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Inhibition of virus-induced age-dependent poliomyelitis by interferon-γ

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Abstract

Age-dependent poliomyelitis (ADPM) is a neuroparolytic disease which results from combined infection of susceptible mice with lactate dehydrogenase-elevating virus (LDV) and murine leukemia virus (MuLV). The present study examined the effects of interferon-γ (IFN-γ) treatment on the incidence of ADPM, replication of LDV and MuLV and anti-LDV immunity. IFN-γ treatment of ADPM-susceptible C58/M mice protected them from paralytic disease, but had no detectable effect on the IgG anti-LDV response or LDV viremia. IFN-γ-mediated protection from ADPM correlated with reduced expression of LDV RNA, but not MuLV RNA, in the spinal cords of C58/M mice. These results confirm that spinal cord LDV replication is the determinant of ADPM and demonstrate that cytokine-mediated inhibition of LDV replication in the central nervous system prevents neuroparalytic disease.

Keywords: Age-dependent poliomyelitis; Lactate dehydrogenase-elevating virus; Interferon-γ

1. Introduction

Age-dependent poliomyelitis (ADPM) is a fatal paralytic disease which develops in certain genetic strains of mice (C58, AKR, C3H/FgBoy, PL/J) after infection with lactate dehydrogenase-elevating virus (LDV) (Murphy et al., 1983; Contag et al., 1989). LDV is an arterivirus which infects macrophages of all mouse strains (Plagemann and Moennig, 1992), but in ADPM-susceptible mice neurons also become LDV-permissive. The susceptibility of mice to ADPM is linked to the presence of replication-competent N-tropic, ecotropic (replication restricted to mouse) murine leukemia virus (MuLV) as well as the ho-

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mozygous presence of the Fv-1^n allele, which permits efficient replication of N-tropic MuLV (Murphy et al., 1983; Contag et al., 1989). The pathogenesis of ADPM seems to be due to a burst of cytocidal LDV replication in anterior horn neurons just prior to onset of symptoms, about 12–25 days p.i. with LDV (Anderson et al., 1995a,b). Infection of glial cells by MuLV is the determinant of neuron permissiveness to LDV replication, but the mechanism by which MuLV acts is unknown (Anderson et al., 1995a,b). Soon after LDV infection of a mouse, there is a brief period of LDV replication in leptomeninges, followed by an interim period of about 10 days, during which LDV replication in CNS is barely detectable. Then, LDV emerges in anterior horn neurons of ADPM-susceptible mice, causing damage or destruction leading to paralysis.

Immunosuppression of younger genetically-susceptible mice with X-irradiation (600 rads) or cyclophosphamide (200 mg/kg) 24 h prior to LDV infection enhances susceptibility to ADPM (Murphy et al., 1983; Contag et al., 1989), but with age, mice progressively lose protective immunity and become naturally susceptible to ADPM. Treatment of mice at various ages with monoclonal anti-LDV antibodies at the time of LDV infection protects them from ADPM, apparently by restricting LDV access to the central nervous system (Harty and Plagemann, 1990). Transfer of syngeneic T-cells from young-resistant to older-susceptible mice also protects against development of ADPM, by an unknown mechanism (Bentley et al., 1983).

LDV causes a persistent infection in all mice, characterized by vigorous IgG anti-LDV responses and circulating infectious virus-IgG complexes (Plagemann and Moennig, 1992; Cafruny and Plagemann, 1982), as well as a transiently-activated LDV-specific cytotoxic T-cell response (Even et al., 1995). Previous studies have demonstrated that both interferon-α/β (IFN-α/β) and interferon-γ (IFN-γ) are also transiently induced during LDV infection (duBuy et al., 1973; Bradley et al., 1991; Plagemann et al., 1995). IFN-α/β appears to have a limited role in LDV infection (Plagemann and Moennig, 1992), but IFN-γ has been shown to be a potent inhibitor of in vitro LDV infection of macrophages (Cafruny et al., 1994). Therefore, we have tested the hypothesis that IFN-γ treatment of ADPM-susceptible mice will alter the course of LDV-induced disease.

2. Materials and methods

2.1. Mice

ADPM-susceptible C58/M mice, originally derived from the colony of Dr W. Murphy (Murphy et al., 1987), were maintained under normal conditions on standard lab chow and water ad lib. CF1 mice (Sasco, Omaha, NE) were used for virus titrations and as virus controls and Swiss mice (Sasco) were used for propagation of virus stocks. Mouse protocols received appropriate approval from the relevant IUCAC.

2.2. Virus

The neuropathogenic LDV-v strain of LDV was isolated from the spinal cord of a paralyzed C58/M mouse which had been infected with the Ib-LDV strain obtained from Dr. W. Murphy (Murphy et al., 1987). LDV-v stocks were prepared from the 24 h post-infection (p.i.) plasma of Swiss mice. Six-month old C58/M or control CF1 mice were infected by intraperitoneal (i.p.) injection of 10^6 ID_{50} LDV-v, 24 h after i.p. injection of 200 mg/kg cyclophosphamide (Sigma).

2.3. Interferon-γ (IFN-γ)

Recombinant murine IFN-γ, 5×10^7 U/mg, was provided by Genentech (South San Francisco). Mice were injected i.p. with 4 μg IFN-γ 4 h prior to LDV infection and thereafter were given 2 μg IFN-γ daily (days 1–7 p.i.) or on alternate days (days 9–19 p.i.), approximating daily doses previously shown to suppress LDV replication (Cafruny et al., 1994).

2.4. Anti-LDV and viremia assays

Mice were bled from the retro-orbital plexus at various times p.i., and blood plasma was assayed

for IgG anti-LDV and viremia. IgG anti-LDV antibodies were determined by indirect fluorescence antibody (IFA) assay as described (Cafruny et al., 1986), using both the homologous (LDV-v) and avirulent (LDV-P; Plagemann and Moennig, 1992) virus strains as target antigens in independent assays. The plasma virion concentration was determined by limiting dilution titration, using four to five mice for each $\log_{10}$ dilution (Plagemann and Moennig, 1992).

2.5. In-situ hybridization for detection of LDV and MuLV RNA

Mice were sacrificed between 17–19 days p.i. and spinal cord tissue pieces were fixed in 10% neutral buffered formalin for 4 h, prior to storage in 80% ethanol. Expression of LDV RNA was determined by in situ hybridization of formalin-fixed spinal cord sections with a random-primed ^{35}S-labeled LDV-specific cDNA probe (437 bp; #4–55; Kuo et al., 1992). Expression of MuLV RNA was determined by in situ hybridization of similarly prepared tissues with either a random- primed ^{35}S-labeled pAKV probe (8 kb; Chattopadhyay et al., 1980) or a 330 bp ecotropic-specific probe prepared by *Sma*I digestion of pAKV (Anderson et al., 1995a; Chattopadhyay et al., 1980). Data were quantitatively estimated by blind scoring of sections, according to the number of cells supporting virus replication: 0, no cells; 1+, average of one or two clusters of granules per low-power field (100×; illustrated in Fig. 3), indicating infection of one or two cells per low-power field; 2+, average of three or four clusters of granules per low-power field; 3+, average of five to ten clusters of granules per low-power field; 4+, average of greater than ten clusters of granules per low-power field.

2.6. Statistical analysis

Statistical analysis was performed using a two-tailed *t*-test as described (Sokal and Rohlf, 1969).

3. Results

3.1. Inhibition of ADPM by IFN-γ

A total of 16 C58/M mice, age 6–7 months, were divided into two age- and weight-matched groups. Of these eight mice received IFN-γ treatment as described above, while the control group received sham injections with the drug vehicle (sterile PBS containing 0.05% bovine serum albumin). All C58/M mice received 200 mg/kg cyclophosphamide 24 h prior to LDV-v infection. Three age- and weight-matched CF1 mice were treated in parallel with the IFN-γ group, receiving cyclophosphamide, IFN-γ and LDV-v. All mice were monitored daily for development of paralytic symptoms, which are recorded in Fig. 1. Paralytic disease was first observed on day 13 p.i. in the control group and by day 19 p.i. all mice in this group displayed paralysis of at least one limb, as well as general wasting ($\approx$50% loss in body weight; lethargy) preceeding paralysis by several days. In contrast, only two of the eight mice in the IFN-γ-treated group displayed paralysis ($P < 0.01$ relative to control group on day 19 p.i.), the onset of which was delayed by 2 days relative to controls (Fig. 1). Furthermore, there was no evidence of wasting in any of the IFN-γ-treated C58/M mice, or in the CF1 mice which failed to develop any paralysis. Mice were sacrificed from all groups between days 17–19 p.i. and tissues were taken for analyses of viremia, IgG anti-LDV and viral RNA. Two C58/M mice from the healthy IFN-γ-treated group were held for observation until day 30 p.i., with no evidence of ADPM.

3.2. Failure of viremia and IgG anti-LDV to correlate with IFN-γ- mediated protection against ADPM

Fig. 2 shows the results of viremia and IgG anti-LDV analyses for all mice. Viremia levels declined in all mice during the study, from a peak at 18 h p.i. of $10^{9.6-9.8}$ ID_{50}/ml, to levels between 17–19 days p.i. of $10^{7.5}$ (C58/M drug vehicle), $10^{7.25}$ (C58/M-IFN-γ group) and $10^{6.0}$ (CF1-IFN-γ) ID_{50}/ml. The absence of a correlation between

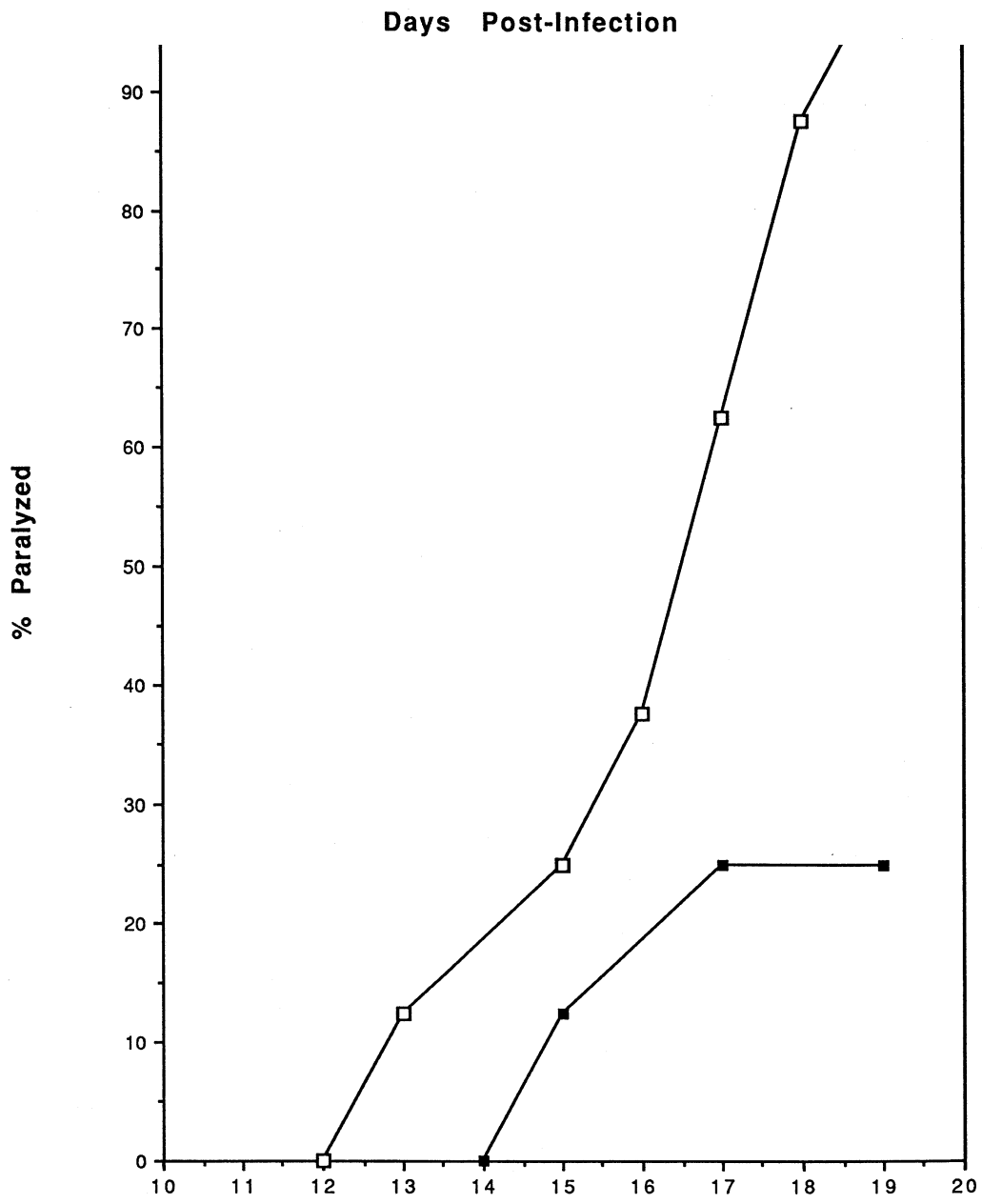
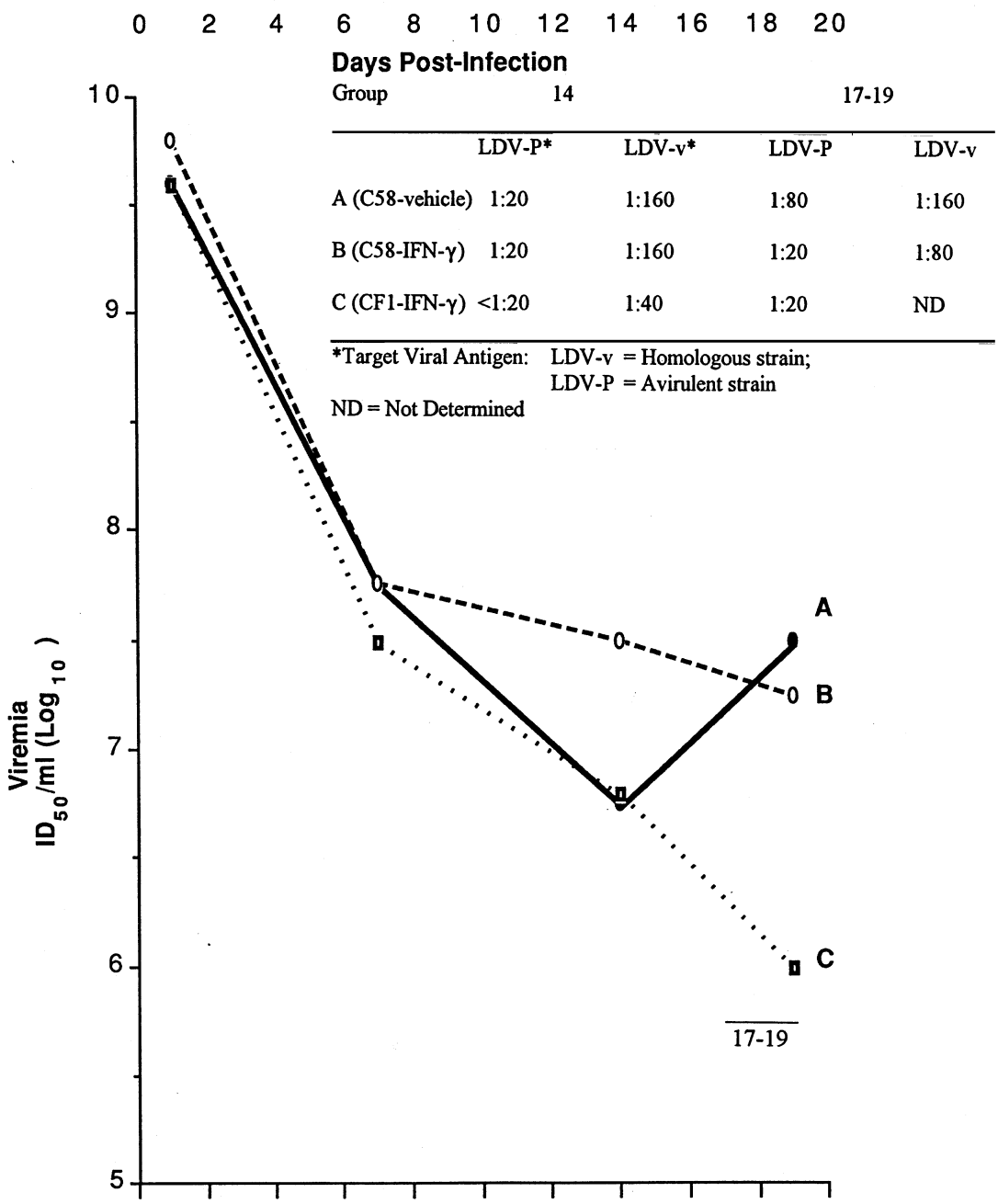


Fig. 1. Interferon-γ treatment of C58/M mice inhibits development of LDV-induced paralytic disease. C58/M mice received recombinant murine IFN-γ (closed squares) or drug vehicle (open squares) according to the dosage schedules listed in Section 2. Development of paralytic disease (% paralyzed) is plotted as a function of days post-infection with LDV-v.



Group	14		17-19	
	LDV-P*	LDV-v*	LDV-P	LDV-v
A (C58-vehicle)	1:20	1:160	1:80	1:160
B (C58-IFN-γ)	1:20	1:160	1:20	1:80
C (CF1-IFN-γ)	<1:20	1:40	1:20	ND

*Target Viral Antigen: LDV-v = Homologous strain; LDV-P = Avirulent strain

ND = Not Determined

Fig. 2. LDV-viremia and plasma IgG anti-LDV antibodies in interferon-γ-treated C58/M and CF1 mice and in control C58/M mice. LDV viremia was determined by limiting dilution assay of plasma pooled from five to six C58/M and three CF1, mice, using four to five mice per $\log_{10}$ dilution. A, C58/M + drug vehicle (control); B, C58/M + IFN-γ; C, CF1 + IFN-γ. The last time point represents samples pooled from mice sacrificed on days 17–19 p.i (bar). Insert: Pooled plasma IgG anti-LDV antibodies reactive with the homologous neurovirulent LDV-v strain and the relatively avirulent LDV-P strain were titrated by indirect fluorescence antibody (IFA) analysis.

Table 1
Detection of spinal cord viral RNA by in situ hybridization

Treatment group	Mouse	Sacrificed at days p.i.	Paralysis	Probe score[a]	
				LDV	MuLV
Control-injected C58	1	–	+	*	*
	2	–	+	*	*
	3	17	+	1+	4+, 3+
	4	17	+	2.5	4, 2
	5	17	+	3	4, 2
	7	18	+	2	3, 1
	11	19	+	3	2, 2
	8	18	+	ND	ND
Mean				2.3	3.4, 2.0
IFN-γ-injected C58	6	17	+	2	3, 1
	9	18	–	0.7	4, 2
	12	19	–	1.5	4, 2
	13	19	–	0.5	3, 3
	14	19	–	0	3, 1
	17	—	+	*	*
	18	36	–	ND[b]	ND[b]
	19	36	–	ND[b]	ND[b]
Mean				0.9	3.4, 1.8
IFN-γ-injected CF1	10	18	–	0	0, 0
	15	19	–	0	0, 0
	16	19	–	0	0, 0

[a] Quantitative ranking on a scale of 0–4+ for hydridization with LDV or MuLV (pAKV, 330 bp *Sma*I fragment)-specific cDNA. For MuLV, data are shown for separate experiments using the random-primed 8 kb pAKV probe or 330 bp *Sma* fragment.
[b]Mice 18 and 19 were kept alive until day 36 p.i. with LDV without evidence of paralysis. All paralyzed mice were sacrificed on the first day of paralysis except for mouse 3 which was sacrificed on the second day of paralysis.
* Mice 1, 2 and 17 died prior to obtaining tissues. ND, not determined.

viremia and progression to ADPM is reinforced by examination of viremia levels on day 14 p.i. which were higher in the protected (IFN-γ) group ($10^{7.5}$ ID_{50}/ml) relative to the ADPM-progressing group ($10^{6.75}$ ID_{50}/ml). In agreement with previous observations (Plagemann et al., 1995), C58/M mice displayed about ten-fold higher levels of viremia by several weeks p.i. relative to those in CF1 mice, presumably due to more efficient LDV replication in their macrophages, the major source of blood LDV. IgG anti-LDV antibodies were determined by IFA, using two target strains (LDV-v and LDV-P) and are displayed in Fig. 2 (insert). Levels of these antibodies were low as expected during the time frame of the experiment, ranging between 1:20 and 1:160 in C58/M mice and failed to display any differences correlating with the clinical outcomes.

3.3. Correlation of LDV RNA expression in spinal cord with ADPM

The expression of LDV and MuLV RNA was determined by in situ hybridization of formalin-fixed spinal cord with virus-specific cDNA probes. Table 1 shows quantitative ranking of the data from these studies, which demonstrated reduced LDV RNA expression in spinal cords from ADPM-protected mice which had received IFN-γ. LDV RNA expression was reduced in one of these mice to undetectable levels, whereas in the others detectable but low LDV RNA levels ap-

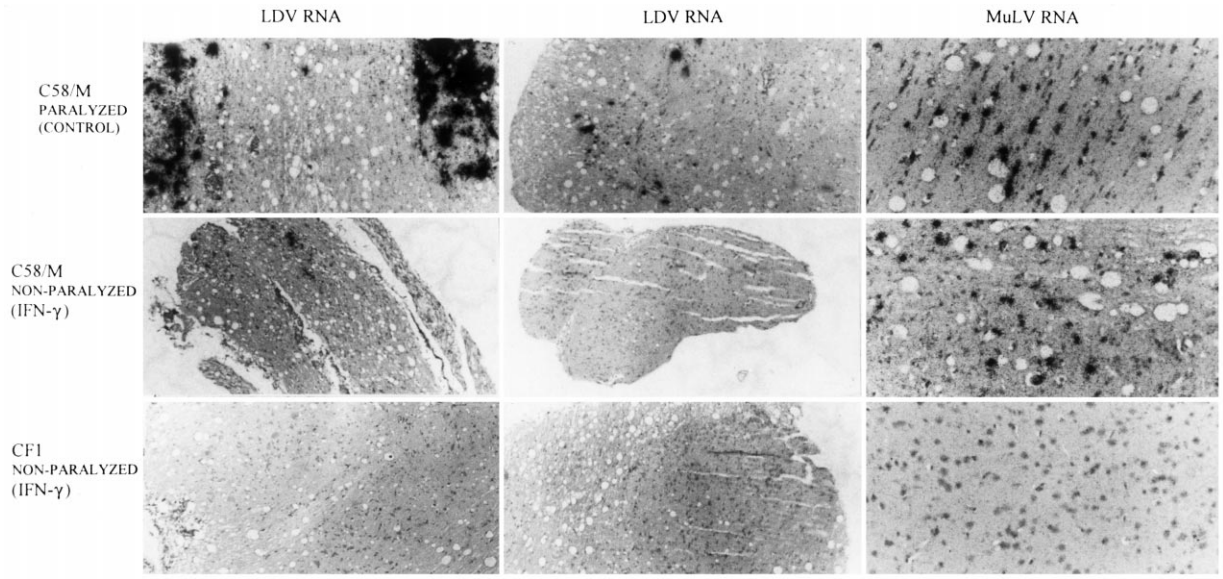


Fig. 3. Detection of spinal cord LDV and MuLV RNA by in situ hybridization. Representative data (100 × magnification) are illustrated for detection of LDV and MuLV RNA in separate C58/M or CF1 mice. The MuLV probe was a 330 bp *Sma*I fragment of pAKV; the LDV probe is described in Section 2. Clinical status and IFN-γ treatment are indicated on the left.

peared. In contrast, all control (sham-injected) C58 mice displayed relatively high levels of spinal cord LDV RNA coincident with paralytic disease. MuLV RNA expression in the same tissues was completely unaffected by IFN-γ treatment and was present in C58/M mice regardless of the clinical status. Fig. 3 illustrates representative in situ hybridization data, demonstrating both fewer and less-dense positive foci of LDV RNA in the spinal cords of IFN-γ-treated C58M mice relative to control C58/M mice, although MuLV RNA expression in these mice was unaffected by IFN-γ. LDV RNA analyses are shown in this figure for two separate mice of each group, indicating the prominant LDV-specific foci in tissues from paralyzed control-treated C58/M mice, but reduced (C58/M) or absent (C58/M; CF1) foci in tissues from IFN-γ-treated mice. Previous studies have established that LDV and MuLV replication are essentially undetectable in ADPM-resistant spinal cord (Plagemann and Moennig, 1992) and the present data (Table 1, Fig. 3) also confirm the absence of LDV, as well as MuLV, RNA in spinal cord of ADPM-non-permissive, genetically resistant IFN-γ-treated CF1 mice. Overall, these results demonstrate LDV-specific viral inhibition by IFN-γ and confirm that LDV, not MuLV, spinal cord replication mediates ADPM.

4. Discussion

The present results show that IFN-γ treatment of ADPM-susceptible C58/M mice inhibits the development of paralytic disease. Both delayed onset and reduced incidence, of ADPM were observed due to IFN-γ treatment. IFN-γ is a normal but apparently transient component of anti-LDV immunity (Bradley et al., 1991; Plagemann et al., 1995; Rowland et al., 1994) and its failure to be maintained during LDV persistence may be related to the disappearance of cytotoxic T-cells during the infection (Even et al., 1995). Our data showing a protective role for exogenous IFN-γ indicate that intrinsic IFN-γ levels are insufficient in C58/M mice to protect against LDV-induced paralysis. Circulating anti-LDV antibodies apparently had no role in IFN-γ-mediated protection from paralysis, since they were low in all mice at 2–3 weeks p.i. regardless of clinical outcome.

IFN-γ failed to suppress LDV viremia, which was also unrelated to the clinical outcome and this finding is in agreement with previous observations that chronic LDV viremia fails to respond to IFN-γ (Cafruny et al., 1994). In contrast, IFN-γ suppresses LDV replication in highly-permissive neonatal macrophages (Cafruny et al., 1994), suggesting that the site-specific anti-viral effects of this cytokine have a developmental basis. It is possible that minor effects of IFN-γ on viremia, below the sensitivity of limiting dilution assays (± 0.20–0.25 $\log_{10}$ in the present study) occur, but in any case are irrelevant to ADPM, since our and other's data clearly show that viremia and ADPM are completely detached, as expected since macrophages supply blood LDV (Plagemann and Moennig, 1992). Rather, our present data demonstrate that IFN-γ inhibits spinal cord LDV RNA expression as well as paralytic disease, confirming that spinal cord LDV replication is the direct link to the pathogenesis of ADPM (Anderson et al., 1995a,b). Although others have observed inhibition of retrovirus production by IFN-γ (Koyanagi et al., 1988), at the doses used in the present study no effect of IFN-γ on MuLV RNA expresssion was observed.

Our observation of IFN-γ-mediated protection from ADPM may explain the mechanism for adoptive transfer of protective T-cells (Bentley et al., 1983), since the T-cells would provide a source of IFN-γ. The transient CTL response would also generate IFN-γ (Plagemann et al., 1995), contributing to the natural immunologic protection in younger mice which, despite meeting virologic and genetic criteria for susceptibility, have a low incidence of LDV-induced paralysis. Furthermore, we propose that local production of IFN-γ, stimulated by initial LDV infection of leptomeninges, may be responsible for the period of delay between leptomeningeal and neuronal LDV infection (Anderson et al., 1995a), although further studies are required to determine the relative importance of other cytokines. Our results do not shed any light on the mechanism by which MuLV infection of glial cells renders anterior horn neurons susceptible to LDV, but the ability of LDV replication in neurons to respond to cytokines would support the search for a possible MuLV-induced cytokine pathway, as suggested earlier (Anderson et al., 1995a).

Both the CNS (Harty and Plagemann, 1990) and the fetus (Broen et al., 1992; Haven et al., 1996) are unique compartments which can be protected immunologically from LDV. Inhibition of LDV transmission from mother to fetus correlates with both maternal anti-LDV antibodies as well as maternal IFN-γ treatment (Broen et al., 1992; Haven et al., 1996). These immune mechanisms are probably distinct with respect to both CNS and fetus: Antibodies are most efficient in protection from ADPM when given prior to LDV infection, so the mechanism of CNS, as well as fetal protection by anti-LDV is probably due to generation of an immune barrier to tissue penetration. Since IFN-γ inhibits neonatal macrophage LDV replication (Cafruny et al., 1994), the fetal and CNS compartments are probably protected by direct cytokine-mediated inhibition of tissue LDV permissiveness, despite having access to the virus (Anderson et al., 1995a,b). In contrast to immunologic protection of specific body compartments, it has not been possible to vaccinate adult mice against LDV (Cafruny et al., 1986). Therefore, our results are of interest to mechanisms of tissue-selective viral protection and the development of potential drugs to combat disease during incurable persistent viral infection.

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