JOURNAL OF VIROLOGY, June 1993, p. 3446–3453
0022-538X/93/063446-08$02.00/0

Vol. 67, No. 6

Protective Anti-Reovirus Monoclonal Antibodies and Their Effects on Viral Pathogenesis

KENNETH L. TYLER,[1]* MARY ANNE MANN,[2] BERNARD N. FIELDS,[2,3] AND HERBERT W. VIRGIN IV[4]*

Departments of Neurology, Medicine, and Microbiology-Immunology, University of Colorado Health Sciences Center and Neurology Service (127), Denver Veterans Administration Medical Center, 1055 Clermont Street, Denver, Colorado 80220[1]; Department of Microbiology and Molecular Genetics,[2] and Department of Medicine, Brigham and Women's Hospital, and Shipley Institute of Medicine,[3] Harvard Medical School, Boston, Massachusetts 02115; and Departments of Medicine, Pathology, and Molecular Microbiology, Washington University School of Medicine, 660 South Euclid Avenue, Box 8051, St. Louis, Missouri 63110[4]

Received 1 February 1993/Accepted 5 March 1993

We used a recently isolated and characterized panel of monoclonal antibodies (MAbs) specific for cross-reactive determinants on reovirus outer capsid proteins to define mechanisms of antibody-mediated protection in vivo. We studied the capacities of MAbs to protect against lethal infection with reoviruses which differ in site of primary replication, route of spread, and central nervous system tropism. We found the following. (i) MAbs specific for each of the viral outer capsid proteins (σ1, σ3, and μ1) and the core spike protein (λ2) were protective under certain circumstances. (ii) In vitro properties of MAbs, including isotype, neutralization of viral infectivity, inhibition of virus-induced hemagglutination, and avidity of binding, were poorly predictive of the capacities of MAbs to protect in vivo. (iii) MAbs did not act at a single stage during pathogenesis to mediate protection; instead, protective MAbs were capable of altering a variety of stages in reovirus pathogenesis. (iv) MAbs protective against one reovirus also protected against other reoviruses that utilized different pathogenetic strategies, suggesting that the viral epitope bound by an antibody rather than the pathogenetic strategy employed by the virus is a critical determinant of antibody-mediated protection in vivo. (v) A prominent mechanism of protective MAb action is inhibition of viral spread through nerves from a site of primary replication (e.g., the intestine or muscle tissue) to the central nervous system.

The immune system plays a critical role in host defense against viral infection. Despite this fact, little is known about how an antibody acts at defined stages during viral pathogenesis to protect a host. Reovirus infection of neonatal mice provides an ideal system for investigating mechanisms of antibody action in vivo because (i) the pathogenesis of a number of reoviruses has been characterized in great detail (17, 19, 21) and (ii) the major reovirus outer capsid proteins (σ3 and μ1) are sufficiently conserved so that monoclonal antibodies (MAbs) specific for these proteins cross-react with reoviruses with a variety of pathogenetic phenotypes (25). These factors allow study of the comparative efficacies of MAbs against different patterns of virus-induced disease.

We have recently isolated and characterized a panel of MAbs specific for the reovirus outer capsid (σ3, μ1, and σ1) and core spike (λ2) proteins that provides an excellent set of reagents for exploring the role of antibodies at different stages in reovirus pathogenesis (25). These MAbs bind to three reoviruses, i.e., serotype 3 Dearing (T3D), serotype 3 clone 9 (T3C9), and serotype 1 Lang (T1L) (25; unpublished data for T3C9), whose pathogenesis has been particularly well studied. After intramuscular (T3D) or oral (T3C9) inoculation, T3 reoviruses spread through nerves to the central nervous system (CNS) (4, 10, 22). By contrast, T1L spreads to the CNS principally through the bloodstream, although abortive neural spread may occur (4, 22). Once in the CNS, T3D and T3C9 produce lethal necrotizing encephalitis with neuronal destruction, while T1L produces ependymitis with hydrocephalus (reviewed in references 17, 19, and 21).

We have previously shown that both polyclonal anti-reovirus sera and a σ1 MAb inhibit entry of T3D and T3C9 into, and growth and spread of these viruses within, the CNS (23, 24). We now report that (i) MAbs specific for each of the reovirus outer capsid proteins and the core spike protein are protective under certain circumstances, (ii) MAb protection does not universally correlate with in vitro properties of MAbs, including isotype, avidity, neutralization, and hemagglutination (HA) inhibition, (iii) protective MAbs act by different mechanisms at a variety of different stages during pathogenesis, and (iv) MAbs protective against one reovirus strain typically protect against all strains, despite differences in pathogenesis among the different viral strains.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used in this report: b-MAb, biotinylated MAb; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; i.c., intracranial; i.m., intramuscular; i.p., intraperitoneal; LD_{50}, dose causing 50% of mice to die; NT, plaque reduction neutralization; PBS, phosphate-buffered saline; p.o., peroral.

Virus. T3D, T3C9, and T1L were from laboratory stocks. The methods used for virus growth, purification, storage, and plaque assay were previously described (23–25). Virus titer was determined by plating serial 10-fold dilutions of freeze-thawed (three times), sonicated organ homogenates on L929 fibroblast monolayers, overlaying them with agar, and detecting plaques with neutral red.

Mice and viral infection. NIH(s) mice were obtained from

* Corresponding authors.

TABLE 1. MAbs and their properties[a]

MAb	Specificity	Isotype	HI[b]	T3D NT[c]	T1L NT[c]
7F4	λ2	IgG2a(κ)	−	−	−
4A3	μ1	IgG2b(κ)	−	−	−
8H6	μ1	IgG2a(κ)	−	−	−
10F6	μ1	IgG2b(κ)	+	−	−
10H2	μ1	IgG2a(κ)	−	−	−
8F12	σ3	IgG2b(κ)	+ (T1L)	−	−
8H1	σ3	IgG3(κ)	+++	30	−
4F2	σ3	IgG2a(κ)	++ (T3D)	−	−
7A1	σ3	IgG2b(κ)	+++ (T3D)	10	−
10C1	σ3	IgG2a(κ)	+++ (T3D)[d]	−	−
5C3	σ3	IgG2b(κ)	−	30	−
10G10	σ3	IgG2a(κ)	+++ (T3D)[d]	10	−
5C6	σ1 (T1L)	IgG2a(κ)	+++ (T1L)	−	<1
G5	σ1 (T3D)	IgG2a(κ)	++ (T3D)	<1	−

[a] See references 2 and 25 for characterization of MAb specificities, isotypes, and HA inhibition.
[b] HI, HA inhibition. −, absent; +, weak; ++, moderate; +++, strong. Human A⁻ erythrocytes were used.
[c] NT, lowest concentration (in micrograms per milliliter) of the affinity-purified MAb that produced ≥80% reduction in plaque number.
[d] HA inhibiting for T1L over a narrow concentration range (25).

,the National Cancer Institute (Frederick, Md.) and maintained in an American Association for the Accreditation of Laboratory Animal Care-approved animal biosafety level 2 facility in accordance with all Federal and University standards. Unless otherwise noted, 1-day-old mice were used and they received 100 μg of a purified MAb (100 μl) by i.p. injection. Mice were inoculated 24 h later with 100 LD_{50}s of T3D (10^3 PFU i.c. or $10^{6.88}$ PFU i.m.). A 10^7-PFU dose of T3C9 (given p.o.) was used since it caused death in 90% of untreated mice (see Fig. 1C). It was not possible to inoculate mice with 100 LD_{50}s of T1L i.c. because of its relative avirulence. The 10^7-PFU dose of T1L (i.c.) selected produced mortality in >50% of the mice and hydrocephalus in >70% of the survivors (see Fig. 1D). We performed i.m., i.c., and p.o. inoculations as previously described (23–25). Protection experiments were performed at least twice, with different litters of mice (n = 13 to 31 per test condition). For T3D and T3C9, mice were checked daily for a minimum of 3 weeks after infection. For studies of T1L-induced hydrocephalus, mice were checked daily for 4 weeks. On day 28 postinfection, survivors were sacrificed and their brains were sectioned in the coronal plane. Hydrocephalus was defined as grossly visible dilatation of the lateral and/or third ventricle. Collection and storage of tissue prior to viral titer determination were done as previously described (23–25). Results of protection experiments were subjected to chi-square analysis, and significance was defined as $P < 0.05$.

Antibodies. Preparation, purification, storage, specificities, isotypes, and functional characterization of the MAbs used were recently reported (Table 1) (2, 25). All MAbs were purified by protein A chromatography, sterilely filtered, and stored at −70°C prior to use.

NT assay. MAb dilutions were made in Joklik's modification of Eagle's minimal essential medium containing 5% heat-fixed fetal calf serum (Hyclone, Ogden, Utah), 2 mM L-glutamine, 1 U of penicillin G per ml, and 1 μg of streptomycin sulfate per ml (cMEM). Diluted MAbs were incubated (60 min, 37°C) with an equal volume of virus (4,000 PFU/ml, cMEM). A 100-μl volume of the MAb-virus mixture was then inoculated in duplicate onto L929 cells, and plaques were assayed as described above. Plaque reduction is reported as the lowest concentration of an affinity-purified MAb (in micrograms per milliliter) producing ≥80% plaque reduction compared with MAb-free controls.

Efficiency of MAb binding. Relative MAb avidity was assessed with both the constant-antigen varying antibody and constant-antibody varying antigen methods (5, 25). For the constant-antigen method, binding of various concentrations of b-MAbs (25) (0.001 to 100 μg/ml) was tested on ELISA plates (Immulon 2; Dynatech, Chantilly, Va.) coated with 1 μg of purified virus per well. For the constant-antibody method, b-MAbs (0.1 μg/ml) were incubated with various concentrations of cesium chloride gradient-purified virus (0.003 to 100 μg/ml). Free (unbound) b-MAb was detected by adding 50 μl of the b-MAb–virus mixture to virus-coated (1 μg per well) plates. Binding of b-MAbs to virus-coated plates was detected with peroxidase-conjugated streptavidin (1:2,500; Jackson Immunoresearch, West Grove, Pa.) with 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid) (Sigma Chemical, St. Louis, Mo.) as the substrate.

RESULTS

In vivo protection. We assessed the protective abilities of MAbs against T3D (given i.m. or i.c.), T3C9 (given p.o.), and T1L (given i.c.) (Fig. 1A to D). Despite variation in route of challenge and strain of virus, MAbs protective in one model were typically protective against all viruses (compare Fig. 1A to D). One of four μl MAbs (8H6; χ^2, $P < 0.0005$ in Fig. 1A to C and $P < 0.01$ in Fig. 1D) and four of seven σ3 MAbs (7A1, 8H1, 10C1, and 10G10; χ^2, $P < 0.0005$ in Fig. 1A to C and $P < 0.005$ in Fig. 1D) were protective regardless of inoculation route or virus strain. The remaining μl MAbs (4A3, 10F6, and 10H2) and σ3 MAb 8F12 were not protective. Two σ3 MAbs (5C3 and 4F2) failed to protect in specific situations. 4F2 was protective against T3D given i.m. or i.c., and T1L given i.c. (χ^2, $P < 0.0005$) but failed to protect against T3C9 given p.o. (Fig. 1C). 5C3 was protective against T3D given i.m. or i.c., and T3C9 given p.o. (χ^2, $P < 0.0005$) but did not prevent T1L-induced hydrocephalus (Fig. 1D). λ2 MAb 7F4 was partially protective against C9 given p.o. (χ^2, $P < 0.0005$) (Fig. 1C). Thus, protection can be mediated by MAbs directed against each of the reovirus outer capsid proteins and the core spike protein, and MAbs protective against one virus are typically (10 of 13) protective, regardless of the virus strain tested or its route of inoculation.

Comparison of in vivo and in vitro antibody activities. Protection did not depend exclusively on protein specificity, since both protective and nonprotective σ3 and μ1 MAbs exist (Fig. 1). There were both protective and nonprotective IgG2a and IgG2b antibodies, suggesting that neither of these isotypes is critical for protection. The one available IgG3 MAb (8H1) was protective. There were no IgG1 MAbs in our panel. We examined the relationship between protection and a variety of in vitro properties of MAbs (compare Fig. 1 and Table 1). There was no correlation between protection and HA inhibition. For example, 5C3 does not show HA inhibition but protects against T3D, and 10F6 does show HA inhibition but is nonprotective. Similarly, there was no consistent relationship between NT and protection. For example, σ3 MAbs 10C1 and 4F2 did not neutralize T3D but protected against i.m. and i.c. challenges (Table 1 and Fig. 1A and B). No σ3 MAb neutralized T1L, yet five of seven protected against T1L-induced hydrocephalus (Table 1 and

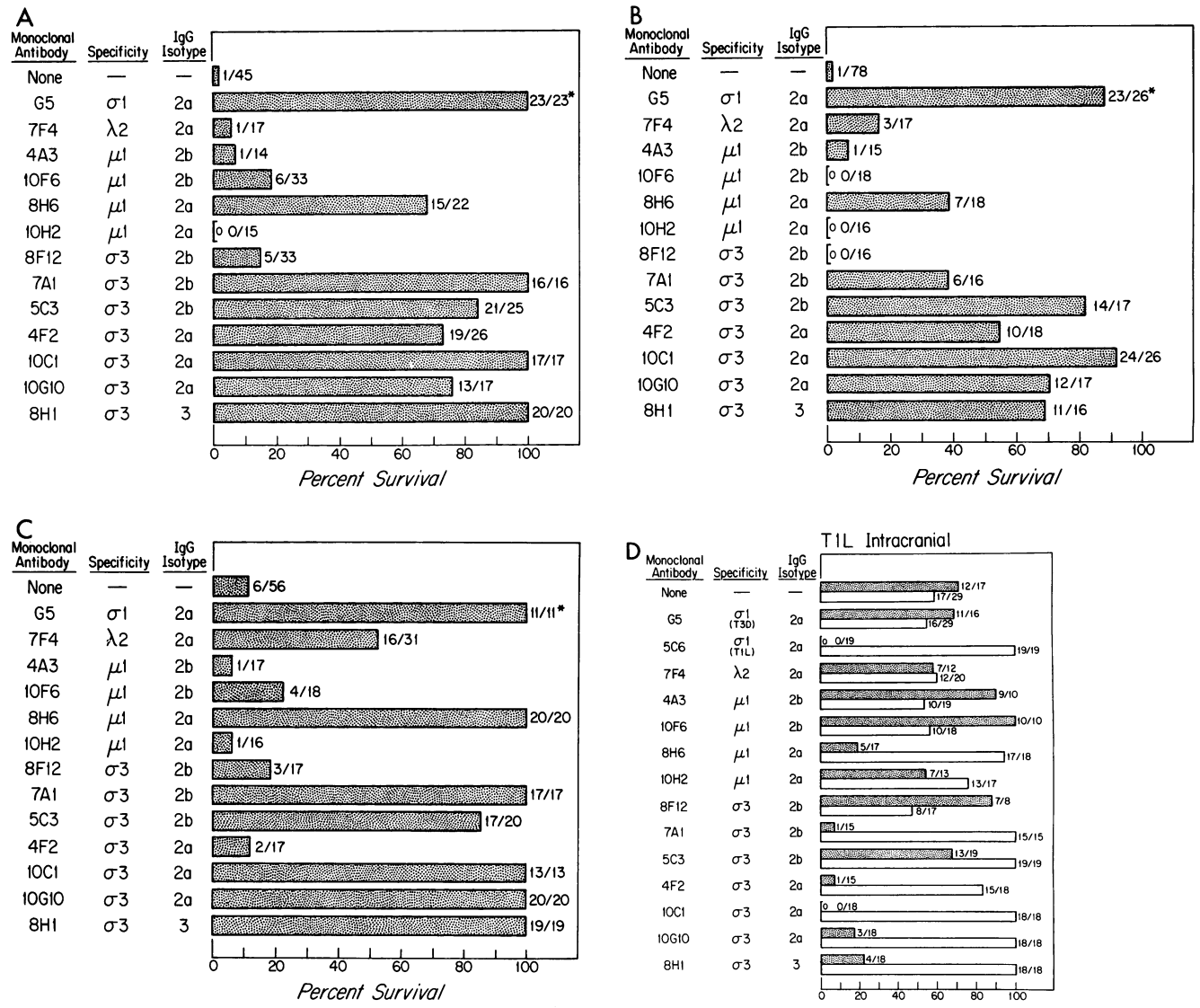

FIG. 1. Protection against challenge with T3D administered i.m. (A), T3D administered i.c. (B), T3C9 administered p.o. (C), or T1L administered i.c. (D). Mice received 100 μg of an affinity-purified MAb i.p. on day 1 of life and were inoculated with virus 24 h later. Panels A to C show percentages of mice surviving 3 weeks after virus challenge. Panel D shows percentages of mice surviving (open bars) and percentages of surviving mice with hydrocephalus (shaded bars). *, G5 data included for comparison purposes (23, 24).

Fig. 1D). Thus, protection is not simply the in vivo analog of in vitro NT or HA inhibition.

Avidity of MAb binding and protective capacity. We next defined the relationship between protective capacity and relative MAb avidity for virus. Avidity was compared under conditions of both constant antigen concentration and constant MAb concentration (5, 25). This method of analysis allows comparison of the relative avidities of different MAbs for an intact virus.

σ3 MAb 4F2 protected against T3D and T1L but not against T3C9 (compare Fig. 1A, B, and D with Fig. 1C). The failure of 4F2 to protect against T3C9 given p.o. may reflect its low avidity for this virus (Fig. 2). Similarly, σ3 MAb 8F12 bound poorly to all three of the viruses assayed and was nonprotective (data not shown). These results suggest that antibody-mediated protection requires a minimal threshold of binding avidity. However, our results also indicate that avidity is not the sole determinant of the protective capacity of a MAb. σ3 MAb 5C3 bound with comparable degrees of avidity to T1L and T3D (Fig. 3) and was protective against T3D but not against T1L-induced hydrocephalus (compare Fig. 1A and B with Fig. 1D). μ1 MAbs 8H6 and 10F6 bound to T3D (Fig. 4) and T3C9 and T1L (data not shown) with comparable avidity, yet 8H6 was protective against each of these viruses and 10F6 was not (Fig. 1). Thus, although MAbs with low binding avidity may fail to protect, protection is not simply a function of the relative avidity with which MAbs bind to a virus.

Mechanisms of action of protective MAbs in vivo. To characterize MAb mechanisms at specific stages in pathogenesis, we collected organs at specific times following viral infection of MAb-treated and control mice. Each of the viral model systems tested allowed evaluation of the effects of MAbs on certain stages in pathogenesis.

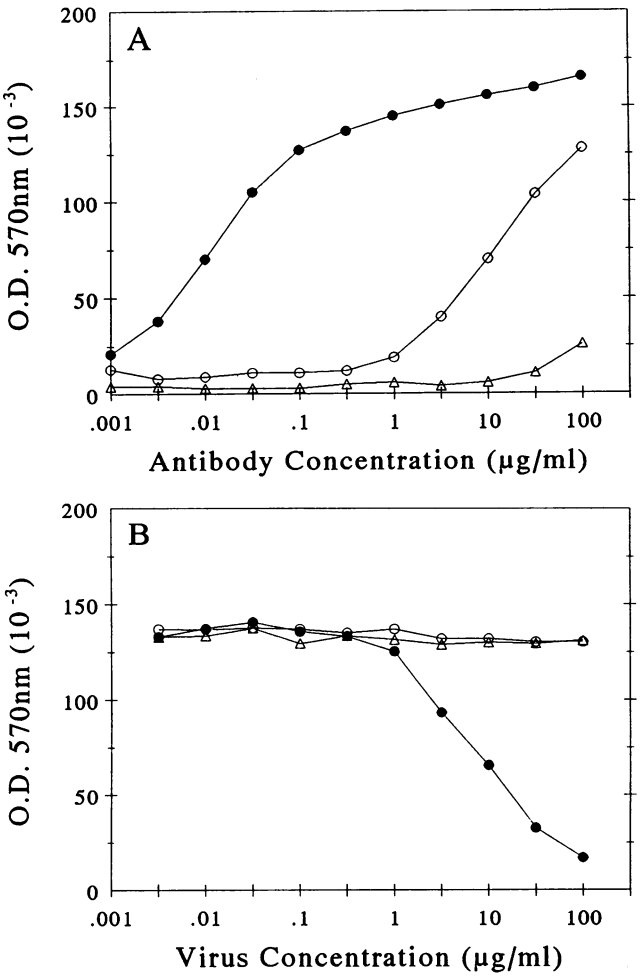

FIG. 2. Relative avidity of σ3 MAb 4F2 for T1L (○), T3D (●), and T3C9 (△). (A) Binding of various concentrations of 4F2 to ELISA plates coated with a constant concentration of purified virus. (B) Capacities of various concentrations of purified viruses to bind a constant concentration of 4F2 in solution. Unbound (free) 4F2 was detected by ELISA. O.D., optical density.

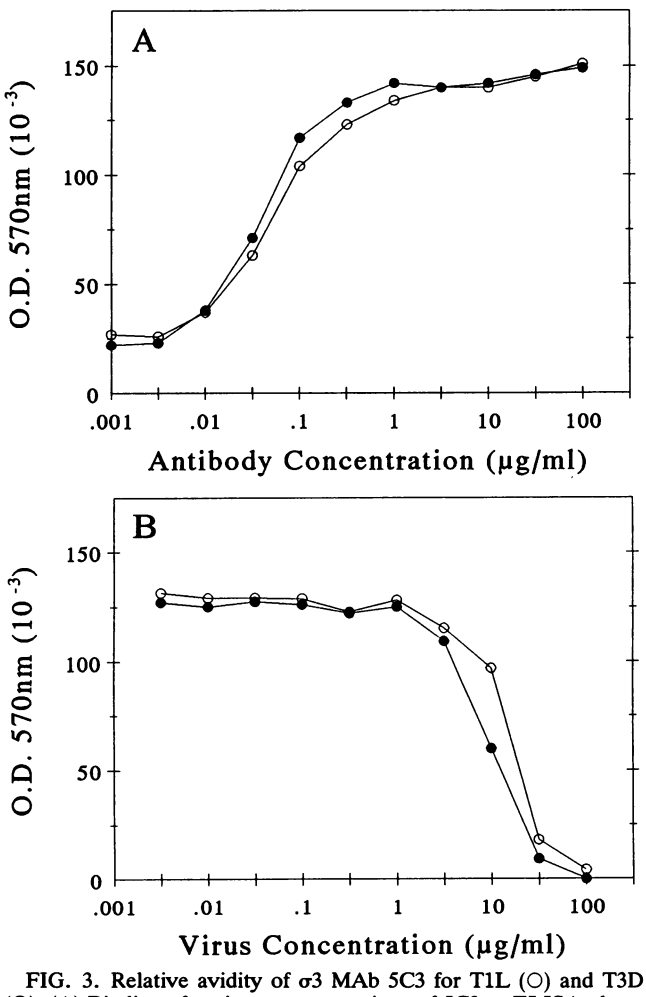

FIG. 3. Relative avidity of σ3 MAb 5C3 for T1L (○) and T3D (●). (A) Binding of various concentrations of 5C3 to ELISA plates coated with a constant concentration of purified virus. (B) Capacities of various concentrations of a purified virus to bind a constant concentration of 5C3 in solution. Unbound (free) 5C3 was detected by ELISA. O.D., optical density.

(i) T3C9. After p.o. inoculation, T3C9 spreads through nerves from the intestine to the CNS to cause encephalitis (10). Neural spread is detectable within 72 to 96 h (10, 23) and is well established by day 5, the time selected for assay (Fig. 5). Spread to visceral organs also occurs, although the pathways of spread have not been defined. No MAb reduced the viral titer in the intestine in this model (Fig. 5A). Despite this, many MAbs both were protective and blocked neural spread of T3C9 to the CNS (Fig. 5A, MAbs G5, 8H6, 7A1, 5C3, 10C1, 10G10, and 8H1). Protective MAbs all reduced brain viral titers to a greater extent than any nonprotective MAb did (Fig. 5A). These same strongly protective MAbs inhibited viremia and blocked virus spread to extraintestinal organs, such as the heart (Fig. 5B). However, reduction in viremia and titer in visceral organs did not always result in protection (Fig. 5B, MAbs 10H2 and 8F12).

(ii) T3D. T3D spreads through nerves from skin and muscle to the CNS, is detected in the spinal cord within 24 h of inoculation, and is present there in high titer by day 3 (22) (Fig. 6). T3D also spreads through nerves within the CNS, as exemplified by its spread from the brain to the retina via the optic nerve (18).

There were several different patterns of action of protective MAbs against i.m. inoculation of T3D (Fig. 6). Some MAbs strikingly decreased the amount of the virus present in the spinal cord without affecting primary replication in muscle tissue (Fig. 6, MAbs G5 and 8H1). Viral titer in the spinal cord reflects both spread of the virus through nerves and subsequent replication of the arriving virus within the spinal cord. Our results indicate that MAbs can inhibit these events independently of any effect on primary replication. By contrast, σ3 MAb 10C1 inhibited primary replication in muscle tissue and markedly reduced the amount of the virus detected in the spinal cord. Other MAbs protected despite only intermediate effects on primary replication, spread through nerves, or growth in the spinal cord (compare Fig. 1A with Fig. 6, MAbs 8H6, 5C3, and 4F2). Thus, protective MAbs appear to act through a variety of different mechanisms to alter pathogenesis, including, but not limited to, inhibition of primary replication, neural spread, and virus growth within the spinal cord.

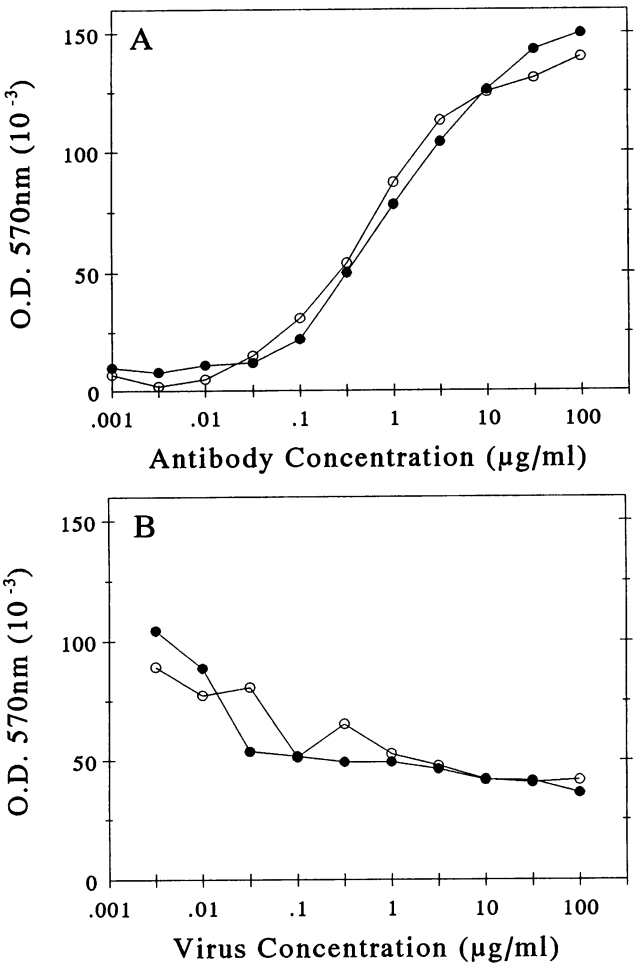

FIG. 4. Relative avidities of μ1 MAbs 10F6 (○) and 8H6 (●). (A) Binding of various concentrations of 10F6 and 8H6 to ELISA plates coated with a constant concentration of purified T3D. (B) Capacities of various concentrations of purified T3D virus to bind a constant concentration of 10F6 or 8H6 in solution. Unbound (free) MAb was detected by ELISA. O.D., optical density.

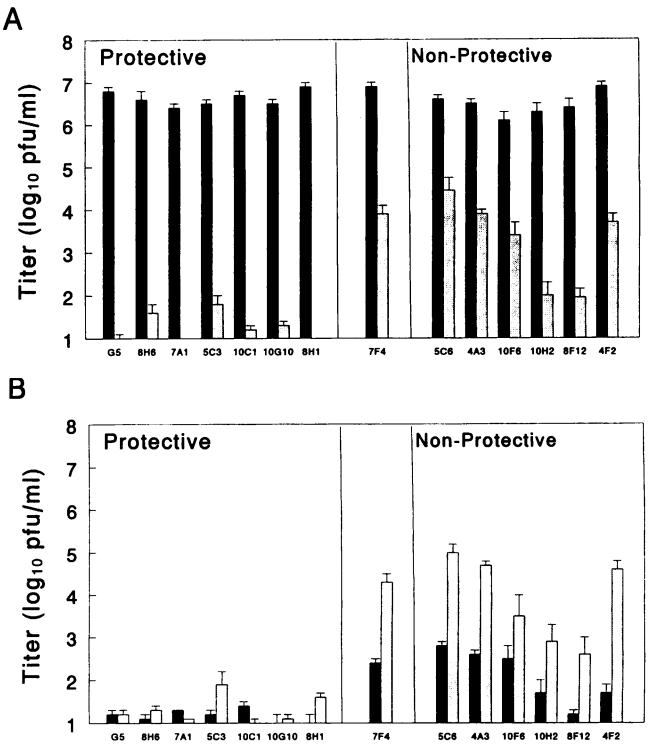

FIG. 5. Virus spread in MAb-treated animals after p.o. administration of T3C9. Mice received 100 μg of affinity-purified MAb i.p. on day 1 of life and were inoculated with virus (10^7 PFU) 24 h later. Specimens were collected 5 days after virus inoculation. There were 7 to 15 mice per group. Panels: A, intestine (■) and brain (▩) titers; B, blood (■) and heart (▩) titers. The values shown are means ± the standard errors. λ2 MAb 7F4 shows an intermediate pattern of protection. MAb 5C6 does not bind to T3C9 and was used as a control.

Several MAbs protected against T3D administered i.c. (Fig. 1B and 7). Surprisingly, there was no universal correlation between protection and decreased viral titer in the brain after i.c. inoculation. For example, μ1 MAb 8H6 was more protective than either 10F6 or 10H2 but less effective at reducing the virus titer in the brain. Protective MAbs significantly reduced the amount of a virus present in the eye, suggesting that inhibition of neural spread of virus within the CNS is an important mechanism of antibody-mediated protection. However, μ1 MAb 10H2 was nearly as effective at reducing the amount of a virus in the eye as was σ3 MAb 4F2 or μ1 MAb 8H6 but was not protective. These findings suggest that factors other than simple reduction of virus growth and spread contribute to protection of the CNS against viral infection.

(iii) T1L. T1L serves as a model for hematogenous spread of a virus to the CNS (22). Once in the CNS, T1L has a tropism for ependymal cells rather than neurons (16, 26, 27). There was a good correlation between MAb-mediated reduction in viremia and inhibition of spread to the CNS in this model. All protective MAbs resulted in inhibition of viremia, in contrast to nonprotective MAbs (Fig. 8A). While some MAbs which decreased viremia inhibit primary replication in muscle tissue (e.g., σ3 MAb 7A1), others reduced viremia with minimal effects on primary replication (e.g., μ1 MAb 8H6 and σ3 MAb 10C1). Thus, prevention of viremia can occur in the absence of inhibition of primary replication.

While control of primary replication was not a prerequisite for protection, MAbs which inhibited the spread of T1L to the CNS after i.m. inoculation (Fig. 8B) generally protected against mortality or hydrocephalus following i.c. inoculation of T1L (Fig. 1D). MAbs which failed to inhibit spread of T1L to the CNS after i.m. inoculation failed to protect against i.c. inoculation of T1L.

DISCUSSION

Evaluation of the mechanisms of MAb action against antigenically related viruses with different pathogenetic phenotypes should improve our understanding of how antibodies act in vivo. We were able to conduct this type of analysis with a recently isolated and characterized (25) panel of MAbs to conserved capsid epitopes of mammalian reoviruses.

Both polyclonal antisera (3, 6, 23, 24) and σ1 MAbs (23, 24) protect mice against lethal infection with reoviruses. A key role for σ1 MAb G5 is blockade of neural spread without

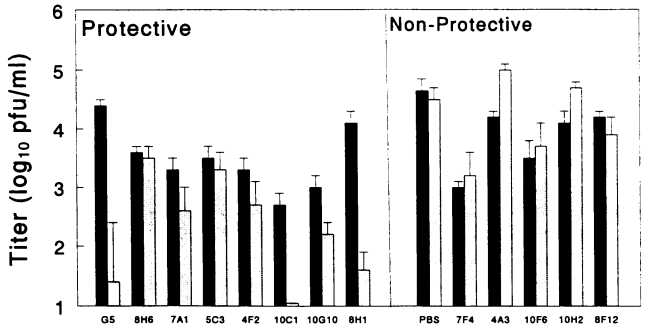

FIG. 6. Virus spread in MAb-treated animals after i.m. administration of T3D. Mice received 100 μg of affinity-purified MAb i.p. on day 1 of life and were inoculated with virus ($10^{6.88}$ PFU) 24 h later. Specimens were collected 3 days after virus inoculation. There were 6 to 12 mice per group. The values shown are mean titers ± the standard errors. PBS was used as a control. Bars: ■, muscle; ▥, spinal cord.

effects on primary replication (23). While anti-σ1 antibody is protective, type-specific polyclonal antiserum also protects mice against heterotypic virus although it is devoid of detectable cross-reacting anti-σ1 antibody (24). This suggested that antibodies specific for proteins other than σ1 are protective against reoviruses. In this report, we show (Fig. 1) that MAbs directed against all of the reovirus outer capsid and core spike proteins are capable of protecting mice against reovirus infection under certain circumstances.

Not all MAbs specific for a particular protein were protective. In some cases, failure to protect may have been the result of low-avidity binding of a MAb to the target virus. For example, σ3 MAb 4F2 protected against T3D but failed to protect against T3C9, for which it has lower binding avidity (Fig. 2). However, there were several examples of MAbs which bound to a virus with equivalent degrees of avidity yet differed in protective capacity (e.g., μ1 MAbs 8H6 and 10F6 [Fig. 1 and 4]), indicating that protection is not merely a function of binding avidity. These findings argue that F_c-mediated effector functions are not required for antibody-mediated protection. Our results are consistent with the idea that above a certain threshold of avidity, MAb

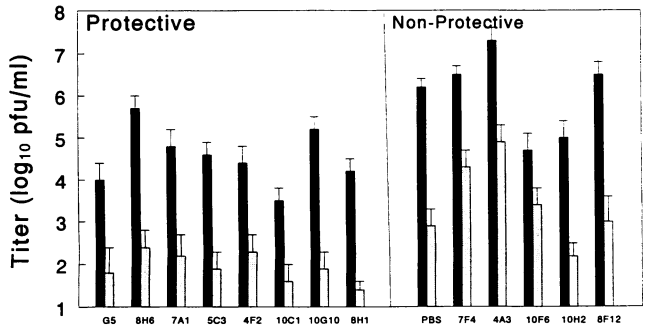

FIG. 7. Virus spread in MAb-treated animals after i.c. administration of T3D. Mice received 100 μg of affinity-purified MAb i.p. on day 1 of life and were inoculated with virus (10^3 PFU) 24 h later. Specimens were collected 5 days after virus inoculation. There were 6 to 10 mice per group. The values shown are mean titers ± the standard errors. PBS was used as a control. Bars: ■, brain; ▥, eye.

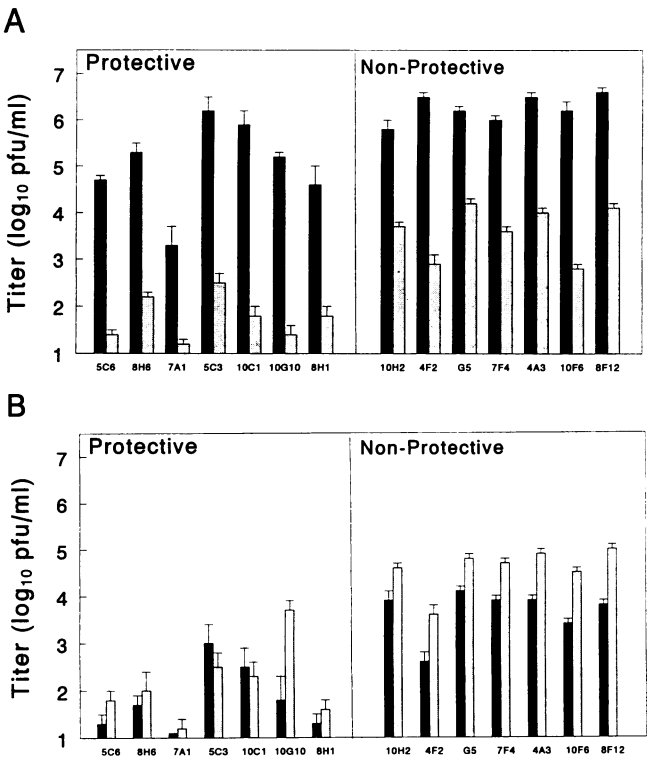

FIG. 8. Virus spread in MAb-treated animals after i.m. administration of T1L. Mice received 100 μg of affinity-purified MAb i.p. on day 1 of life and were inoculated with virus (10^7 PFU) 24 h later. Specimens were collected 3 days after virus inoculation. There were seven to nine mice per group. Panels: A, muscle (■) and blood (▥); B, spinal cord (■) and brain (▥). The values shown are mean titers ± the standard errors. MAb G5 does not bind to T1L and was used as a control.

effectiveness in vivo is determined by the epitope recognized rather than how efficiently that epitope is bound.

The in vitro properties of MAbs are poor predictors of the ability to provide protection in vivo. We examined a number of in vitro properties of MAbs, looking for a correlation with protection. Protection did not correlate with MAb isotype, HA-inhibiting capacity, or NT titer (Table 1). None of our non-σ1 MAbs showed NT for T1L, yet six of them protected against T1L-induced hydrocephalus (Fig. 1D). These results highlight the fact that protection is not simply the in vivo analog of in vitro NT (24). The capacity of MAbs that do not show NT to protect in vivo has been repeatedly demonstrated in other viral systems (1, 7, 9, 14, 15, 24). These results suggest that use of in vitro neutralization assays for selection of candidate target epitopes for vaccines or use of in vitro NT as a screening test for potentially efficacious vaccines is not an optimal strategy.

Reassortant genetic techniques have been used to demonstrate that the proteins encoded by certain reovirus gene segments play critical roles at defined stages in pathogenesis. For example, the σ1 cell attachment protein, encoded by the S1 gene segment, is critical in determining CNS tropism and route of spread (20). The μ1 protein, encoded by the M2 gene segment, is a determinant of neurovirulence for serotype 3 reoviruses (8). Thus, different capsid proteins are involved in distinct aspects of pathogenesis in vivo. We

hypothesized that MAbs specific for these individual proteins might act at different stages in pathogenesis to mediate in vivo protection. This possibility is supported by our finding that protective MAbs could act at a variety of different stages in pathogenesis, including primary replication, entry of a virus into the nervous system, and spread and growth of the virus within the nervous system. For example, (i) several MAbs inhibited primary replication of T1L or T3D in skeletal muscle tissue (Fig. 6 and 8A), (ii) MAbs inhibited neural spread from the intestine to the CNS (Fig. 5A), (iii) MAbs inhibited hematogenous spread of T1L (Fig. 8A), (iv) MAbs decreased viral titers at secondary sites of infection, such as the heart and brain (Fig. 5B and 8B), and (v) MAbs inhibited disease expression (hydrocephalus) following i.c. inoculation of virus (Fig. 1D). These differences in MAb action may reflect the differing roles of reovirus proteins at distinct stages in viral pathogenesis.

A key finding was that protective MAbs differed strikingly in their mechanisms of action in vivo, despite sharing the essential capacity to protect against disease. For example, all σ3 MAbs except 8F12 protected against T3D inoculated i.m. (Fig. 1A). Some of these MAbs inhibit primary replication in muscle tissue, whereas others do not. Some MAbs are potent inhibitors of neural spread of virus to or growth within the spinal cord, while others have only modest effects. A strong correlation exists between the capacity of MAbs to block spread to the CNS and their capacity to protect (Fig. 5A and 8B). Similarly, many MAbs inhibited T1L viremia but in some cases this was associated with decreased primary replication while in others it was not. We conclude from our studies that MAbs which bind to a virus, or even to the same protein on the virus, differ in their mechanisms of action at defined stages of pathogenesis in vivo.

Another striking finding was the failure of any of the 14 MAbs tested to decrease the amount of the virus present in the intestine after p.o. inoculation of T3C9 (Fig. 5A). We did not study the effects of MAbs on viral replication in the intestine following low-dose virus inoculation. However, we have previously shown that systemic administration of high doses of a protective σ1 MAb fails to inhibit viral primary replication in the intestine (23). Systemic antibody also fails to control intestinal infection with rotaviruses, another member of the family *Reoviridae* (12). The failure of systemic IgG to decrease the viral titer in the intestine while successfully protecting against systemic disease and death parallels findings obtained with poliovirus (13). These results are consistent with the view that secretory IgA and/or immune cells, rather than systemic IgG, play the predominant role in controlling viral infections at mucosal surfaces (reviewed in reference 11).

The same family of MAbs protected against all of the reoviruses used in these experiments. This was true although these viruses differed dramatically in their patterns of pathogenesis. This suggests that regardless of the pathogenetic strategy employed by a particular virus or differences in MAb effects at a given stage of pathogenesis, one mechanism of MAb action may be binding to epitopes essential for critical functions common to all reoviruses.

ACKNOWLEDGMENTS

Research support came from PHS Program Project grant 2 P50 NS16998 from the NINCDS (B.N.F. and H.W.V.), RAGS and Merit grants from the Department of Veterans Affairs (K.L.T.), and the Shipley Institute of Medicine (B.N.F.). H.W.V. is a Burroughs Wellcome Young Investigator in Virology.

Elaine Freimont maintained L cells and provided essential general laboratory support services.

REFERENCES

1. **Boere, W. A. M., B. J. Benaissa-Trouw, T. Harmsen, T. Erich, C. A. Kraaijeveld, and H. Snippe.** 1985. Mechanisms of monoclonal antibody-mediated protection against virulent Semliki Forest virus. J. Virol. **54**:546–551.
2. **Burstin, S. J., D. R. Spriggs, and B. N. Fields.** 1982. Evidence for functional domains on the reovirus type 3 hemagglutinin. Virology **117**:146–155.
3. **Cuff, C. F., E. Lavi, C. K. Cebra, J. J. Cebra, and D. Rubin.** 1990. Passive immunity to fatal reovirus serotype 3-induced meningoencephalitis mediated by both secretory and transplacental factors in neonatal mice. J. Virol. **64**:1256–1263.
4. **Flamand, A., J.-P. Gagner, L. Morrison, and B. N. Fields.** 1991. Penetration of the nervous systems of suckling mice by mammalian reoviruses. J. Virol. **65**:123–131.
5. **Friguet, B., A. F. Chaffotte, L. Djavadi-Ohaniance, and M. E. Goldberg.** 1985. Measurements of true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. J. Immunol. Methods **77**:305–319.
6. **Gaulton, G., A. Sharpe, D. Chang, B. N. Fields, and M. Greene.** 1986. Syngeneic monoclonal internal image anti-idiotypes as prophylactic vaccines. J. Immunol. **137**:2930–2936.
7. **Grosfeld, H., B. Velan, M. Leitner, S. Cohen, S. Lustig, B.-E. Lachmi, and A. Shafferman.** 1989. Semliki Forest virus E2 envelope epitopes induce a nonneutralizing humoral response which protects mice against lethal challenge. J. Virol. **63**:3416–3422.
8. **Hrdy, D. L., D. Rubin, and B. N. Fields.** 1982. Molecular basis of reovirus neurovirulence: role of the M2 gene in avirulence. Proc. Natl. Acad. Sci. USA **791**:1298–1302.
9. **Mester, J. C., and B. T. Rouse.** 1991. The mouse model and understanding immunity to herpes simplex virus. Rev. Infect. Dis. **13**(Suppl. 11):S935–S945.
10. **Morrison, L., R. Sidman, and B. N. Fields.** 1991. Direct spread of reovirus from the intestinal lumen to the central nervous system through vagal autonomic nerve fibers. Proc. Natl. Acad. Sci. USA **88**:3852–3856.
11. **Murphy, B. R., and R. M. Chanock.** 1990. Immunization against viruses, p. 469–502. *In* B. N. Fields and D. M. Knipe (ed.), Fields' virology. Raven Press, New York.
12. **Offit, P. A., and H. F. Clark.** 1985. Protection against rotavirus-induced gastroenteritis in a murine model by passively acquired gastrointestinal but not circulating antibodies. J. Virol. **54**:58–64.
13. **Ogra, P. L.** 1984. Mucosal immune response to poliovirus vaccines in childhood. Rev. Infect. Dis. **6**(Suppl. 2):S361–S368.
14. **Rector, J. T., R. N. Lausch, and J. E. Oakes.** 1982. Use of monoclonal antibodies for analysis of antibody-dependent immunity to ocular herpes simplex virus type 1 infection. Infect. Immun. **38**:168–174.
15. **Schmaljohn, A. L., E. D. Johnson, J. M. Dalrymple, and G. A. Cole.** 1982. Non-neutralizing monoclonal antibodies can prevent lethal alphavirus encephalitis. Nature (London) **297**:70–72.
16. **Tardieu, M., and H. Weiner.** 1982. Viral receptors on isolated murine and human ependymal cells. Science **215**:419–421.
17. **Tyler, K. L.** 1991. Pathogenesis of reovirus infections of the central nervous system. Semin. Neurosci. **3**:117–124.
18. **Tyler, K. L., R. Bronson, K. Byers, and B. N. Fields.** 1985. Molecular basis of viral neurotropism: experimental reovirus infection. Neurology **35**:88–92.
19. **Tyler, K. L., and B. N. Fields.** 1988. Reovirus infections of the central nervous system, p. 11–19. *In* R. T. Johnson and G. Lyon (ed.), Virus infections of the developing nervous system. Kluwer Academic Publishers, Dordrecht, The Netherlands.
20. **Tyler, K. L., and B. N. Fields.** 1990. Pathogenesis of viral infections, p. 191–240. *In* B. N. Fields and D. M. Knipe (ed.), Fields' virology. Raven Press, New York.
21. **Tyler, K. L., and B. N. Fields.** 1990. Reoviruses, p. 1307–1328. *In* B. N. Fields and D. M. Knipe (ed.), Fields' virology. Raven Press, New York.

22. **Tyler, K. L., D. A. McPhee, and B. N. Fields.** 1986. Distinct pathways of viral spread in the host determined by reovirus S1 gene segment. Science **233**:770–774.
23. **Tyler, K. L., H. W. Virgin IV, R. Bassel-Duby, and B. N. Fields.** 1989. Antibody inhibits defined stages in the pathogenesis of reovirus serotype 3 infection of the central nervous system. J. Exp. Med. **170**:887–900.
24. **Virgin, H. W., IV, R. Bassel-Duby, B. N. Fields, and K. L. Tyler.** 1988. Antibody protects against lethal infection with the neurally spreading reovirus type 3 (Dearing). J. Virol. **62**:4594–4604.
25. **Virgin, H. W., IV, M. A. Mann, B. N. Fields, and K. L. Tyler.** 1991. Monoclonal antibodies to reovirus reveal structure/function relationships between capsid proteins and genetics of susceptibility to antibody action. J. Virol. **65**:6772–6781.
26. **Weiner, H., D. Drayna, D. Averill, and B. N. Fields.** 1977. Molecular basis of reovirus virulence: role of the S1 gene. Proc. Natl. Acad. Sci. USA **74**:5744–5748.
27. **Weiner, H., M. Powers, and B. N. Fields.** 1980. Absolute linkage of virulence and central nervous system tropism of reoviruses to viral hemagglutinin. J. Infect. Dis. **141**:609–616.