JOURNAL OF VIROLOGY, Apr. 1986, p. 107–115
0022-538X/86/040107-09$02.00/0

Vol. 58, No. 1

Monoclonal Antibody Cure and Prophylaxis of Lethal Sindbis Virus Encephalitis in Mice

JEFF STANLEY, SUSAN J. COOPER, AND DIANE E. GRIFFIN*

Departments of Medicine and Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received 7 August 1985/Accepted 10 December 1985

Neuroadapted Sindbis virus (NSV) causes acute encephalitis and paralyzes and kills adult mice unless they are treated with primary immune serum after infection. To study the nature and specificity of curative antibodies, we gave mice 30 different monoclonal antibodies (MAbs) against Sindbis virus (SV) 24 h after lethal intracerebral inoculation of NSV. By the time of MAb treatment, NSV replication in the brain had been well established (7.5×10^7 PFU/g). Seventeen MAbs directed against multiple biological domains on the NSV E1 and E2 envelope glycoproteins prevented paralysis and death. Anticapsid MAbs failed to protect. Altogether, 15 of 17 curative MAbs either neutralized NSV infectivity or lysed NSV-infected cells with complement, but neither ability was necessary or sufficient to guarantee recovery. All 5 protective anti-E2 MAbs neutralized NSV infectivity; 6 of 10 protective anti-E1 MAbs neutralized NSV; 4 did not. Plaque assay or immunohistochemical staining showed that neutralizing and nonneutralizing curative MAbs decreased NSV in the brain, brainstem, and spinal cord. Despite high neutralization titers, hyperimmune anti-SV and anti-NSV mouse sera prevented only 6 and 30% of deaths, respectively, while primary immune sera prevented 50 (SV) and 90% (NSV) of deaths. Secondary intravenous immunization with a live virus apparently diminished, obscured, or failed to boost a class of protective antibodies. When separate mouse groups were given these 30 MAbs 24 h before lethal intracerebral inoculation of NSV, a slightly different set of 17 neutralizing or nonneutralizing anti-E1 and anti-E2 antibodies protected. Two nonneutralizing MAbs and hyperimmune anti-SV serum, which had failed to promote recovery, prophylactically protected 100% of the mice. The antibody requirements or mechanisms of prophylaxis and recovery may differ.

Togaviruses cause sporadic, epidemic, and potentially fatal meningoencephalitis (34). Survival from encephalitis confers immunity to reinfection (21), just as vaccines and immune sera prevent primary infections (14, 31). Because togaviral infections induce abundant neutralizing, hemagglutination-inhibiting, and complement-fixing cytolytic antibodies (8, 34), hyperimmune sera have been used to treat established experimental encephalitis (40); yet the precise roles that antibodies of each biological function play in natural recovery from togaviral infections are unknown.

Neuroadapted Sindbis virus (NSV) causes acute encephalitis and paralyzes and kills adult mice at an intracerebral (i.c.) 50% lethal dose (LD_{50}) of 2 to 20 PFU (17, 38). Twenty-four hours after i.c. inoculation, when brain virus exceeds 10^7 PFU/g, passive transfer of primary immune serum, but not immune spleen or lymph node cells, prevents 60 to 90% of deaths (17, 18). Since sera with similar NSV neutralization titers differ markedly in their protective abilities, neutralizing antibodies alone cannot account for recovery. The nature and viral specificity of these curative serum antibodies and how they prevent death are unknown.

Sindbis virus (SV), from which NSV was derived by mouse brain passage, is an alphavirus, the genus which includes the more virulent arthropod-borne viruses of eastern, western, and Venezuelan equine encephalomyelitis (17, 34). SV contains a single molecule of positive-stranded RNA, which encodes three structural proteins: a 30-kilodalton icosahedral nucleocapsid protein C and two distinct, roughly 50-kilodalton envelope glycoproteins, E1 and E2 (15). Monospecific antiserum to E1 inhibits virus-induced hemagglutination, and antiserum to E2 neutralizes infectivity (11), but monoclonal antibodies (MAbs) to either glycoprotein can inhibit hemagglutination, neutralize infectivity, or both (9, 35, 36, 38). E2 may be an important determinant of virulence (30, 38).

Recently Schmaljohn et al. (35) showed that neutralizing and nonneutralizing anti-envelope glycoprotein MAbs given 24 h before infection can prevent death from lethal NSV encephalitis in mice. Protective MAbs decrease maximal virus in brains at 72 h by up to 10^5-fold, effectively preventing the full establishment of encephalitis. Antibodies given before infection mimic prior vaccination. They may protect by a different mechanism than do antibodies which promote recovery from established encephalitis.

We have used a new array of 30 anti-SV MAbs (38) to answer four questions. (i) Can neutralizing and nonneutralizing MAbs, like primary SV-immune serum, promote recovery from established lethal NSV encephalitis? (ii) Does antibody curative ability correlate with the antigen target, isotype, neutralization titer, hemagglutination inhibition titer, or the ability to lyse NSV-infected cells by antibody-dependent, complement-mediated cytolysis (ADCMC)? (iii) Do antibodies promote recovery by decreasing virus in the brain or spinal cord? (iv) Do the antibody requirements for recovery and immunoprophylaxis differ? We show that anti-E1 and anti-E2 MAbs displaying diverse biological properties can prevent paralysis and death from established, otherwise fatal NSV encephalitis in mice.

MATERIALS AND METHODS

Mice. Four- to five-week-old BALB/c An NrIBR mice of either sex were used (Charles River Breeding Laboratories, Inc., Wilmington, Mass.).

* Corresponding author.

Virus. NSV was twice plaque purified from BHK-21 cells (17, 38). Stock NSV contained 1.5×10^9 PFU/ml and killed 4-week-old mice with an i.c. LD_{50} of 2 PFU.

MAbs and sera. Thirty MAbs against SV strain AR-339 were isolated and characterized as described before (38). Primary mouse immune sera were obtained 10 to 16 days after intravenous (i.v.) injection of 10^7 PFU of either SV or NSV. Hyperimmune sera were obtained 3 days after 10^7-PFU i.v. secondary boosts at 1 month.

ADCMC. L-929 cells were infected with NSV (10 to 20 PFU per cell) for 45 to 60 min, trypsinized, and labeled for 1 to 2 h at 37°C with ^{51}Cr (100 μCi/10^7 cells; Amersham Corp., Arlington Heights, Ill.). Washed cells were suspended in Eagle minimal essential medium (MEM) with 1% heat-inactivated fetal bovine serum and distributed at 2×10^5 cells per 100 μl for each well to 96-well, flat-bottomed microtiter plates (Costar, Cambridge, Mass.). After infection (10 to 11 h), 100 μl of MAb ascites (diluted 1:10 to 1:3,000 in MEM) and 100 μl of freshly diluted (1:7) neonatal rabbit or guinea pig complement (Pel Freez, Brown Deer, Wis.) were added to duplicate wells. After 60 min at 37°C, 100 μl was harvested from each well and counted, and the replicates were averaged. Controls consisted of MEM, MEM plus complement, anti-visna virus MAb ascites (matched for isotype) plus complement, and 1% sodium dodecyl sulfate (total lysis). Percent specific lysis was calculated as $(E - B)/(T - B) \times 100$, where E is counts released per minute by the experimental MAb, B is background counts released per minute by MEM or MEM plus complement, and T is counts released per minute after total lysis. Each MAb was tested two to six times, and the percent specific lysis observed at optimal MAb dilution in each experiment was averaged.

Protection. Mice were inoculated i.c. with 10 LD_{50}s (20 PFU) of NSV in 0.03 ml of Hanks balanced salt solution with 1% fetal bovine serum. Mice were observed for paralysis and death for 21 days. To test recovery, we injected mice i.v. with a single 0.2-ml dose of MAb ascites, anti-SV mouse serum, or anti-NSV mouse serum 24 h after NSV inoculation. To test prophylaxis, we gave mice the same i.v. dose of ascites or sera 24 h before NSV inoculation. MAb ascites fluids contained 5 to 20 mg of immunoglobulin per ml. Anti-visna virus MAb ascites that were matched for isotype, normal mouse serum, and phosphate-buffered saline (PBS; pH 7.4) were passively transferred as negative controls.

CNS virus titrations. On days 1, 3, and 5 after NSV i.c. inoculation, brains and spinal cords were excised, frozen, thawed, homogenized in Hanks balanced salt solution, and serially diluted in Hanks balanced salt solution with 2% fetal bovine serum. Central nervous system (CNS) virus titers were determined by plaque formation on BHK-21 cells (38). Brain and spinal cord NSV titers from three mice were averaged at each time point.

Immunohistochemistry. Primarily biotinylated immunoglobulins were prepared by the method of Leary et al. (22). Infected mice were perfused with periodate-lysine-paraformaldehyde-glutaraldehyde (16). Brains and spinal cords were excised, fixed for 12 h in periodate-lysine-paraformaldehyde-glutaraldehyde, dehydrated, paraffin embedded, and cut into 8-μm sections. Tissues were immunoperoxidase stained by the avidin-biotin-complex method of Hsu et al. (19) as modified by Moench and Griffin (29) with three exceptions: (i) sections were blocked for 30 min in PBS with 2% normal mouse serum, (ii) biotinylated MAbs were diluted 1:50 to 1:250 and incubated for 1 h, and (iii) no secondary antibody was needed.

Statistical analysis. The percentage of survivors in anti-SV MAb treatment groups was compared with the same percentage in control groups treated with isotype-matched, anti-visna virus MAb by means of Fisher's exact two-tailed test, with $P < 0.05$ as the level of minimal significance.

RESULTS

Monoclonal antibodies. Nearly all MAbs bound purified NSV strongly in an enzyme-linked immunosorbent assay (Table 1). In all, 13% were directed against nucleocapsid C, 37% were directed against glycoprotein E1, 40% were directed against glycoprotein E2, and 10% were directed against the E1-E2 heterodimer. About half of the anti-E1 and anti-E2 MAbs neutralized NSV infectivity or inhibited NSV-induced hemagglutination. Nonneutralizing MAbs were defined by the failure to reduce viral plaques by 80% at ascites dilutions exceeding 1:10 (80% plaque reduction neutralization titer [PRNT] ≤ 1.0).

Five anti-E1 MAbs, five anti-E2 MAbs, and SV-immune and NSV-immune sera caused ≥10% specific lysis of NSV-infected L cells in the presence of complement (Table 1). Anti-C MAbs, anti-visna virus MAbs, and normal serum did not lyse infected cells. ADCMC was independent of neutralization and hemagglutination inhibition. A total of 4 nonneutralizing MAbs (3 against E1 and 1 against E2) lysed NSV-infected cells; 6 of 12 IgG-neutralizing MAbs (4 against E1 and 1 each against E2 and the E1-E2 heterodimer) did not. This suggests that neutralization epitopes may or may not be displayed on infected cells in a manner favoring cytolysis or that these antibodies may not fix or activate complement efficiently.

Recovery from lethal encephalitis. To test whether antibodies could promote full recovery from established lethal encephalitis, we gave mice a single dose of MAb ascites or serum 24 h after NSV i.c. inoculation. At the time of antibody treatment, NSV replication in the brain and spinal cord was well established, averaging 7.5×10^7 and 6.3×10^6 PFU/g of tissue, respectively (Fig. 1).

The recovery data show that 98% of 52 infected mice treated with control anticapsid MAbs, anti-visna virus MAbs, or PBS became paralyzed and died (Table 2). By contrast, 82% of 152 infected mice recovered when treated with 17 of the 26 MAbs directed against the NSV envelope glycoproteins. Eleven antiglycoprotein MAbs prevented 100% of deaths. In all, 10 of 11 anti-E1, 5 of 12 anti-E2, and 2 of 3 anti-E1-E2 heterodimer MAbs promoted recovery. Protective MAb isotypes included all immunoglobulin G (IgG) subclasses and one IgM antibody.

Of 17 recovery-promoting MAbs, 12 (70%) neutralized NSV. All 5 protective anti-E2 MAbs but only 6 of 10 protective anti-E1 MAbs blocked infectivity. Five nonneutralizing MAbs prevented deaths, four were targeted against E1, and three lysed NSV-infected cells with complement. Nearly all protective anti-E2 MAbs but only half of the protective anti-E1 MAbs mediated ADCMC. Eight noncytolytic MAbs promoted recovery; all but two neutralized virus. Finally, all recovery-promoting anti-E2 MAbs inhibited hemagglutination, but only 60% of protective anti-E1 MAbs did.

Despite high neutralization titers and marked cytolytic ability, hyperimmune anti-SV and anti-NSV mouse sera (collected 3 days after secondary i.v. immunization) prevented only 6 and 30% of deaths, respectively. Primary immune anti-SV and anti-NSV sera protected better, preventing 50 and 90% of deaths ($P < 0.05$ and $P < 0.002$, respectively, compared with hyperimmune sera). Secondary

TABLE 1. Biological properties of MAbs and hyperimmune sera against NSV

MAb clone or control[a]	Isotype[b]	ELISA (log_{10})[c]	80% PRNT (log_{10})[d]	HI (log_{10})[e]	ADCMC (%)[f]
Anti-capsid C					
1	IgG2A	>5.7	<1.0	–	–
2	IgG2A	ND[g]	<1.0	–	ND
3	IgM	>5.7	<1.0	–	–
4	IgM	4.0	≤1.0	–	–
Anti-E1					
101	IgG2A	5.0	1.0	3.4	28.2
102	IgG2A	5.7	1.0	–	–
103	IgG2A	4.0	2.6	–	61.7
104	IgG2A	>5.7	≤1.0	4.9	22.9
105	IgG3	>5.7	>3.5	5.7	–
106	IgG2B	>5.7	4.1	4.0	–
107	IgG2B	>5.7	3.2	3.4	11.0
108	IgG1	>5.7	3.6	–	–
109	IgG2A	>5.7	≤1.0	–	25.0
110	IgG2B	>5.7	2.6	ND	–
111	IgG2B	>5.7	1.0	4.8	–
Anti-E2					
201	IgG3	>5.7	>3.2	4.9	51.5
202	IgG3	>5.7	>3.8	4.9	64.3
203	IgG2A	5.7	<1.0	ND	ND
204	IgM	≤1.0	<1.0	–	ND
205	IgG2A	4.0	2.3	2.8	15.2
206	IgG2A	>5.7	≤1.0	4.8	20.4
207	IgA	5.7	≤1.0	–	–
208	IgA	4.0	2.3	–	–
209	IgG3	>5.7	>4.2	6.0	10.6
210	IgG3	5.0	3.5	2.8	–
211	IgA	5.0	<1.0	ND	ND
212	IgG1	ND	<1.0	ND	ND
Anti-E1-E2					
301	IgG2A	5.0	3.5	–	–
302	IgM	>5.7	<1.0	–	–
303	IgA	>5.7	<1.0	–	–
Controls					
Anti-visna virus MAbs	IgA	–	<1.0	–	–
	IgM	–	<1.0	–	–
	IgG2A	–	<1.0	–	–
Mouse sera					
SV[h] primary immune		4.0	ND	ND	82.7
SV[h] hyperimmune		5.0	3.7	3.7	57.8
NSV primary immune		4.0	ND	ND	75.8
NSV hyperimmune		4.0	3.4	ND	60.5
Normal		–	<1.0	–	–

[a] Specificity determined by immunoprecipitation of radiolabeled pure virus or infected cell lysates or by protein immunoblot with pure SV (38). Three MAbs were directed against the E1-E2 heterodimer.

[b] Determined by an enzyme-linked immunosorbent assay with the use of isotype-specific rabbit anti-mouse immunoglobulin sera.

[c] ELISA, Enzyme-linked immunosorbent assay. Greatest log_{10} dilution of ascites or sera binding purified NSV to an optical density at 492 nm of ≥0.5.

[d] PRNT, Plaque reduction neutralization titer. Greatest log_{10} dilution of ascites or sera reducing 100 NSV plaques on BHK-21 cells by 80%.

[e] HI, Hemagglutination inhibition. Greatest log_{10} dilution of ascites or sera completely inhibiting NSV-induced hemagglutination (<2.5 was considered negative [–]).

[f] The percent specific NSV-infected L-cell lysis caused by ascites or sera with added complement; <10% was considered negative (–).

[g] ND, Not done.

[h] Strain AR-339.

immunization apparently diminished, obscured, or failed to boost the recovery-promoting antibodies found in primary immune serum.

The timing of MAb transfer was critical. As little as 4 μg of anti-E2 neutralizing immunoglobulin protected 100% of the mice if given 24 h after lethal virus i.c. inoculation (Table 3). Even a 1,000-fold greater immunoglobulin dose could not prevent death if treatment were delayed until 48 or 72 h, though NSV brain titers rose only slightly from 24 to 72 h (Fig. 1).

All six protective MAbs that were tested by plaque assay decreased NSV in brains 10- to 200-fold and in spinal cords 100- to 100,000-fold compared with virus titers in mice given nonprotective MAbs (Fig. 2, day 5). Neutralizing (105, 202, and 209) and nonneutralizing (101, 106, and 109) recovery-promoting MAbs decreased CNS virus equally. Immunoperoxidase staining for NSV (Fig. 3) showed that the protective, neutralizing anti-E2 MAb 209 markedly decreased viral antigens in the pons, medulla oblongata, and (not shown) cortex.

Prophylaxis of lethal encephalitis. By giving mice a single dose of ascites or serum 24 h before lethal NSV i.c. inoculation, we tested whether these MAbs could prevent deaths (Table 2). Eighty percent of infected mice died when treated before infection with anti-C MAbs, anti-visna virus MAbs, PBS, or normal serum. By contrast, 99% of infected mice survived when treated prophylactically with 17 of 26 anti-envelope glycoprotein MAbs; 16 of 17 MAbs prevented all deaths. All 11 anti-E1 MAbs, 6 of 12 anti-E2 MAbs, and 2 of 3 anti-E1-E2 heterodimer MAbs protected. Prophylaxis data from IgM MAbs 4 and 302, showing partial but statistically insignificant protection, were difficult to interpret because control anti-visna virus IgM MAbs nonspecifically prevented half of the deaths. The nature and universality of the nonspecific effect of IgM given before infection is unknown.

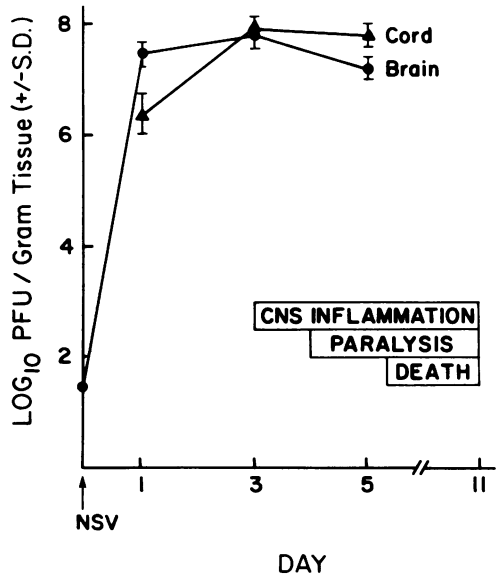

FIG. 1. Acute lethal NSV encephalitis. Mice were inoculated i.c. with 10 LD_{50}s (20 PFU) of NSV and sacrificed, and their brains and spinal cords were assayed for virus on BHK-21 cells. Each point is the average from three mice.

TABLE 2. Immunotherapy of lethal NSV encephalitis[a]

Exptl and control	Antigen	Isotype	Recovery (MAb after NSV)			Prophylaxis (MAb before NSV)			Biological activities		
			No. of mice	% Paralyzed	% Dead	No. of mice	% Paralyzed	% Dead	N[b]	C[c]	HI[d]
Protective											
Recovery and prophylaxis											
108	E1	IgG1	12	58	42	6	0	0	+	−	−
101	E1	IgG2A	6	0	0	11	0	0	−	+	+
102	E1	IgG2A	10	30	30	0	0	0	−	−	−
103	E1	IgG2A	6	0	0	6	0	0	+	+	−
104	E1	IgG2A	6	0	0	6	0	0	−	+	+
109	E1	IgG2A	6	0	0	6	0	0	−	+	−
106	E1	IgG2B	6	0	0	6	0	0	+	−	+
107	E1	IgG2B	6	0	0	6	16	16	+	+	+
110	E1	IgG2B	6	0	0	6	0	0	+	−	+[e]
105	E1	IgG3	10	0	0	11	0	0	+	−	+
201	E2	IgG3	6	0	0	6	0	0	+	+	+
202	E2	IgG3	6	0	0	6	0	0	+	+	+
209	E2	IgG3	24	4	4	12	0	0	+	+	+
210	E2	IgG3	12	17	17	6	0	0	+	−	+
301	E1-E2	IgG2A	9	11	11	6	0	0	+	−	−
Recovery											
205	E2	IgG2A	17	41	35	11	45[f]	45[f]	+	+	+
302	E1-E2	IgM	4	0	0	6	16[f]	16[f]	−	−	−
Prophylaxis											
111	E1	IgG2B	5	100	100	6	0	0	−	−	+
206	E2	IgG2A	6	100	100	6	0	0	−	+	+
Nonprotective											
3	C	IgM	6	100	100	10	80	80	−	−	−
4	C	IgM	6	100	83	6	50[f]	16[f]	−	−	−
1	C	IgG2A	6	100	100	6	100	100	−	−	−
2	C	IgG2A	6	100	100	ND[g]	ND	ND	−	ND	−
207	E2	IgA	10	100	100	6	83	83	−	−	−
208	E2	IgA	6	100	100	12	83	83	+	−	−
211	E2	IgA	6	100	100	ND	ND	ND	−	ND	ND
204	E2	IgM	6	100	100	ND	ND	ND	−	ND	−
212	E2	IgG1	5	80	80	4	75	75	−	ND	ND
203	E2	IgG2A	5	100	100	4	50[f]	50[f]	−	ND	ND
303	E1-E2	IgA	6	100	100	6	100	100	−	−	−
Controls											
PBS			6	100	100	6	100	100	−	−	−
Anti-visna virus MAbs		IgA	6	100	100	6	100	100	−	−	−
		IgM	6	100	100	6	67	50	−	−	−
		IgG2A	10	100	100	6	83	83	−	−	−
Mouse sera											
SV primary immune			10	50	50	ND	ND	ND	ND	+	ND
SV hyperimmune			16	94	94	6	0	0	+	+	+
NSV primary immune			10	10	10	ND	ND	ND	ND	+	ND
NSV hyperimmune			10	70	70	6	0	0	+	+	ND
Normal			6	100	100	6	83	83	−	−	−

[a] Mice were given 0.2 ml of MAb ascites or sera i.v. either 24 h after (recovery) or 24 h before (prophylaxis) i.c. inoculation of 10 LD_{50}s (20 PFU) of NSV. Paralysis and death within 21 days were noted.
[b] N, Neutralizes infectivity.
[c] C, Mediates ADCMC.
[d] HI, Inhibits hemagglutination.
[e] Hybridoma supernatant fluid inhibited hemagglutination; ascites was not titered.
[f] Statistically insignificant protection compared with isotype-matched anti-visna virus MAb control ($P > 0.05$, Fisher's exact two-tailed test).
[g] ND, Not done.

The set of MAbs which promoted recovery differed slightly from the set which prophylactically protected. A total of 15 MAbs (10 anti-E1, 4 anti-E2, and 1 anti-E1-E2 heterodimer) protected both before and after NSV i.c. inoculation. However, two nonneutralizing MAbs (anti-E1 MAb 111 and anti-E2 MAb 206) given before infection prevented all deaths, but no deaths were prevented after infection. Interestingly, SV- and NSV-hyperimmune sera, which had failed to promote recovery when given after infection, prophylactically protected 100% of mice.

TABLE 3. Effect of time of MAb treatment upon recovery[a]

MAb	Dilution	Time of MAb transfer (h)	No. of mice	% Dead
Anti-E2 (209)	None	24	6	0
	1:10	24	6	0
	1:100	24	6	0
	1:1,000[b]	24	6	0
	None	48	6	67[c]
	None	72	4	100
Anti-visna virus	None	24	6	100

[a] 10 LD_{50}s of NSV administered i.c. and followed 24 to 72 h later by a 0.2-ml i.v. injection of ascites undiluted or diluted in PBS.
[b] Approximately 4 μg of immunoglobulin.
[c] $P > 0.05$, insignificant protection.

DISCUSSION

We have shown that single doses of 19 neutralizing or nonneutralizing anti-SV MAbs directed against the E1 and E2 envelope glycoproteins can prevent paralysis and death from established lethal NSV encephalitis in mice if given 24 h before or after infection. Four anticapsid MAbs failed to protect.

The recovery arm of our study differs from previous works showing postinfection antibody-mediated protection from encephalitis caused by alphaviruses (3, 6, 31, 40), flaviviruses (7), herpesviruses (12), and rabies virus (33). In those experiments antibodies were transferred after peripheral rather than i.c. inoculation of viruses and prevented the full establishment of CNS infection, presumably by neutralizing blood-borne virus before its entry into the brain or spinal cord (3, 7). Many protective MAbs are ineffective once a virus invades the CNS (23, 26, 28). By inoculating NSV directly into the brain 24 h before MAb transfer, we allowed NSV replication to reach 7.5×10^7 PFU/g of brain before MAb treatment. Our protocol, like those of recent protection studies on neurotropic influenza A (13) and mumps (39) viruses, more clearly distinguishes antibody-promoted recovery from prevention.

Mechanisms and topography of protection. NSV replicates rapidly in the mouse CNS after i.c. inoculation, and mild viremia persists until 96 h, when serum-neutralizing antibodies are first detected (17, 18; Fig. 1). The brain virus titer falls slightly, but the immune response fails to eradicate NSV before death at 7 to 10 days (17, 18). To prevent paralysis and death, we transferred the MAbs within 24 h of NSV i.c. inoculation, well before anti-NSV antibodies are normally detectable. Protective MAbs against i.c. inoculated mumps (39) and influenza A (13) viruses and against peripherally inoculated St. Louis encephalitis (26) and herpes simplex (12, 26) viruses must similarly be given within 24 to 72 h of infection. Early passively transferred MAbs shorten the humoral immune reaction time of the mouse. Virus assay (Fig. 2) and immunoperoxidase staining (Fig. 3) suggest that protective MAbs, neutralizing or not, promote recovery by either preventing or slowing early NSV spread in the brainstem or spinal cord or by accelerating NSV clearance from the CNS.

Knowledge of the in vitro biological abilities of a MAb does not necessarily imply knowledge of the mechanism by which it promotes recovery in vivo. Nonetheless, 88% of our curative MAbs modulated at least one important antiviral activity potentially related to protection—neutralization, hemagglutination inhibition, or ADCMC (Table 2). Rare protective MAbs against NSV (e.g., 102) or against the related Semliki Forest alphavirus (6) show none of these activities.

The NSV E1 and E2 glycoprotein biological domains modulating recovery and prophylaxis were topographically mapped (Fig. 4). Nearly all anti-E1 MAbs targeted against all four E1 domains prevented death; no nonprotective NSV E1 domain was detected by these MAbs or in prophylactic protection experiments by Schmaljohn and colleagues (10, 35, 36). Fewer anti-E2 MAbs protected, and these MAbs were topographically restricted for recovery (domains II_A and III) and prophylaxis (domains II_A, III, and II_B).

Four types of antibodies promoted recovery: (i) neutralizing cytolytic (35%; prototype MAbs 103 and 201), (ii) neutralizing noncytolytic (35%; prototype MAbs 105 and

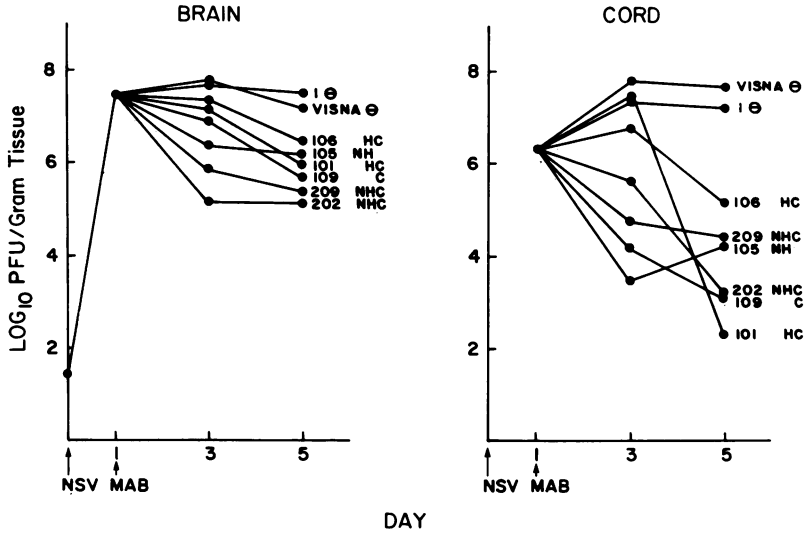

FIG. 2. Reduction of CNS virus by protective MAbs. Mice were treated with nonprotective (Θ) (anti-C or anti-visna virus MAbs) or protective neutralizing and nonneutralizing MAbs 24 h after 10 LD_{50}s of NSV i.c. NSV in the brains and spinal cords of three mice in each treatment group was determined by plaque assay. MAb properties are marked as neutralizing (N), hemagglutination inhibiting (H), or ADCMC mediating (C).

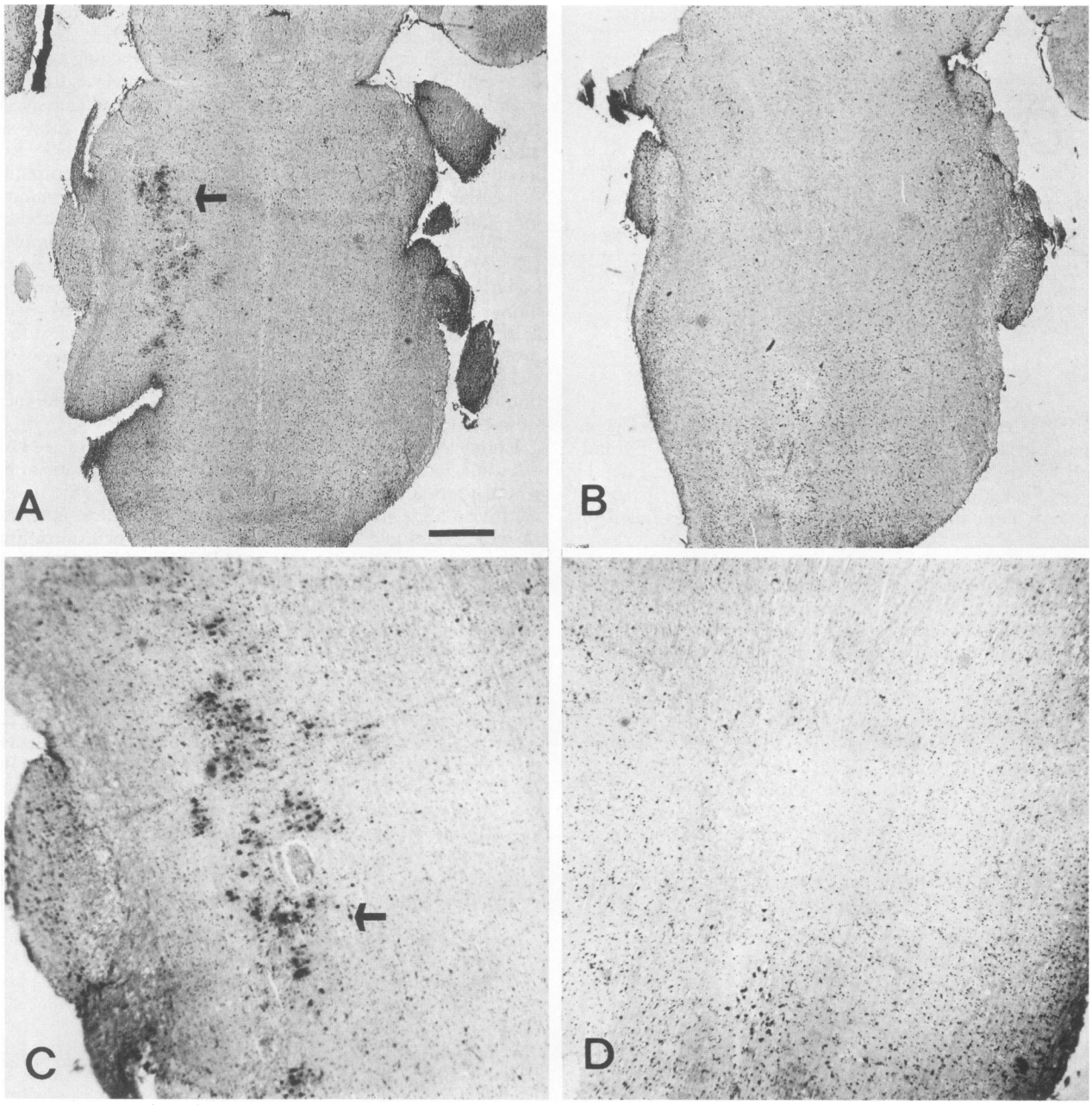

FIG. 3. Immunoperoxidase staining for NSV capsid antigen in brainstem. Twenty-four hours after 10 LD_{50}s of NSV were inoculated i.c., mice were given either nonprotective visna virus MAb (A and C) or protective anti-E2 MAb 209 (B and D). Five days after infection the pons and medulla oblongata of the unprotected mouse (A and C) showed diffuse NSV antigens (black reaction product, arrows) nearly absent from the MAb-protected brainstem (B and D). Magnification for horizontal sections A and B, ×10; for C and D, ×35. Bar, 1 mm.

210), (iii) nonneutralizing cytolytic (18%; prototype MAb 101), and (iv) nonneutralizing noncytolytic (12%; prototype MAb 102). For two MAbs, neutralizing or cytolytic ability was insufficient to guarantee recovery; when given after infection, neutralizing MAb 208 (anti-E2 IgA) and cytolytic MAb 206 (anti-E2 IgG2A) prevented no deaths. Two neutralizing, nonprotective IgG2A MAbs have also been isolated against St. Louis encephalitis flavivirus (26). We conclude that (i) protective anti-E2 MAbs are topographically restricted and predominantly neutralizing, hemagglutination inhibiting, and cytolytic; and (ii) protective anti-E1 MAbs are targeted against all topographical biological domains and may or may not neutralize NSV, inhibit hemagglutination, or lyse NSV-infected cells.

Schmaljohn and colleagues (10, 35, 36) have shown that prophylactically protective, nonneutralizing SV MAbs are directed against multiple conserved E1 epitopes that are displayed on NSV-infected cells but that are cryptic on intact virions (36). This suggests that protection by nonneutralizing MAbs might be due to ADCMC. However, genetically C5-deficient mice and cobra venom factor C3-depleted mice are still completely protected by their non-

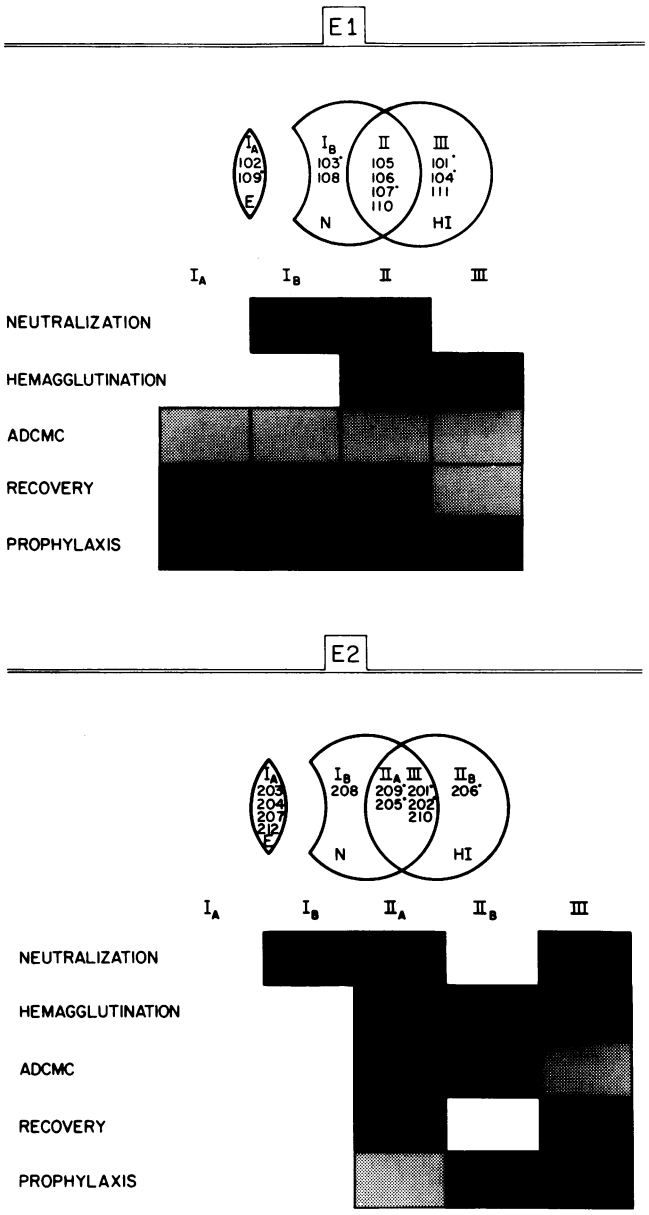

FIG. 4. NSV topography of protection. Biological domains of NSV envelope glycoproteins E1 and E2 were mapped with the use of MAbs to neutralizing (N), hemagglutination-inhibiting (HI), or enzyme-linked immunosorbent assay-binding (E) sites only. MAbs mediating complement-dependent lysis of NSV-infected cells (ADCMC) are marked by an asterisk. Recovery MAbs prevented death if given 24 h after lethal NSV inoculation; prophylaxis MAbs prevented death if given 24 h before inoculation. ■, all MAbs; ▨, some but not all MAbs. Each domain may contain more than one discrete epitope.

neutralizing MAbs (10, 35) as they are by anti-SV immune serum (17), so ADCMC alone cannot explain in vivo protection. Nonneutralizing anti-envelope glycoprotein MAbs have also been shown to prophylactically prevent fatal encephalitis from peripherally inoculated Semliki Forest (4, 5), western equine encephalitis (20), Venezuelan equine encephalitis (25, 27, 32), St. Louis encephalitis (26), herpes simplex (1), and vesicular stomatitis (23, 35) viruses. Protection by anti-herpes simplex virus type 2 MAbs correlates best with the ability to mediate antibody-dependent cellular cytotoxicity (1). This cytotoxicity requires Fc-receptor-bearing mononuclear cells (24, 37). Nonneutralizing MAbs against Semliki Forest (4) and vesicular stomatitis (23) viruses, nonneutralizing and some neutralizing MAbs against Venezuelan equine encephalitis virus (27), and immune serum against SV (17) require intact Fc portions of their immunoglobulin fractions to protect. Anti-SV serum protection also requires a cyclophosphamide-sensitive, non-T-cell population (17). Antibody-dependent cellular cytotoxicity would explain why Fc fragments are obligatory and yet why protection is unaffected by complement depletion. Neutralizing $F(ab')_2$ fragments from some protective alphaviral MAbs may (4) or may not (27) continue to protect. This suggests that neutralizing alphaviral MAbs may protect by at least two different mechanisms, one requiring an intact Fc portion of the immunoglobulin.

Prophylaxis versus recovery. Nearly all neutralizing and nonneutralizing anti-E1 or anti-E2 MAbs which prevent death do so whether given before or after infection. But the antibody requirements or mechanisms of prophylaxis and recovery may differ slightly. When given 24 h before infection, two nonneutralizing MAbs (111 and 206) and hyperimmune SV serum prevented 100% of deaths, but when given 24 h after infection, the MAbs and serum prevented virtually no deaths (Table 2). Lefrancois similarly found that none of his prophylactically protective MAbs protected mice against fatal vesicular stomatitis virus encephalitis if MAb treatment was delayed just 2 h after viral i.v. inoculation (23). MAb-mediated recovery may require more antibodies, higher avidity antibodies, or antibodies directed more precisely at critical biological epitopes on intact virions or infected cell surfaces.

Implications for vaccines and immunotherapy. Our results, together with other recent MAb protection studies (4–6, 10, 20, 25–27, 35, 36), suggest two alphaviral vaccine strategies and the potential benefits of immunotherapy. Because alphaviruses show E1 RNA homology, some anti-E1 MAbs cross-protect without cross-neutralizing by binding conserved E1 epitopes displayed on infected cell surfaces (2, 8, 10, 20, 35, 36). E2 is the least genetically conserved alphaviral structural protein (2, 15) and predominantly elicits type-specific antibodies (34). Though multiple MAbs against either E1 or E2 can prevent death from encephalitis, neutralizing anti-E2 MAbs tend to protect more efficiently than anti-E1 MAbs (4, 5, 32). This suggests that infectious attenuated alphaviral vaccines might induce both neutralizing and cross-protective antibodies (35). Synthetic E2 vaccines might elicit highly efficient neutralizing antibodies but protect only against the homologous virus or strain. Synthetic E1 vaccines might protect, albeit less efficiently, against a wider range of alphaviruses.

Togavirus infections of the heart and brain can kill domestic animals and humans (21, 34). Early treatment with hyperimmune serum has sometimes been beneficial in experimental infections (40). Our results suggest that for mice, single pharmacologically reasonable doses of anti-E1 or anti-E2 MAbs can reduce NSV in the CNS, prevent paralysis, and promote full recovery from even vigorously established, otherwise fatal viral encephalitis.

ACKNOWLEDGMENTS

We thank Querubin Mendoza, Marcia Lyons, and Joseph Giffels for technical help; Linda Kelly and Nancy Michel for typing the manuscript; Diane Summers and Nancy Held for artwork; and Hazel Reddick and Gloria Barber for caring for our mice. J.S. thanks

Thomas R. Moench and Joshua M. Farber for sharing ideas; Theresa A. Pluth, Winifred Hampton Stanley, and Howard Stanley for their encouragement; and John A. Bartlett and Richard T. Johnson for support.

These experiments were supported by Public Health Service grants NS-18596 and NS-07000 from the National Institutes of Health.

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