

SPONTANEOUS RECOVERY OF RATS FROM EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IS DEPENDENT ON REGULATION OF THE IMMUNE SYSTEM BY ENDOGENOUS ADRENAL CORTICOSTEROIDS

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Experimental allergic encephalomyelitis (EAE)¹ is a paralytic disease that can be induced in a number of animal species by evoking immune responses to antigens in central nervous system (CNS) myelin, and has been studied as a model for multiple sclerosis in man (1). In Lewis rats EAE can be induced either by immunization with guinea pig myelin basic protein (MBP) in CFA (active EAE) or by the intravenous injection into naive syngeneic recipients of spleen cells from animals with active EAE, after *in vitro* culture of the splenocytes with MBP (passive EAE). The ascending paralysis characteristic of EAE is caused by the action of CD4⁺ T lymphocytes that produce focal edema in the CNS by increasing vascular permeability (2-4). In both active and passive EAE, animals develop a transient paralysis, and recover completely within 4-5 d of its onset (5). The mechanisms responsible for this spontaneous recovery, which may be similar to the acute remissions occasionally seen in multiple sclerosis, are still poorly understood. Various mechanisms have been proposed, including: suppressor cells (T lymphocytes [6-9], B lymphocytes [10], and macrophages [11]), anti-T lymphocyte idiotype responses (12), serum suppressor factors (13-16), production of immunosuppressive factors by glial cells (17-19), regulation by IFN- γ (20), and neuroendocrine-mediated immunoregulation (21, 22). None of these mechanisms has been shown directly to be necessary for spontaneous recovery from EAE. It has, however, been demonstrated that CD8⁺ T lymphocytes are not required (23-25). Here we demonstrate that endogenously produced corticosterone plays an essential role in the spontaneous recovery of rats from EAE.

Materials and Methods

Animals. Lewis strain rats, 2-4 mo old, from the specific pathogen-free animal house in the MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, were used throughout. They were housed in a daylight cycle-controlled animal room. Animals were

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¹ *Abbreviations used in this paper:* ACTH, adrenocorticotrophic hormone; CNS, central nervous system; DTH, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; MBP, guinea pig myelin basic protein.

age and sex matched within each experiment. All procedures were performed under ether anesthesia.

EAE Induction. Animals were immunized with 50 μg guinea pig MBP (prepared as in reference 26, but without the cation exchange chromatography step), emulsified in 100 μl CFA (10 mg/ml mycobacterium tuberculosis H37Ra in IFA [Difco Laboratories, Detroit, MI]), and given subcutaneously into the hind footpads (active EAE). Alternatively, the disease was transferred to naive syngeneic recipients by spleen cells from these animals. The spleens were taken on days 11–13 after immunization and single cell suspensions were prepared. The cells were cultured at a concentration of 2×10^6 viable leukocytes/ml in RPMI 1640 medium (Gibco Ltd., Paisley, Scotland) containing 5% heat-inactivated FCS, 2.5×10^{-5} M 2-ME, and 2 $\mu\text{g}/\text{ml}$ MBP for 72 h in 5% CO_2 in air at 37°C. The cells were harvested and washed three times with PBS containing 0.2% BSA. 5×10^7 viable leukocytes were then injected intravenously (27, 28). Animals were scored daily for clinical signs of disease on a scale from 0 to 5 depending on severity: 0, normal; 1, limp tail; 2, hind limb paresis; 3, unilateral hind limb paralysis; 4, bilateral hind limb paralysis; and 5, bilateral hind limb paralysis and incontinence.

Blood Cell Counts. Animals were bled from a cut in the tail between 9:00 a.m. and 10:00 a.m. The total number of nucleated cells per ml of blood was determined using an electronic cell counter (Coulter Electronics Inc., Hialeah, FL) and differential counts were done on Giemsa-stained smears (100 cells were counted on each smear).

Corticosterone Measurement. Animals were caged in pairs. Between 9:00 a.m. and 10:00 a.m. both animals in the cage were anesthetized simultaneously and bled from a cut in the tail within 2 min. This procedure has been reported not to elevate blood steroid levels significantly within this time interval (29). The level of corticosterone in the serum was determined by RIA (30) using a rabbit antiserum raised against corticosterone 21 hemisuccinate conjugated to BSA (Sigma Chemical Co., St. Louis, MO). This antiserum, which was a gift from Dr. C. Kenyon, MRC Blood Pressure Unit, Western Infirmary, Glasgow, shows a 7% crossreaction with progesterone and 6% crossreaction with deoxycorticosterone (C. Kenyon, personal communication).

Adrenalectomy and Steroid Replacement. Animals were bilaterally adrenalectomized or sham adrenalectomized by blunt dissection through a single dorsal incision. Animals were checked at postmortem for residual adrenal tissue, which was never found. Adrenalectomized animals (and the controls) were given 0.9% saline to drink. Replacement steroid therapy was given by inserting implants of corticosterone mixed with cholesterol (Sigma Chemical Co.) in varying proportions, subcutaneously in the flank (31). All of the steroid pellets used weighed 100 mg at the start of the experiment and had a uniform shape.

Delayed-type Hypersensitivity (DTH). Spleens were removed from animals immunized with 100 μg OVA (Sigma Chemical Co.) in FCA 11 d previously and cells were cultured as for adoptive transfer of EAE, but with 40 $\mu\text{g}/\text{ml}^{-1}$ OVA instead of MBP. 5×10^7 viable leukocytes were injected intravenously into syngeneic recipients 14 d after immunization with MBP in FCA, FCA only, or physiological saline. 2 d later each animal was injected subcutaneously in one ear with 40 μg OVA in 20 μl PBS, and in the contralateral ear with 40 μg BSA in 20 μl PBS. 48 h later, the thickness of each ear was measured in triplicate using a "Quick-tester" micrometer gauge (H. C. Kroplin, Federal Republic of Germany) and a mean score for each ear was determined (2).

Results

Animals with EAE Are Lymphopenic. Spontaneous recovery from EAE presumably involves a shut down in the activity of the cells that cause the pathology in the CNS, and possibly a block in the generation of these cells or their traffic to the CNS. In active EAE the effector cells that are generated in the lymph nodes draining the site of immunization (hind footpads) must enter the bloodstream via the thoracic duct to reach the CNS (32). When the output of cells from the thoracic ducts of animals immunized with MBP in CFA was studied, it was found that the traffic of

cells into the blood from the thoracic duct was reduced fourfold during the time when the animals were paralyzed (Table I). The relative proportions of cell subsets present, defined by labeling with mAbs and flow cytofluorimetry, did not change with time (data not shown). However, because the thoracic duct is the main source of lymphocytes entering the blood (32), it was anticipated that this fall would be accompanied by a peripheral lymphopenia. Table I shows that in both active and passive EAE there was a fall in the PBL count, similar in magnitude to the drop in thoracic duct lymphocyte output. Fig. 1 shows the changes in peripheral blood leukocyte counts with time after immunization with MBP in CFA. Lymphocyte counts were normal before and after clinical disease but were markedly depressed during the episode of paralysis. Circulating neutrophil numbers increased by three- to fourfold within 5 d of immunization with MBP in CFA (as has been reported previously [33]), a change which was also seen in animals given the adjuvant only (data not shown). During EAE neutrophil numbers fell to normal levels but were again elevated after recovery. This temporary reversal of the neutrophilia was not seen in animals immunized with FCA only. Another lymphoid compartment that was depleted of cells during EAE was the spleen (Table I). The spleens of animals immunized with OVA in FCA were also hypocellular but not to such a degree.

Rats with EAE Have Elevated Serum Levels of Corticosterone. In rats corticosterone is known to cause lymphopenia and changes in lymphocyte traffic (34, 35). Accordingly, a series of experiments was carried out to determine the serum level of this hormone in rats developing EAE. The results, shown in Fig. 2, demonstrate that animals with signs of the disease had markedly elevated levels of the hormone, the increase occurring just before the onset of paralysis with its peak at the time of maximum clinical disease. In both active and passive EAE, animals started to recover at the time when corticosterone levels were maximal. As the severity of disease declined so did the amount of steroid present, basal levels being measured by 20 d after immunization in active EAE or 8 d after cell transfer in passive EAE. In active EAE 34% (67 of 195) of the animals suffered from a second mild episode of paralysis (this was never seen in passive EAE). The onset of this relapse correlated well with the return of normal serum corticosterone levels, always occurring at 22–25 d after immunization. This single relapse at this time has also been reported elsewhere (36, 37) and it has been shown that adrenalectomy during recovery from EAE accelerates the onset of this relapse (21).

A criticism that could be made of measurement of steroid levels by bleeding serially under ether anesthesia is that the stress of bleeding is the most important factor in raising steroid production. To confirm that the elevated steroid levels were not an anesthetic stress artifact, animals were immunized with MBP in FCA and caged individually. They were bled by decapitation without anesthesia within 20 s of handling. The mean corticosterone level in four animals bled on day 7 after immunization was 6.1 ± 4.9 nM, and in unimmunized controls bled on the same day the value was 2.2 ± 2.0 nM. Of four immunized animals bled on day 14, two showed clinical signs of paralysis with serum corticosterone levels of 259 nM and 316 nM and two had no clinical signs with corticosterone levels of 1.7 and 20.7 nM. Unimmunized controls on day 14 had corticosterone levels below the threshold of detection of the assay. Comparing these results with those illustrated in Fig. 2, it is evident that repeated episodes of anesthesia and bleeding did induce some elevation in serum

TABLE I
Lymphopenia in EAE

Immunological compartment	Normal animals ¹	Animals with clinical EAE ²	Animals immunized with FCA only ³	p 1v2*	p 2v3*
	<i>n</i>	<i>n</i>	<i>n</i>		
Nucleated cells recovered from the thoracic duct after overnight collection (17 h) in active EAE	4.28 ± 0.35 × 10 ⁸ (10)	1.06 ± 0.12 × 10 ⁸ (3)	-	<0.001	-
Lymphocytes/ml of peripheral blood in active EAE	1.12 ± 0.11 × 10 ⁷ (6)	0.38 ± 0.06 × 10 ⁷ (7)	1.30 ± 0.21 × 10 ⁷ (3)	<0.001	<0.001
Lymphocytes/ml of peripheral blood in passive EAE	1.10 ± 0.06 × 10 ⁷ (4)	0.35 ± 0.04 × 10 ⁷ (5)	-	<0.001	-
Nucleated cells recovered from the spleen	3.51 ± 0.16 × 10 ⁸ (23)	2.06 ± 0.12 × 10 ⁸ (34)	2.90 ± 0.14 × 10 ⁸ † (13)	<0.001	<0.001

The values given are means ± SE. The values for thoracic duct lymphocyte output for normal animals were for animals immunized with MBP in FCA that had not yet developed clinical signs of EAE. In all other cases the "normal animals" were unimmunized. The measurements for animals with clinical EAE were made at the time of maximum clinical disease, and for FCA immunized animals, 11-13 d after immunization.

* By student's *t* test.

† These animals were immunized with 100 µg OVA in FCA and there was a significant reduction in spleen cell numbers when compared with normal animals (0.01 < *p* < 0.02).

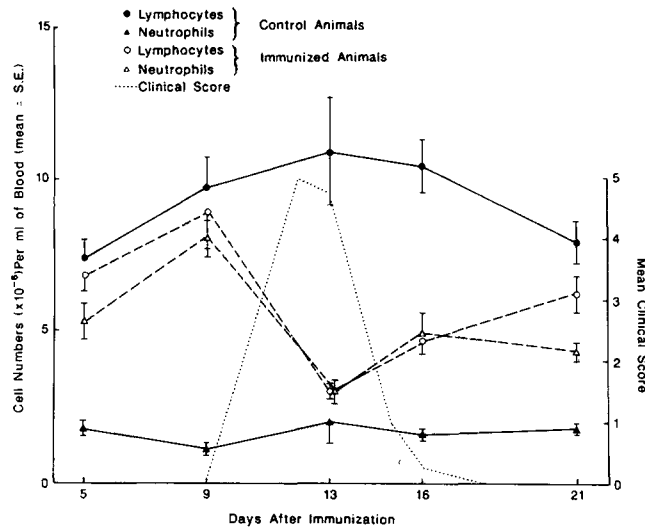


FIGURE 1. Peripheral blood white cell counts in EAE. On day 0 groups of four animals were immunized with 50 μ g MBP in FCA or 100 μ l saline (controls). The animals were bled from a cut in the tail between 9:00 a.m. and 10:00 a.m. and the total number of nucleated cells per ml of blood was determined using an electronic cell counter (Coulter Electronics Inc.). Differential counts were done on Giemsa-stained smears. This experiment has been repeated twice with similar results.

corticosterone, but that this effect did not account for the very high levels found in animals with EAE.

Adrenalectomized Animals Do not Recover from EAE. To determine whether the increase in endogenous corticosterone production in EAE was essential for recovery from the disease, animals were bilaterally adrenalectomized 3 d before immunization or cell transfer, and given subcutaneous steroid implants to maintain normal resting serum corticosterone levels. In both actively and passively induced EAE, adrenalectomized animals showed clinical signs earlier than sham-operated controls and always died (Fig. 3). Disease developed very rapidly in adrenalectomized rats so that an animal showing only a limp tail at one time point could be severely paralyzed 12 h later. For this reason the terminal clinical scores for animals that died overnight are not known. However, rats that were observed with terminal disease were always severely paralyzed. Histological examination of the spinal cords of these animals showed inflammatory changes typical of EAE. In pilot experiments the maintenance dose of corticosterone was not given and 8 of 10 animals immunized with MBP in FCA died within 48 h of immunization. These animals had much more severe footpad edema than sham-adrenalectomized controls and it is likely that death was due to susceptibility to the lethal effects of IL-1 and TNF (produced after immunization with the adjuvant) subsequent to adrenalectomy, as was recently reported (38).

Steroid Replacement Therapy Restores Recovery from EAE in Adrenalectomized Animals. Having demonstrated an essential role for the adrenal gland in preventing fatalities due to EAE, it remained to be shown that it mediated its protective effect by the production of increased levels of serum corticosterone rather than by the release of other corticosteroid hormones, or hormones from the adrenal medulla. To address this point steroid levels in adrenalectomized rats were manipulated by subcutaneous corticosterone implants. Animals were either adrenalectomized or sham operated as above and maintenance steroid implants were inserted 3 d before immunization (see above) followed by an extra subcutaneous implant of varying corticosterone content

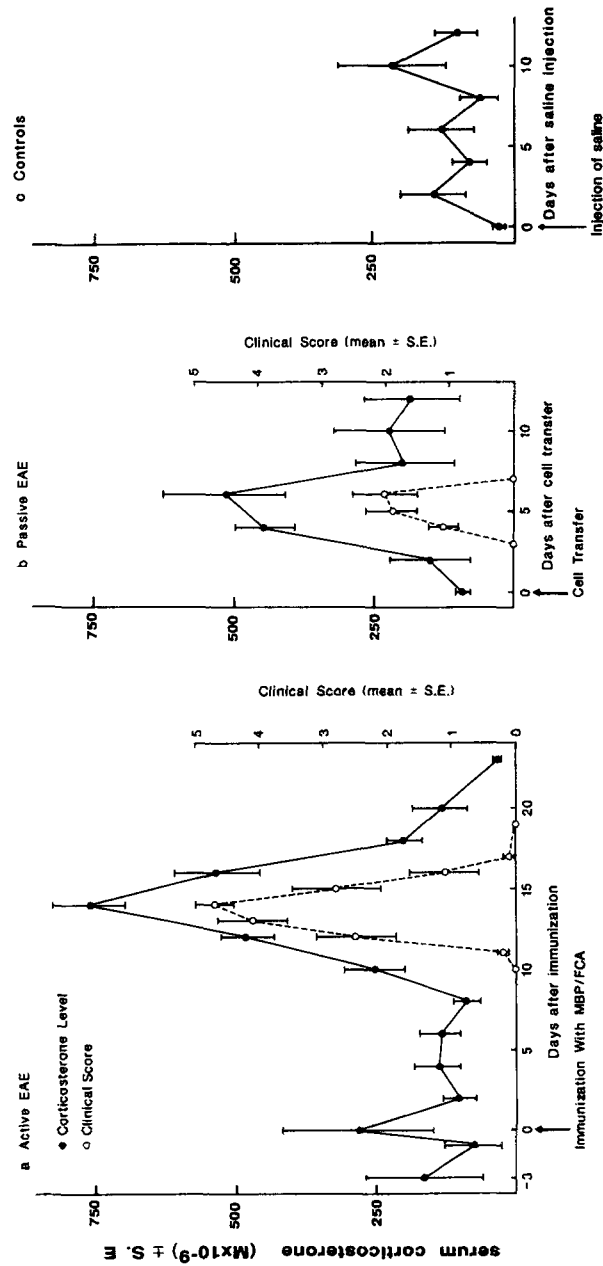


FIGURE 2. Changes in serum corticosterone levels in EAE. Active EAE was induced by immunization with 50 μ g MBP in FCA on day 0 (n, 8; data pooled from two experiments). For passive EAE spleen cells from MBP/FCA-primed donors were cultured in vitro with MBP and 5×10^7 cells were injected intravenously into naive recipients on day 0 (n, 5). Controls were given the same volume of saline intravenously (n, 4). Animals were bled from a cut in the tail vein within 2 min of ether anesthesia between 9:00 a.m. and 10:00 a.m. Corticosterone was measured by RIA.

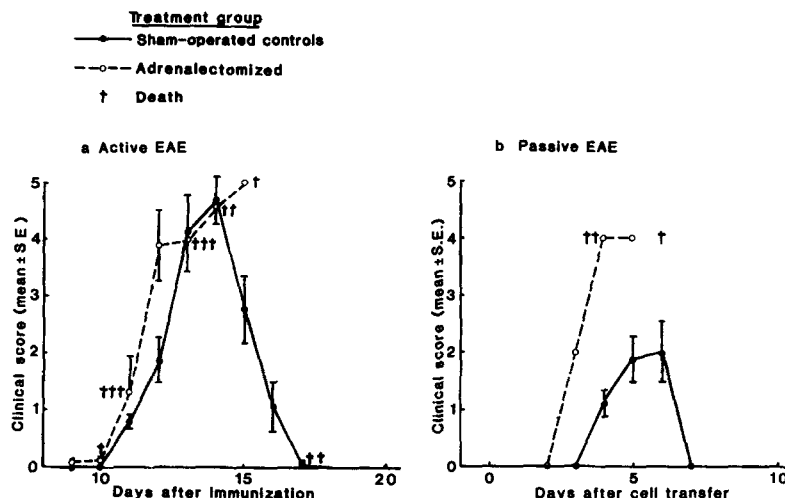


FIGURE 3. Adrenalectomy prevents recovery from EAE. Active EAE was induced by immunization with 50 μ g MBP in FCA on day 0. For passive EAE spleen cells from MBP/FCA-primed donors were cultured in vitro with MBP and 5×10^7 cells were injected intravenously into naive recipients on day 0. Animals were bilaterally adrenalectomized or sham operated 3 d before immunization or cell transfer, normal basal levels of serum corticosterone being maintained in adrenalectomized rats by subcutaneous steroid implants. For active EAE there were nine control animals and 10 adrenalectomized. For passive EAE there were five control animals and three adrenalectomized. The mean clinical scores apply only to surviving animals.

9 d after immunization (the time at which steroid levels in intact animals started to rise) (Fig. 4). When serum corticosterone levels similar to those produced endogenously in EAE were achieved, the animals either suffered from a single episode of paralysis from which they recovered completely or, in the case of one animal, failed to develop clinical signs of EAE (Fig. 4 c). If lower levels were achieved the animals still died (Fig. 4, a and b) and at higher levels the disease was suppressed in all cases (Fig. 4 d).

In a second series of experiments using steroid replacement, the additional 100% corticosterone pellet was inserted on the day when animals first showed definite clinical signs of disease (a score ≥ 1) rather than on day 9. When steroids were given at this later time 2/4 animals showed clinical signs and recovered and two died (Fig. 5).

In all of the above experiments steroid-treated adrenalectomized animals that recovered from EAE were observed at least until day 30 after immunization and showed no deterioration in condition, except for one animal in Fig. 4 d that died unaccountably, but did not have signs of paralysis.

In the adrenalectomized animals shown in Fig. 4, a steep rise in serum corticosterone was seen either just before death (Figs. 4 a and 5 a), or when clinical disease was observed (Fig. 4 c). At postmortem these animals were confirmed to have no residual adrenal tissue and the subcutaneous steroid pellets had not broken up, thus ruling out these two possible sources of increased levels of serum corticosterone. An alternative explanation could be a change in the pharmacokinetics of corticosterone due to the acute retention of urine, with subsequent hydronephrosis seen in male rats with EAE (21, and our own observations).

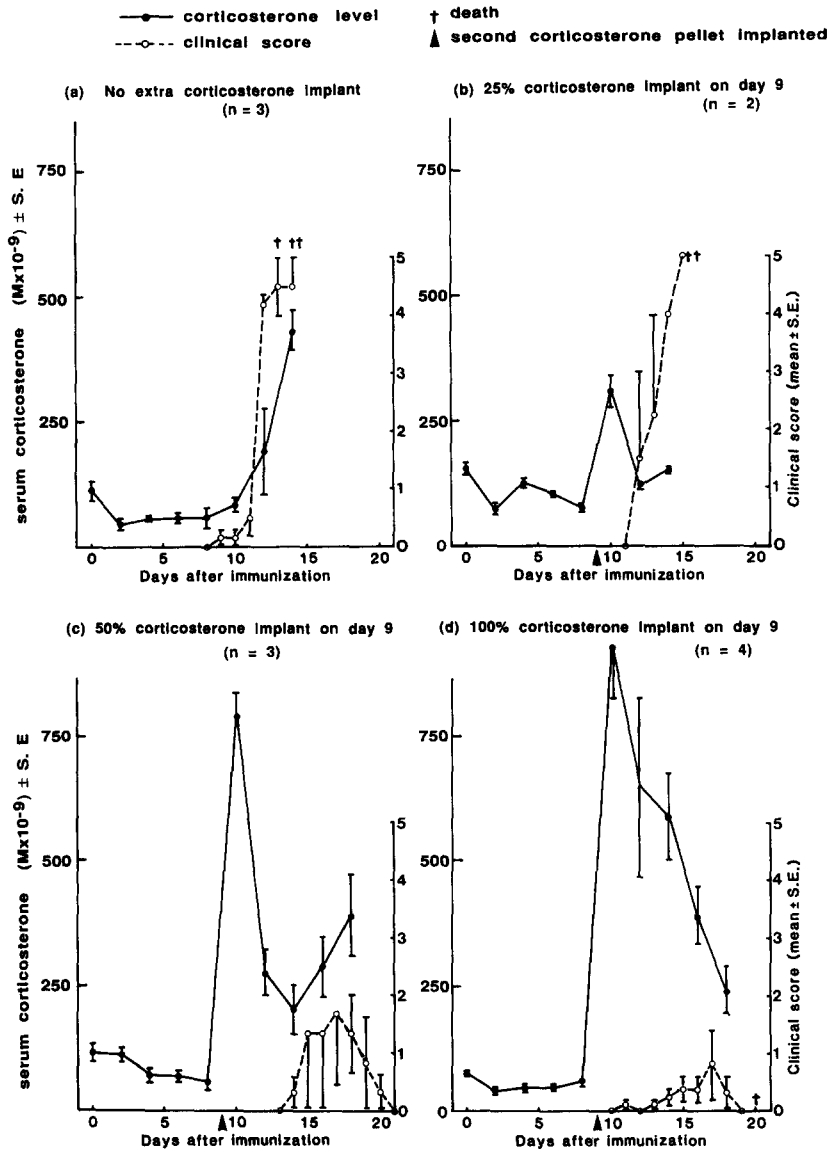


FIGURE 4. The effect of corticosterone therapy in adrenalectomized animals. Animals were adrenalectomized and given a 25% corticosterone in cholesterol implant (25 mg corticosterone in 75 mg cholesterol) subcutaneously to maintain normal basal steroid levels, 3 d before immunization with 50 μ g MBP in FCA (day 0). Additional implants of the same size with increasing corticosterone content were inserted on day 9 after immunization, the time at which steroid levels started to rise in control animals (Fig. 2). The mean clinical scores apply only to surviving animals.

DTH Responses to OVA Are Inhibited in Animals with EAE. The immunosuppressive effects of steroids are not antigen specific (34, 39), and experiments were carried out to determine whether rats with EAE demonstrated a generalized hyporeactivity to antigenic challenge. Table II shows that the adoptive transfer of DTH to OVA

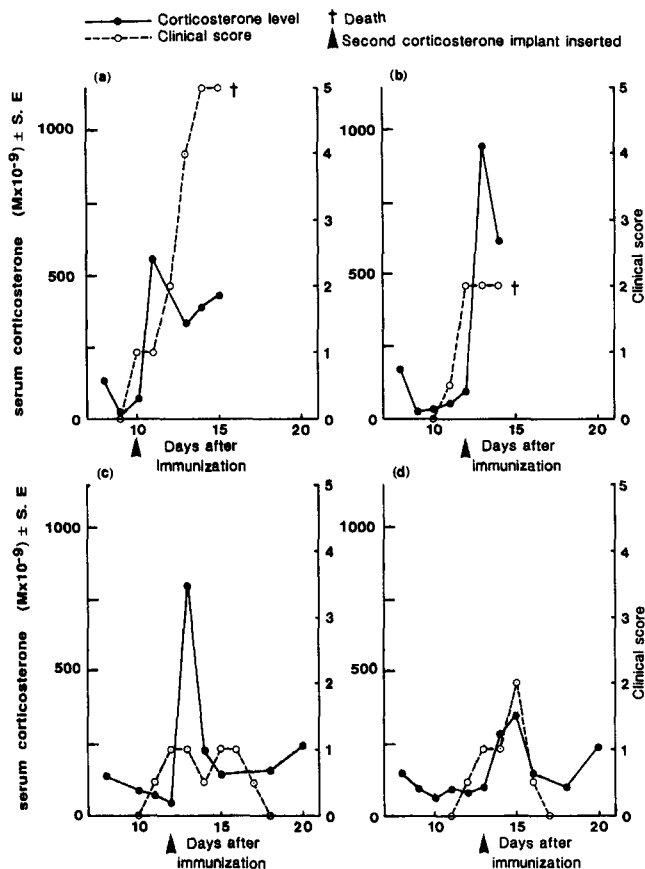


FIGURE 5. The effect of corticosterone therapy given to animals after the onset of paralysis. Animals were adrenalectomized and given a subcutaneous steroid implant to maintain normal basal steroid levels as in Fig. 4. On the first day that animals showed definite clinical signs of EAE (a score ≥ 1) a second 100-mg 100% corticosterone implant was inserted.

was significantly suppressed in animals with EAE. The antibody response to SRBC in these animals was not suppressed (data not shown), a finding that is not inconsistent with steroid-mediated immunosuppression (39).

Discussion

The data presented demonstrate the essential role of endogenous corticosterone in modulating the severity of EAE. It is evident that the elevated levels of the hormone that develop spontaneously during the course of the disease not only prevent the disease being a lethal one, but produce a sufficient level of immunosuppression to induce complete remission of clinical signs. It could be argued that the elevated steroid levels are necessary but not sufficient for the self cure reaction to occur and that some other immunoregulatory mechanism, involving suppressor cells or one of the other processes listed in the introduction, play an equally essential role. Although this more complex interpretation cannot be completely dismissed, the observation that rats with EAE made very depressed DTH responses to OVA indicates that the immunosuppression does not display antigen specificity. The finding that

TABLE II
The DTH Response to OVA Is Inhibited in EAE

Exp.	Increase in ear thickness when challenged with OVA			p 1v3*	p 2v3*
	Control animals ¹	FCA immunized ²	MBP in FCA immunized ³		
	<i>n</i>	<i>n</i>	<i>n</i>		
1	0.43 ± 0.045 (4)	0.42 ± 0.067 (4)	0.22 ± 0.07 (3)	<0.01	<0.02
2	-	0.26 ± 0.032 (5)	0.17 ± 0.080 (5)	-	<0.05

On day 14 after immunization 5×10^7 OVA cultured cells were transferred to each animal. 2 d later the animals were challenged with OVA in the left ear and BSA in the right ear, and ear swelling was measured after 48 h. The values shown are the mean difference in thickness between the left and right ears \pm 1 SD. In Exp. 1, male animals were used; and in Exp. 2, females were used, which may explain the difference in the absolute values recorded between the experiments. The mean clinical scores for the MBP-immunized animals on the day of cell transfer were 5 for Exp. 1, and 3.6 for Exp. 2. ¹ Untreated; ² Immunized with 100 μ l FCA; ³ Immunized with 50 μ g MBP in FCA.

* The differences between the groups were tested for statistical significance by student's *t* test.

EAE can be prevented by the prior administration of corticosterone (Fig. 4) also supports this view as does the finding that rats exposed to environmental stress develop less severe paralytic signs (40). Steroids have been given successfully as chemotherapy in EAE (33, 41) and multiple sclerosis (42), and adrenocorticotrophic hormone (ACTH) has been used to suppress EAE in guinea pigs (43).

The possibility that the endogenous production of corticosterone was responsible for recovery from EAE has been proposed previously (21), but seems not to have gained general acceptance. Earlier studies showed that animals with EAE developed a partial splenic and thymic atrophy, associated with adrenal hypertrophy (33). Thymic atrophy was found to be associated with elevated levels of corticosterone in the blood (21). These important results, while indicating that corticosterone release in EAE was associated with changes in lymphoid tissue and with reduced blood lymphocyte counts, did not establish that these steroid-induced changes were instrumental in bringing about the remission of signs of paralysis.

The site of action of this steroid response is still not clear. Steroids can regulate the immune response in a number of ways, including the inhibition of production of IL-1 and IL-2 (44, 45), effects on lymphocyte traffic (35), and the depletion of lymphocytes (corticosterone is lympholytic in rats) described earlier (34). Steroids, therefore, have the capacity to inhibit the activity of effector cells in the CNS, and to prevent the generation and supply of fresh activated cells.

Although the pathogenesis of EAE is probably not due to a DTH reaction in the CNS (2), a DTH response does have some essential features similar to the development of EAE; i.e., the localization of antigen-specific effector cells in a nonimmune organ, and their activation to secrete lymphokines. As already noted, the DTH response to an antigen not involved in EAE (OVA) was suppressed during the time when animals were recovering from EAE and had elevated steroid levels. It has also been reported that DTH responses to MBP are suppressed in animals with clinical signs of EAE (46, 47), and that responses to mycobacterial purified protein derivative are also suppressed, although the data were not shown (47). Steroids are known to interfere with the development of DTH responses at several levels (34, 35, 39, 44, 45), and it is reasonable to suggest that corticosterone modulates the course of

EAE by interfering with the same processes. Steroids have also been shown to have a direct effect on the permeability of the blood brain barrier (48), the breakdown of which appears to be important in the pathogenesis of EAE (4), and it is possible that this effect contributes to the recovery phase of EAE.

The elevated steroid levels in EAE may be due directly to the stress of the disease, or possibly subsequent to lymphokine release in the CNS. In both this paper and reference 21, elevated steroid levels were first recorded before animals were paralyzed but after the appearance of inflammation in the CNS. Several inflammatory mediators, most notably IL-1, have been shown to be capable of increasing adrenocortical steroid production by action on either the anterior pituitary, increasing the release of ACTH (49); or neurones in the paraventricular nucleus of the anterior hypothalamus, by increasing the release of corticotropin releasing factor (50, 51). In addition lymphocytes have the capacity to produce ACTH (52), so in principal these cells may act directly on the adrenal cortex although this action remains to be demonstrated. EAE may therefore be an example of an autoimmune response that is regulated by the immunoneuroendocrine feedback loop proposed by several investigators (53-55).

In regulation of EAE by exogenous steroids, it appears to be important that steroid levels in adrenalectomized animals rise early in the disease course as is the case in intact animals. At levels of corticosterone that allowed 100% survival when they were achieved before the onset of clinical signs, 2/4 animals died when the implants were inserted after the onset of paralysis. Such findings may have implications for the treatment of acute exacerbations of disease in patients with multiple sclerosis.

After spontaneous recovery from actively induced EAE, animals become refractory to further attempts to induce the disease (5). During this phase animals have normal steroid levels, which suggests that a steroid-independent mechanism is involved at this stage. There seems to be a window of several days between the return of normal resting corticosterone levels and the development of the long-term refractory state. A single second episode of paralysis is often observed at this time (36, 37) and EAE can be reinduced by immunization on days 21 and 28 after immunization but no later than this (56). Treatment of Lewis rats with a low dose of cyclosporine A (57) or with cyclophosphamide (58) during the development of EAE results in a chronic relapsing and remitting disease course where animals recover spontaneously from each episode of paralysis but the long-term refractory state fails to develop. The finding that the long-term refractory phase does not depend on chronically elevated levels of corticosterone and that rats treated with low doses of immunosuppressive drugs tend to relapse, provides compelling evidence that the acute recovery from a monophasic episode of EAE depends on a different immunoregulatory mechanism to that responsible for the subsequent refractory phase.

Summary

Lewis rats with experimental allergic encephalomyelitis (EAE), induced either by the subcutaneous injection of guinea pig myelin basic protein (MBP) or by the adoptive transfer of MBP-primed spleen cells, suffer from a single episode of paralysis from which they recover spontaneously. Animals developing EAE were found to have greatly elevated levels of corticosterone in the blood. This endogenous increase in steroid production was accompanied by lymphopenia and depressed delayed-type hyper-

sensitivity responses to OVA, indicating that rats with EAE are immunosuppressed in an antigen-nonspecific fashion. Adrenalectomized rats given subcutaneous implants of corticosterone to maintain basal steroid levels invariably died when EAE was induced. However, if the steroid replacement therapy was adjusted to mimic the hormone levels that were observed in intact rats developing EAE, then the disease followed a nonfatal course closely resembling that seen in the nonadrenalectomized controls. Replacement therapy that achieved serum corticosterone levels slightly higher than those found in intact rats with EAE virtually suppressed the disease completely.

It is concluded that endogenous corticosterone release in rats with EAE plays an essential role in the spontaneous recovery that is observed in this condition. However, the subsequent refractory phase that is characteristic of rats that have recovered from EAE induced by active immunization with MBP is not associated with chronically elevated corticosterone levels. This finding is discussed in the light of other data that suggest that unlike the spontaneous recovery, the refractory state has an immunological basis rather than an endocrinological basis.

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