Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Neuroimmunology, 8 (1985) 79–92
Elsevier

JNI 00242

Enhancement of Encephalomyeloradiculitis in Mice Sensitized with Spinal Cord Tissue and Infected with Lactate Dehydrogenase-Elevating Virus

William G. Stroop [1,2,*] and Margo A. Brinton [2]

[1] *Departments of Neurology and Pathology, University of Utah School of Medicine, Neurovirology Research Laboratory, Veterans Administration Medical Center, Salt Lake City, UT and* [2] *The Wistar Institute of Anatomy and Biology and Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA (U.S.A.)*

(Received 11 May, 1984)
(Revised, received 21 August, 1984)
(Accepted 21 August, 1984)

Summary

C57BR/cdJ mice develop encephalomyeloradiculitis following peripheral inoculation of the C strain of lactate dehydrogenase-elevating virus (LDV-C). We investigated the effect of subcutaneous administration of syngeneic spinal cord homogenate mixed with phosphate buffered saline or emulsified in complete or incomplete Freund's adjuvant 1 week before or after inoculation of LDV-C on the incidence and severity of central nervous system lesions. C57BR/cdJ mice developed an acute histological allergic encephalomyelitis when given 2 weekly sensitizing injections of homogenate in complete Freund's adjuvant. When peripheral inoculation of LDV-C was substituted for one of the two sensitizing injections, a higher percentage of mice developed lesions, and the lesions were more severe and persisted for much longer periods of time. This same lesion-enhancing effect was not observed if the mice were sensitized with homogenate suspended in buffer or emulsified in incomplete Freund's adjuvant. Interestingly, mice sensitized with complete Freund's

This work was supported by the medical research service of the Veterans Administration Medical Center, National Research Service Award, NS-07180 in Neurovirology and Microbiology from the National Institute of Neurological and Communicative Disorders and Stroke, and in part by Public Health Service Grant NS-110036.

* Please address all correspondence and requests for reprints to: William G. Stroop, Ph.D., Neurovirology Research Laboratory (151B), Veterans Administration Medical Center, 500 Foothill Drive, Salt Lake City, UT 84148, U.S.A., Phone (801) 582-1565, ext. 1147.

adjuvant alone before or after infection with LDV-C also developed intense central nervous system lesions, suggesting that the mycobacterial component of the adjuvant was the critical element in enhancing the lesions.

Key words: *Central nervous system – Experimental autoimmune encephalomyelitis – Lactate dehydrogenase-elevating virus*

Introduction

The C strain of lactate dehydrogenase-elevating virus (LDV-C) has been shown to be the etiologic agent of an age-dependent paralytic poliomyelitis in C58 mice (Brinton 1981; Martinez et al. 1979, 1980; Nawrocki et al. 1980; Stroop et al. 1982) that are immunosuppressed by the aging process (Murphy et al. 1970) or in young iatrogenically immunosuppressed C58 mice (Duffy et al. 1976; Martinez et al. 1980). Recently we discovered that LDV-C can produce encephalomyeloradiculitis in C57BR/cdJ mice (Stroop and Brinton 1983). Following intraperitoneal inoculation of LDV-C, approximately 50% of young C57BR/cdJ mice developed encephalomyeloradiculitis, and the inflammatory lesions could be detected for many weeks after infection. Development of lesions in C57BR/cdJ mice was independent of immunosuppression and was not age-related, sex-linked or exclusively controlled by the H-2 histocompatibility locus (Stroop and Brinton 1983). The central nervous system (CNS) lesions consisted of infiltration of inflammatory cells into the leptomeninges or around blood vessels in the white matter; demyelination was not a prominent feature.

The CNS lesions in LDV-C infected C57BR/cdJ mice were similar in distribution to the lesions observed during the early stages of experimental autoimmune encephalomyelitis (EAE) produced in SLJ/J mice given 2 weekly injections of isogeneic spinal cord homogenate in complete Freund's adjuvant (Brown and McFarlin 1981; Brown et al. 1982). Because of the similar neuropathology of LDV-C-induced encephalomyeloradiculitis in C57BR/cdJ mice and acute EAE in SJL/J mice, we investigated the possibility that administration of spinal cord homogenate to C57BR/cdJ mice before or after intraperitoneal infection with LDV-C would alter the incidence or severity of the CNS lesions produced.

Materials

Mice

Four to six-week-old male or female C57BR/cdJ mice were obtained from the Jackson Laboratory, Bar Harbor, ME.

Virus

The LDV-C strain of LDV, which was previously designated as the age-depen-

dent polioencephalomyelitis (ADPE) (Martinez et al. 1979) or the immune polioencephalomyelitis (IPE) agent (Sager et al. 1973) was used throughout these studies. Preparation of the stock virus has been previously described (Stroop and Brinton 1983), and contained 10^9 median infectious doses/ml of plasma.

Preparation of syngeneic spinal cord homogenate (SSCH)

Twenty-four C57BR/cdJ mice approximately 2–7 months of age, were killed by cervical dislocation and their spinal cords were removed and placed in phosphate-buffered saline on ice. 1.55 g of spinal cord tissue was homogenized in sterile distilled water using Ten Broeck homogenizers until a total of 3.8 ml of spinal cord mixture was prepared. The mixture was placed into horizontal glass tubes and frozen at −70°C. The lyophilyzed SSCH was ground into a fine powder and stored in a desiccator at −70°C until used.

Adjuvants

Incomplete Freund's adjuvant (IFA) and *M. tuberculosis* H37 RA were obtained from Difco Laboratories, Detroit, MI, U.S.A.

Methods

Preparation of SSCH for injection

SSCH was emulsified in complete Freund's adjuvant (SSCH/CFA) as a mixture containing 1.0 mg SSCH, 0.03 mg *M. tuberculosis* H37RA, 0.15 ml IFA and 0.15 ml phosphate-buffered saline, pH 7.4. SSCH emulsified in incomplete Freund's adjuvant (SSCH/IFA) was prepared identically, except the *M. tuberculosis* was omitted. SSCH for injection (SSCH) was prepared by mixing 1.0 mg of SSCH with 0.3 ml of phosphate-buffered saline, pH 7.4.

Injection of mice

Groups of C57BR/cdJ mice were given subcutaneous injections of SSCH, SSCH/IFA, or SSCH/CFA. A total of 0.3 ml of material per mouse was injected per sensitization, with 0.1 ml being delivered at each of 3 sites along the flanks or back. Groups of mice (Table 1) were sensitized once with SSCH, SSCH/IFA or SSCH/CFA; other groups of mice received 2 weekly sensitizations with SSCH, SSCH/IFA, or SSCH/CFA. Other mice were inoculated interperitoneally with 10^7 median infectious doses of LDV-C, and then, 1 week later, injected subcutaneously with SSCH, SSCH/IFA, or SSCH/CFA. Conversely, mice inoculated with SSCH, or SSCH/IFA, or SSCH/CFA were inoculated 7 days later with 10^7 median infectious doses of LDV-C. Control groups of mice received subscutaneous injections of 0.3 ml of CFA or IFA; other control mice were sensitized twice with CFA or 2 injections of IFA injections, separated by 1 week. One group of mice was inoculated intraperitoneally with 10^7 median infectious doses of LDV-C alone. The groups of mice injected under each protocol is shown in Table 1. Day 0 of the infection was defined as the day that mice received their first injection. Mice were observed daily for the presence of neurologic signs.

TABLE 1

Accumulative central nervous system histopathology in LDV-C infected C57BR/cdJ mice sensitized with syngeneic spinal cord homogenate [a]

Condition	Group	Injection protocol [b]		Inflammation of		Day lesions first seen [c]	Average grade of lesions [d]
		First injection	Second injection	Brain	Spinal cord		
SSCH and adjuvant	A	SSCH/CFA	–	4/17 (24) [e]	5/17 (29)	6	2+
	B	SSCH/CFA	SSCH/CFA	3/6 (50)	4/6 (67)	18	2+
	C	SSCH/IFA	–	0/10	0/10		
	D	SSCH/IFA	SSCH/IFA	0/2	0/2		
SSCH alone	E	SSCH	–	0/14	0/14		
	F	SSCH	SSCH	0/14	2/14 (14)	20	1+
Adjuvants alone	G	CFA	–	0/10	0/10		
	H	CFA	CFA	0/10	0/10		
	I	IFA	–	0/7	0/7		
	J	IFA	IFA	0/3	0/3		
Virus, SSCH, and adjuvant	K	LDV-C	SSCH/CFA	11/14 (79)	10/14 (71)	18	3+
	L	SSCH/CFA	LDV-C	10/12 (83)	7/12 (58)	18	3+
	M	LDV-C	SSCH/IFA	0/14	3/14 (21)	20	1+
	N	SSCH/IFA	LDV-C	0/13	3/13 (23)	20	1+
Virus and SSCH	O	LDV-C	SSCH	3/14 (21)	5/14 (36)	20	1+
	P	SSCH	LDV-C	4/13 (31)	2/13 (15)	20	2+
Virus and adjuvant	Q	LDV-C	CFA	4/6 (67)	4/6 (67)	18	1+
	R	CFA	LDV-C	4/6 (67)	5/6 (83)	18	2+
	S	LDV-C	IFA	0/6	0/6		
	T	IFA	LDV-C	0/6	1/6 (17)	35	1+
Virus alone	U	LDV-C	–	2/10 (20)	4/10 (40)	25	2+

[a] Data were combined for all days post first injection.

[b] Mice were given 10^7 median infectious doses of LDV-C intraperitoneally or sensitized with syngeneic spinal cord homogenate (SSCH) in phosphate-buffered saline, or emulsified in complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (IFA). The second injection was given 1 week later.

[c] Day 0 was defined as the day of the first injection.

[d] The degree of inflammation was scored as described in the Methods.

[e] Numbers in parentheses indicate the percent of animals with inflammatory lesions.

Histology

At 6–10, 20–25, 35, and 45 days after their first injection, mice from each group were anesthetized with ether and killed by perfusion with 10% buffered formalin (Fisher Scientific Co., Pittsburg, PA). CNS tissues were removed and immersion fixed in formalin for 24 h. The tissues were cut into 3-mm blocks, embedded in paraffin and cut into 6-μm sections. 3–5 serial sections were cut at each of 2 levels separated by 60–80 μm, and stained with either hematoxylin and eosin (H&E) or Luxol-fast blue (LFB) stain for myelin. At least 30–60 μm of tissue from each block was examined from each mouse studied. Two–4 mice were killed at each time point is indicated in the results. The lesions were scored on a 0–4 + scale as follows: 1 +, small perivascular cuffs of inflammatory cells in white matter parenchyma and/or mild leptomeningitis; 2 +, more numerous lesions per section or more levels of CNS involved; 3 +, severe white matter myelitis with some necrosis and/or moderate meningitis; 4 +, very severe leptomeningitis and/or severe necrotizing white matter myelitis.

Statistics

Comparison between groups of animals was performed by the Fisher exact probability tests and/or a Chi-square analysis (Swinscow 1976).

Results

Production of acute histologic EAE in C57BR/cdJ mice

C57BR/cdJ mice were sensitized with SSCH emulsified in CFA (SSCH/CFA); or subjected to 2 sensitizations with SSCH/CFA, separated by 1 week. In similar protocols, mice were also sensitized with SSCH emulsified in IFA (SSCH/IFA). None of the mice injected under any protocol exhibited neurologic signs characteristic of EAE.

Mice sensitized once or twice with SSCH/CFA developed CNS inflammatory lesions consisting of perivascular infiltrates of inflammatory cells, which occasionally invaded the white matter parenchyma; perivenular demyelination was not prominent (Fig. 1). Histologic evidence of EAE was first observed in recipients of one injection of SSCH/CFA 6 days after infection. Mice sensitized twice with SSCH/CFA developed a slightly higher incidence of lesions than recipients of one sensitization (Table 1, group A vs B, Brains, $P = 0.034$). A few animals in both groups developed inflammatory lesions of the brain or spinal cord gray matter (Fig. 1).

Sixty-seven percent of the mice twice sensitized with SSCH/CFA (group B, Table 1) developed inflammatory CNS lesions, whereas only 14% of the mice twice sensitized with SSCH in phosphate-buffered saline developed lesions (group F, Table 1), which was statistically significant (group B vs group F, $P = 0.004$).

Mice sensitized with 1 or 2 injections of SSCH/IFA (groups C, D, Table 1) did not develop lesions. Additionally, all control mice sensitized with 1 or 2 injections of CFA or IFA always appeared clinically normal, and did not develop CNS lesions (groups G, H, I and J, Table 1).

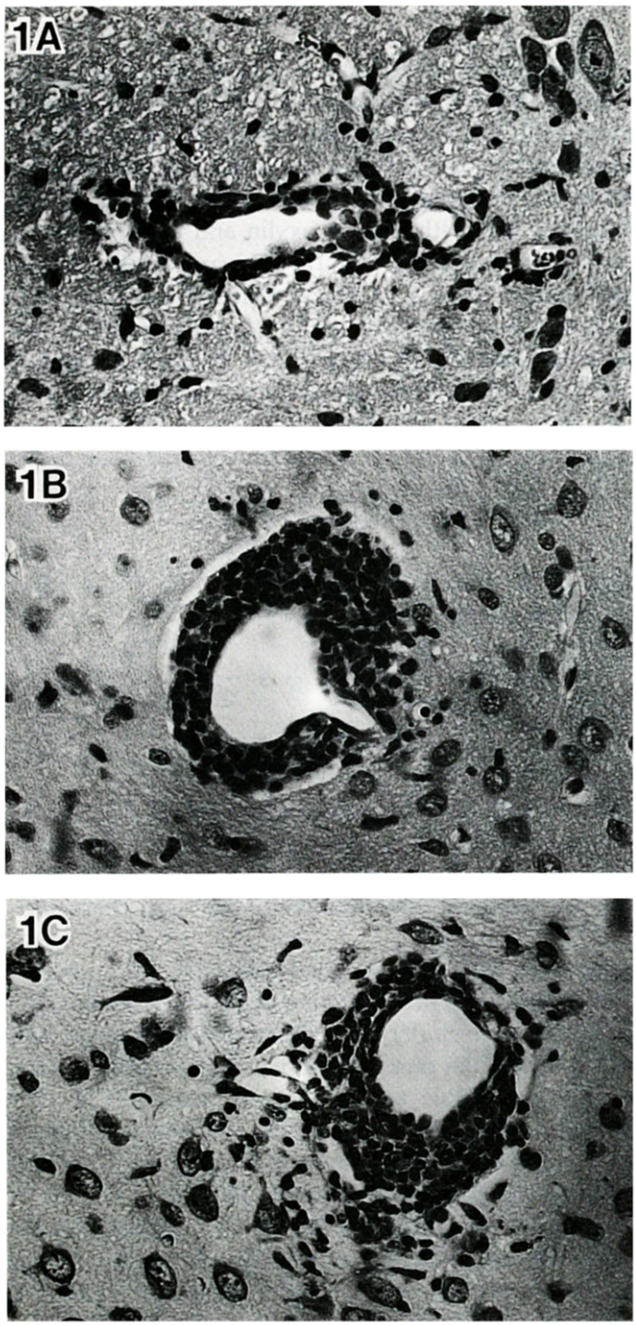

Fig. 1. Histopathology of the CNS in C57BR/cdJ mice sensitized with SSCH and CFA. *A*: A perivascular cuff of inflammatory cells in the posterior white matter of a thoracic segment of spinal cord, 18 days after sensitization with SSCH/CFA (H&E, ×820). *B*: Large inflammatory cuff in the cerebral cortex of a mouse twice sensitized with SSCH/CFA, 25 days later (H&E, ×820). *C*: Adjacent section to *B* demonstrates the absence of prominent demyelination (LFB, ×820).

Taken together, these results indicate that C57BR/cdJ mice, although resistant to the development of clinical signs, develop inflammatory lesions characteristic of EAE when given 2 sensitizing injections of SSCH/CFA separated by 1 week.

Production of central nervous system inflammation in C57BR/cdJ mice sensitized with SSCH before or after infection with LDV-C

It was next of interest to determine if infection with LDV-C could replace one of the SSCH/CFA sensitizing injections. Groups of mice were infected with LDV-C either 1 week before or after subcutaneous injection of SSCH/CFA, SSCH/IFA, or SSCH in phosphate-buffered saline (Table 1). Again, none of the mice exhibited neurologic signs.

When mice were injected with LDV-C before sensitization with SSCH/CFA, 79% developed inflammatory lesions of the CNS (group K, Table 1) and when mice were injected with LDV-C after sensitization, 83% developed CNS lesions (group L, Table 1). Lesions were largely confined to the brain or spinal cord white matter or leptomeninges, but occasionally consisted of scattered inflammatory cells in gray matter (Fig. 2). Injection of LDV-C 1 week before or 1 week after sensitization with SSCH/CFA (groups K and L, Table 1) produced a significantly higher percent of lesions than was seen with one sensitization of SSCH/CFA alone (group A vs K, $P = 0.003$; A vs L, $P = 0.002$, Table 1).

When LDV-C was inoculated 1 week before (group M, Table 1) or after (group N, Table 1) sensitization with SSCH/IFA, no significantly greater incidence of lesions was observed than in recipients of 1 or 2 sensitizations of SSCH/IFA (groups B, C, respectively, Table 1).

Central nervous system inflammation in C57BR/cdJ mice injected with adjuvant before or after infection with LDV-C

The role of CFA in the injection protocol was also investigated. Mice were injected with either CFA or IFA 1 week before or 1 week after intraperitoneal inoculation with LDV-C (Fig. 3, Table 1). Approximately 67–83% of mice injected with LDV-C before or after sensitization with CFA developed lesions (groups Q and R, Table 1). Only 1 animal inoculated with LDV-C after sensitization with IFA developed lesions (group T, Table 1). The total incidence of lesions seen in recipients of LDV-C before or after sensitization with IFA (groups S and T) was statistically identical to the incidence of lesions seen in mice inoculated with LDV-C alone (group U).

Comparison of LDV-C-induced CNS lesions with SSCH plus adjuvant-induced CNS lesions

When mice were sensitized with 2 injections of SSCH/CFA separated by 1 week, there was no statistically significant increase in the percent of animals that developed CNS lesions (group B, Table 1), as compared to recipients of LDV-C alone (group U, Table 1). However, mice which received LDV-C 1 week before (group K, Table 1) or 1 week after (group L, Table 1) sensitization with SSCH/CFA did demonstrate a significantly higher incidence of CNS lesions than mice inoculated

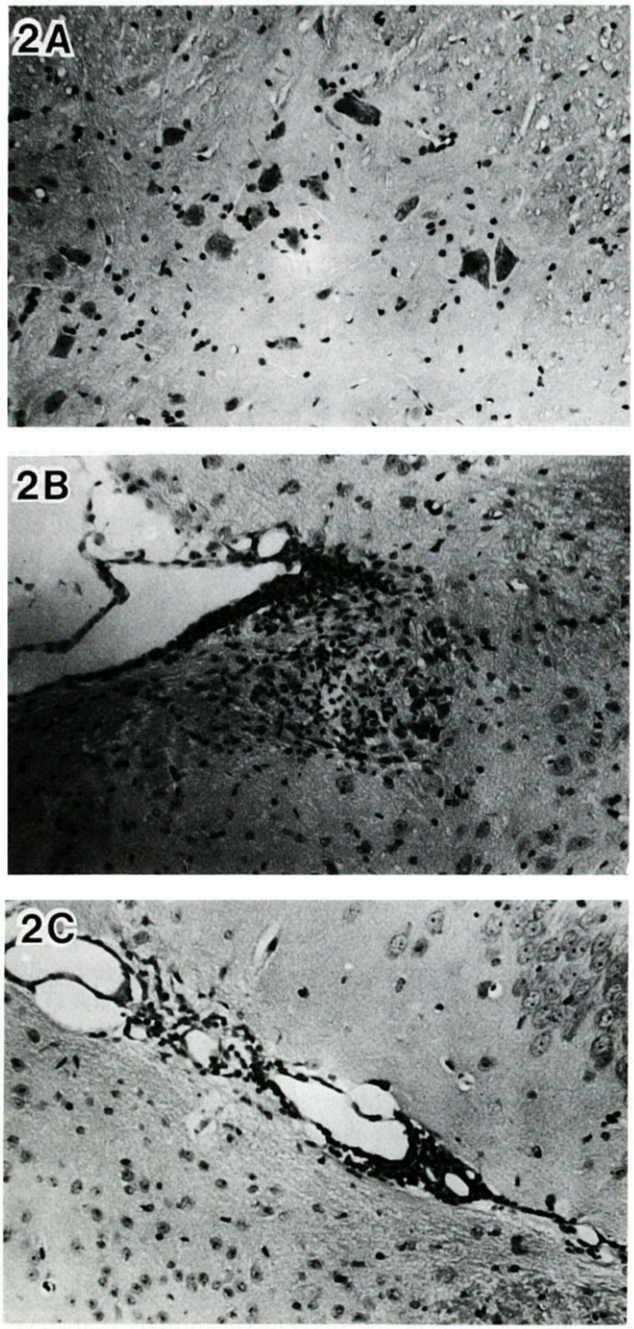

Fig. 2. Histopathology of the CNS in C57BR/cdJ mice sensitized with SSCH/CFA before and after infection with LDV-C. *A*: Diffuse infiltration of the anterior horn of a lumbar segment of spinal cord in a mouse infected with LDV-C 1 week before sensitization with SSCH/CFA, 25 days after infection (H&E, ×328). *B*: Infiltration of the ventral thalamus in a mouse sensitized with SSCH/CFA 1 week before infection with LDV-C, 18 days after sensitization (H&E, ×328). *C*: Infiltration of the fimbria of the hippocampus in a mouse subjected to the protocol described in *B*, 25 days after sensitization (H&E, ×390).

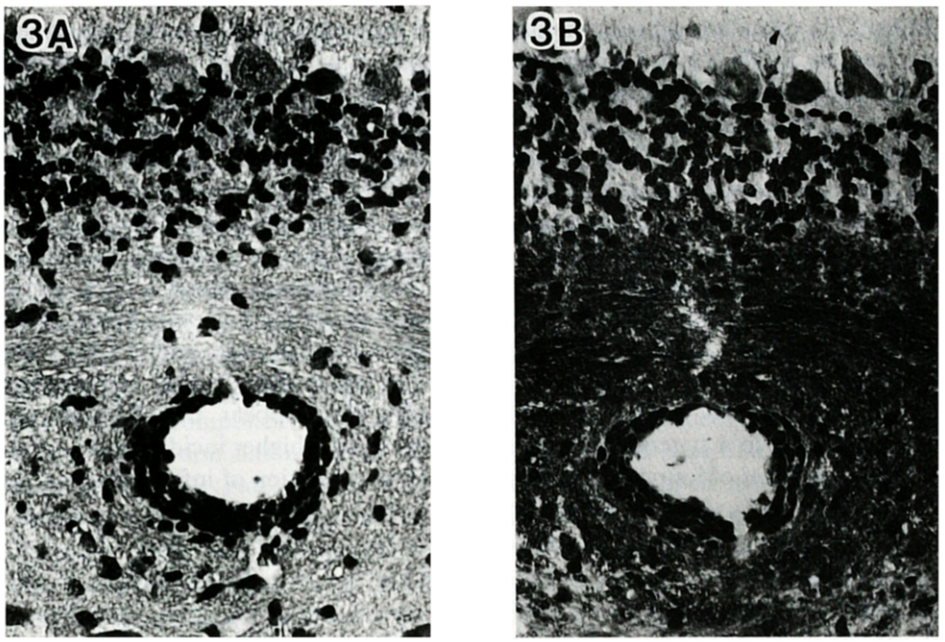

Fig. 3. Histopathology of the CNS in C57BR/cdJ mice sensitized with CFA 1 week before infection with LDV-C. *A*: Perivascular cuff of inflammatory cells in cerebellar white matter, 25 days after sensitization with CFA (H&E, ×820). *B*: Adjacent section to *A* demonstrates the absence of pronounced perivascular demyelination (LFB, ×820).

with LDV-C alone (group K vs group U, $P = 0.007$; group L vs group U, $P = 0.002$).

Together, these results indicate that the incidence of CNS lesions observed in recipients of LDV-C alone is statistically equivalent to the percent of lesions observed in recipients of 2 injections of SSCH/CFA (Table 1). Mice sensitized with SSCH/CFA 1 week before or 1 week after intraperitoneal inoculation of LDV-C developed a significantly higher percent of lesions as compared to recipients of LDV-C alone, but developed approximately the same percent of lesions as mice receiving 2 injections of SSCH/CFA (Table 1). This finding indicated that LDV-C could substitute for 1 of 2 weekly injections of SSCH/CFA.

Although recipients of CFA 1 week before or 1 week after inoculation of LDV-C developed about the same incidence of lesions as recipients of 2 sensitizations of SSCH/CFA and recipients of SSCH/CFA 1 week before or after inoculation with LDV-C, there was a difference in the number of animals that developed lesions at later times after the first injection. Recipients of CFA 1 week before or 1 week after inoculation with LDV-C had fewer lesions at 35 days after their first injection than recipients of 2 injections of SSCH/CFA (data not shown). Also, a greater number of mice that received SSCH/CFA 1 week before or after inoculation of LDV-C had lesions at 35 days after their first injection than recipients of 2 injections of

SSCH/CFA (data not shown). Similarly, at 35 days after their first injection, more recipients of SSCH/CFA 1 week before inoculation with LDV-C had CNS lesions than recipients of CFA alone before (group R) or after inoculation with LDV-C (Table 1). These results indicated that infection with LDV-C before or after sensitization with SSCH/CFA caused more long lasting CNS inflammation than that seen in mice infected with LDV-C before or after sensitization with CFA.

It appeared that mice that developed acute histologic EAE had milder, and more self-limiting CNS lesions than mice that received an injection of LDV-C in place of 1 of the 2 injections of SSCH/CFA. LDV-C alone produced milder lesions overall than the lesions produced in mice injected with both LDV-C and SSCH/CFA (Groups K and L), and lesions appeared in mice injected with both LDV-C and SSCH/CFA at an earlier day after the first injection than lesions appeared in recipients of LDV-C only (Table 1). It would thus appear that LDV-C and SSCH/CFA act in a synergistic manner to (i) produce a higher incidence of lesions, (ii) an earlier onset of lesions, and (iii) cause a longer duration of inflammatory CNS involvement.

Discussion

A portion of C57BR/cdJ mice infected with LDV-C develop a relatively mild CNS inflammatory disease which is not sex-linked, age-dependent, nor exclusively controlled by the H-2 histocompatibility locus (Stroop and Brinton 1983). Other strains of mice, closely related to C57BR/cdJ mice have been found to develop a very low level of very mild leptomeningeal or CNS white matter inflammation following intraperitoneal inoculation with LDV-C (Stroop and Brinton 1983). In this study, we have shown that if C57BR/cdJ mice are sensitized with SSCH in CFA before or after intraperitoneal inoculation with LDV-C, a larger number of animals develop more severe inflammatory CNS lesions. We have also shown that C57BR/cdJ mice, although resistant to the development of clinical signs, can develop acute histologic EAE.

Brown et al. (Brown and McFarland 1981; Brown et al. 1982) demonstrated that SJL/J mice were susceptible to develop relapsing EAE when 2 injections of SSCH were given in CFA 1 week apart. C57BR/cdJ mice, when sensitized twice with SSCH/CFA developed CNS inflammatory lesions consistent with acute EAE. In contrast to EAE induction in SJL/J mice, no clinical signs were produced in C57BR/cdJ mice, and the relapsing course described for similarly treated SJL/J mice did not occur.

C57BR/cdJ mice inoculated with 1 or 2 injections of CFA or IFA did not develop histologic lesions, whereas 14% (2/14) of mice that received 2 injections of SSCH developed minor CNS inflammatory lesions. When SSCH was emulsified in CFA and given in a 1- or 2-dose regimen, a much higher incidence of more severe CNS lesions were observed. These results indicated that C57BR/cdJ mice were susceptible to develop acute histologic EAE when *M. tuberculosis* was included in the sensitizing regimen. When LDV-C was substituted for one of the two injections

of SSCH/CFA, an even higher incidence of more severe and more long-lasting CNS inflammation was observed than in mice inoculated only with LDV-C or with 1 or 2 injections of SSCH/CFA. When LDV-C was used in conjunction with CFA, an incidence of lesions was observed similar to that seen in recipients of 2 injections of SSCH/CFA. These data suggested that *M. tuberculosis* was the critical element in the sensitizing inoculum responsible for the lesion-enhancing effect when injected into an LDV-C infected mouse or when given to a mouse prior to LDV-C infection. This was confirmed by the observation that when SSCH in phosphate-buffered saline was administered before or after infection, an incidence of lesions was produced consistent with the incidence of lesions produced by LDV-C infection.

The role of *M. tuberculosis* in the construction of an adjuvant in the production of EAE in mice, guinea pigs and rats has been known for many years. The mycobacterial component of CFA appears critical to the induction of EAE, although its mode of action has not been completely elucidated (Lee and Schneider 1962; Shaw et al. 1962; Paterson 1976). In contrast, when animals are immunized with syngeneic brain tissue or mylein basic protein in IFA, little or no EAE is produced (Paterson 1976), and animals fail to develop EAE when subsequently challenged with encephalitogenic proteins in CFA (Alvord et al. 1965; Swierkosz et al. 1975) due to the induction of suppressor T cells (Swierkosz et al. 1975; Welch and Swanborg 1976; Swierkosz and Swanborg 1977). Killen and Swanborg (Killen and Swanborg 1982) found that rats sensitized with a dose of myelin basic protein in IFA produced EAE effector cells in lymph node and spleen, suggesting that mycobacteria were not required for the activation of EAE effector cells. However, mycobacteria may be responsible for an amplification of effector cells generated through production of interleukin 1 by macrophages which in turn activates T helper cells (Gery et al. 1972; Killen and Swanborg 1982; Oppenheim et al. 1982). Helper cells, which produce interleukin 2 (Larsen et al. 1980; Smith et al. 1980) may activate the T effector cells responsible for EAE production (Killen and Swanborg 1982; Ortiz-Ortiz and Weigle 1982). Also because LDV-C replicates in macrophages in vitro (Brinton-Darnell et al. 1975) and probably replicates in fixed macrophages in vivo, it is possible that interleukin production itself is also directly affected by LDV infection.

It is also possible that replication of LDV, an RNA virus, is related to the enhanced incidence of lesions observed in LDV-infected recipients of CFA. Double stranded RNA has been shown to be a potent adjuvant in inducing EAE in guinea pigs (Gumbiner et al. 1973), and poly A:U, a synthetic polyribonucleotide, can substitute for the mycobacteria in CFA (Paterson 1973; Paterson and Drobish 1974).

Mice given SSCH in IFA before or after infection with LDV-C developed a low percent of mild CNS lesions. If the mechanism of LDV-induced CNS inflammation was autoimmune due to some antigenic similarity between CNS and LDV antigens, analogous to production of EAE in animals when sensitized to autologous or heterologous CNS encephalitogens, one would expect that administration of SSCH/IFA before infection with LDV/C would prevent lesion development. The fact that mice sensitized with SSCH/IFA 1 week before infection with LDV-C developed lesions, suggests that antigenic similarity between LDV-C and SSCH does

not form the basis for an autoimmune mechanism by which LDV-C produced CNS inflammation.

The relationship between EAE and virus infection of the CNS has been investigated in numerous ways. Massanari and colleagues (Massanari et al. 1979; Massanari 1981) found that a portion of hamster injected with measles virus before sensitization with neuroantigen emulsified in CFA and pertussis vaccine developed EAE faster than hamsters given the sensitizing injection alone. In our studies, we also found that antecedent LDV-C infection not only caused lesions to develop slightly earlier, but also made the lesions more intense.

In a study of the role of herpes simplex virus (HSV) infection of rats sensitized with guinea pig spinal cord homogenate emulsified in CFA, Hochberg et al. (1977) found that an intracranial injection of HSV could substitute for an injection of the encephalitogen in their EAE protocol. These results are similar to the results we obtained when C57BR/cdJ mice were given LDV-C 1 week before or 1 week after SSCH/CFA. Recently, Watanabe et al. (1983) have found that reactive T cells are generated against myelin basic protein during the course of coronavirus infections in Lewis rats. Infection of rats with murine coronavirus JHM produces a late onset demyelinating encephalomyelitis due to lytic replication of the virus in oligodendrocytes. When lymphocytes from infected rats were stimulated in vitro with myelin basic protein, and adoptively transferred to naive recipient rats, lesions and mild clinical signs consistent with EAE were produced. It is possible that replication of LDV-C in the CNS may cause the release of neural antigens, in a manner similar to JHM infection. These antigens may then participate in the EAE process to hasten and worsen the CNS lesions observed, perhaps through production of effector T cells.

Acknowledgements

We thank Drs. Lucy B. Rorke, Neal Nathanson, and Jan Tuttleman for their helpful discussions. We thank Anita Jackson for preparation of some of the histologic sections, and Terrie Larsen for superb editorial and secretarial assistance.

References

Alvord, Jr., E.C., C.-M. Shaw, S. Hruby and M.W. Kies, Encephalitogen-induced inhibition of experimental allergic encephalomyelitis — Prevention, suppression and therapy, Ann. N.Y. Acad. Sci., 122 (1965) 333–345.

Barnard, C.C.A. and P.R. Carnegie, Experimental autoimmune encephalomyelitis in mice — Immunologic response to mouse spinal cord and myelin basic proteins, J. Immunol., 114 (1975) 1537–1540.

Brinton, M.A., Genetically controlled resistance to flavivirus and lactate dehydrogenase-elevating virus-induced disease, Curr. Top. Microbiol. Immunol., 92 (1981) 1–14.

Brinton-Darnell, M., J.K. Collins and P.G.W. Plagemann, Lactate dehydrogenase-elevating virus replication, maturation and viral RNA synthesis in primary mouse macrophage cultures, Virology, 65 (1975) 187–195.

Brown, A.M. and D.E. McFarlin, Relapsing experimental allergic encephalomyelitis in SJL mouse, Lab. Invest., 45 (1981) 278–284.

Brown, A.M., D.E. McFarlin and C.S. Raine, Chronologic neuropathology of relapsing experimental allergic encephalomyelitis in the mouse, Lab. Invest., 46 (1982) 171–185.

Duffy, P.S., D. Martinez, G.D. Abrams and W.H. Murphy, Pathogenetic mechanisms in immune polioencephalomyelitis — Induction of disease in immunosuppressed mice, J. Immunol., 116 (1976) 475–481.

Gery, I., R.K. Gershon and B.H. Waksman, Potentiation of the thymocyte response to mitogens, Part 1 (The responding cell), J. Exp. Med., 136 (1972) 128–142.

Gumbiner, C., P.Y. Paterson, G.P. Youmans and A.S. Youmans, Adjuvanticity of microbacterial RNA and poly A:U for induction of experimental allergic encephalomyelitis in guinea pigs, J. Immunol., 110 (1973) 309–312.

Hochberg, F.H., J.R. Lehrich and B.G.W. Aranson, Herpes simplex infection and experimental allergic encephalomyelitis, Neurology, 27 (1977) 584–587.

Killen, J.A. and R.H. Swanborg, Autoimmune effector cells, Part 3 (Role of adjuvant and accessory cells in the in vitro induction of autoimmune encephalomyelitis), J. Immunol., 129 (1982) 759–763.

Larsson, E.-L., N.N. Iscove and A. Coutinho, Two distinct factors are required for induction of T cell growth, Nature (Lond.), 283 (1980) 664–666.

Lee, J.M. and H.A. Schneider, Critical relationships between the constituents of the antigen-adjuvant emulsion affecting experimental allergic encephalomyelitis in a completely susceptible mouse genotype, J. Exp. Med., 115 (1962) 157–167.

Martinez, D., B. Wolanski, A.A. Tytell and R.G. Davlin, Viral etiology of age-dependent polioencephalitis in C58 mice, Infect. Immunol., 23 (1979) 133–139.

Massanari, R.M. Acceleration of experimental allergic encephalomyelitis in hamsters with antecedent virus infection, Clin. Immunol. Immunopath., 19 (1981) 457–462.

Massanari, R.M., P.Y. Paterson and H.L. Lipton, Potentiation of experimental allergic encephalomyelitis in hamsters with persistent encephalitis due to measles virus, J. Infect. Dis., 139 (1979) 297–303.

Murphy, W.H., M.R. Tam, R.L. Lansy, M.R. Abell and C. Kauffman, Age dependence of immunologically induced central nervous system disease in C58 mice, Cancer Res., 30 (1970) 1612–1622.

Nawrocki, J.F., L.R. Pease and W.H. Murphy, Etiology role of lactic dehydrogenase virus infection in age-dependent neuroparalytic disease in C58 mice, Virology, 103 (1980) 259–264.

Oppenheim, J.J., B.N. Stadler, R.P. Siraganian, M. Mage and B. Mathieson, Lymphokines — Their role in lymphocyte responses. Properties of interleukin 1, Fed. Proc., 41 (1982) 257–262.

Ortiz-Ortiz, L. and W.O. Weigle, Activation of affector cells in experimental allergic encephalomyelitis by interlukin II (I.L. II), J. Immunol., 128 (1982) 1545–1550.

Paterson, P.Y., Experimental autoimmune encephalomyelitis — Induction, pathogenesis and suppression. In: P.A. Meicher and H.J. Muller-Eberhard (Eds.), Textbook of Immunopathology, Grune and Stratton, New York, 1976, p. 179.

Paterson, P.Y., Adjuvants, cell-mediated immune responses and autoimmune diseases, J. Reticuloendothelial Soc., 14 (1973) 426–440.

Paterson, P.Y. and D.G. Drobish, Adjuvanticity of a synthetic polynucleotides for induction of experimental allergic encephalomyelitis in guinea pigs, J. Immunol., 113 (1974) 1942–1946.

Sager, M.A., J.M. Lawton and W.H. Murphy, Serum transmissibility of immune polioencephalomyelitis in C58 mice, J. Immunol., 110 (1973) 219–226.

Shaw, C., E.C. Alvord, Jr., W.J. Fahlberg and M.W. Kies, Adjuvant-antigen relationships in the production of experimental 'allergic' encephalomyelitis in the guinea pig, J. Exp. Med., 115 (1962) 169–179.

Smith, K.A., L.E. Lachman, J.J. Oppenheim and M.F. Favata, The functional relationship of the interlukin, J. Exp. Med., 151 (1980) 1551–1556.

Stroop, W.G. and J.R. Baringer, Persistent slow and latent virus infections, Progr. Med. Virol., 28 (1982) 1–43.

Stroop, W.G. and M.A. Brinton, Mouse strain specific central nervous system lesions associated with lactate dehydrogenase-elevating virus infection, Lab. Invest., 49 (1983) 334–345.

Swierkosz, J.E. and R.H. Swanborg, Suppressor cell control of unresponsiveness to experimental allergic encephalomyelitis, J. Immunol., 115 (1975) 631–633.

Swierkosz, J.E. and R.H. Swanborg, Immunoregulation of experimental allergic encephalomyelitis — Conditions for induction of suppressor cells and analysis of mechanisms, J. Immunol., 119 (1977) 1501–1526.

Swinscow, T.D.V., Statistics at Square One, British Medical Association, London, 1976, pp. 54–57.

Watanbe, R., H. Wege and V. Ter Meulen, Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis, Nature (Lond.), 305 (1983) 150–153.

Welch, A.M. and R.H. Swanborg, Characterization of suppressor cells involved in regulation of experimental allergic encephalomyelitis, Europ. J. Immunol., 6 (1976) 910–917.