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Monoclonal Antibody Protection from Age-Dependent Poliomyelitis: Implications Regarding the Pathogenesis of Lactate Dehydrogenase-Elevating Virus

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Over 90% of cyclophosphamide-treated, 6- to 7-month-old C58/M mice developed fatal paralytic disease after infection with a virulent strain of lactate dehydrogenase-elevating virus (LDV), with a mean onset of paralysis of about 16 days. Passive immunization with polyclonal antibodies or with a group of anti-LDV monoclonal antibodies (MAbs) with single-epitope specificity 1 day before or at the time of LDV infection prevented the development of paralytic disease without interfering with the replication of LDV in permissive macrophages, the primary host cells of LDV. In situ hybridization of spinal cord sections with an LDV-specific cDNA probe indicated that the MAb specifically prevented the cytocidal infection of motor neurons by LDV without blocking the infection of smaller nonneuronal cells in the spinal cord. The protective antibodies recognize at least two different epitopes on the glycoprotein of LDV, VP-3. Passive immunizations with other anti-LDV MAbs, which recognize at least three other epitopes on VP-3 of LDV, afforded no protection. In contrast to the protective effect of anti-LDV MAb injection before or at the time of LDV infection, their administration postinfection exerted relatively little protection, though it delayed the appearance of paralytic symptoms. However, repeated injections of MAbs until at least 7 days postinfection also afforded a high degree of protection. The results indicate that protective MAbs may interfere with two stages in the development of LDV-induced paralytic disease. When administered at the time of LDV infection, they prevent the initial infection of spinal cord motor neurons. After this initial event, repeated injections of MAb are required to inhibit the spread of LDV between neurons until the endogenous production of protective anti-LDV antibodies in these mice.

Age-dependent poliomyelitis (ADPM) is a motor neuron disease which occurs in certain mouse strains after acute infection by lactate dehydrogenase-elevating virus (LDV) (6, 13, 14). Susceptibility to disease is dependent on a number of predisposing conditions, including the presence of multiple proviral copies of endogenous N-tropic, ecotropic murine leukemia virus (MLV) and of the *Fv-1*$^{n/n}$ genotype, which permits the replication of these endogenous viruses after birth (13, 14, 17). C58/M and AKR/Boy mice are susceptible to the disease, but susceptibility does not develop until about 6 months of age, and at this age it is dependent on cyclophosphamide (CY) treatment or X-irradiation of the mice before LDV infection (13, 14).

ADPM results from LDV infection and destruction of ventral horn motor neurons in the spinal cords of susceptible mouse strains (1, 5–8). Cytocidal LDV infection of neurons appears to be dependent upon the antecedent expression of MLV in these neurons (6–8). Accumulation of endogenous MLV RNA specifically in the target motor neurons, which occurs naturally with age and may be enhanced by CY treatment and X-irradiation, correlates with the susceptibility of neurons to cytocidal infection by LDV (6–8). This correlation suggests that the etiology of ADPM involves a novel interaction at the level of the motor neuron between two normally nonpathogenic viruses in the generation of neurological disease.

LDV primarily replicates in a subpopulation of mouse macrophages and generally causes a lifelong persistent infection without overt disease manifestations (15, 19, 20). The infection of macrophages in C58 and AKR mouse strains is identical to that seen in other mouse strains which are not susceptible to ADPM (4). The persistent infection of mice is maintained by cytocidal replication of LDV in permissive macrophages as they arise from presumably nonpermissive precursor cells (15, 16, 19, 20). Although mice mount an immunological response to LDV, as determined by plasma anti-LDV antibodies, virus is not eliminated from the host (3, 9, 15). In addition, passive immunization of mice with neutralizing or nonneutralizing polyclonal or monoclonal anti-LDV antibodies has no significant protective effect against LDV infection (4, 10, 12). To date, all evidence indicates that humoral and cellular immune responses do not play a significant role in controlling LDV viremia in infected animals (15). Thus, LDV has apparently evolved mechanisms of persistence which allow the virus to evade immune surveillance.

The lack of effectiveness of anti-LDV antibodies in controlling LDV replication in macrophages contrasts with their ability to interfere with the development of ADPM in 6- to 7-month-old C58 mice that have been rendered susceptible by CY treatment or X-irradiation (10, 14). We and others have previously determined that polyclonal anti-LDV antibodies can prevent ADPM in these mice when present at the time of LDV infection (10, 14). We demonstrated that the protective antibodies were primarily directed to the envelope glycoprotein of LDV, VP-3, and that they prevented ADPM without affecting peripheral LDV replication, as determined by plasma LDV titers. The protective antibodies appear to specifically prevent the infection of motor neurons by LDV and thus prevent neurological disease (10). In the present study, we have analyzed a battery of anti-LDV

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TABLE 1. Effects of CY treatment on incidence of ADPM, LDV replication, and anti-LDV antibody formation[a]

Treatment	Plasma LDV (ID_{50}/ml) at day p.i.			No. of mice paralyzed/total	Onset of paralysis (day[s] p.i.)		Anti-LDV antibody titer at day p.i.	
	1	2	5		Individual mice	Mean ± SEM	8	12
None	$10^{9.5}$	$10^{9.5}$	$10^{7.5}$	0/10			128	1,024
CY	$10^{10.5}$	$10^{8.5}$	$10^{8.0}$	7/7	15, 15, 15, 16, 16, 16, 17	16 ± 0.3	<32	<32

[a] Where indicated, one group of 6- to 7-month-old C58/M mice was injected intraperitoneally with 200 mg of CY per kg. One day later these mice and a group of untreated mice were injected with 10^6 ID_{50} of LDV_v. The mice were monitored for symptoms of paralysis for 63 days p.i. Five mice of each group were bled at intervals (1, 2, 5, 8, and 12 days p.i.), and their plasma was pooled and assayed selectively for infectious LDV and anti-LDV antibodies.

monoclonal antibodies (MAbs) for the ability to protect against ADPM. We have identified five protective MAbs which are specific for VP-3 and neutralize LDV in vitro. Previous studies indicated that these protective MAbs recognize a different epitope on VP-3 than the protective polyclonal antibodies do (12). Furthermore, our results suggest that the protective MAb can interfere at two different stages in the development of ADPM. Implications of these findings for the mechanism of LDV pathogenesis are discussed.

MATERIALS AND METHODS

Mice. C58/M male or female mice were bred in the animal facility at the University of Minnesota. The breeding nucleus was originally obtained from W. Murphy (University of Michigan). Unless otherwise indicated, mice were 6 to 7 months of age at the initiation of experiments. Swiss mice were obtained from BioLabs (St. Paul, Minn.).

Virus. LDV_v is a neurovirulent strain of LDV originally isolated from an infected C58/M mouse and reisolated from a paralyzed C58/M mouse 10 days postinfection (p.i.) (8). LDV_v used in the present experiments was a pool of plasma from 1-day-infected C58/M mice with a titer of about $10^{9.5}$ 50% infectious doses (ID_{50}) per ml. LDV titers were estimated by an endpoint dilution assay in Swiss mice (18). For experiments, C58/M mice were infected with LDV by intraperitoneal injection of 10^6 ID_{50} LDV_v per mouse.

Antibody protection experiments. C58/M mice were inoculated intraperitoneally with CY (200 mg/kg of body weight) to render them susceptible to ADPM and 1 or 2 days later were infected with LDV_v and then observed for paralytic symptoms over a period of at least 40 days. When indicated, the mice were injected via the lateral tail vein with the indicated amounts of anti-LDV MAbs 1 day before or at various times after infection with LDV.

MAbs. Isolation and characterization of the anti-LDV MAbs generated to Formalin-inactivated LDV has been previously described (12). The MAbs were used either as a dilution of ascites fluid or in the form of protein G-purified MAb. The MAbs were purified from ascites fluid by protein G (Genex Corp., Gaithersburg, Md.) chromatography by the supplier's instructions. Protein concentrations were determined by BCA assay (Pierce Chemicals, Rockford, Ill.).

Anti-LDV antibody titration. Anti-LDV antibodies were quantitated by indirect fluorescent antibody staining of cultures of peritoneal macrophages from adult Swiss mice infected for 8 h with LDV as described previously (3, 12). The antibody titer was expressed as the reciprocal of the highest twofold dilution of plasma or of MAb preparation that yielded recognizable staining of 3 to 10% of the total macrophages in LDV-infected cultures without staining any cells in uninfected cultures.

In situ hybridization. In situ hybridization of sections of aldehyde-fixed spinal cord tissues was performed as previously described by using a ^{125}I-labeled, 1.6-kb, LDV-specific cDNA clone (6–8, 10).

RESULTS

Importance of CY treatment in rendering 6- to 10-month-old C58/M mice susceptible to ADPM. Previous work indicated that treatment of 6- to 10-month-old C58/M mice with CY or X-irradiation before LDV infection greatly increased the incidence of paralytic disease in these mice (6, 8, 13, 14), but the contributions of different potential mechanisms to this effect have not been entirely resolved. Previous work showed that both treatments enhanced the expression of endogenous ecotropic MLV in spinal cord motor neurons of 6- to 7-month-old C58/M mice, thus probably enhancing their susceptibility to cytocidal infection by LDV (6, 8). Other recent experiments, however, have indicated that the immunosuppressive effects of CY and X-irradiation are at least equally important in rendering the mice susceptible to ADPM (J. T. Harty et al., unpublished data). In this scenario, untreated LDV-infected C58/M mice rapidly mount an anti-LDV immune response, which specifically protects the motor neurons from LDV infection in spite of the expression of ecotropic MLV in these cells. This conclusion is in part derived from the data in Table 1, from the fact that protective anti-LDV antibodies have been demonstrated in 7- to 10-day-infected mice (10, 13) and from the results presented in later sections.

The results in Table 1 show that anti-LDV antibodies, as measured by fluorescence antibody titration, rapidly appeared in 6- to 7-month-old C58/M mice after LDV infection and that none of the mice developed paralytic disease. In contrast, all of the mice injected with CY 1 day before LDV infection succumbed to the disease. In the latter mice, anti-LDV antibody formation was inhibited for at least 12 days. It is known, however, that mice generally recover their immune function 10 to 12 days after a single administration of CY (see reference 15). As in previous experiments (4, 15), the CY treatment had little effect on plasma LDV levels during the first 5 days p.i. (Table 1).

Protective anti-LDV MAbs are epitope-specific and do not affect peripheral LDV replication. We and others have previously demonstrated that when present at the time of infection, polyclonal anti-LDV antibodies obtained from persistently infected mice of various strains can protect C58/M mice from ADPM (10, 14). The protective capacity of the antisera did not correlate with the presence of LDV-specific neutralizing activity (10). LDV-specific, nonneutralizing MAbs which were raised against glutaraldehyde-inactivated LDV alone or in combination did not afford any protection from ADPM (11), presumably because of epitope loss or alteration after fixation of the virus.

We have continued these analyses by using both neutral-

TABLE 2. Protection of C58/M mice from ADPM by MAbs raised to Formalin-inactivated LDV[a]

Antibody	Neutralizing activity[b]	No. of mice paralyzed/total	Onset of paralysis (day[s] p.i.)	
			Individual mice	Mean ± SEM
None		15/15	12, 13, 13, 14, 14, 15, 15, 15, 15, 15, 15, 17, 19, 19, 26	16 ± 0.9
eIMP	+	3/21	22, 23, 26	24 ± 1.2
159-7	+	0/6		
159-12	+	2/15	17, 26	22 ± 4.5
159-16	+	1/5	26	26
159-18	+	2/11	19, 40	30 ± 11
159-19	+	0/6		
159-3	−	4/4	12, 12, 14, 19	14 ± 1.7
159-4	−	5/5	12, 12, 13, 14, 16	13 ± 0.7
159-5	−	3/5	14, 16, 19	16 ± 1.5
159-14	−	6/6	13, 13, 15, 15, 16, 16	15 ± 0.6

[a] Groups of 6- to 7-month-old C58/M mice were treated with 200 mg of CY per kg. One day later the mice were injected i.v. with 0.5 ml of a 1:2 dilution of ascites fluid containing the indicated MAb or with 0.5 ml of a 1:3 dilution of ether-extracted polyclonal anti-LDV antibodies obtained from 90-day-infected mice (eIMIP). The mice were infected with 10^6 ID_{50} of LDV_v 1 day after antibody injection. Animals were observed for paralysis until 45 days p.i.

[b] Neutralizing activity by anti-LDV antibodies was assessed by incubating LDV with anti-LDV antibodies in vitro followed by infectivity titration in mice as previously described (3).

izing and nonneutralizing anti-LDV MAbs raised against Formalin-inactivated LDV. All five of the neutralizing MAbs we obtained after immunization with Formalin-inactivated LDV reacted with the same epitope on VP-3, as demonstrated by reciprocal competitive binding assays in conjunction with Western immunoblot analyses (12). Four nonneutralizing antibodies to Formalin-inactivated LDV represent three additional epitope specificities (12; data not shown). Each of these MAbs, as well as polyclonal anti-LDV antibodies (eIMP), was injected intravenously (i.v.) into CY-treated C58/M mice 1 day prior to LDV infection. Infection of CY-treated C58/M mice in the absence of anti-LDV antibody resulted in 100% incidence of paralysis, whereas the polyclonal anti-LDV antibodies (eIMP) reduced the incidence of disease to 14% (Table 2). Nonneutralizing MAbs 159-3, -4, -5, and -14 did not significantly reduce the incidence of disease (MAbs 159-3 and 159-5 recognize the same epitope, and the cumulative incidence of disease with these two MAbs was 78%). A cocktail of the four nonneutralizing MAbs also failed to protect mice from ADPM (data not shown). In contrast, all five neutralizing MAbs exhibited significant protective capacity against ADPM. Their protective effect was comparable to that effected by the polyclonal antibodies (eIMP). Furthermore, the few mice that succumbed to ADPM after injection of protective antibodies developed paralytic symptoms relatively late, compared with their unprotected companion mice. Since the epitope recognized by the protective MAbs is poorly recognized, if at all, during a natural infection of mice (12), at least two epitopes on the envelope glycoprotein of LDV, one (or more) recognized by polyclonal anti-LDV antibodies generated in infected mice and the other defined by the neutralizing MAbs raised to Formalin-inactivated LDV, can elicit antibodies which are protective against ADPM. The protective anti-LDV MAbs, which recognize a single epitope on VP-3, have previously been shown not to affect peripheral LDV replication in macrophages, as determined by plasma LDV levels in passively immunized BALB/c mice (12). This finding has been extended to CY-treated 6-month-old C58/M mice. The LDV titers in the plasma of the latter mice were about the same 1 and 7 days p.i. (about 10^9 and 10^7 ID_{50} per ml, respectively) whether or not the mice were injected with 200 μg of protein G-purified MAb 159-12 1 day before LDV infection, a dose that is sufficient to almost completely protect these mice against ADPM (see below).

In order to determine the efficiency of the MAbs in protecting mice from ADPM, MAb 159-12 was purified from high-titer ascites fluid with protein G, and various doses (1, 10, 50, 200, and 400 μg per mouse) were inoculated i.v. into groups of CY-treated C58/M mice prior to LDV infection. MAb protection was concentration dependent; maximal protection was achieved at an inoculum size of 200 μg of protein G-purified MAb 159-12 (only 2 of 12 mice became paralyzed at 18 and 19 days p.i.).

Specific protection of motor neurons by anti-LDV MAbs. The mechanism of protection from ADPM by polyclonal anti-LDV antibodies had previously been assessed by in situ hybridization analyses of spinal cord tissues from protected mice (10). In these studies, the polyclonal anti-LDV antibodies specifically inhibited LDV infection of target motor neurons without preventing infection of nonneuronal cells. Since the protective MAbs recognize an epitope that is not recognized by protective polyclonal anti-LDV antibodies obtained from infected mice, we similarly compared LDV replication in the spinal cord of MAb-protected and control mice. In this experiment, 24 mice were treated with CY, and 12 of these animals were injected with 200 μg of protein G-purified MAb 159-12 prior to LDV infection of all animals. Three mice from each group were sacrificed 16 days p.i., and their spinal cords were analyzed for LDV RNA-containing cells by in situ hybridization. The remaining mice were observed for the development of paralytic symptoms. All three mice in the control group exhibited frank hind limb paralysis when sacrificed at 16 days p.i. In total, 10 of 11 mice in the control group developed paralysis, with a mean onset time of 17 ± 2 days p.i. (a single mouse died from unknown causes at 6 days p.i.), whereas none of the MAb-protected mice exhibited paralysis at termination of the experiment 40 days p.i.

Figure 1 illustrates representative results of the in situ hybridization analyses. In a section of the spinal cord from a paralyzed mouse of the control group (panel A), several large motor neurons containing LDV RNA can be seen, as well as the characteristic inflammatory cells associated with cytocidal LDV infection of motor neurons (8, 14). In contrast, only smaller nonneuronal cells containing LDV RNA were found in sections of spinal cords from protected mice at 16 days p.i. The representative spinal cord section in panel B, for example, shows several uninfected large motor neurons and one LDV RNA-containing smaller nonneuronal

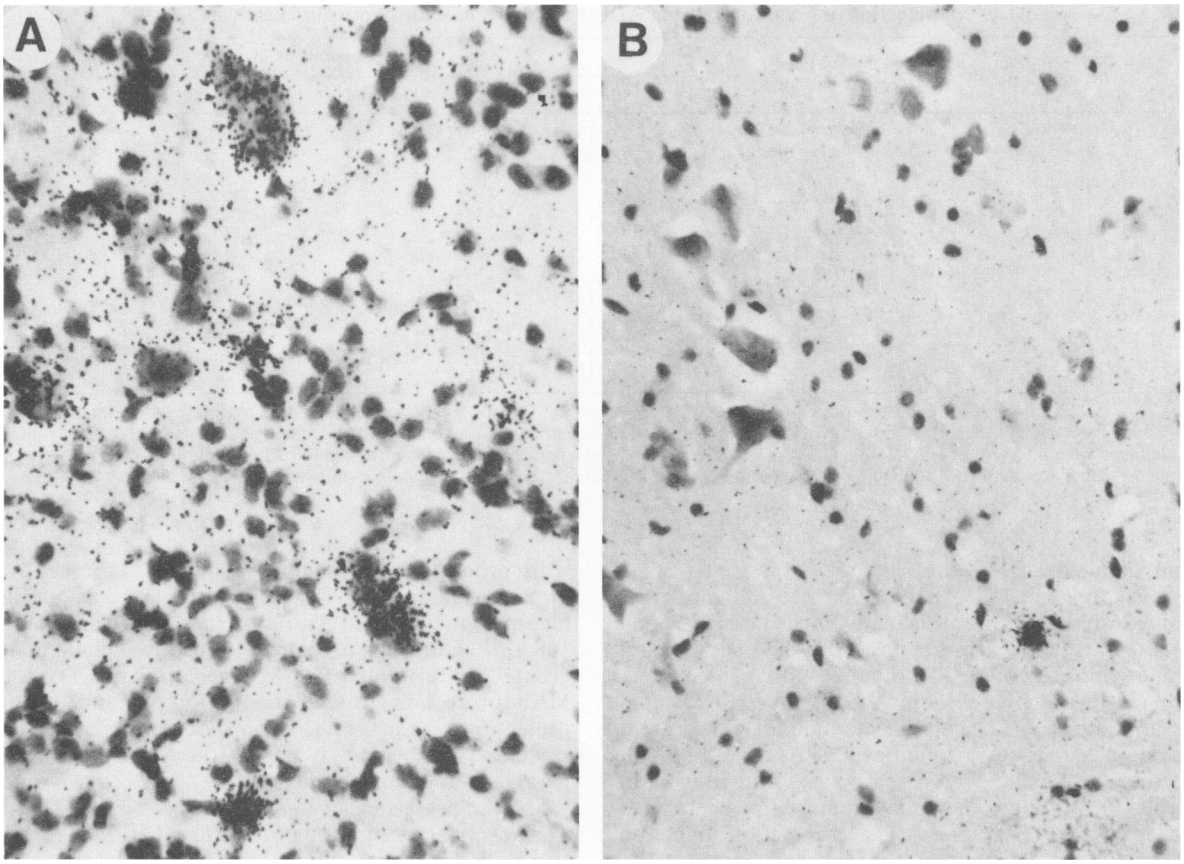

FIG. 1. Presence of LDV RNA-containing motor neurons and nonneuronal cells in the spinal cords of paralyzed (A) and anti-LDV MAb-protected (B) C58/M mice. CY-treated 6-month-old C58/M mice were injected i.v. with 200 μg of protein G-purified MAb 159-12 1 day prior to infection with LDV_v (B) or were infected with LDV_v without prior MAb treatment (A). The mice were sacrificed 16 days p.i., at which time the mice without MAb treatment had developed hind limb paralysis, whereas those that had been treated remained without symptoms (see text). The spinal cords were fixed in a solution composed of paraformaldehyde, lysine, and sodium periodate (PLP), and sections thereof were analyzed by in situ hybridization using a 1.6-kb LDV-specific cDNA probe, as described in Materials and Methods and in reference 5. Magnification, ×400 (A) and ×320 (B).

cell. Furthermore, the inflammatory infiltrates normally associated with LDV infection of motor neurons were not detected in spinal cords of protected mice. Thus, the protective MAbs, in a fashion similar to the protective polyclonal anti-LDV antibodies (10), function to specifically prevent LDV infection of ventral horn motor neurons but do not prevent the infection of nonneuronal cells.

Protective capacity of MAbs at various times p.i. In order to assess the ability of the protective MAbs to interfere with LDV at different stages in the pathogenic infection, the MAbs were administered to CY-treated C58/M mice at various times pre- and postinfection. In the experiment depicted in Table 3, animals were injected i.v. with MAb 159-12 at the indicated times p.i. In this experiment, the MAb afforded protection when mice were inoculated 1 day prior to or at the time of infection, whereas the MAbs administered in identical doses as early as 12 h p.i. had relatively little effect. However, although single injections of MAb at 0.5, 1, or 2 days p.i. decreased the incidence of disease only slightly (if at all), they delayed the onset of disease (19 to 25 days p.i.), compared with that observed in control animals (~16 days p.i.). Two inoculations of MAb, at 2 and 4 days p.i., also did not decrease the incidence of disease but further delayed the onset of paralysis. In contrast, three inoculations of MAb 159-12 at 2, 4, and 7 days p.i. completely prevented the onset of neurological disease up to 60 days p.i. (Table 3) or greatly reduced the incidence of ADPM (Table 4). Table 3 is a composite of two independent experiments which yielded comparable results.

The data in Table 3, together with the results described already, suggest that LDV is accessible to the effects of the protective MAb at two temporally distinct stages in the pathogenic infection. The most plausible explanation is that anti-LDV antibodies present at the time of infection specifically prevent the initial infection of motor neurons in the spinal cord, whereas the same antibodies when administered 0.5 to 4 days p.i. delay the onset of neurological disease by transiently inhibiting the spread of LDV within the spinal cord. The effect is only transient, perhaps because of either the short half-life of the passively transferred anti-LDV antibodies or its inefficient entry into the central nervous system. Other experiments have shown that the half-life of passively transferred MAb 159-12 in blood was 1 to 2 days in CY-treated C58/M mice (data not shown). Multiple inoculations of the MAb, beginning 2 days p.i., are capable of protecting the mice more effectively, presumably by inhibiting or delaying the spread of LDV among neurons in the

TABLE 3. Effect of time of administration of MAb on protection of C58/M mice from ADPM[a]

Antibody and time of administration p.i. (day[s])	No. of mice paralyzed/ total (%)	Onset of paralysis (day[s] p.i.)	
		Individual mice	Mean ± SEM
None	11/12 (96)	13, 14, 15, 15, 16, 16, 16, 17, 18, 18, 21	16 ± 0.7
MAb 159-12			
−1	5/28 (18)	16, 17, 19, 19, 26	19 ± 1.7
0	2/6 (33)	17, 21	19 ± 2
+0.5	5/7 (71)	18, 18, 20, 20, 21	19 ± 0.6
+1	4/6 (67)	20, 20, 21, 28	22 ± 1.9
+2	9/11 (82)	20, 23, 23, 23, 24, 24, 24, 28, 32	25 ± 1.2
+2, +4	4/5 (80)	23, 25, 26, 39	28 ± 3.6
+2, +4, +7	0/12 (0)		

[a] The experiment was performed as described in Table 1, footnote a, except that MAb 159-2 was inoculated i.v. at the indicated times p.i. Animals were observed for paralysis until 60 days p.i.

spinal cord until host protective antibodies arise after the CY inhibition subsides.

This hypothesis was tested by administering both the protective MAb and the CY in multiple doses. As shown in Table 4, multiple doses of the MAbs at 2, 4, and 7 days p.i. afforded protection from ADPM if a single dose of CY was given 2 days before LDV_v infection. In contrast, an additional CY inoculation at 5 days p.i. abrogated the protective effect of multiple administrations of the MAb. Subsequent inoculations of protective MAbs at 10, 13, and 16 days p.i. were not capable of preventing ADPM after two injections of CY at 2 days preinfection and 5 days p.i.

DISCUSSION

The identification of MAbs with the capacity to protect C58/M mice from ADPM has allowed further dissection of the pathogenesis of LDV-induced neurological disease. First, these experiments confirm the ability of antibodies to interfere with events involved specifically in the pathogenesis of viral disease without functioning to eliminate the virus from the host. Protection can be achieved by using antibodies to at least two epitopes of VP-3 of LDV: one recognized by the neutralizing MAbs and one or more recognized by polyclonal anti-LDV antibodies formed during a natural infection of mice. On the other hand, MAbs to three other epitopes of VP-3 are nonprotective.

Second, these experiments suggest that protective MAbs can interfere with two temporally distinct stages in ADPM progression. When present before or at the time of LDV infection, they seem to prevent the initial infection of a limited number of motor neurons, most likely via axonal transport from the periphery (8). On the other hand, anti-LDV antibody administration p.i. seems to temporarily impede the spread of LDV among neurons. This conclusion is indicated by the findings that a single administration of anti-LDV MAbs p.i., though slightly protective, delayed the onset of paralysis and that complete protection was achieved by multiple antibody treatments after infection. For protection, MAbs must be administered until endogenous host protective antibodies arise. This hypothesis is supported by the finding that an additional injection of CY at 5 days p.i. abrogated the protective capacity of multiple MAb treatments, which were protective when only a single CY injection was administered before infection. The reason for the failure of additional injections of protective MAbs to overcome the effect of the second CY treatment is not clear. This finding may signify the requirement of additional factors in protection, such as protective T cells (13, 14), that are also eliminated by the CY treatment. That the LDV infection of motor neurons spreads only slowly through the spinal cord from initial foci of infection is indicated by a slow but progressive increase in total LDV RNA in the spinal cord of mice developing ADPM, as well as by results from in situ hybridization analyses of spinal cord sections of these mice (6, 8). It is this stage that seems to be prevented by anti-LDV antibodies endogenously generated in mice that are not immunosuppressed by CY treatment and do not develop paralytic disease (Table 1).

It is unclear whether the protective MAbs function in the same fashion to prevent disease when present at the time of infection or when interfering with the spread of the virus in the spinal cord, but this seems likely because both processes are inhibited by the same antibodies exhibiting different epitope specificities. Furthermore, in each case both types of antibodies appear to protect in a similar fashion by preventing LDV infection of motor neurons without inhibiting infection of nonneuronal cells, perhaps microglial cells, and without affecting LDV replication in the periphery. These data suggest that protection may result from a cell type-specific (i.e., neuron-specific) neutralization of LDV infectivity by the anti-LDV antibodies. One interpretation of these results suggests that LDV may utilize an alternate cellular receptor for the infection of neuronal cells which differs from that used for the infection of macrophages (6). Although the identity of the cell surface receptor for LDV on macrophages is not known (2), the requirement for antecedent endogenous MLV expression in neurons for LDV infection suggests that either an MLV protein or a neuron-specific protein(s) coordinately produced with MLV expression is rendering neurons susceptible to LDV infection by functioning as the surrogate LDV receptor on neurons (6). Preliminary analyses have demonstrated the presence of MLV gp70

TABLE 4. Effect of multiple CY injections on the ability of MAb to protect C58/M mice from ADPM[a]

Day(s) (p.i.) of CY administration	Day(s) (p.i.) of MAb 159-12 administration	No. of mice paralyzed/total (%)	Onset of paralysis (day[s] p.i.)	
			Individual mice	Mean ± SEM
+2	None	5/5 (100)	13, 13, 13, 15, 15	14 ± 0.5
−2	+2, +4, +7	2/6 (33)	22, 27	25 ± 2.5
−2, +5	+2, +4, +7	4/4 (100)		
−2, +5	+2, +4, +7, +10, +13, +16	5/6 (83)	19, 19, 19, 19, 19	19 ± 0

[a] The experiment was performed as described in Table 1, footnote a, except that some mice received a second inoculation of CY (200 mg/kg) at 5 days p.i. Protein G-purified MAb 159-12 was injected at the indicated days p.i. Animals were observed for paralysis for 60 days p.i.

in the spinal cords of 9-month-old C58/mice for at least 5 days after CY treatment (C. Contag, unpublished data).

The concept of tissue-specific protection from virally induced disease may be important in vaccine design, particularly with respect to diseases of the central nervous system, which may require the interaction of multiple predisposing factors, including age-related effects on gene expression and immune status.

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