

ANTIBODY INHIBITS DEFINED STAGES IN THE
PATHOGENESIS OF REOVIRUS SEROTYPE 3 INFECTION
OF THE CENTRAL NERVOUS SYSTEM

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While it has long been known that IgG protects mice from a variety of viral infections, the mechanisms by which antibody acts *in vivo* have proved elusive. Two issues are of particular interest. First, while antibody inhibits central nervous system (CNS)¹ infection with a number of viruses, the specific sites at which antibody acts against neurally spreading viruses have not been defined. Second, the contribution of systemic antibody (IgG), as opposed to mucosal antibody (IgA), to control of viral replication in the intestine remains unclear. We are using the mammalian reoviruses as a model system to investigate these issues. Our strategy is to use mAbs of known specificity in a viral system with well-defined pathogenesis in order to identify stages in viral pathogenesis at which antibody acts.

The pathogenesis of CNS infection after inoculation of neonatal mice with reovirus serotype 3 Dearing (T3D) has been studied in detail (reviewed in reference 1). After intramuscular or footpad (FP) inoculation, T3D replicates initially within skeletal muscle (2). Virus then spreads to the CNS via peripheral nerves using fast axonal transport (2). Once within the CNS, T3D infects neurons (3-5). After intracerebral (IC) inoculation, T3D replicates and spreads to the eye to infect the retinal ganglion cells (6). Both the capacity of T3D for neural spread and its tropism for neurons are determined by the viral S1 gene (2, 4, 6) which encodes the viral cell attachment protein $\sigma 1$ and, in addition, a nonvirion-associated protein (7, 8).

Reoviruses can produce infection in mice after oral inoculation (9-11). T3D, a laboratory-adapted virus, has lost the ability to cause lethal CNS infection after peroral

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¹ *Abbreviations used in this paper:* CNS, central nervous system; FP, footpad; PO, peroral; IC, intracerebral; ISC, inferior spinal cord; SSC, superior spinal cord; LD₅₀, dose causing 50% of mice to die; $\sigma 1$, the σ -1 reovirus capsid protein; PFNS, pyrogen free normal saline; PFU, plaque forming unit; asc, ascites; T3D, reovirus serotype 3 Dearing; T3C9, reovirus serotype 3 clone 9; mar, monoclonal antibody resistant.

(PO) infection (9). However, reovirus serotype 3 clone 9 (T3C9, 12) differs from T3D in that it is lethal in neonatal mice after PO infection (Hrdy, D. B., unpublished data), and can be transmitted from infected to uninfected neonatal mice, presumably via the fecal-oral route (10). Thus, T3C9 provides a useful model for viruses such as polio for which neural invasion follows primary enteric infection (13).

Once in the CNS, both T3D and T3C9 produce a lethal necrotizing meningoencephalomyelitis (3-5, 14). CNS infection is accompanied by extensive neuronal destruction and associated inflammation in cortex, hippocampus, and thalamus. Variants of T3D that are resistant to the neutralizing $\sigma 1$ mAb produced by the G5 hybridoma ($\sigma 1$ mAbG5, 15) have attenuated neurovirulence (16), and a restricted pattern of neuropathologic injury compared with wild-type virus (17). This provides additional evidence for the primary role played by the $\sigma 1$ protein in determining viral neurotropism and neurovirulence.

Thus, previous work has identified several distinct stages in the pathogenesis of CNS infection with reoviruses: (a) replication at a primary site (skeletal muscle [T3D], or intestine [T3C9]), (b) spread to the CNS via nerves (T3D) or through as yet undefined pathways (T3C9), (c) spread and growth of infectious virus within the CNS, and (d) neuronal destruction and induction of pathology. We have recently shown that antibody protects neonatal mice from lethal infection with T3D (18). Polyclonal rabbit antibody to T3D, neutralizing and nonneutralizing anti- $\sigma 1$ mAbs, and polyclonal rabbit antibody to determinants other than $\sigma 1$ all protect mice from lethal T3D infection. Antibody-mediated protection occurs even after establishment of extensive CNS infection.

We wished to better understand at which specific stage(s) in the pathogenesis of T3C9 and T3D infection antibody exerts its protective effect. In this paper, we demonstrate that antibody to the $\sigma 1$ protein: (a) has minimal effect on primary replication of virus in extraneural tissues such as intestine or muscle, (b) blocks the neural spread of T3D from muscle to spinal cord, (c) blocks spread of virus from the alimentary tract to CNS following PO inoculation, (d) inhibits spread of infectious virus within the CNS, (e) inhibits replication within the CNS, and (f) significantly alters the pattern of T3D induced neuropathology.

Materials and Methods

Animal Inoculations and Tissue Sampling. Neonatal NIH Swiss [NIH(s)] mice born of pregnant mothers obtained from the National Cancer Institute (Frederick, MD) were used in all experiments. Mice were injected with virus on the day after birth unless otherwise noted. IC, hindlimb FP, and PO injections were carried out as previously described (9, 18). Unless otherwise noted all experiments used 100 times the IC LD₅₀ or 100-250 times the FP LD₅₀ of T3D in order to assure that the majority of mice would die from viral infection in the absence of antibody administration. The LD₅₀ for T3D in NIH(s) mice is 4.8 log₁₀ plaque-forming units (PFU) per mouse after FP inoculation, and 1 log₁₀ PFU/mouse after IC inoculation (18). The expected mean day of death after receiving these doses of T3D are 9-10 d after IC and 10-12 d after FP inoculation. At various times after infection, mice were killed and tissues samples including brain, eye, superior and inferior spinal cord (SSC and ISC), muscle, and intestine (from duodenum to rectum) were collected into 1.0 ml of gel saline and frozen at -70°C before assay (18). Antibody or control solutions (100 μ l vol) were injected intraperitoneally as previously described (18). Either 200 μ g of affinity-purified $\sigma 1$ mAbG5, a 1:1 dilution of anti-T3D serum, or undiluted ascites was used in all experiments. 200 μ g of $\sigma 1$ mAbG5 is fourfold more antibody than would be expected to protect 100% of mice from

FP inoculation with $100 \times LD_{50}$ of T3D (18). Previous experiments showed that the half-life of $\sigma 1mAbG5$ in neonatal mice was >3 d (unpublished data). Unless otherwise noted pyrogen-free normal saline (PFNS; Travenol Inc., Deerfield, IL) was used for control injections.

Virus and Viral Assays. Reovirus T3C9 (12), T3D, and variant AI7 were derived from laboratory stocks. Viruses were doubly plaque purified and then passaged twice on L929 cells. Second passage virus was used for animal infection. The amount of infectious virus present in tissues was assayed as previously described (18). Briefly, tissue samples were frozen and thawed three times ($-70^{\circ}C$, $37^{\circ}C$), and then sonicated for 15–30 s. Serial 10-fold dilutions in gel saline were plated in duplicate on L cell monolayers and overlaid with medium containing agar. After 7 d, plaques were visualized using neutral red (Fisher Scientific, Pittsburgh, PA). Vial titers are reported as plaque forming units per milliliter. The data in Fig. 1–5, 8, and 9 are presented as the mean and standard error of the mean.

To ensure that neutralizing antibody in tissues from antibody-treated mice did not interfere with our ability to detect infectious virus, we mixed known amounts of T3D with sonicated brain tissue from antibody-treated mice, incubated this mixture at $37^{\circ}C$ for 60 min, and then assayed the mixture for infectious virus. Tissue from antibody-treated animals did not decrease viral titer in these mixtures (data not shown), demonstrating that antibody present in tissue was not responsible for diminution of viral titers in antibody-treated animals.

Production, Purification, and Assay of Antibodies. Production, purification, and assay of serum, ascites, and mAb were performed as previously described (18). Polyclonal antibody to T3D (anti-T3D serum) was raised in rabbits immunized with pure T3D. mAb to the T3D $\sigma 1$ protein ($\sigma 1mAbG5$) was prepared by protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) chromatography of tissue culture supernatant from the G5 cell line. $\sigma 1mAbG5$ -containing ascites was obtained from pristane-primed BALB/c mice (National Cancer Institute, Frederick, MD) bearing the G5 cell line. G5-containing ascites was equivalent to $144 \mu g/ml$ of purified $\sigma 1mAbG5$ by ELISA using purified T3D as antigen (data not shown). Control ascites was generated using the 8D3 cell line that did not produce antibody to T3D (15, data not shown). Both T3D immune serum and $\sigma 1mAbG5$ had significant neutralizing activity against T3D and T3C9 (15, 18, our unpublished data).

Tissue Pathology. Tissue samples were collected in 10% buffered formalin (Fisher Scientific, Medford, MA), embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were examined by K. L. Tyler and H. W. Virgin after covering all identifying signs and randomizing slides from different experimental groups. Areas of cortex, meninges, hippocampus, and thalamus were separately evaluated, and scored for the presence of necrosis, inflammation, and viral inclusions.

Results

Effect of $\sigma 1mAbG5$ Administered before T3D Infection. 24 h after receiving either $\sigma 1mAbG5$ or PFNS IP, mice were inoculated with T3D in the footpad (FP) (Fig. 1). In PFNS-treated mice, virus spread from hindlimb muscle to spinal cord and brain. Viral titers in the ISC were $>3 \log_{10}$ higher than those in the SSC and brain at day 3, reflecting the neural spread of T3D (2). Pretreatment of mice with $\sigma 1mAbG5$ significantly altered the pathogenesis of T3D infection. There was marked inhibition of the spread of virus from muscle to the CNS (Fig. 1). $\sigma 1mAbG5$ significantly ($>5 \log_{10}$ at day 10) inhibited spread of virus to the ISC and brain. Thus, administration of $\sigma 1mAbG5$ before infection with T3D inhibited both entry of virus into the ISC, and subsequent spread of virus to SSC and brain.

Effect of $\sigma 1mAbG5$ Administered at the Same Time as T3D Infection. Mice were inoculated with T3D in the FP and simultaneously given either $\sigma 1mAbG5$ or PFNS (Fig. 2). $\sigma 1mAbG5$ inhibited entry of virus into the CNS. Treatment with $\sigma 1mAbG5$ reduced viral titer in both ISC and SSC by 2–5 \log_{10} . $\sigma 1mAbG5$ also inhibited spread of virus from spinal cord to brain, with viral titers in brain $>5 \log_{10}$ lower in

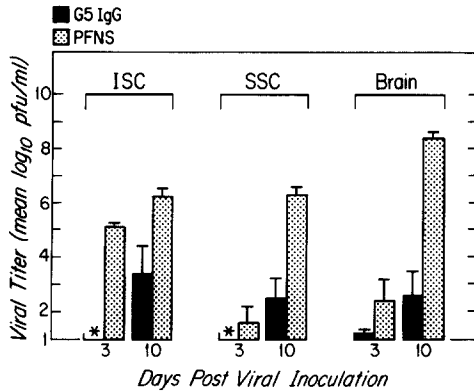


FIGURE 1. Effect of antibody administered before viral inoculation. Mice were given either $\sigma 1mAbG5$ or PFNS IP. 24 h later mice were inoculated with T3D in the hindlimb FP. 3 and 10 d after infection, samples of ISC, SSC, and brain were collected and assayed for virus (three to five mice per data point). Only upward error bars are shown for clarity. (*) <10 PFU/ml.

$\sigma 1mAbG5$ -treated animals by 5 after infection. Thus, antibody administered simultaneous to viral inoculation inhibited entry of virus into, and subsequent spread within, the CNS.

Effect of $\sigma 1mAbG5$ Administered after Virus has Reached the CNS. The experiments above show that antibody present early in infection acts to prevent viral entry into the CNS. Antibody can also protect mice subsequent to viral entry into the CNS (18). To define sites of antibody action late in infection, we infected mice with T3D in the FP and delayed $\sigma 1mAbG5$ transfer by 24 h (Fig. 3). After the first 48 h, the amount of virus in the ISC of $\sigma 1mAbG5$ -treated animals declined sharply compared with controls. In addition, $\sigma 1mAbG5$ markedly inhibited spread from ISC to SSC and brain. Similar results were obtained with polyclonal anti-T3D serum (data not shown). Thus, both monoclonal and polyclonal antibodies to T3D inhibited spread of virus within the CNS, and decreased virus titer in the ISC.

Effect of $\sigma 1mAbG5$ on T3D Infection of Muscle. To determine whether failure of virus to spread from muscle to ISC was due solely to inhibition of viral replication in muscle, we examined the effect of antibody on T3D infection of muscle. $\sigma 1mAbG5$ was administered prior to (Fig. 4 A), at the same time as (Fig. 4 B), or after (Fig. 4 C), FP infection with T3D. $\sigma 1mAbG5$ did not significantly alter viral titer in muscle until late in infection (see day 10, Fig. 4 A).

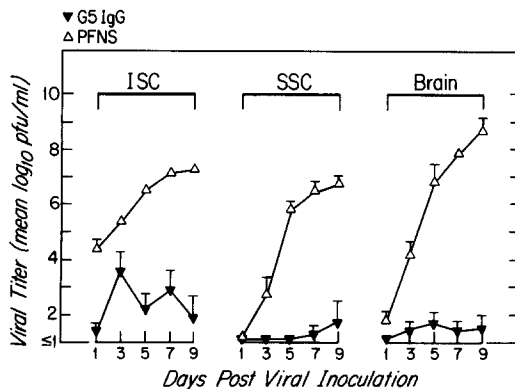


FIGURE 2. Effect of antibody administered at the time of viral inoculation. Mice were given either $\sigma 1mAbG5$ or PFNS IP. At the same time, mice were inoculated with T3D in the hindlimb FP. At the indicated times postinfection samples of ISC, SSC, and brain were collected and assayed for virus (five to seven mice per data point). Only upward error bars are shown for clarity.

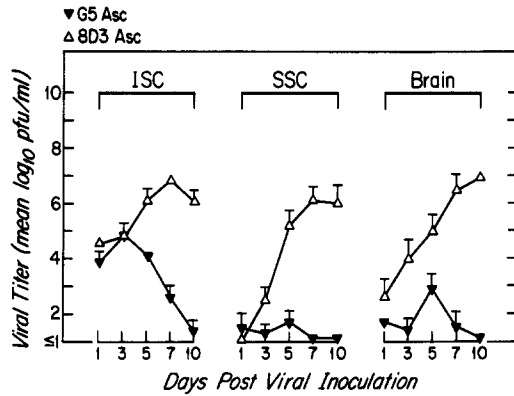


FIGURE 3. Effect of antibody administered after viral inoculation. Mice were inoculated in the hindlimb FP with T3D. 24 h later mice were given σ 1mAbG5-containing or control (8D3) ascites. At the indicated times, samples of ISC and SSC and brain were collected and assayed for virus (two to four mice per data point). Only upward error bars are shown for clarity.

To assess the effect of antibody on primary replication in a more sensitive experiment, we inoculated mice with a low dose of T3D (3 log₁₀ PFU) into the FP, and assayed viral titer in the presence and absence of antibody at a time (day 6) when viral growth in muscle would be maximal (Tyler, K. L., unpublished data) (Fig. 4 D). There was no significant difference in viral titers between σ 1mAbG5 treated and control muscles.

We conclude that σ 1mAbG5-mediated inhibition of viral entry into and spread within the CNS, is not due to decrease in viral titer at the primary site of infection.

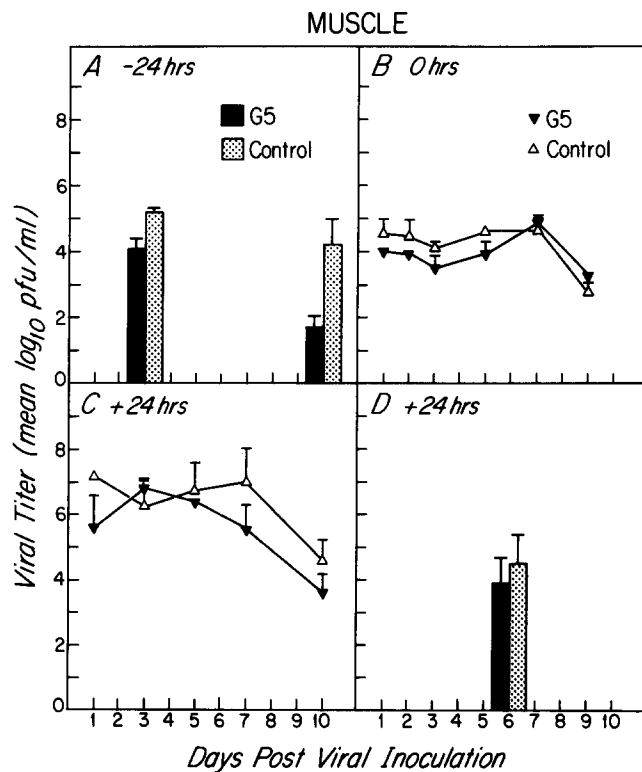


FIGURE 4. Effect of antibody on viral titer in muscle. Mice were infected with T3D in the FP and at the indicated times after infection the leg musculature was assayed for virus. (A) Mice were given σ 1mAbG5 or PFNS IP followed 24 h later by viral infection (three to five mice per data point). (B) mice were given σ 1mAbG5-containing ascites or PFNS IP concurrent with viral infection (5.7 log₁₀ PFU/mouse inoculated, three to nine mice per data point). (C) Mice were given σ 1mAbG5 containing or control (8D3) ascites 24 h after FP infection with T3D (two to four mice per data point). (D) Mice were given σ 1mAbG5 or PFNS IP 24 h after footpad infection with 1,000 PFU of T3D (nine mice per data point). Only upward error bars are shown for clarity.

Effect of $\sigma 1mAbG5$ after IC Inoculation with T3D. To further define the effect of $\sigma 1mAbG5$ on spread and growth within the CNS, we inoculated mice IC with T3D and 24 h later administered either $\sigma 1mAbG5$ or PFNS IP (Fig. 5). In mice given PFNS, virus replicated in brain, and spread from brain to eye, SSC, ISC, and hind-limb muscle. $\sigma 1mAbG5$ almost completely inhibited spread of virus to these sites, and inhibited viral growth in the brain (Fig. 5).

Effect of $\sigma 1mAbG5$ on Neuropathology Produced by T3D. To determine the effect of $\sigma 1mAbG5$ on T3D-induced neuropathology we inoculated mice IC with T3D, and 24 h later gave them either $\sigma 1mAbG5$ IP ($n = 36$) or no treatment ($n = 11$). Untreated mice were killed when moribund (day 9–10). All animals given $\sigma 1mAbG5$ appeared healthy, and 16 were killed on days 9–10 for comparison with the untreated group. Additional $\sigma 1mAbG5$ -treated mice were killed at day 16–17 ($n = 11$), and day 21–22 ($n = 9$).

By day 9, untreated mice had substantial necrosis of cortex (8/11), hippocampus (6/9), and thalamus (9/10). Almost all animals showed marked perivascular and meningeal inflammation in the cortex (9/11), hippocampus (7/9), and thalamus (10/10). In contrast, there was no significant neuronal necrosis in $\sigma 1mAbG5$ -treated mice (1 of 16 mice had mild cortical and thalamic necrosis). Fig. 6 shows sections of hippocampus on days 9–10 in the presence (A), and absence (B), of $\sigma 1mAbG5$ treatment. 2 of 16 $\sigma 1mAbG5$ -treated mice had inclusions within neurons in the hippocampus. At day 9–10, $\sigma 1mAbG5$ -treated mice had evidence of mild perivascular inflammation, and 6 of 16 mice had mild inflammation, confined primarily to the perihippocampal area.

$\sigma 1mAbG5$ -treated mice killed at 16–17 d after infection had no evidence of either tissue necrosis or viral inclusions. However, almost all mice had prominent perivascular inflammation involving the hippocampus (9/11, Fig. 7 B), and thalamus (6/11), but completely sparing the cortex (0/11). Similarly, 9 of 11 mice showed significant perihippocampal meningeal inflammation (Fig. 7 B). This inflammatory response was still present on day 22, and was accompanied in some mice by hippocampal necrosis (4/9).

Thus, $\sigma 1mAbG5$ eliminated neuronal necrosis and significantly attenuated the initial T3D-induced inflammatory response. A prominent inflammatory response

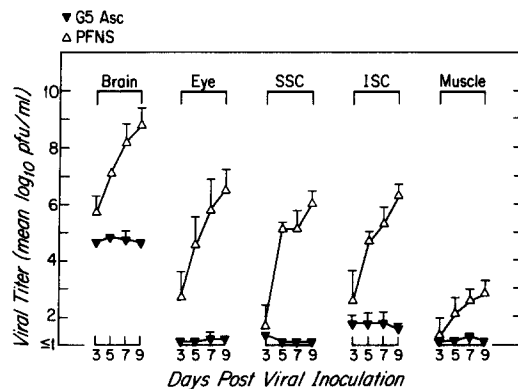


FIGURE 5. Effect of antibody administered after IC inoculation. Mice were inoculated with $3 \log_{10}$ PFU of T3D IC. 24 h later mice received either $\sigma 1mAbG5$ -containing ascites or PFNS IP. At the indicated times, samples of brain, eye, ISC, SSC, and muscle were collected and assayed for virus (controls: two to three mice per data point; antibody-treated: five to seven mice per data point). Only upward error bars are shown for clarity.

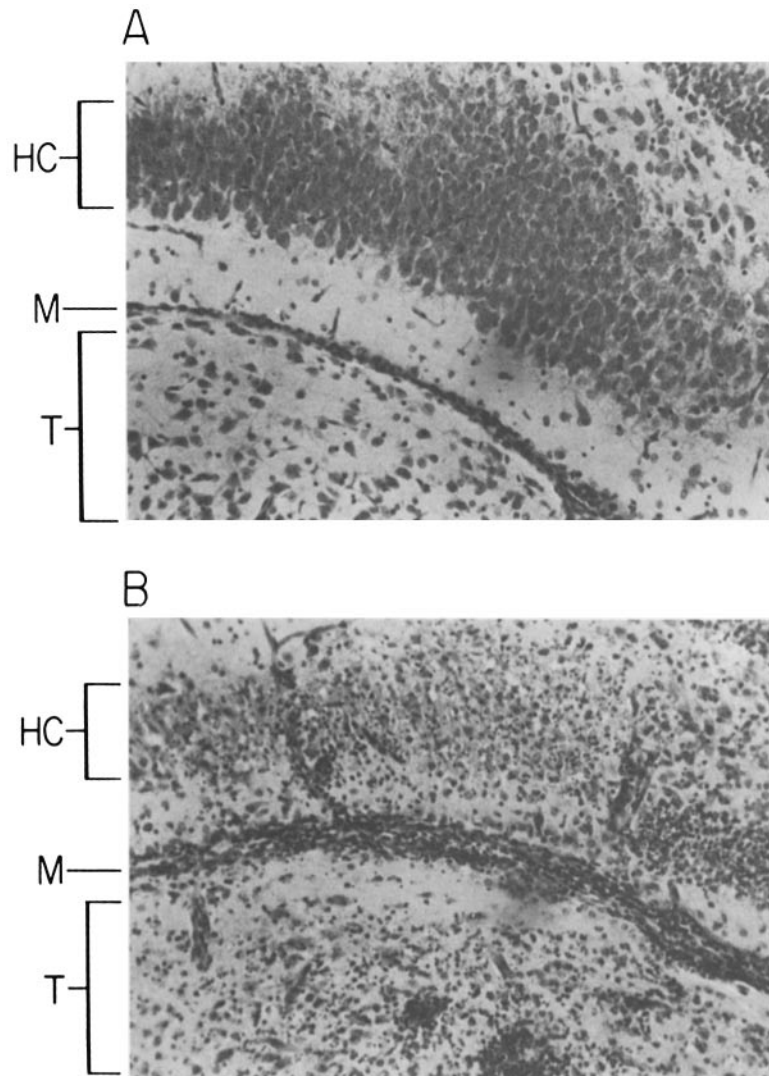


FIGURE 6. Effect of antibody on neuropathology induced by T3D. Mice were inoculated with $3 \log_{10}$ PFU of T3D IC. 24 h later mice were given either no treatment or either $\sigma 1mAbG5$ or PFNS IP. Photomicrographs of coronal brain sections stained with hematoxylin and eosin are shown (original magnification, $\times 100$). (A) Day 10, $\sigma 1mAbG5$ treatment. (B) Day 10, PFNS treatment. HC, hippocampus; M, meninges; T, thalamus.

did appear by day 16 in $\sigma 1mAbG5$ -treated animals and gradually waned over the ensuing week. This late inflammatory response was limited almost entirely to the perihippocampal region, and was associated with some delayed neuronal necrosis.

Absence of Detectable $\sigma 1mAbG5$ -resistant Variants In Vivo. The neuropathology observed in both $\sigma 1mAbG5$ -treated mice and mice infected with $\sigma 1mAbG5$ mAb-resistant (mar) variants of T3D is restricted to the limbic system (17) (Fig. 7). To determine

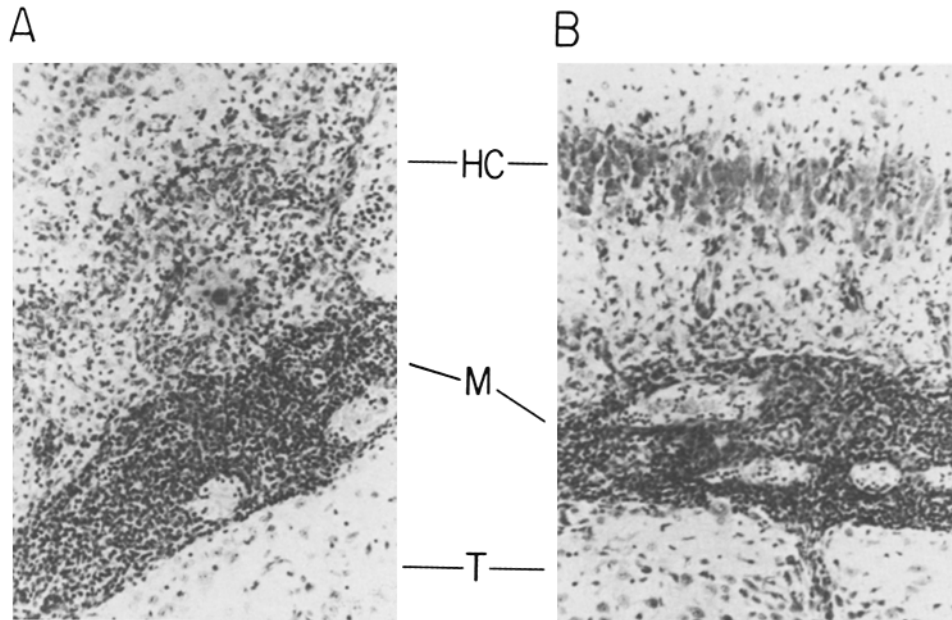


FIGURE 7. Effect of antibody on late neuropathology induced by T3D. Mice were inoculated with either $3 \log_{10}$ PFU of T3D or $6.3 \log_{10}$ PFU of variant AI7 IC. 24 h later mice were given either $\sigma 1mAbG5$ IP or left untreated. Photomicrographs of coronal brain sections stained with hematoxylin and eosin are shown (original magnification, $\times 100$). (A) Day 10, variant AI7 infected given no treatment. (B) Day 16, T3D infected given $\sigma 1mAbG5$ treatment. HC, hippocampus; M, meninges, T, thalamus.

whether antibody treatment resulted in the selection of mar mutants in vivo, we studied 40 doubly plaque-purified viral isolates from brains of $\sigma 1mAbG5$ -treated mice obtained 9–10 d after infection. We found that all isolates (40/40) remained sensitive to neutralization with a concentration of $\sigma 1mAbG5$ ($1 \mu\text{g/ml}$) that neutralizes T3D, but not variants selected using $\sigma 1mAbG5$ (data not shown, 15, 16, 18). Thus, the small amounts of virus detected in brains of $\sigma 1mAbG5$ -treated animals 9–10 d after inoculation presumably represent wild-type T3D virus that escaped antibody blockade, and not in vivo selected mar mutants.

Effect of $\sigma 1mAbG5$ on the Pathogenesis of T3C9 Infection. T3C9 is a serotype 3 reovirus that produces encephalitis after PO infection, providing a model for studying the effect of systemic antibody on CNS infection produced after viral entry via the gastrointestinal tract. Mice were inoculated PO with $7 \log_{10}$ PFU of T3C9, and 24 h later were given PFNS, $\sigma 1mAbG5$, or anti-T3D serum IP. All mice ($n = 12$) receiving PFNS died after a mean of 10.4 ± 1.5 d. In contrast, none of the mice receiving $\sigma 1mAbG5$ ($n = 11$), or anti-T3D serum ($n = 12$) died over a 30-d period.

To study the effect of antibody on spread of T3C9 to the CNS after the establishment of intestinal infection, mice were inoculated with $7 \log_{10}$ PFU of T3C9 PO and 24 h later were given PFNS or $\sigma 1mAbG5$ IP (Fig. 8). $\sigma 1mAbG5$ blocked T3C9 viremia and completely inhibited entry of T3C9 into spinal cord and brain, but only slightly reduced T3C9 titer in intestine (day 9).

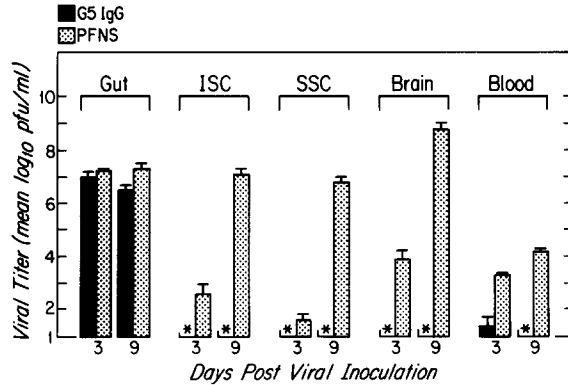


FIGURE 8. Effect of antibody administered after PO inoculation with T3C9. Mice were inoculated with 7 log₁₀ PFU of T3C9 PO. 24 h later mice received either σ 1mAbG5 or PFNS IP. At the indicated times samples of intestine, ISC and SSC, brain, and blood were collected and assayed for virus (three mice per data point). Only upward error bars are shown for clarity. (*) < 10 PFU/ml.

We next assessed the role of antibody administered before PO infection with T3C9 (Fig. 9). Mice were given either σ 1mAbG5 or PFNS IP, and 24 h later were inoculated with 6.4 log₁₀ PFU of T3C9 PO. σ 1mAbG5 prevented entry of T3C9 into the CNS without reducing the amount of virus in intestinal tissue (Fig. 9 B). To assess the influence of antibody on intestinal primary replication in a more sensitive experiment, mice were inoculated PO with 2.8 log₁₀ PFU of T3C9 IP 24 h after receiving either σ 1mAbG5 or PFNS IP (Fig. 9 A). σ 1mAbG5 had no effect of primary replication and yet completely blocked the spread of virus from the intestine to the CNS.

Thus, antibody protects mice from lethal PO infection with a serotype 3 reovirus. This protection involves prevention of spread of virus from the intestinal primary site to the CNS in the absence of any effect on growth of virus at the primary site.

Discussion

In this paper we define stages at which an IgG mAb specific for the reovirus cell attachment protein σ 1 acts to protect mice against lethal reovirus-induced CNS disease (see Fig. 10). Antibody inhibited entry of virus into the CNS from extraneural sites of primary replication (either muscle or intestine), but had only minimal effects on viral replication at these sites. Antibody administered after virus had already

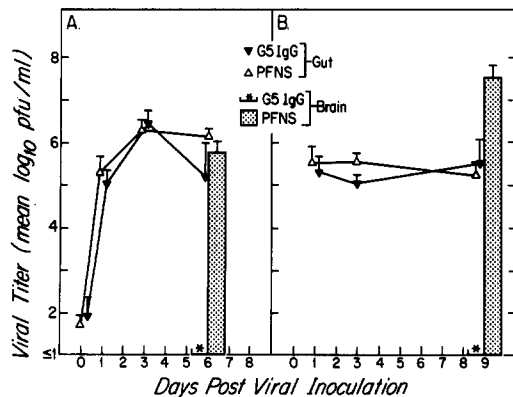


FIGURE 9. Effect of antibody administered before PO inoculation with T3C9. Mice were given either σ 1mAbG5 or PFNS IP. 24 h later mice were inoculated with either 2.8 log₁₀ PFU (A), or 6.4 log₁₀ PFU (B) of T3C9 IP. At the indicated times, samples of intestine and brain were collected and assayed for virus (four to seven mice per data point). Only upward error bars are shown for clarity. (*) < 10 PFU/ml.

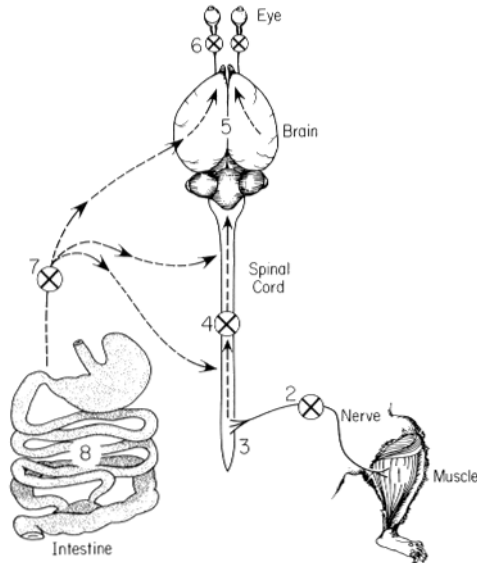


FIGURE 10. Sites of antibody action. (1) Antibody has minimal effects on viral titer in muscle early after infection. (2) Antibody inhibits spread of virus from muscle to inferior spinal cord. (3) Antibody blocks entry into and/or growth within the inferior spinal cord. (4) Antibody blocks spread of virus from inferior spinal cord to superior spinal cord and brain. (5) Antibody inhibits growth of virus in the brain, and limits pathology to the hippocampus and perihippocampal tissues. (6) Antibody blocks spread from brain to eye. (7) Antibody blocks spread of virus from the intestine to brain and spinal cord. (8) Antibody has no effect on primary replication in the intestine.

invaded the CNS, prevented subsequent growth and spread of virus. Antibody also prevented the development of necrotizing cortical encephalitis and limited T3D-induced pathologic changes to the limbic system.

Effect of Antibody at the Primary Site of Infection. Although for some viral infections antibody-mediated inhibition of entry into the CNS is accompanied by inhibition of primary replication (19), this is not invariably true. For example, neither immunization with killed poliovirus vaccine nor passive transfer of antibody eliminates gastrointestinal shedding of poliovirus, yet both clearly protect against the development of paralytic poliomyelitis (20–24). In contrast, immunity established after immunization with Sabin oral poliovirus vaccine, or following recovery from natural infection with poliovirus, results in both protection from CNS disease and decreased shedding of virus from the intestine (20, 25). These results have been used to argue that local immunity, rather than systemic IgG, plays a predominant role in limiting viral shedding from the alimentary tract, although a role for systemic antibody has also been suggested (21, 26). Our studies clearly show that although systemic IgG may be extremely effective in preventing viral spread to the CNS, even a high dose of protective IgG has no effect on replication of virus in the intestine.

Effect of Antibody on Entry of Virus into the CNS. T3D spreads from muscle to CNS via nerve, a capacity conferred by the T3 S1 gene (2) encoding the outer capsid protein $\sigma 1$. $\sigma 1$ mAbG5 given before, or at the same time as, infection with T3D inhibited entry of virus into the CNS (Figs. 1 and 2; Fig. 10, site 2), without significantly decreasing viral titer in muscle (Fig. 4; Fig. 10, site 1), clearly showing that IgG can inhibit spread of virus through nerves. Antibody inhibits entry of herpes virus into the CNS (19), and prevents the development of paralytic poliomyelitis after inoculation of neurally spreading poliovirus strains (27), providing additional examples of inhibition of neural spread (Fig. 10, site 2).

Effect of Antibody on Growth and Spread within the CNS. Antibody protects mice after

significant CNS infection with T3D has been established (18), indicating that antibody-mediated protection must involve mechanisms in addition to inhibition of viral entry into the CNS. We now show that $\sigma 1$ mAbG5 inhibited both growth and spread of virus within the CNS (Figs. 2, 3, 5). Similar phenomena have been seen with other neurotropic viral infections (28–32). Thus, inhibition of viral growth and spread within CNS tissue (Fig. 10, sites 3–6) is an important mechanism of antibody-mediated protection against neurotropic viral infection.

Effect of Antibody on Virus-induced Pathology. Studies with several viruses have shown that antibody can either prevent or alter the nature of virally induced tissue injury (33–35). In our experiments, $\sigma 1$ mAbG5-treated animals had several major alterations in CNS pathology (Figs. 6 and 7). There was marked reduction and delay in tissue injury, and in the appearance of T3D-induced inflammatory responses. The inflammatory response, when present, was topologically restricted to perihippocampal tissues.

There are several possible explanations for the restricted pattern of injury and inflammation seen in our experiments. First, the cells in the hippocampus may be more vulnerable to injury by T3D or G5-selected variants than cortical neurons. A similar selective vulnerability of the hippocampus has been seen following a variety of CNS insults including hypoxia (36). Second, the hippocampus may be the site of early replication for T3D after IC inoculation and antibody may inhibit spread of virus from this site to distant regions such as cortex. Finally, it is possible that a determinant on the $\sigma 1$ protein is critical to causing cortical injury and this determinant is lacking in G5-selected variants of T3D, or directly inhibited by $\sigma 1$ mAbG5 in vivo.

Mechanism of Antibody Action at Defined Stages in Pathogenesis. In this study we have defined specific pathogenetic steps at which antibody acts to protect mice from CNS infection. The precise mechanism(s) by which antibody acts at these steps are not understood. A role for complement, antibody-dependent cell-mediated cytotoxicity, or the Fc portion of Ig has been proposed but never unequivocally documented in vivo (reviewed in reference 18). Alternatively, it has been assumed that the action of antibody in vivo is related to in vitro functions such as neutralization (i.e., plaque reduction). A lack of correlation between in vitro neutralization and protection has been repeatedly demonstrated for a wide variety of viral infections (reviewed in reference 18), clearly indicating that in vivo protection is not simply an analogue of in vitro neutralization. Studies limited to antibodies that are “neutralizing” in vitro may not be relevant to understanding protective immune responses in vivo. Similarly, vaccine development strategies based on the induction of neutralizing antibody may not lead to protection of the host.

Another possible mechanism for the prevention of neural spread and protection provided by $\sigma 1$ mAbG5 is inhibition of critical in vivo functions of the $\sigma 1$ molecule such as attachment of T3D to neurons (4), or spread within nerves (2). In rats, the S1 gene determines the pattern of clearance of reovirus T1 from the blood stream after intravenous inoculation, and antibody to the T1 $\sigma 1$ protein alters this clearance (37). This suggests that antibody to the T1 $\sigma 1$ protein may alter the pathogenesis of T1 infection by binding to and inhibiting the normal function of a viral capsid protein.

Antibody could also be acting at intracellular sites to inhibit key steps in the life

cycle of the virus or the movement of the virus within cells (38, 39). Antibody has been demonstrated inside nerve cells after passive transfer (40), and could therefore act at this site to prevent viral growth or spread. These observations suggest that antibody may act across the cell membrane, or within neural cells, to inhibit critical steps in viral replication or assembly.

Further work will be needed to define the specific mechanisms by which antibody acts at defined sites in the pathogenesis of CNS infection. We are currently characterizing a panel of mAbs to capsid proteins other than $\sigma 1$ in order to study similarities or differences in the *in vivo* sites of action of these antibodies compared with sites identified in this paper for a mAb to the $\sigma 1$ protein. In addition, the fact that immune cells also protect from lethal infection with T3D and T3C9 (Virgin, H. W., and K. L. Tyler, unpublished data) opens up the possibility that we will be able to define and contrast the specific sites at which cells and antibody act to combat viral infection.

Summary

The mammalian reoviruses provide a model for studying specific aspects of the immunopathogenesis of viral infection. We have used two serotype 3 reoviruses to define stages in the pathogenesis of central nervous system (CNS) infection at which a mAb specific for the reoviral cell attachment protein $\sigma 1$ ($\sigma 1$ mAbG5) acts to protect mice against lethal disease. $\sigma 1$ mAbG5 administered either before or at the time of footpad inoculation with reovirus T3D prevented entry of T3D into the CNS. $\sigma 1$ mAbG5 also inhibited the spread of reovirus T3C9 from the gastrointestinal tract to the CNS after peroral inoculation with T3C9. These effects occurred in the absence of a significant effect of $\sigma 1$ mAbG5 on primary replication in skeletal muscle (T3D) or the gastrointestinal tract (T3C9). $\sigma 1$ mAbG5 administered after T3D had reached the spinal cord inhibited subsequent spread of infectious virus from spinal cord to brain. Even after direct intracerebral inoculation of T3D, $\sigma 1$ mAbG5 prevented both growth in the brain and spread of infectious virus from brain to eye, spinal cord, and muscle. Treatment with $\sigma 1$ mAbG5 after intracerebral inoculation with T3D prevented neuronal necrosis and resulted in a delayed and topographically restricted inflammatory response. We detected no antibody-resistant T3D variants *in vivo* after treatment with $\sigma 1$ mAbG5. We conclude that systemic IgG does not play a significant role at the primary site of infection with reoviruses, while it clearly acts to prevent infection of the CNS and extension of infection with the CNS. Further study will be directed to defining what components of the immune system do act at primary sites of infection, and to defining the mechanisms by which antibody acts at defined stages in pathogenesis.

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