JOURNAL OF VIROLOGY, Feb. 1985, p. 651–657
0022-538X/85/020651-07$02.00/0

Vol. 53, No. 2

Neutralizing Monoclonal Antibodies to Theiler's Murine Encephalomyelitis Viruses

SORACHAI NITAYAPHAN, MARCIA M. TOTH, AND RAYMOND P. ROOS*

Department of Neurology, University of Chicago Medical Center, Chicago, Illinois 60637

Received 25 June 1984/Accepted 10 October 1984

Theiler's murine encephalomyelitis viruses (TMEV) are serologically related picornaviruses which cause both enteric and neurological disease in mice. The biological activities of TMEV vary between the two different TMEV subgroups (TO and GDVII) and with different passage histories of the same TMEV strain (e.g., mouse brain-passed versus tissue culture-passed DA strain of the TO subgroup). We raised neutralizing monoclonal antibodies (mAbs) against tissue culture-passed DA and GDVII strains of TMEV. We produced two mAbs against the DA strain which neutralized all members of the TO subgroup, but not the GDVII subgroup strains (GDVII and FA); these two DA mAbs reacted similarly with both mouse brain-passed DA and tissue culture-passed DA. Of six neutralizing GDVII mAbs, four reacted only to GDVII and FA, whereas two neutralized TO strains as well. These mAbs demonstrate the presence of TMEV group-specific as well as subgroup-specific neutralization and substantiate the division of TMEV into two distinct subgroups. On Western immunoblots one of the two DA mAbs reacted against isolated DA VP1, two GDVII mAbs (which were TMEV group specific) reacted against isolated GDVII VP1 and DA VP1, and the other DA mAb and four other GDVII mAbs required an intact virion conformation for reactivity. An analysis of the epitopes recognized by these mAbs may elucidate sites important in TMEV biological activities.

The biochemical determinants of in vivo biological activities of viruses are poorly understood. Theiler's murine encephalomyelitis viruses (TMEV), a group of serologically related picornaviruses, provide a particularly attractive experimental model system to help elucidate this issue because of the easily manipulated murine experimental host. TMEV produce several types of neurological disease in mice; the biological activities of the viruses (neurovirulence, neurotropism, persistence, and demyelination) vary between the two different TMEV subgroups (TO and GDVII) (13, 19) and between different passage histories of the same TMEV strain (e.g. mouse brain-passed versus tissue culture-passed DA strain of the TO subgroup) (10, 15, 30).

There is some disagreement in the literature regarding the extent of biochemical difference between the two TMEV subgroups. Lorch et al. (18, 19a) claim that nonstructural and structural proteins of all TMEV strains are extremely similar by two-dimensional protein gel electrophoresis and tryptic peptide mapping, whereas Lipton et al. (17) recently reported some differences between proteins of the two subgroups by two-dimensional gel electrophoresis. The RNase T1 oligonucleotide two-dimensional maps of the two subgroups substantially differ from one another (19, 25); however, Lorch et al. (19a) found that the double-stranded RNAs of DA and GDVII had very similar denaturation maps by electron microscopy.

Monoclonal antibody (mAb) technology offers a powerful tool to define differences between the two TMEV subgroups and to delineate viral epitopes critical in determining important in vivo biological activities. We chose to select for and to specifically evaluate neutralizing mAbs because the neutralization epitopes are major antigenic sites and because they play a key role in host immune defenses. We describe two DA strain mAbs and six GDVII strain mAbs that display either TMEV group-specific or subgroup-specific neutralization, supporting the separation of TMEV into two subgroups (13). Several of the TMEV-neutralizing mAbs require an intact virion conformation as an antigenic target, whereas the other neutralizing mAbs are directed against VP1. These epitopes may function in other subgroup-specific biological activities, such as neurovirulence, persistence, and demyelinating activity.

MATERIALS AND METHODS

Viruses. Strains belonging to the two TMEV subgroups, the relatively less virulent TO subgroup (which includes the DA, TO, WW, and Yale strains) and the highly virulent GDVII subgroup (the GDVII and FA strains), were used in the study. Tissue culture-adapted DA and GDVII (obtained from J. Lehrich) and FA, TO, WW, and Yale (obtained from H. Lipton) were grown in BHK-21 cells to prepare stocks. The growth, purification, and plaque assay of TMEV have been previously described (13, 25–27). Mouse brain-passed DA virus (DAMB) was propagated in suckling C3H/HeJ mouse (Jackson Laboratories) brain.

Preparation of hybridoma cell lines. To prepare DA-neutralizing mAbs, 3-week-old SJL/J mice (Jackson Laboratories) were inoculated intracerebrally with 1.3×10^5 PFU of tissue culture-passed DA virus (DATC). Fusion was performed after approximately 6 months, when mice were significantly weak due to chronic demyelination; for a second fusion, the animals were intravenously and intraperitoneally boosted with 5 μg of purified DATC 3 days before the fusion. To prepare GDVII-neutralizing mAbs, 8×10^6 PFU of GDVII were mixed in an equal volume with complete Freund adjuvant (Difco Laboratories) and inoculated into the footpads of 6-month-old BALB/c mice (Jackson Laboratories). One month later the same amount of virus was mixed in an equal volume with incomplete Freund adjuvant (Difco Laboratories) and inoculated subcutaneously. One month

* Corresponding author.

after this, and 3 days before fusion, 4×10^7 PFU of GDVII were inoculated intraperitoneally.

The spleen cells were fused with murine SP2/0-Ag14 myeloma cells according to the method of McKearn et al. (20). Hybrids were selectively grown in Dulbecco modified minimal essential medium with 20% agammaglobulinemic horse serum with hypoxanthine-aminopterin-thymidine and screened by an enzyme-linked immunosorbent assay (ELISA) (28) and a microneutralization test (4). Hybridomas of interest were cloned twice by limiting dilution on rat thymocyte feeder layers. Ascites was produced in nude mice (Harlan Sprague-Dawley Inc.) by injecting 2×10^7 hybridoma cells intraperitoneally after 2,6,10,14-tetramethylpentadecane (Pristane) priming.

Monoclonality of the antibodies was verified by isoelectric focusing on polyacrylamide gel plates (LKB Instruments Inc.) followed by immunofixation as previously described (24), except that anti-mouse immunoglobulin G (IgG) serum (heavy and light chain specific) (Cooper Biomedical-Cappel) was used as the overlay. The immunoglobulin subclass was determined by immunodiffusion against anti-mouse IgG subclass antiserum (Miles Laboratories) or by a "dot" immunoblot with immunoperoxidase staining. For the latter assay, 2 μl of hybridoma supernatant was directly applied to nitrocellulose paper, dried, and processed for immunostaining as described before (29), with the following modifications. The first overlay was the mouse subclass antiserum and the second overlay was peroxidase-conjugated goat anti-rabbit IgG serum (Cooper Biomedical-Cappel). The immunoblot was developed with diaminobenzidine and hydrogen peroxide.

Neutralization and hemagglutination (HA) tests. Plaque-reduction neutralization tests were performed as previously described (13). The mAb was generally run at serial 10-fold dilutions against 100 PFU of virus. The neutralization titer of the mAb corresponded to the reciprocal of the highest dilution which neutralized 50% of the plaques. Control hybridoma supernatants and ascites were always run in the assay.

A micro-HA test was performed with twofold dilutions of virus mixed with 0.4% human type O erythrocytes in phosphate-buffered saline. After incubation at 4°C for 2 h, 4 HA units were determined. The HA inhibition (HI) titer of the mAb was determined by mixing twofold dilutions of mAb with 4 HA units of the virus for 1 h at room temperature followed by addition of erythrocytes and a further 2-h incubation at 4°C. The HI titer was the reciprocal of the highest dilution which inhibited HA.

Immunocytochemical staining. Unfixed frozen brain sections from moribund suckling mice infected with DAMB were analyzed by a peroxidase-antiperoxidase staining technique as previously described (23), with the following modifications. The first overlay was mouse mAb, the second overlay was goat anti-mouse IgA, IgM, and IgG serum (Cooper Biomedical-Cappel), and the third overlay was mouse peroxidase-antiperoxidase (Accurate Chemical and Scientific Corp.). Frozen brain sections from uninoculated mice were used as a control.

Western blots. Western blots were used to delineate the epitope of the mAbs. For experiments with DATC and GDVII, purified DATC viral capsid proteins or proteins from TMEV-infected BHK-21 cells were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12.5% polyacrylamide gels (9), transferred to nitrocellulose paper by electroelution, and processed for immunoblotting as previously described (29), with the following modifications. The first overlay of the blot was mouse antibody and the second overlay was peroxidase-conjugated sheep anti-mouse IgG serum (heavy and light chain specific) (Cooper Biomedical-Cappel). The immunoblot was developed with diaminobenzidine and hydrogen peroxide. Prestained protein molecular weight standards (Bethesda Research Laboratories) were also electrophoresed and blotted to verify the identity of virion proteins. In one experiment the electrophoresed gel was renatured as previously described (3) and then transferred.

Western blots were also used to delineate the epitopes of DA mAbs on DAMB. For some experiments, DAMB was concentrated and partially purified by means of affinity column chromatography. IgG from one DA mAb was ammonium sulfate precipitated, purified on a DEAE–Affi-Gel Blue (Bio-Rad Laboratories) column, and then coupled to cyanogen bromide-Sepharose 4B beads (Pharmacia Fine Chemicals, Inc.). A 10% clarified brain homogenate from moribund DAMB-infected C3H/HeJ suckling mice was passed through an mAb cyanogen bromide-Sepharose column. The column was extensively washed, and then the beads from the column were boiled for 3 min in sample buffer and quickly centrifuged; the supernatant was subjected to SDS-PAGE and processed for a Western immunoblot as described above. In other experiments the unpurified DAMB brain homogenate was directly run on an SDS-polyacrylamide gel and then immunoblotted.

RESULTS

Preparation and preliminary characterization of mAbs. Spleens from DATC chronically infected and GDVII-immunized mice were separately fused with mouse myeloma cells. A summary of our fusion results immediately follows, and the details of the fusion are given in the three paragraphs which follow. Two DA-neutralizing mAbs (DA mAb1 and -2) and six GDVII mAbs (GDVII mAb 1 to 6) were cloned (see Table 1) and then inoculated into nude mice to produce ascites fluid. Isoelectric focusing with immunofixation demonstrated the monoclonality of the mAbs; all of the mAbs had unique isoelectric focusing patterns. By immunodiffusion or dot immunoblot assay, the subclass of the mAb was identified (see Table 1); one mAb could not be characterized regarding the subclass, presumably due to deficiencies in the typing antiserum.

In the first fusion, with spleens from DATC chronically infected SJL/J mice, approximately 1,000 hybridomas grew and were screened by an ELISA. Thirteen ELISA-positive hybridomas were identified, but only one (DA mAb1) was found to have neutralizing activity against DA. The other 12 hybridomas were believed to be false-positive clones since they non-specifically bound to many "irrelevant" proteins.

The second fusion involved DATC chronically infected SJL/J mice which had been boosted with virus. Because of large numbers of false-positives obtained with the ELISA, we screened with both a microneutralization and an HI test. Of approximately 150 hybridomas which grew and were screened, one DA-neutralizing mAb (DA mAb2) and one HI mAb (without neutralizing activity) were identified and cloned.

For the GDVII fusion, with spleens from GDVII-hyperimmunized BALB/c mice, 2,000 hybridomas grew and were screened by a microneutralization test. Of 50 neutralizing hybridomas identified, 12 were cloned and studied. Six of the 12 mAbs (GDVII mAb 1 to 6) had a unique isoelectric focusing pattern and were studied further. The reasons for the more successful fusion from spleens of mice inoculated

TABLE 1. Properties of TMEV-neutralizing mAbs[a]

mAb	Neutralization and HI activity[b]		Western blot reactivity[c]	
	TO (DA)	GDVII	DA	GDVII
DA mAb1	+ (>10^5)	−	−	−
	+ (>2^{12})	−		
DA mAb2	+ (>10^3)	−	+ (VP1)	−
	−	−		
GDVII mAb1 and -2	+ (>10^3)	+ (>10^3)	+ (VP1)	+ (VP1)
	ND	ND		
GDVII mAb3–6	−	+ (>10^1–10^3)	−	−
	ND	ND		

[a] The IgG subclasses of the mAbs are as follows: DA mAb1, IgG3; DA mAb2, not determined; GDVII mAb1 and -2, IgG2a; GDVII mAb3, -4, -5, and -6, IgG2a, IgG3, IgG2b, and IgG1, respectively. The + sign signifies activity; the − sign signifies no activity. ND indicates that the assay was not done.

[b] The neutralization and HI titer is indicated for multiple strains of the TO and GDVII subclasses. The particular strains tested are given in the text. The titers of the individual mAbs were identical for all strains tested within the same subgroup.

[c] Western blot reactivity to either DA or GDVII viral proteins; see text for details.

with GDVII rather than with DA may relate to the different strains of mice which were involved (BALB/c versus SJL/J), the more efficient hyperimmunization of the GDVII-inoculated mice, or increased technical expertise for this third fusion.

Activity of mAbs against tissue culture-passed TMEV. The results of plaque reduction neutralization tests against tissue culture-passed TMEV strains are shown in Table 1. DA mAb1 neutralized all of the four TO subgroup strains (DA, TO, WW, Yale) and DA mAb2 neutralized all of the three TO subgroup strains (DA, TO, WW) which were tested. There was no evidence of neutralization, however, to the GDVII subgroup strains (GDVII, FA). Thus, the two neutralizing DA mAbs were TO subgroup specific. DA mAb1 had a high titer of HI activity to all members tested (DA, TO, WW, Yale) of the TO subgroup of viruses but not to the GDVII subgroup (Table 1). In contrast, DA mAb2 did not inhibit HA of DA (the only strain tested), demonstrating that the mAbs were directed against different epitopes. The variable relationship between HI and neutralization was supported by our finding of another DA mAb which had HI activity but no neutralizing activity.

All six GDVII-neutralizing mAbs cross-reacted with FA, the other member of the GDVII subgroup. Two GDVII mAbs (GDVII mAb1 and -2) neutralized the DA and WW strains (the only TO subgroup members tested), whereas the other four GDVII mAbs did not, demonstrating both TMEV group-specific and subgroup-specific neutralization.

Biochemical characterization of epitopes on tissue culture-passed TMEV. To biochemically delineate the neutralizing epitopes, we used Western immunoblots. For the DA mAbs, purified DATC virion capsid proteins were separated by SDS-PAGE and transferred to nitrocellulose paper (Fig. 1). Lane A shows the viral capsid proteins on an amido black-stained blot. Lane B, which was overlaid with DA mAb2 followed by peroxidase-conjugated anti-mouse IgG serum, showed strong immunoperoxidase staining of VP1. In contrast, DA mAb1 ascites failed to stain the blotted capsid proteins (lane C). Renaturation of the gel before transfer also failed to demonstrate immunostaining by DA mAb1 (data not shown). The absence of staining by DA mAb1 indicates that this mAb does not react with SDS-disrupted virion structural polypeptides. In other experiments, we found that DA mAb1 immunoprecipitated undisrupted whole virus (data not shown), confirming DA mAb1 reactivity with intact virus.

For GDVII mAbs, proteins from GDVII-infected BHK-21 cells were separated by SDS-PAGE and transferred to nitrocellulose paper (Fig. 2). Lane A, which was overlaid with a GDVII hyperimmune serum, shows staining of a number of proteins, including VP1 and VP2. GDVII mAb1 and -2 (lanes B, C) stained VP1, whereas the other four GDVII mAbs (lanes D to G) failed to stain any of the viral proteins. GDVII mAb1 and -2 cross-reacted with DA as demonstrated by immunostaining of DA VP1 (Fig. 3B) (and cross-neutralization of DA); however, DA mAb2, which reacted against DA VP1, did not immunostain GDVII VP1 (Fig. 3A).

Activity of DA mAbs against DAMB. Since the biological activities of DA differ depending on the passage history (10, 15, 30), we were interested in determining whether DA mAb1 and DA mAb2 reacted against DAMB, as well as DATC. Both mAbs neutralized plaques formed by DAMB on L cells with as high a titer as with DATC. The reaction of the mAbs against DAMB in vivo was confirmed by immunocytochemical studies. Both mAbs stained brain tissue sections from DAMB-infected suckling mice as demonstrated by an immunoperoxidase reaction (Fig. 4). The staining showed a similar cytoplasmic distribution with both mAbs. There was no staining of uninfected mouse brain or spinal cord sections. This latter observation was of interest since an autoimmune contribution to a chronic murine demyelinating disease which develops after DA intracerebral inocula-

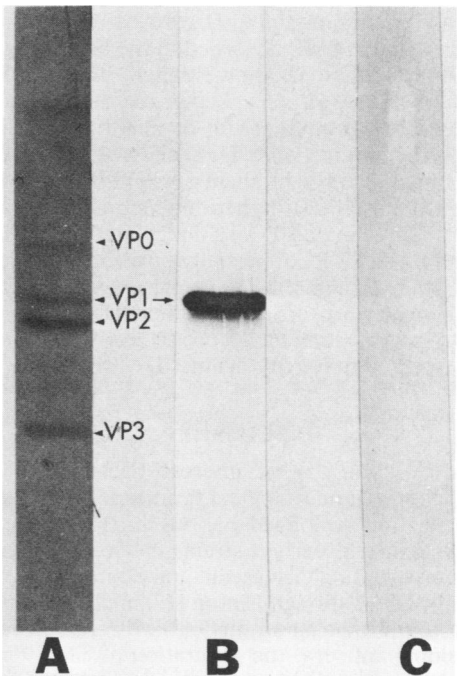

FIG. 1. Immunoblot analysis of DA mAbs. DATC virion capsid proteins were run on SDS-PAGE, blotted to nitrocellulose paper, and then amido black stained (A) or overlaid with DA mAb2 (B) or DA mAb1 (C). Staining of VP1 is seen with DA mAb2. A contaminating protein is seen in lane A above VPO.

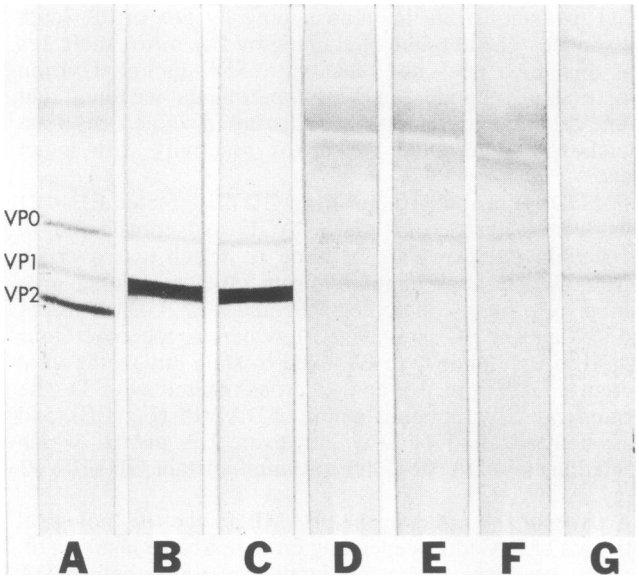

FIG. 2. Immunoblot analysis of GDVII mAbs. Proteins from GDVII-infected BHK-21 cells were run on SDS-PAGE, blotted to nitrocellulose paper, and overlaid with GDVII hyperimmune serum (A) or GDVII mAb1 to -6 (B to G). Staining of VP1 is seen with GDVII mAb1 and -2.

tion has been hypothesized (on the basis of the briskness of the immune reaction at the time of the demyelination and the results of immunosuppression studies) (10, 11, 14); the lack of staining of normal tissue demonstrates that these two mAbs do not apparently cross-react with normal tissue.

To delineate the epitopes of mAbs on DAMB, virion capsid proteins were Western blotted. DAMB virus was partially purified by passing DAMB-infected brain homogenate over a column containing DA mAb1 coupled to Sepharose beads. Both DAMB adsorbed to the beads and unpurified DAMB brain homogenate were then run on SDS-PAGE and immunoblotted (Fig. 5). Lanes overlaid with control mAb (lanes E, J), normal serum control (lanes F, K), and DA mAb1 supernatant (lanes D, I) showed staining of only mouse IgG heavy and light chains, whereas DA mAb2 also stained VP1 (lanes C, H); hyperimmune mouse serum from chronically demyelinated DATC-inoculated mice (lanes B, G) stained VP1 and VP2 of partially purified DAMB, as well as several other proteins in the crude DAMB homogenate. Thus, both mAbs are directed against DAMB as well as DATC; DA mAb1 immunoadsorbs DAMB, whereas DA mAb2 has specific reactivity against DAMB VP1.

DISCUSSION

The TMEV group is of interest because the viruses produce a variety of neurological syndromes, certain TMEV strains persist for prolonged periods in the mouse central nervous system, and certain strains cause an experimental chronic demyelinating disease in mice similar to multiple sclerosis (10, 11). Although Lipton (12) and we (unpublished data) have found that all strains of TMEV cross-neutralize with polyclonal antisera, the separation of TMEV into two subgroups has been advocated on the basis of the following differences in biological properties (13). The TO subgroup, typified by the DA strain, grows to a relatively low titer, produces small plaques (13), appears ultrastructurally to line up on infected cell membranes in tissue culture (8), and produces a chronic progressive demyelinating disease in weanling mice with persistence of the virus for months (10, 11). The GDVII subgroup is highly virulent, grows to high titer, produces large plaques (13), forms crystalline arrays of virions in tissue culture (8), and produces acute encephalomyelitis with no virus persistence in weanling mice (13).

We produced two mAbs against the DA strain which reacted by neutralization (and by HI as well in the case of DA mAb1) to all members tested of the TO subgroup and not to the GDVII subgroup strains. Of six neutralizing GDVII mAbs, four reacted only to GDVII and FA, whereas two neutralized TO subgroup strains as well. These mAbs demonstrate the presence of TMEV group-specific as well as subgroup-specific neutralization and substantiate the division of TMEV into two distinct subgroups.

We investigated whether the DATC mAbs cross-reacted with DAMB because DAMB and DATC have different phenotypes. DAMB produces a biphasic disease in weanling animals with an initial, early acute polioencephalomyelitis followed by late demyelination; DATC only produces a late demyelinating disease (10, 15, 30). In addition, DAMB has a high 50% lethal dose in suckling mice with a relatively low 50% tissue culture infectivity dose, whereas DATC has a high 50% tissue culture infectivity dose with a relatively low 50% lethal dose (27, 30). In vitro DAMB plaque reduction neutralization tests and immunocytochemical studies demonstrated that the epitopes of the two mAbs were present on DAMB as well as DATC and indicated that DA mAb1 and -2 do not distinguish between DATC and DAMB. It is of course possible that the epitopes of the two mAbs are not present on the whole DAMB virus population since this heterogeneous virus stock is obtained from infected mouse brain without subsequent plaque purification.

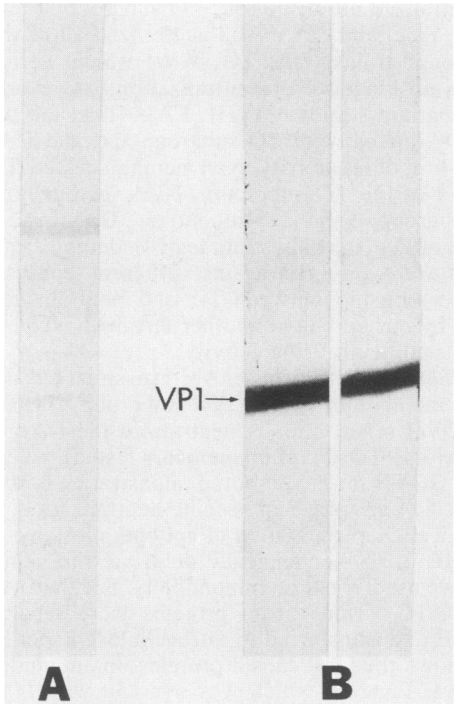

FIG. 3. Immunoblot analysis of cross-reactivity. GDVII (A) and DATC (B) virion capsid proteins were run on SDS-PAGE, blotted to nitrocellulose, and overlaid with DA mAb2 (A) or GDVII mAb1 and -2 (B). Staining of VP1 is seen with GDVII mAbs.

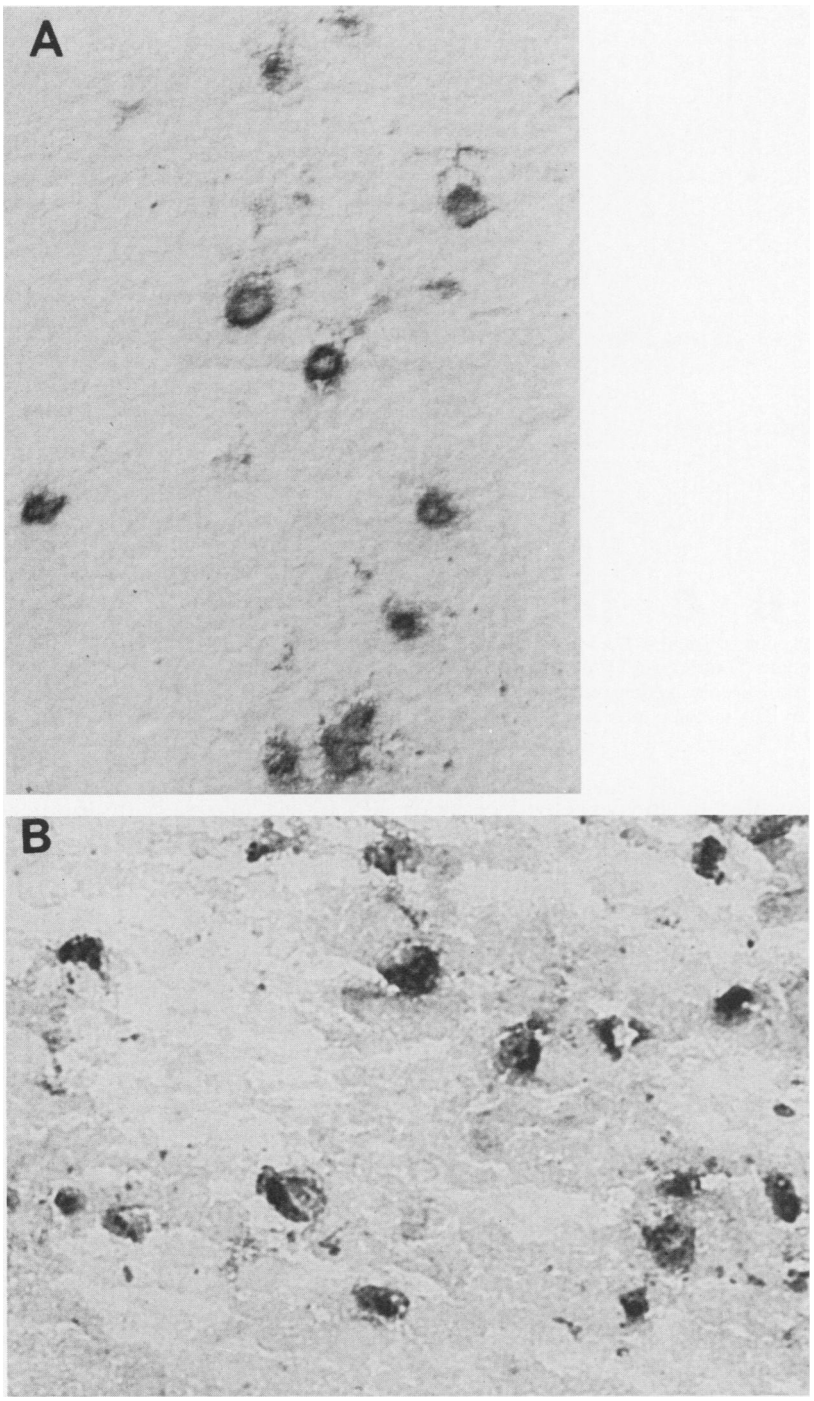

FIG. 4. Immunoperoxidase staining of DAMB with mAbs. Frozen sections of brains of suckling mice, inoculated 5 days previously with DAMB intracerebrally, were overlaid with DA mAb1 (A) or DA mAb2 (B) and then processed with a peroxidase-antiperoxidase immunochemical staining technique. Cytoplasmic staining antigen is seen in neural cells.

We used Western immunoblots to characterize the neutralization epitopes. One DA mAb and two GDVII mAbs reacted with isolated VP1, whereas the other DA mAb and the four other GDVII mAbs required an intact virion conformation. Our data demonstrate at least four antigenic sites for TMEV neutralization: two different sites, one unique to DA and one unique to GDVII, are present only on the intact virion; a third site is present on isolated DA VP1 but not GDVII VP1; and a fourth is present on both isolated GDVII VP1 and DA VP1. Sites present on DA virus are presumably present on all members of the TO subgroup, and those present on GDVII virus are presumably on both members of the GDVII subgroup.

The potential importance of TMEV-neutralizing antigenic sites in determining persistence, virulence, and demyelination is suggested by our finding that these biological properties segregate into the same subgroups defined by several of the neutralizing mAbs. It is not difficult to envision how the

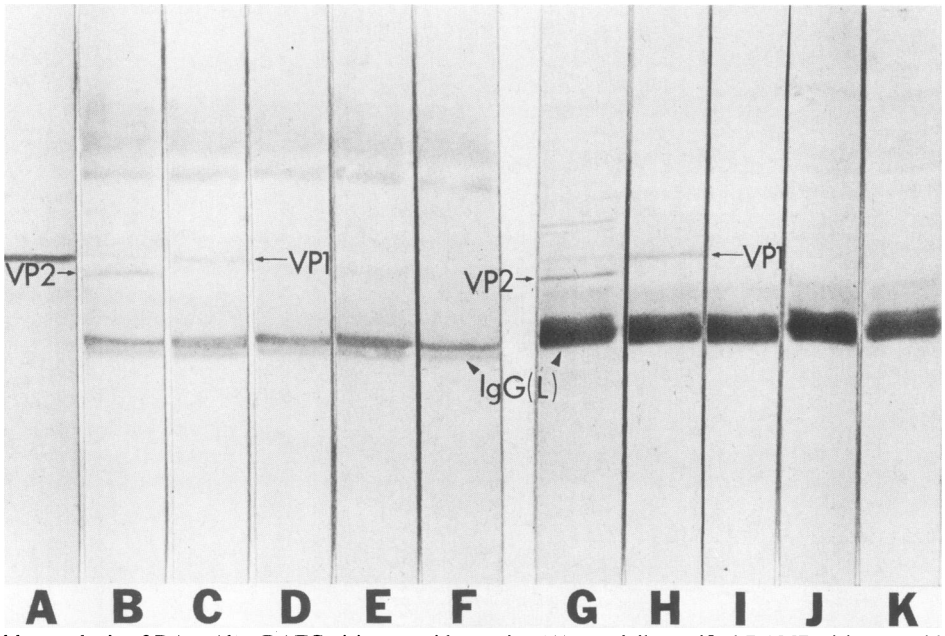

FIG. 5. Immunoblot analysis of DA mAbs. DATC virion capsid proteins (A), partially purified DAMB virion capsid proteins (B to F), or a crude DAMB brain homogenate (G to K) were run on SDS-PAGE, blotted to nitrocellulose paper, and overlaid with: DA mAb2 (A, C, H), hyperimmune mouse serum from chronically demyelinated DATC-inoculated mice (B, G), DA mAb1 (D, I), control mAb (E, J), control serum (F, K). The staining of VP1 by DA mAb2 is present in all three DA preparations. Mouse IgG heavy- and light [IgG(L)]-chain background staining is evident in lanes B to K.

neutralization epitope might affect the in vivo activity of the virus. A change in the neutralization antigenic site of DA in infected mouse brain might permit this mutant virus to escape immune clearance and persist, even in the face of the large amounts of locally produced antibody known to be present (16). The neutralization site might also be important in neural cell binding (as with foot-and-mouth disease virus [1], another picornavirus) and thereby affect critical biological activities. A neutralization site important in virus binding could lead to the production of anti-idiotypic antibody that would bind to neural cells (such as oligodendrocytes) and conceivably produce an immune-mediated (or immune-amplified) demyelination (22). Our plan is to isolate virus variants resistant to the mAbs; a change in the biological properties of the virus would suggest that the epitope is important in a particular biological activity. We also plan to determine the action of the TMEV-neutralizing mAbs in in vivo infections. Our preliminary studies show that DA mAb2 is unable to neutralize mice in vivo, although the mAb is effective in vitro (S. Nitayaphan, M. M. Toth, and R. P. Roos, manuscript in preparation).

Our neutralizing mAb findings can be compared with the extensive studies of neutralization of poliovirus, another member of the enterovirus genus. In contrast to our TMEV results, only one poliovirus-neutralizing mAb reacts with isolated VP1 (2), whereas many poliovirus-neutralizing mAbs require an intact virion conformation for reactivity (4, 21). The epitopes of several of the poliovirus mAbs have been localized to one (21, 31) or two (6) sites on VP1. The biochemical characterization of mutants resistant to neutralizing mAbs identified an eight-amino acid sequence of VP1 as an "immunodominant" site for neutralization of poliovirus type 3 (7, 21); an analogous neutralization site for a mAb was found on VP1 of poliovirus type 1 (31). ELISA studies, using synthetic peptides, confirmed this site on poliovirus type 1 and demonstrated a second distinct site on VP1 for neutralizing mAbs (6). In addition to these (murine hybridoma) immunodominant neutralization sites, other neutralization sites are presumably present since synthetic peptides containing sequences from regions of poliovirus VP1, VP2, and VP3 will each "prime" a polyclonal neutralizing antibody response in certain laboratory animals (6). There is also evidence that inoculation of laboratory animals with purified poliovirus VP1, VP2, or VP3 leads to low levels of polyclonal neutralizing antibody (references cited in reference 5).

The varied results of these poliovirus studies suggest that the antigenic target of a neutralizing antibody partly depends on the immunization scheme, antibody screening assay, and animal species used. It is possible that the poliovirus-neutralizing mAbs prepared from murine hybridomas (and the polyclonal neutralizing antisera prepared in laboratory animals) give a limited or even distorted picture of the immune response against this primate-specific virus. The infrequent occurrence of poliovirus-neutralizing mAbs with reactivity against isolated VP1 may therefore relate to the fact that poliovirus hybridomas were prepared from rodents which had never had on-going infection. In contrast, the TMEV hybridomas, which display a much higher frequency of mAbs reactive against isolated VP1, were prepared from infected mice, a more natural situation; our TMEV findings are similar to those made with foot-and-mouth disease virus, where murine hybridoma studies have demonstrated two neutralization sites on isolated VP1 and one conformation-specific site (1). Perhaps human hybridomas prepared from individuals after poliovirus infection or live poliovirus vaccination would more accurately reflect the natural poliovirus immune response than would rodent hybridomas.

ACKNOWLEDGMENTS

This research was supported by grants from the National Multiple Sclerosis Society (MS: RG 1512-A-1) and the Kroc Foundation.

S.N. received support from the Supreme Command Headquarters of The Royal Thai Armed Forces.

The advice of E. Ehrenfeld, B. G. W. Arnason, and D. Richman and the secretarial help of Peggy Peach and Gretchen Hendrie are gratefully acknowledged.

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