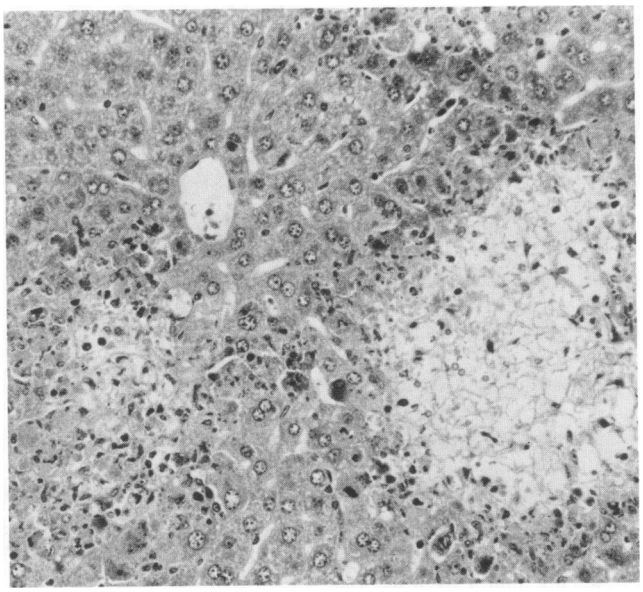

FIG. 4. Severe destruction of hepatocytes with poor cell infiltration on day 4 postinfection in a control infected mouse treated with ascitic fluid without MAb. Hematoxylin and eosin stain; magnification, ×350.

mice, but they also had more remarkable infiltration of mononuclear cells. Thus, MAb 10-1:D2 may not be effective in preventing initial seeding of virus into tissues but might act at a later stage in inhibiting the spread of virus from initial lesions.

Hasony and Macnaughton (3) reported that mice could be immunized with E2 antigen, but not with the E1 or NP, against infection with MHV-3. In the present studies, however, one of the anti-NP MAbs as well as the MAb to E2, with or without VN activity, protected mice from lethal challenge. Such a role of anti-NP antibodies in immunity has not yet been described with any enveloped-virus infections. Recently, Buchmeier et al. (2, 21) and Wege et al. (24) showed that neutralizing MAb to E2 of MHV-JHM immunized animals against lethal MHV-JHM infection. In studies with other enveloped viruses, nonneutralizing MAbs directed against viral glycoprotein immunized mice against lethal infection (1, 8, 9, 14, 16).

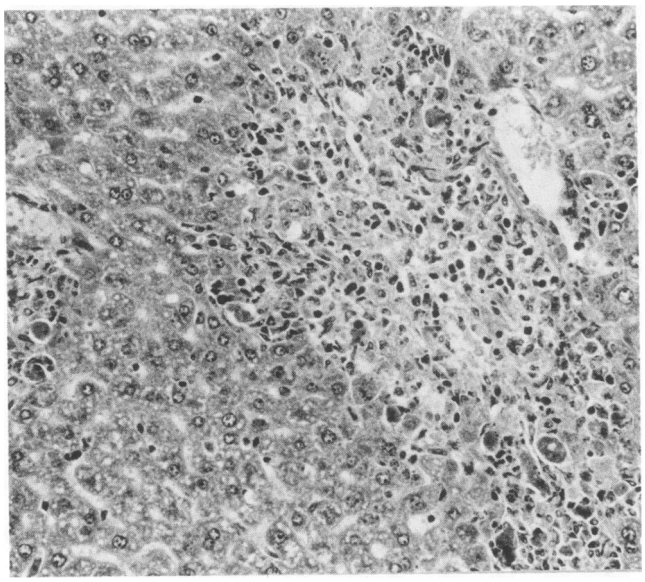

FIG. 3. Focal hepatic necrosis accompanied by mononuclear and polymorphonuclear cell infiltration in an MAb 10-1:D2-treated and infected mouse on day 4 postinfection. Hematoxylin and eosin stain; magnification, ×350.

The MAb to MHV is assumed to bind with the surface of infected host cells, causing cytolysis as in the case of either antibody-dependent cellular cytotoxicity or cytotoxicity mediated by complement-dependent antibody as has been described for various virus infections (13). Although there have been no reports of NP expression on MHV-infected cell surfaces, it might be proposed that anti-NP MAb 10-1:D2 recognizes some NP-related antigens appearing on the surfaces of MHV-2-infected cells, as in case of influenza A virus infection (22, 23, 26), resulting in cytolysis. The severer inflammatory response in the presence of effective MAbs may be due to this type of cytolysis, whereas the inflammatory cells themselves might have an inhibitory effect on virus spreading.

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