

Δ^9 -Tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis

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Summary

Since multiple sclerosis (MS) is believed to be an immune-mediated disease, it follows that its therapies should be directed towards modulating the immune system. Current MS treatments, which include the use of exogenous steroids that are immunosuppressive, do not meet therapeutic objectives. Δ^9 -Tetrahydrocannabinol (THC), an active component of marijuana, has been shown to be immunosuppressive. To test THC's ability to suppress an immune-mediated disease, experimental autoimmune encephalomyelitis (EAE), the laboratory model of MS, was used. Lewis rats and strain 13 guinea pigs were administered THC either before inoculation for EAE or treated with THC after injection. Control animals received placebo. The effect of dose, in addition to the timing of treatment, was also investigated. All animals treated with placebo developed severe clinical EAE 10–12 days post-injection (d.p.i.) and more than 98% died by 15 d.p.i. THC-treated animals had either no clinical signs or mild signs with delayed onset (13–15 d.p.i.) with survival greater than 95%. Examination of central nervous system tissue revealed a marked reduction of inflammation in the THC-treated animals. Therefore, as THC has been shown to inhibit both clinical and histologic EAE, it may prove to be a new and relatively innocuous agent for the treatment of immune-mediated diseases.

Introduction

Current treatments of multiple sclerosis (MS) include the use of immunosuppressive and syn-

thetic drugs (Kastrukoss et al., 1978; Basten et al., 1980; Ellison and Myers, 1980; Bornstein et al., 1982), plasmapheresis (Weiner and Dawson, 1980), and hyperbaric oxygen (Fischer et al., 1983). However, all present therapies fall short of ideal goals (McFarlin, 1983; Silberberg, 1984; Traugott and Raine, 1984). Since the effectiveness of each of these therapies has proven to be limited (McFarlin, 1983), it is clear that new and more effec-

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tive treatments are necessary and that these should be focused on modulating the immune system. The reason for this comes from mounting evidence that there may be an abnormality in the regulation of the immune system in MS patients and that this disease is believed to be immune-mediated (McFarlin and McFarland, 1982; Traugott and Raine, 1984). Many studies have reported alterations in the functions of humoral and cell-mediated (CMI) immunity in MS patients (reviewed in McFarlin and McFarland, 1982).

For some time, attention has been directed to the possible effects marijuana and its components may have on the immune system (Munon and Fehr, 1983; Holliter, 1986). Much of the evidence supporting the hypothesis that marijuana can affect the immune system comes from studies that examined changes in susceptibility or resistance of animals to infectious diseases (Bradley et al., 1977). Additionally, studies have investigated the effects of marijuana and its major component, Δ^9 -tetrahydrocannabinol (THC), on the function of lymphocytes in vitro (Martin, 1986). THC can affect many cellular functions including inhibition of nucleic acid and protein synthesis in cultured lymphocytes (Nahas et al., 1976), suppression of primary (Lefkowitz and Chiang, 1975) and secondary (Baczynsky and Zimmerman, 1983) antibody responses of mouse spleen cells, and inhibition of phytohemagglutinin- or antigen-induced T cell blastogenesis (Lau et al., 1976). THC has also been shown to suppress natural killer cell cytotoxicity (Patel et al., 1985) and directly affect macrophage functions (Gaul and Mellors, 1975). Therefore, the possible use of THC as a treatment for immune-mediated demyelination may be indicated.

Materials and methods

Preventive protocol

THC was administered to Lewis rats (200–250 g) in an emulsion containing 1 g of purified THC (Research Triangle Park) suspended in 1.5 ml sesame oil, 0.5 ml Tween 80, and 198 ml of distilled water giving a final THC concentration of 5 mg/ml. As a control, an emulsion of sesame oil, Tween 80, and water was prepared with the volume

of THC being compensated for by an additional 1 ml of water. The control emulsion is referred to as 'vehicle'. Beginning as early as 10 days before inoculation for EAE, Lewis rats received a volume of THC equivalent to 1 mg/kg body weight via an oral-gastric tube (p.o.). Thereafter, the volume of THC was increased every 2 days to reach a test dosage of 1, 2, 5, 10, 15, or 25 mg/kg of body weight/day (mg/kg/day) for each group (five rats/group). Control animals received equivalent volumes of either vehicle or saline.

Induction of experimental autoimmune encephalomyelitis (EAE)

(a) *Rats.* Lyophilized guinea pig MBP was dissolved in isotonic saline at a concentration of 1 mg/ml. To this, an equal volume of complete Freund's adjuvant (CFA) containing 10 mg of killed *Mycobacterium tuberculosis* was added and the mixture was emulsified. Rats were injected subcutaneously, while under mild anesthesia, with 0.2 ml of this emulsion.

(b) *Guinea pigs.* Strain 13 guinea pigs (450–550 g) were injected subcutaneously, while under mild ether anesthesia, in the nuchal area with 0.5 ml of an emulsion containing 0.25 ml of a 50% (w/v) suspension of bovine white matter homogenate in saline and 0.25 ml of CFA containing 10 mg of killed *M. tuberculosis*/ml.

Suppressive protocol

(a) *Rats.* Since 5 mg/kg/day of THC was found to be the smallest effective dose, to test the ability of THC to suppress EAE, animals were treated p.o. with this amount commencing 1, 3, 5, 7, and 9 days post-inoculation (d.p.i.). Control animals were given equal volumes of vehicle.

(b) *Guinea pigs.* THC treatment was initiated 7 d.p.i. in five animals. The treatment was administered by intraperitoneal (i.p.) injection of THC emulsified in a 10% solution (v/v) of polyethylene glycol (PG) in ethanol. Each animal received 0.5 ml of the THC/PG suspension containing 5 mg of THC daily. Control animals received either 0.5 ml of PG alone (five animals) or 0.5 ml of isotonic saline (five animals).

Clinical evaluation

The clinical course of acute EAE in the Lewis rat and strain 13 guinea pig is highly predictable

and it can be adequately monitored by examining the animals daily. The animals were scored from 0 to 7 (rats) and 0 to 5 (guinea pigs). The scores were: 0 = normal; 1 = flaccid tail (rats); 2 = generalized atonia (1 for guinea pigs); 3 = ataxia (2 for guinea pigs); 4 = paraparesis or incontinence; 5 = paraparesis and incontinence (3 for guinea pigs); 6 = a moribund state (4 for guinea pigs); and 7 = death (5 for guinea pigs).

Histology

At the end of each experiment (ranging from 9 to 16 d.p.i.), animals were killed by administration of sodium pentobarbital and perfused through the heart with 10% formalin. Formalin-fixed tissues were sliced into approximately 1 mm pieces, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E). Coded slides were examined and scored (0 = normal; 1 = meningeal hypercellularity or one perivascular cuff with a non-invasive margin per high power field; 2 = meningeal hypercellularity and one perivascular cuff per high power field, or two to three perivascular cuffs per field without meningeal hypercellularity; 3 = inflammatory cells extending from cuffs into central nervous system (CNS) parenchyma; 4 = diffuse inflammation in either white or gray matter; and 5 = inflammation extending throughout entire tissue section with or without primary demyelination).

Results

Clinical findings

Preventive protocol. The initial rat experiment showed that THC (25/mg/kg/day) can prevent the full development of clinical EAE (Figs. 1 and 2). Specifically, it was observed that THC caused a lower rate of body weight increase than that experienced by both saline and vehicle controls (Fig. 3). This reduction in gain of weight provided an excellent internal control showing that THC was in fact affecting the animals. Similar changes in body weight of rats caused by THC have previously been reported in studies that examined the effect of this drug on development (Fujimoto et al., 1982). Unlike other EAE studies in which the percentage change in body weight after inocula-

tion for disease provides an index of clinical status, the effect of THC in this regard precludes such use. However, as illustrated in Fig. 4, while vehicle and saline control animals developed clinical signs of EAE by 11 d.p.i., THC-treated Lewis rats exhibited a delayed onset (12 d.p.i. minimal signs and 13 d.p.i. definite signs). The severity of clinical signs was significantly ($P < 0.01$) reduced in the THC-treated group.

Dose. Our initial studies used a THC dosage of 25 mg/kg/day because this amount of drug was shown to be most effective in modulating endocrine functions in rats without being toxic (Gaul and Mellors, 1975; Karniol et al., 1975; Fujimoto et al., 1982; Patel et al., 1985). As 25 mg/kg/day represents a much higher dose of THC than is used clinically in humans (Karniol et al., 1975; Petro and Ellenberger, 1981; Clifford, 1983), dose titration studies were conducted.

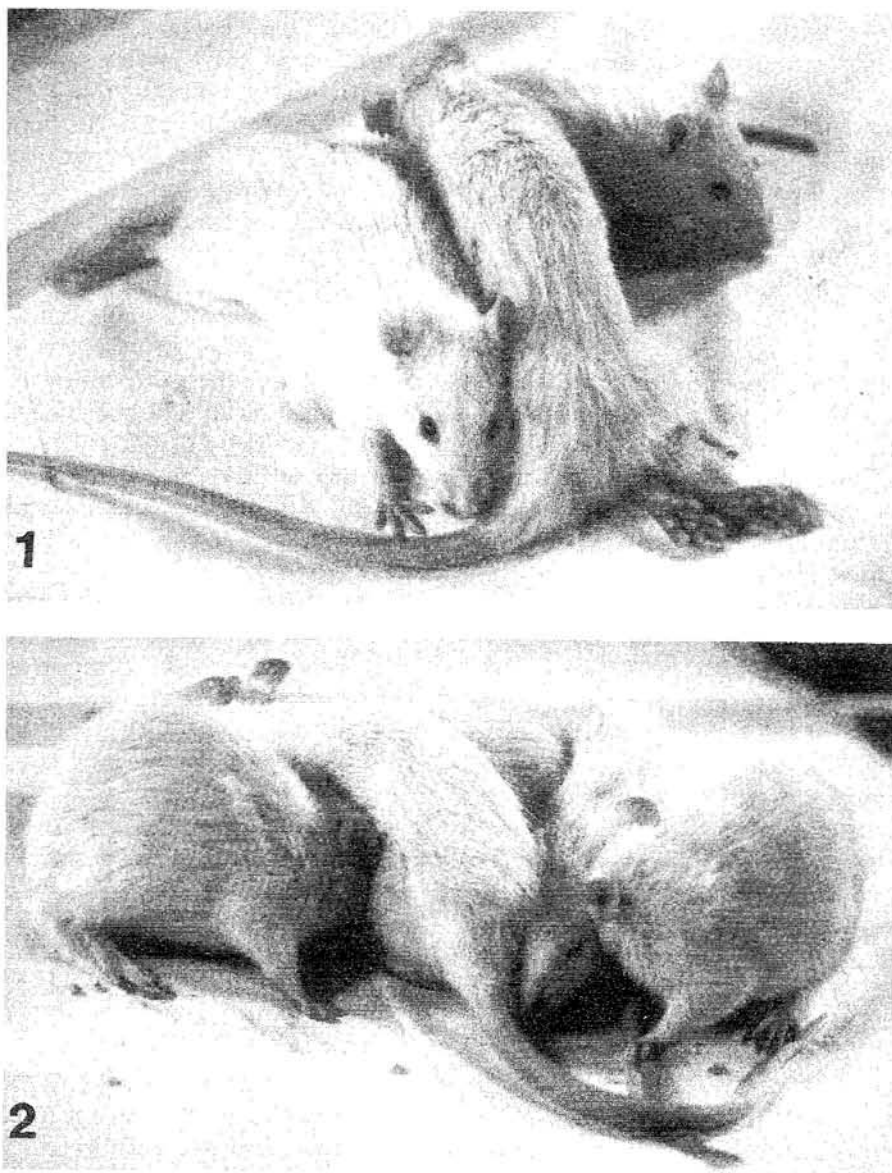
The smallest dose of THC which was as effective as higher amounts in suppressing EAE was 5 mg/kg/day (Fig. 5). At 5 mg/kg/day, the animals did better clinically because the characteristic weight loss induced by high levels of THC was absent.

Although some animals had a delayed onset of clinical signs with lower doses of THC (three of five animals at 2 mg/kg/day and two of five animals at 1 mg/kg/day), their eventual clinical score was as high as vehicle-treated control animals.

Suppressive protocol

(a) Rats. Treatment of EAE was commenced after sensitization (Fig. 6) for disease as this protocol has more relevance to MS than does prophylaxis. Five animals per group were started on either 5 mg/kg/day of THC or vehicle at 1, 3, 5, 7, or 9 d.p.i. Control animals were given vehicle at these same time points.

All of the vehicle-treated control rats developed clinical signs of EAE between 10 and 12 d.p.i. By 16 d.p.i., 24 of 25 control animals had died of EAE-related complications. In contrast, with the exception of rats started on THC 9 d.p.i., beginning treatment of animals at the earlier time points corresponded with a delayed onset of clinical signs and significantly reduced ($P < 0.001$) at all time points. One animal started on THC 7 d.p.i. died



Figs. 1 and 2. Photographs of Lewis rats treated either with vehicle alone (Fig. 1) or with THC (Fig. 2). Vehicle-treated animals show typical clinical signs of EAE: hindlimb paraparesis, atonic tail, and incontinence. In contrast, THC-treated rats (Fig. 2) have normal posture and no neurological signs. Both sets of animals were photographed 11 days post-inoculation.

without exhibiting any neurological or other signs of EAE.

(b) *Guinea pigs.* Results of this study showed that strain 13 guinea pigs receiving i.p. injections of either PG or saline developed clinical signs of

EAE commencing 10 d.p.i. (Fig. 7). Clinical severity reached a maximum (mean = 5.0) by 16 d.p.i. at which time all the animals were moribund. In contrast, guinea pigs receiving THC/PG had a delayed onset of clinical signs, beginning 11–12

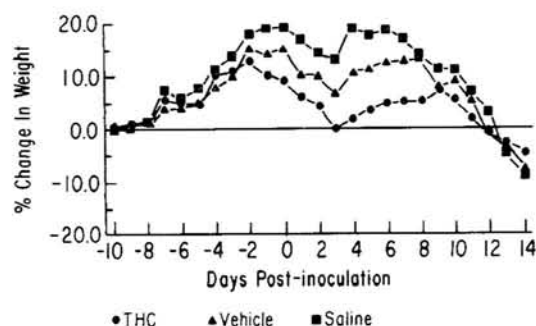


Fig. 3. Weight chart for representative Lewis rats treated with THC (circles), vehicle (triangles), or saline (squares) from 10 days before inoculation for disease.

d.p.i., and had significantly reduced signs by 16 d.p.i. (mean = 2.0) when compared to control groups ($P < 0.01$).

Histology

(a) *Rats*. Spinal cord sections taken from all control animals revealed extensive inflammation (mean histologic score = 4.2) in both white and gray matter areas (Figs. 8 and 9). Equivalent sections from THC-treated animals showed significantly less (mean histologic score = 1.2) inflammation (Figs. 10 and 11).

(b) *Guinea pigs*. Histologic evaluation revealed that PG- and saline-treated guinea pigs had extensive, multifocal inflammatory lesions extending

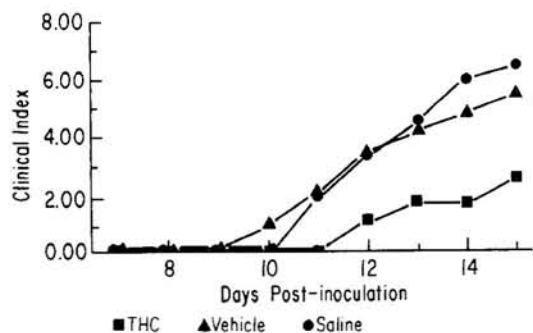


Fig. 4. Representative vehicle and saline control animals developed clinical signs of EAE by 10 and 11 d.p.i. respectively; THC-treated Lewis rats exhibited a delayed onset (12 d.p.i. marginal signs and 13 d.p.i. definite signs). Control vehicle- and saline-treated animals were moribund (clinical index greater than 6) by 15 d.p.i. while THC-treated rats had a mean clinical index of 2.75.

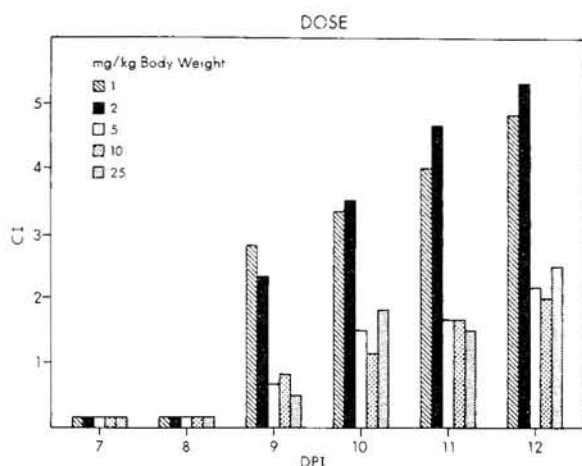


Fig. 5. Lewis rats were administered different doses of THC commencing before inoculation for EAE. The results shown here for representative animals from each group indicate the clinical status of the respective dosage groups from 7 to 12 d.p.i. 5 mg/kg body weight was found to be the lowest effective dose to suppress the clinical expression of EAE.

throughout the neuroaxis (mean histologic score = 4.7). These lesions were comprised of diffuse perivascular mononuclear cell and lymphocyte infiltrates and meningitis. However, THC-treated animals had reduced inflammation (mean histo-

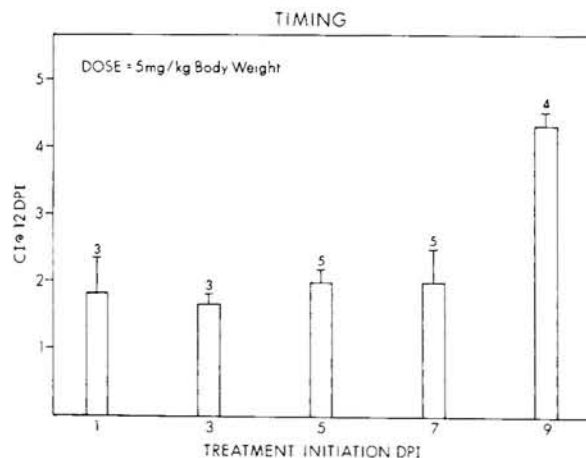


Fig. 6. Clinical signs at 12 d.p.i. Treatment was begun at different times after inoculation for EAE. Commencing as late as 7 d.p.i., THC could suppress the clinical expression of EAE. Animals were treated with 5 mg/kg body weight. The numbers over each standard error flag indicate the number of animals in each group.

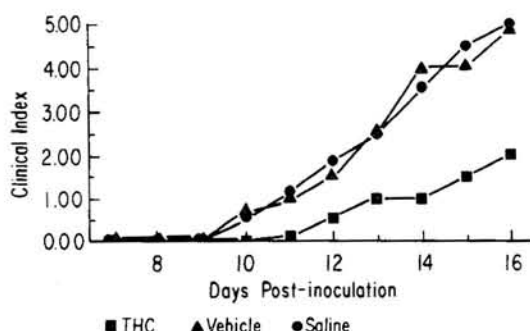


Fig. 7. Strain 13 guinea pigs receiving i.p. injections of either polyethylene glycol or saline developed clinical signs of EAE commencing 10 d.p.i. Clinical severity reached a maximum (mean = 5.0) by 16 d.p.i. at which time all animals were moribund. In contrast, guinea pigs receiving THC had a delayed onset of clinical signs, beginning 11–12 d.p.i., and had significantly reduced signs at 16 d.p.i. (mean = 2.0).

logic score = 2.2) restricted to the lumbar spinal cord and consisting of discrete perivascular cuffs with no infiltration of the surrounding parenchyma and some hypercellularity of the meninges.

Discussion

The results reported here show that THC is an effective drug in the prevention and suppression

of EAE. Although some THC-treated animals did develop clinical signs of EAE and had histopathologic changes consistent with this disease, the fact that they did not succumb to disease-related causes as did control vehicle-treated animals underscores THC's efficacy in treating this immune-mediated disease.

Since our interests were focused on determining if THC might prove to be of some benefit in treating animals with EAE, we increased the antigen dose with which the animals were sensitized to create a model with an unambiguous objective end point — life or death. In fact, with rare exception the THC-treated animals either did not develop acute-phase clinical signs of EAE or recovered without observable neurological deficits. In contrast, vehicle-treated animals had a disease-related mortality rate of greater than 90% and surviving animals showed significant post-acute-phase neurological morbidity. In experiments in which antigen dose was not maximized, THC-treated animals developed mild signs (clinical score 1.8) while control animals were paraparetic and incontinent (clinical score greater than 4). An interesting observation not reported here was that, when the odd THC-treated animal had neurological signs of EAE (clinical score greater than 3), the recovery

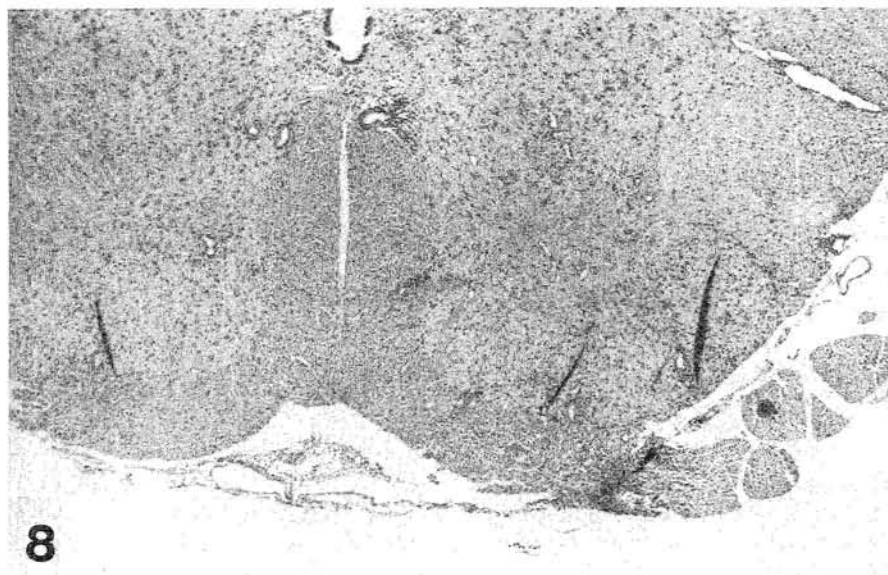


Fig. 8. Section through the thoracic spinal cord of a Lewis rat inoculated for EAE and treated with vehicle (12 d.p.i.). Diffuse and extensive inflammation is seen throughout this section. 250 ×.

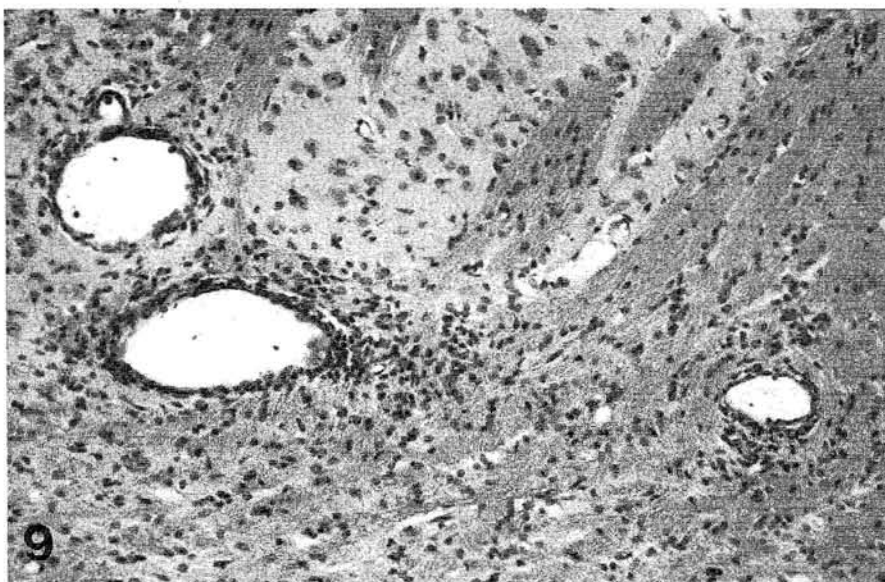


Fig. 9. Higher power (1000 $\times$) view of Fig. 8. Multiple perivascular cuffs and parenchymal inflammation are seen.

time to 'normal' was shorter than in vehicle-treated animals with equivalent clinical scores. We are currently investigating this point.

CNS tissue from THC-treated animals which developed clinical signs showed corresponding and correlative inflammation along the neuroaxis.

Other studies have documented that the intensity and the rostral progression from lumbar towards cervical spinal cord of inflammation correlated with both the degree of neurological signs and time course of acute EAE in rats and guinea pigs (Paterson et al., 1981; Raine and Traugott, 1985).

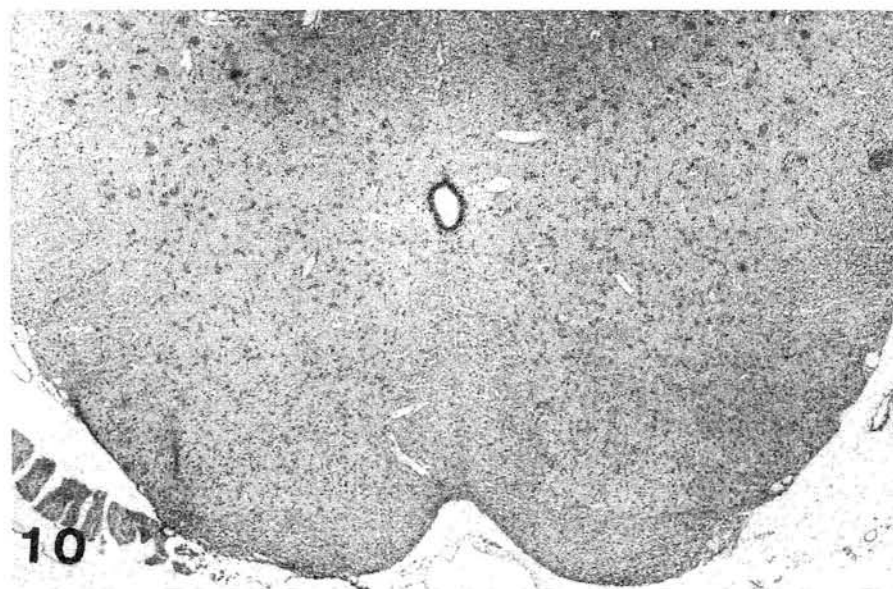


Fig. 10. Section through thoracic spinal cord of a Lewis rat 12 d.p.i. and treated with THC. Significant less inflammation is noted when this photomicrograph is compared to Fig. 8. 250 $\times$.

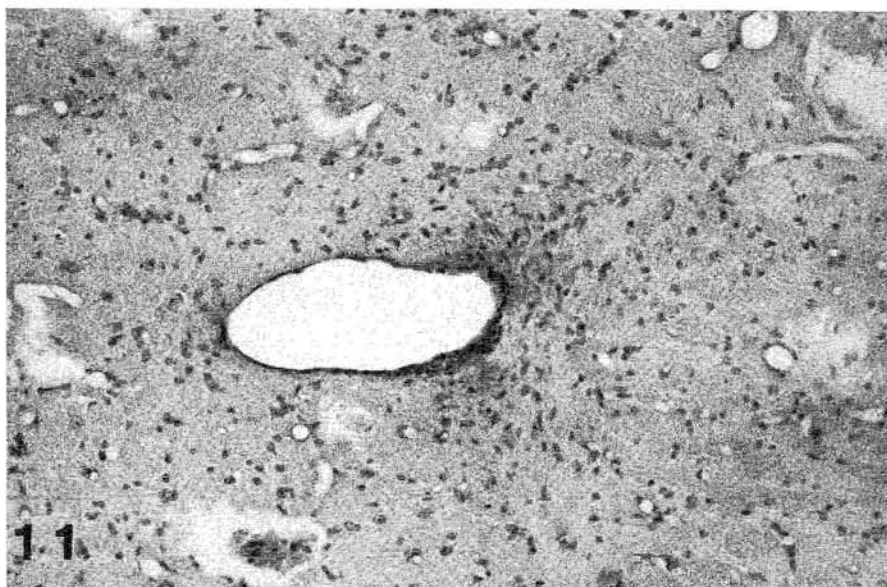


Fig. 11. High power (1000 $\times$) area of Fig. 10. Non-invasive perivascular inflammatory cuff is shown.

We found that this was also true in THC-treated animals in that tissue from animals with marginal signs of EAE had discrete non-invasive perivascular inflammatory cuffs in the lumbar spinal cord with reduced pathology in more rostral levels.

Although neither the clinical nor pathologic observations point clearly to the mechanism by which THC inhibits the development of EAE, these data in conjunction with other preliminary results indicate that THC may exert a direct effect on lymphocyte function and migration. Ongoing studies are examining an approximate 40% reduction in spleen cell responses to concanavalin A when cells from THC-treated animals are compared to vehicle-treated EAE-sensitized rats. Spleen cells from THC animals also responded to myelin basic protein (MBP) while spleen cells from vehicle animals did not. These two observations when taken together may indicate that THC is non-specifically immunosuppressive and may alter lymphocyte migration or sequestration. That is, in THC-treated animals MBP-responsive cells remain in the peripheral pool while in vehicle-treated animals these cells may have infiltrated into the CNS.

However, defining THC's mode of action in EAE may prove difficult because it has been shown

to cross the blood-brain barrier and intercalate itself into every plasma membrane system studied (Weiner and Dawson, 1980). Hence, THC could render myelin, oligodendrocytes, neurons, or astrocytes structural or functionally more resistant to pathologic insult. In addition, since it is believed that a component of EAE pathophysiology may involve antigen presentation at the level of the vascular endothelium, THC solubility in endothelial membranes could affect this proposed function as well.

In summary, although THC is not the only drug which may be effective in treating immune-mediated diseases, it may provide a new and effective approach towards treating a defined population of patients more effectively.

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