Experimental allergic encephalomyelitis

I. Optic nerve and central nervous system manifestations

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Experimental allergic encephalomyelitis (EAE) was produced in six adult rhesus monkeys. The animals were evaluated serially by ocular, ophthalmoscopic, fluorescein fundus angiographic, pupillary, visual evoked potential, neurologic, cerebrospinal fluid (CSF), and hematologic examinations and by postmortem detailed histopathologic examination. All the animals developed acute EAE. Four of the monkeys, surviving longer than 1 month, developed chronic relapsing EAE and were sacrificed 3 to 14 months after sensitization. All 12 eyes developed acute optic neuritis (with variable degrees of optic disc edema and visual loss). Later on, all the eyes of animals with chronic EAE developed optic atrophy with total or almost total blindness. Histopathologic examination of the optic nerve and central nervous system revealed inflammatory infiltrates, extensive demyelination, and axonal degeneration, without inflammation in the retina or optic nerve head (i.e., nonmyelinated neural tissue). Relapsing EAE was reflected in episodic increases of CSF proteins and pleocytosis. The various findings are correlated.

Key words: optic nerve, brain, demyelination, optic neuritis, multiple sclerosis, encephalomyelitis
normalities. This is the first study in which animals were evaluated in detail over a long period with a variety of clinical and laboratory parameters. We report observations in animals with acute optic nerve and neurological lesions, as well as in animals with chronic relapsing EAE.

Materials and methods

EAE was produced in six adult rhesus monkeys weighing 3.3 to 4.4 kg.

Sensitization regimen. Homologous spinal cord tissue was prepared as a 33% suspension (w/v) in sterile physiologic buffered saline and was homogenized through a double-hubbed syringe with complete Freund's adjuvant (BCG, 4 mg/ml). Five animals were sensitized by a single intradermal injection of 0.1 ml of the sensitizing inoculum over the dorsal thorax, between the scapulae. The first animal (No. 5) to be sensitized received five 0.1 ml injections at the same site simultaneously. Because the subsequent neurologic disease was acute and rapidly fatal, the sensitizing dose was reduced to a single injection.

Evaluation. All monkeys were examined serially, including prior to sensitization and immediately before sacrifice. The following investigations were conducted:

1. Complete ocular examination, including slit-lamp examination, and testing for the pupillary light reaction and visual impairment.
Fig. 2. Left eye of monkey 1 on day 35. Fundus photograph (A) and fluorescein angiogram (B) during the late phase showing ODE (reflexes are photographic artifacts).

Results

Onset. All the animals developed clinical and histopathologic evidence of EAE. Clinically, the optic nerve involvement was seen within 2½ to 3½ weeks after the sensitization, except in one eye where it was seen on day 31. Simultaneous bilateral optic nerve involvement was seen in four animals. The central nervous system (CNS) lesions developed 2½ to 3½ weeks after sensitization in animals 3 to 6 and after 5 weeks in animals 1 and 2. Clinically, the optic nerve was involved 2 to 12 days before the CNS involvement in animals 1 to 3; in animals 4 and 5 the CNS was involved 1 to 7 days before the optic nerve; in animal 6 the two developed simultaneously.

Optic disc changes

Initial changes. These consisted essentially of optic disc edema (ODE) (Figs. 1 and 2) and associated changes. The findings are summarized in Table I.

Later changes. The ODE in eyes of animals that survived long enough (all except monkeys 5 and 6) subsided and was replaced by optic atrophy (Table I). The eyes fell into two groups.
Table I. Optic disc changes

<table>
<thead>
<tr>
<th>Animal</th>
<th>Eye</th>
<th>Optic disc edema</th>
<th>Optic atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Appearance (day)</td>
<td>Reached maximum</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
<td>&lt;31</td>
<td>34 4+</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>&lt;31</td>
<td>26 3+</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>&lt;38</td>
<td>72 3+*</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>38</td>
<td>65 4+</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>&lt;19</td>
<td>24 3+</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>19</td>
<td>24 2+</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>&lt;28</td>
<td>28 1+</td>
</tr>
<tr>
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<td></td>
<td>28</td>
<td>49 1+</td>
</tr>
<tr>
<td>5</td>
<td>R</td>
<td>31</td>
<td>31 ±</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>&lt;31</td>
<td>2+</td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>17</td>
<td>20 1+</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>&lt;17</td>
<td>17 1+</td>
</tr>
</tbody>
</table>

Grade: 1+ = mild; 2+ = moderate; 3+ = marked; 4+ = severe; ± = equivocal.

*In this eye the ODE was 2+ on day 51, resolved to 1+ on day 67, but recurred to reach 3+ on day 72.
†The animal became comatose on day 17 and developed severe seizures. The animal was placed on 50% Dextrose i.v. and Decadron, and these led to subsiding of ODE.

Table II. Histopathological changes

<table>
<thead>
<tr>
<th>Animal</th>
<th>Eye</th>
<th>Optic nerve</th>
<th>Brain and spinal cord EAE changes*</th>
<th>Survival time (days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Infiltration*</td>
<td>Demyelination</td>
<td>Axonal loss</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
<td>Minimal</td>
<td>Extensive</td>
<td>Almost total</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>Minimal</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>Moderate</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>Moderate</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>Moderate</td>
<td>Focal</td>
<td>Extensive</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>Moderate</td>
<td>Focal</td>
<td>Extensive</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>Marked</td>
<td>Focal in anterior part</td>
<td>None</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>Marked</td>
<td>Focal in anterior part</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>R</td>
<td>Minimal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>Minimal</td>
<td>None</td>
<td>None</td>
</tr>
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</table>

*Mild = up to 10 perivascular lesions per section; moderate = 11 to 100 perivascular lesions per section; marked = >100 perivascular lesions per section.

GROUP A. Six eyes in three animals with 2+ to 4+ ODE (monkeys 1 through 3) belonged to this group. In these animals, as the ODE resolved, optic atrophy developed and was progressive for many weeks. Usually the more marked the ODE, the greater the optic atrophy.

GROUP B. Two eyes of animal 4 developed no more than 1+ ODE (Fig. 1, A) and yet developed 4+ optic atrophy.

Pupil. The pupil size and light reaction were noted in all eyes on several occasions during the study. These were simple clinical observations, and no effort was made to quantify the reaction to light. In general, a nonreactive and dilated pupil showed a good
Fig. 3. Sequential VEP changes during the course of EAE in monkey 4. A, On day 72, VEP in the left eye became unrecordable at 40° and 35° C and was present at 37° C though its latency might be delayed. B, Same pattern was observed in the right eye a week later.
correlation with a flat VEP, but an apparently defective light reaction frequently accompanied a normal-looking VEP and sometimes an increased latency of the VEP. Optic disc changes and pupillary reaction did not always correlate exactly. For example, in at least two of the animals (Nos. 2 and 4) poor pupillary reaction preceded the ODE by a few days. On the other hand, in some eyes the pupil reacted fairly reasonably in spite of early mild ODE or optic atrophy. However, once the optic disc showed either marked ODE or marked optic atrophy, the pupillary reaction was markedly impaired or lost and the pupil was dilated.

**VEP changes.** The initial negative peak with latency of 50 to 70 msec, often preceded by a small positive component, was the most consistent VEP component. Because of fluctuating latency from day to day, it was not possible to determine definitely when VEP became abnormal during the course of EAE though there was an overall latency prolongation with development of demyelination. The most reliable indication of abnormal VEP was therefore flattening of VEP or absence of measurable negative peak. Nonetheless, the effect of temperature on VEP latency was consistent within the same day; the higher the temperature, the shorter the latency in the normal state. The variability in VEP amplitude was even worse, so that it was considered unreliable.

The VEP changes were correlated with those of the optic disc and optic nerve in these animals.

**Correlation with ODE**

**At the time ODE first appeared.** In all the eyes ODE preceded the development of any gross VEP defect except that (1) in both eyes of monkey 4 there was a transient increase in VEP latency 3 days before the onset of ODE, but the VEP was apparently normal on the day ODE developed, and (2) in animal 1 the exact sequence of changes could not be ascertained because the first postsensitization VEP recording and fundus examination were unfortunately not conducted till day 31, when they revealed a flat VEP with 3+ ODE in the right eye and 1+ ODE in the left eye.

**At the time ODE was most marked.** In six eyes (both eyes of monkeys 1 to 3) with 2+ to 4+ ODE, the VEP was flat. In the remaining six eyes, where ODE at its maximum was only 1+ to 2+ (Table I), the VEP showed no gross abnormality.

**During the entire period of presence of ODE.** The VEP showed no gross abnormality in one eye (right eye of animal 5) and no abnormality for over 4 weeks initially, with progressively worsening ODE in two eyes (of animal 2). It showed a transient abnormality for a short period during the course of ODE in seven eyes (only at 40° and 35° C in both eyes of animals 3 and 4, increased latency in both eyes of animal 6, and flat VEP in the left eye of animal 5), and was flat throughout the course of ODE in two eyes of animal 1.

**Correlation with optic atrophy.** Although ultimately all the eyes with well-developed optic atrophy had a flat VEP, three patterns emerged during the earlier stages.

VEP abnormality appeared simultaneously with the onset of optic atrophy. This was seen in two eyes (in left eye of monkey 3 and right eye of monkey 4). The VEP was abnormal at first only at 40° and 35° C but later became persistently flat (Fig. 3).

VEP abnormality appeared before the onset of optic atrophy. This was seen in five eyes. Four of these eyes (monkeys 1 and 2) had 3+ to 4+ ODE and retinal vascular changes (Tables I and II), which could be a factor in VEP abnormality. The fifth eye (left eye of monkey 4) had only 1+ ODE at its maximum.

Optic atrophy appeared a few days before gross VEP changes. This was seen in only one eye (right eye of monkey 3). This eye showed a transient VEP abnormality only at high temperature (40° C) when ODE was at its peak (3+), but the VEP showed no gross abnormality during the early or resolution phases of ODE or at onset of optic atrophy.

**VEP pattern during the period between resolution of ODE and onset of optic atrophy.** Of the eight eyes that developed optic atrophy (monkeys 1 to 4), four eyes (monkeys 3 and 4) had optic discs that looked normal during the period between resolution of ODE and onset of optic atrophy. During this period the VEP showed no gross change in two eyes (animal 3); it was normal at 37° C.
Figs. 4 to 8. Photomicrographs of longitudinal sections of five optic nerves.

Fig. 4. Left optic nerve in monkey 6 on day 29 showing minimal cellular infiltration and normal myelinated nerve fibers. The animal was treated with systemic dexamethasone (Decadron; Merck, Sharpe, and Dohme, West Point, Pa.) (Luxol fast blue stain.)

Correlation of VEP changes recorded just before death and histopathologic changes in the optic nerve. The VEP was recorded in all eyes a short period before sacrificing the animal—an exceptional opportunity provided by this study. In four eyes (monkeys 5 and 6) the VEP was recordable; two of these eyes (monkey 6) showed minimal cellular infiltration without any demyelination or loss of nerve fibers in the optic nerve (Fig. 4) whereas the other two eyes (animal 5) showed marked cellular infiltration (Figs. 5 and 6), with only a few foci of demyelination and almost normal axons in the nerve. In eight eyes (monkeys 1 to 4) with extensive demyelination and loss of nerve fibers in the optic nerve and with minimal to moderate inflammatory cellular infiltration in the nerve (Figs. 7 and 8), the VEP was invariably flat.

Visual disturbance. For obvious reasons, it was not possible to assess satisfactorily any mild visual loss in the monkeys. Our observations are based on indirect evidence from the VEP and pupillary changes and the behavior of the animal. These indicated that all animals developed a variable degree of visual loss during the initial involvement of the optic nerve. The visual loss varied from mild disturbance to complete blindness. Four animals (1 to 4) that were followed for a prolonged period after the resolution of the initial attack were either totally blind or almost blind. In between the initial and the final visual loss, some eyes recovered a variable degree of visual function, some apparently to normal.

Neurological and systemic findings. Weight loss and/or lethargy were observed in all animals and often preceded the onset of neurologic signs. Ataxia occurred in five of the
Fig. 5. Right optic nerve in monkey 5 on day 31, during acute EAE, showing marked inflammatory cellular infiltration, most marked in the septa. Myelinated nerve fibers are dark bundles in B and C. C, Partial demyelination with focal marked cellular infiltration around central retinal vein (V) (arrows). A, Central retinal artery; (A, Hematoxylin-eosin stain; B and C, luxol fast blue stain.)
Fig. 6. Left optic nerve in monkey 5 on day 31 during acute EAE, showing marked inflammatory cellular infiltration: (A) in peripheral part of the nerve and dural sheath (D), and (B) in central part of the nerve with marked perivascular exudation, particularly around central retinal vein (V), and partial demyelination. A, Central retinal artery. (Luxol fast blue stain.)

Six animals and often progressed to paresis or paralysis. Paresis occurred most frequently in the hind limbs but was occasionally manifest as hemiparesis or quadriparesis. Additional findings included coma, clonic/tonic seizures, nystagmus, ptosis, conjugate eye movement, and incontinence. One animal died during the acute phase of EAE (monkey 6), and another (monkey 5) was found moribund and was sacrificed on day 31 after sensitization. Monkey 5 received multiple injections of the sensitizing inoculum, which may have accounted for its more fulminating illness. Four monkeys survived the initial paralytic episode and recovered in 10 days (animals 1 and 3), 20 days (animal 4), and 50 days (animal 2). There was no well-defined correlation between onset, severity, or duration of clinical neurologic manifestations and optic nerve changes.

The cerebrospinal fluid (CSF) and peripheral blood were examined serially after sensitization. The number of circulating white cells was generally increased during the acute paralytic episode of EAE. The CSF protein (50 to 214 mg/dl) and leukocytes (5 to 95 mm$^3$) were also elevated during the initial phase of the illness. With one exception (monkey 5), lymphocytes comprised the major portion of CSF leukocytes (70% to 100%). Levels of CSF protein and leukocytes fluctuated considerably after the initial paralytic episode.

Two of four animals that survived longer than 1 month (monkeys 1 and 4) developed clinical relapses of EAE. Relapses occurred 1 to 2 weeks after recovery from the initial paralytic episode, and paralytic signs persisted for 30 to 50 days. Monkey 1 developed an additional relapse 70 days later. During remissions the animals appeared normal. If the presence of abnormal numbers of leukocytes and increased protein concentrations in the CSF were considered an index of subclinical relapses of EAE, all four animals that survived the initial paralytic episode had at least one to three subclinical relapses, with monkey 1 showing at least five clinical/subclinical relapses. One eye showed a definite recurrence of ODE (see Table I, right eye of animal 2). The disease was monophasic in the remaining eyes.
Fig. 7. Left optic nerve in monkey 1 with chronic EAE on day 433, showing optic atrophy and extensive demyelination with few patches of myelin remaining (dark areas in B and C, arrows), focal cellular infiltration around central retinal vein (V) in A and minimal cellular infiltration in the rest of the nerve. A, Central retinal artery. (A, Hematoxylin and eosin stain; B and C, luxol fast blue stain.)
Histopathologic changes

Optic nerve. Histopathologic changes of EAE were particularly prominent in the optic nerve (Table II). These involved primarily the myelinated part of the optic nerve (i.e., posterior to the lamina cribrosa, Fig. 5, C), with only secondary atrophic changes involving the nonmyelinated optic nerve head. Histologic changes consisted of cellular infiltration, demyelination, and loss of axons. The inflammatory infiltrates were usually located around small venules and arterioles beneath the pia mater and in the septa of the optic nerve (Figs. 5 and 6). Occasionally, foci of mononuclear cells were noted around the central retinal vessels, usually the central retinal vein (Fig. 5, C). The infiltrates usually contained lymphocytes, histiocytes, and glial cells. In many instances histiocytes were engorged with myelin debris. During the acute inflammatory reactions (monkey 5), polymorphonuclear leukocytes were also present in infiltrates (Figs. 5 and 6). In the left eye of monkey 5 there was also marked exudation in the center of the anterior part of the optic nerve (Fig. 6, B); in addition, the dural sheath of the optic nerve and orbital tissues surrounding the sheath often contained inflammatory infiltrates (Fig. 6, A). Although the infiltration was dense and diffuse during the acute stage (5), it was usually mild to moderate and focal in animals followed for 92 to 433 days (monkeys 1 to 4, Fig. 7). In monkey 6 there was

Fig. 8. Left optic nerve in monkey 2 on day 227, showing a few scattered fragments of nerve fibers (dark lines, arrow). (Bodian stain.)
only sparse infiltration (Fig. 4), presumably because of systemic corticosteroids given to it. Animals 4 and 1, which survived for 308 and 433 days, respectively, had minimal infiltration of the optic nerve, apparently because of resolution of the inflammatory process. None of the eyes showed evidence of inflammatory infiltration in the retina or around the vessels in the retina or optic disc.

Focal areas of demyelination, usually located around inflammatory infiltrates and prominent beneath the pia and around the central retinal vessels, were seen among animals sacrificed before 100 days after sensitization (monkeys 3 and 5, Figs. 5, C, and 6, B). Optic nerves of animals sacrificed after 200 days (monkeys 1, 2, and 4) were almost totally devoid of myelin (Fig. 7). The only exception was monkey 6, which died 29 days after sensitization and showed no demyelination (Fig. 4).

Axons were extensively damaged in four monkeys with optic atrophy (1 to 4). Only fragments of axons were seen here and there throughout the entire length of the optic nerve (Fig. 8). Ascending optic atrophy involved the optic nerve fibers in the retina.

Central nervous system. Histologic evidence for EAE was observed in the CNS tissues of all animals examined (Table II). The number and extent of the infiltrates varied with the duration between sensitization and time of death. Although inflammatory infiltrates were found most frequently about small venules within the white matter of the brain, they were also noted in the gray matter and throughout the neuraxis. Infiltrates usually consisted of histiocytes, lymphocytes, and glial cells. In animal 5, sacrificed during the acute phase of the disease, numerous polymorphonuclear leukocytes were also present in the infiltrates, often accompanied by focal necrosis. Eosinophils were occasionally noted in the inflammatory infiltrates of some animals. Histopathologic changes were sparse and focal in long-surviving animals but nevertheless indicated ongoing inflammation within CNS tissue.

Perivascular infiltrates were occasionally observed within peripheral nerves in the cauda equina. No evidence for peripheral neuritis was noted in the sections of neurons taken from the brachial or iliac plexus, however. The remainder of the histopathologic examination was unremarkable except for scattered granulomas located in lungs, liver, spleens, and occasionally kidneys of four animals.

Discussion

A chronic relapsing type of EAE has been produced in juvenile guinea pigs and hamsters. Relapsing neurologic disease has also been reported in rhesus monkeys, none of which survived longer than 6 months. In the present study, three of the four rhesus monkeys with chronic relapsing EAE were sacrificed 9 to 14 months after sensitization, and all four animals might have survived for their natural life. All four animals surviving the initial episode of EAE were either blind or almost blind by the time they were sacrificed. The animals had up to five clinical and/or subclinical relapses of neurologic disease. One animal had a relapse of optic neuritis in one eye whereas all the other eyes demonstrated an inexorable progression to marked optic atrophy and massive demyelination after the initial attack of optic neuritis. This explains the absence of relapses and remissions of optic neuritis. The changes occurring in the CSF during acute EAE have been described by Kabat et al. and Behan et al.; however, they did not describe the fluctuations in CSF protein and cells seen in chronic EAE.

The primary cause of visual loss in EAE is optic neuritis involving only the myelinated part of the nerve. ODE is secondary to optic neuritis involving the retrolaminar region; the more marked the neuritis, the more marked the ODE. The ODE was not due to any rise of CSF pressure, since no significant rise occurred. Although marked ODE generally went along with severe optic atrophy (Table I), this was not always the case; for example, both eyes of monkey 4 developed 4+ optic atrophy in spite of only 1+ ODE (Fig. 1, A), which suggests that the main inflammation was in the posterior part of the
optic nerve as in retrobulbar neuritis. Clinically, ODE in demyelinating disease is usually called "papillitis" or "neuroretinitis." All histopathologic studies on optic neuritis in EAE have clearly demonstrated that the inflammatory process stops abruptly where the myelinated nerve fibers stop behind the lamina cribrosa, with no inflammation at all in the optic nerve head or the retina or vitreous. Thus both "papillitis" and "neuroretinitis" are incorrect terms.

The association of pain on extraocular movement or palpation of the eyeball with optic neuritis is well known. The histopathologic examination of the optic nerve during acute phases of EAE clearly revealed an inflammatory reaction in the dural sheath, occasionally extending into surrounding tissues (Fig. 6, A). Histologic lesions of EAE appear in the CNS several days before the onset of clinical signs, and mononuclear infiltration is presumed to account for myelin degeneration. Similarly in the present study, inflammatory reactions preceded clinical abnormalities in the optic disc, VEP, or pupil. It would seem reasonable to state that the pain, indicative of dural sheath inflammation, constitutes an early clinical sign of optic neuritis.

We tried to correlate the VEP abnormalities with the ophthalmoscopic and histopathologic findings of the optic nerve lesions. Because a flat VEP was considered the most reliable index of abnormality, it could be argued that we did not detect early, mild abnormalities. Moreover, the use of a checkerboard stimulus instead of the white flash used by us could further improve the sensitivity of the method. The findings of the present study on VEP have to be evaluated in the light of these limitations. Although there was good correlation between the flat VEP and optic atrophy with extensive demyelination and loss of nerve fibers (but inflammatory cellular infiltration of the optic nerve), mild focal demyelination and ODE were seen without any gross VEP abnormality.

Thus the primary ocular lesion in EAE is optic neuritis, which involves the myelinated part of the optic nerve and is accompanied by demyelination and degeneration of axons.

The clinical and pathologic findings are similar to those seen in demyelinating disease of the optic nerve in man.

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REFERENCES


