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Research Article

Necrosis and myelomalaic lesions in acute experimental allergic encephalomyelitis in guinea pigs

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ABSTRACT

Background: Multiple Sclerosis (MS) and its animal model Experimental Allergic Encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system, in which the myelin sheath has been considered to be the primary target for many years. However, an increasing number of reports have focused on neurodegenerative aspects of the disease pathogenesis. Damage to axons is taken as a key factor of disability in multiple sclerosis, but its pathogenesis is largely unknown. Axonal injury is believed to occur as a consequence of demyelination and was recently shown to be a feature even of the early disease stages. It is evident that the crucial distinction between primary and secondary demyelination depends on the preservation or the destruction of the axons and the neuronal elements.
Methods: EAE was induced in the adult healthy guinea pigs by weekly intradermal injections of homologous whole brain and spinal cord antigen together with complete Freund's adjuvant into the foot pad of the animal. The animals were observed for clinical features of the disease after injection.
Results: The histological observation revealed two stages of EAE; an initial inflammatory stage followed by demyelination. The inflammatory lesions were focal and invariably related to blood vessels. The inflammatory lesions consisted of perivascular cuffings with lymphocytes and mononuclear cells in the perivascular space and surrounding parenchyma. Perivascular demyelination was restricted to that part of the white matter which was infiltrated by mononuclear cells. The fibres in demyelinating lesions were demyelinated. Perivascular demyelination is followed by patchy demyelination and large plaques of demyelination. Neuronal and axonal damage, necrosis, tissue degeneration and cavity formation were seen in those animals which died during the acute phase of the disease. These changes were found in the spinal cord, brainstem and cerebellum.
Conclusion: The changes observed in results lead to the conclusion that the acute EAE with severity of disease is no more a primary demyelinating disease.

Keywords: EAE, MS, Inflammation, Demyelination, Necrosis, Neurodegeneration

INTRODUCTION

EAE has been considered an experimental model for demyelinating disease in man, especially for acute disseminated encephalomyelitis.[1] The histopathology of Experimental Allergic Encephalomyelitis (EAE) is characterized by perivascular infiltrates of mononuclear inflammatory cells. The fundamental types of lesions were found to be focal and invariably related to venules, veins and small arterioles. Older lesions show greater or lesser degree of parenchymal invasion by mononuclear cells from vessels possessing cuffs. The notable changes which occur during EAE include fibrin deposition and accumulation of inflammatory cells around blood vessels.[2] The associations among acute disseminated

encephalomyelitis, acute hemorrhagic necrotizing leukoencephalopathy, and Multiple Sclerosis (MS) have been used to promulgate the concept of a spectrum of demyelinating disease in man.The question of whether axon pathology is a critical event in the formation of multiple sclerosis lesions, or just a consequence of demyelination in later plaque development? Invasion of the neural parenchyma by inflammatory cells is accompanied by demyelination but not usually by necrosis. However, animals with very severe EAE may suffer axonal destruction, hemorrhage, exudation of fibrin, necrosis thrombosis of vessels, and focal infarction. Hyperacute EAE has been described as a close replica of acute hemorrhagic necrotizing leukoencephalopathy in man. Necrosis of the spinal cord was produced by administering tilorone to rats before or during the incubation period of EAE.[3] Necrosis would probably occur in every animal with the exceptionally severe hyperacute form of EAE but not in ordinary EAE. It is not fully explained whether axonal and neuronal damage occurs in the acute phase of the disease. The present study was undertaken in order to see whether the axonal and neuronal damage and necrosis is a regular event in the severity of the acute phase of EAE.

METHODS

Adult healthy guinea pigs of both sexes weighing 400 to 500 grams were used for the study. Animals were acclimatized to the laboratory conditions prior to the experimentation. The animals were given free access to water and standard diet during acclimatization and throughout the experimental period. EAE was induced by weekly intradermal injections of homologous whole brain and spinal cord antigen together with complete Freund's adjuvant in the ratio of 1:1 into the foot pad of the animal.

Nine parts by weight of homologous whole brain and spinal cord tissue was mixed with ten parts by weight of freshly prepared normal saline. The normal saline and homologous CNS tissue was homogenized by a homogenizer to a fine emulsion (homogenate). The homogenate was collected in a sterilized sample tube and preserved in the freezer for further use. The homogenate and Freund's complete adjuvant were (at laboratory temperature) thoroughly mixed in the ratio of 1:1 just before injection. 0.5 ml of homologous whole brain and spinal cord antigen + complete Freund's adjuvant was injected intradermally with a fine syringe needle into the foot pads of the animal.

Clinical observations or assessment

The weight and rectal temperature of the animals was recorded daily before the diet was given. The clinical assessment of the animals was made daily according to standard method of Keith and McDermott (1980) [4] and the following clinical symptoms were graded as follows

- Weight loss
- Mild paraperesis (weakness of one or both hind limbs)
- Moderate paraperesis (slight dragging of hind legs) with fecal impaction and urinary retention and ataxia
- Severe paraperesis or paraplegia (prominent dragging of hind limbs and pronounced ataxia)
- Moribund state
- Death

Histological

Experimental animals were grouped into three groups of five animals each. Animals of first group received one injection, animals of second group received two injections and the animals of third group received three injections at weekly intervals. The animals were sacrificed at random on the days 2, 4 and 6 after first, second and third injection from each group. All the animals were sacrificed by an over dose of ether anesthesia. Thorax of the animal was opened immediately and perfused with 15 ml of formol ammonium bromide intracardially, introduced through left ventricle and a vent in the right atrium allowing outlet. The spinal cord and brain was dissected out and fixed in formol ammonium bromide for three days. The fixed CNS tissue was processed for paraffin sectioning. Paraffin sections of 15 micrometer were made and stained with:

a) Luxol Fast Blue and neutral red
b) Haematoxylin and eosin
c) Phosphotungstic Acid Haematoxylin (PTAH)

The stained sections were examined under light microscope.

One set of control animals were injected with 0.5 ml of complete Freund's adjuvant alone and the other set were injected with 0.5 ml of freshly prepared normal saline to compare with CFA injected ones. The control animals were sacrificed with the experimental animals of each group.

RESULTS

Onset of the disease symptoms marked by body weight loss and rise in temperature appeared between day 9 and 14 after first injection. The period between the first injection and the onset of clinical symptoms is the latent period of EAE. A latent period of 9 to 14 days was observed in this study. The clinical severity progressed in the succeeding days.

Mild inflammatory lesions were seen in the lower segments of spinal cord on the day 13 and 14 after first injection. With the progress of clinical severity of the disease, the inflammatory lesions increased and extended into higher segments of the spinal cord and brain stem.

Perivascular demyelination was observed in the lower segments of the spinal cord as early as day 18. On the succeeding days the inflammatory lesions were gradually replaced by demyelination. After 24th and 26th day onwards demyelination was seen in spinal cord, brainstem and cerebellum.

On day 15 after first injection (day 8 after second injection) focal linear inflammatory lesions with lymphocytes infiltrating the lesion site (Figure 1). Lymphocytic infiltration in and around the blood vessels at the junction of lateral funiculus and lateral grey column of the spinal cord with the vacuolated appearance in the neuropil (Figure 2).

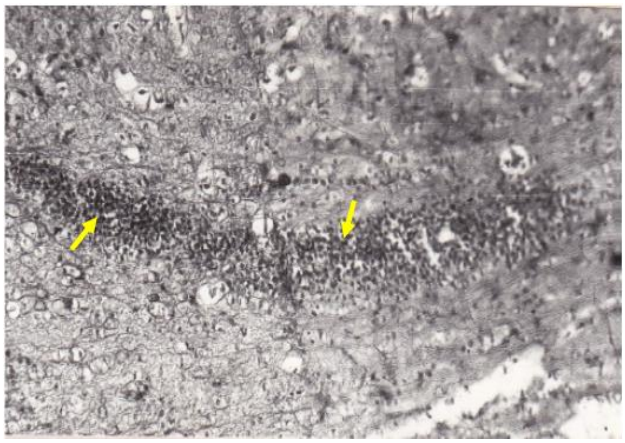

Figure 1: Brainstem showing focal linear inflammatory lesion with lymphocytes (arrows) infiltrating the lesion site. Luxol fast blue and neutral red x168.

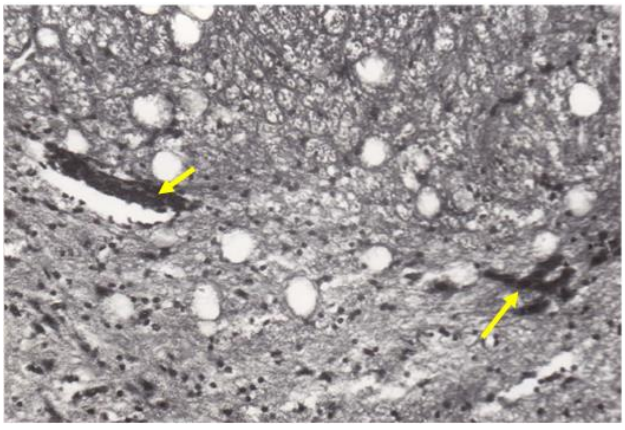

Figure 2: TS of spinal cord showing perivascular lymphocytic infiltration around a vessel (arrow) at the junction of grey and white matter with vacuolated appearance of neuropil H and E x268.

Out of five animals, two died on day 8 after second injection. The CNS of these two animals showed profound histopathologic changes which included perivascular lymphocytic infiltration, perivascular cuffings in grey and white matter and cavity formation (Figure 3). The perivascular cuffings showed fibrin deposition in the walls of the vessels and in the surrounding parenchyma (Figure 4). Diffuse cellular infiltration was found surrounding the perivascular cuffings in the grey matter of the spinal cord (Figure 3, 4). Spinal cord showed necrosis, cellular degeneration and softening of the tissue in and around the necrotic area and small vacuolations towards the dorsal aspect of the spinal cord (Figure 5, 6). A large cavity was found in the vermis of the cerebellum, affecting all the three layers of the cerebellar cortex and extending into the subjacent white matter (Figure 7). Brain stem also showed cavity in the ventral portion and a smaller one in its lateral portion (Figure 8). The cerebral aqueduct was found to be full of inflammatory cells (Figure 9, 10). Large scale necrotic areas were also seen which consisted of packed masses of rounded macrophages, with the destruction of the surrounding parenchyma (Figure 11); lymphocytic aggregation was found in its vicinity.

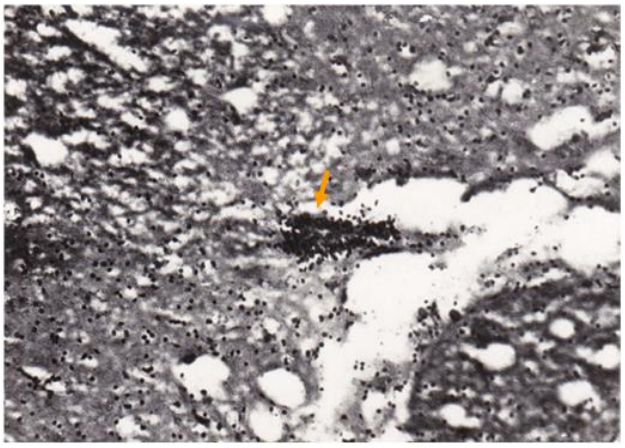

Figure 3: TS of spinal cord showing perivascular cuffing (arrow) and cellular infiltration in the surrounding grey matter with tissue degeneration and vacuolated spaces in the neuropil. Luxol fast blue and neutral red x163.

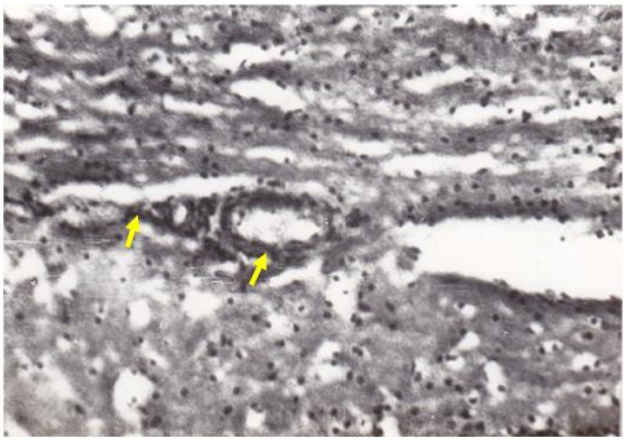

Figure 4: TS of spinal cord showing perivascular cuffing with fibrin deposition in the perivascular space (arrow). PTAH stain. x256.

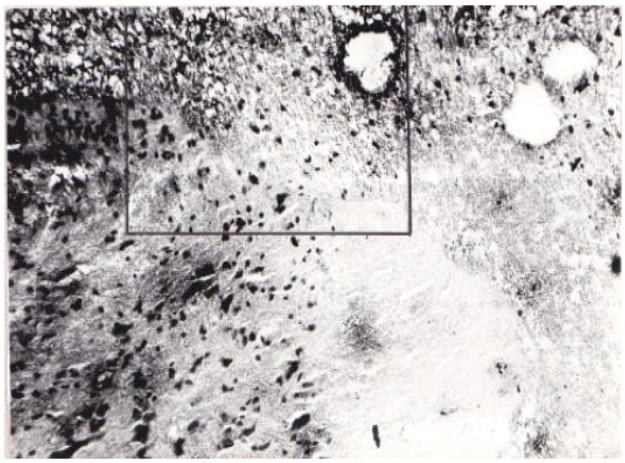

Figure 5: TS of spinal cord showing necrosis, cellular degeneration, softening of the tissue and vacuolations with the cavity formation. Hematoxylin eosin x268.

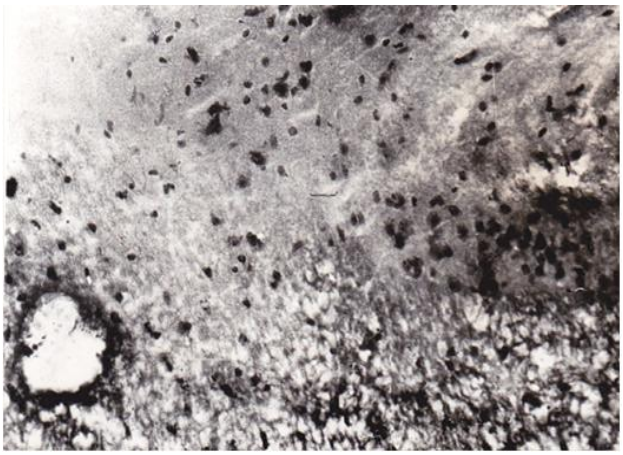

Figure 6: A portion of the Figure 5 with higher magnification showing hyperchromic area around vacuolation and softening of the neuropil. Hematoxylin eosin x423.

Figure 7: Section of vermis of cerebellum showing a large cavity. Luxol fast blue and neutral red x64.5.

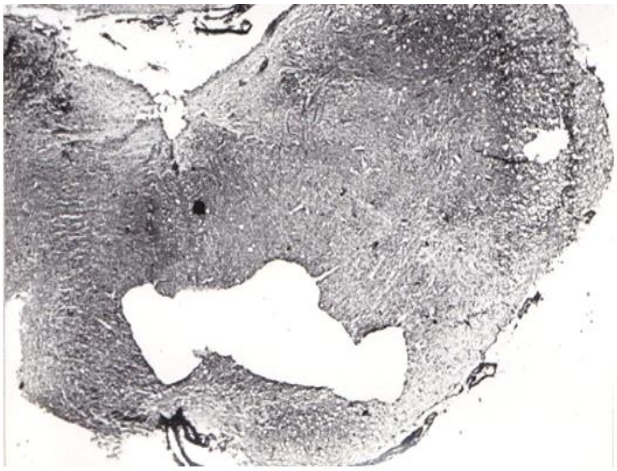

Figure 8: TS of brainstem showing a large cavity in its ventral portion and a small cavitation in its lateral portion. L F B and neutral red x20.45.

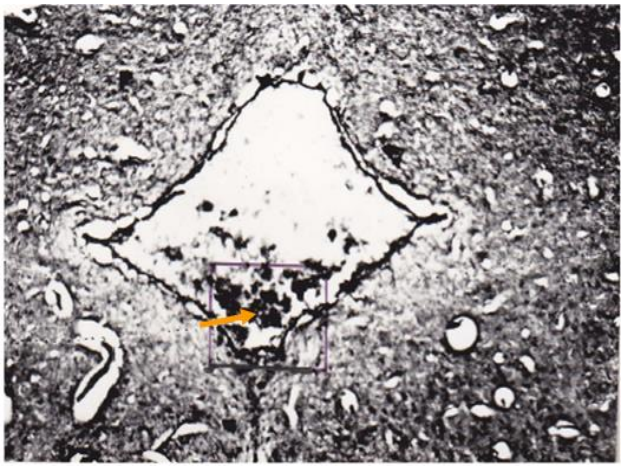

Figure 9: TS of mid brain showing inflammatory cells in the cerebral aqueduct. (arrow) Luxol fast blue and neutral red x42.3.

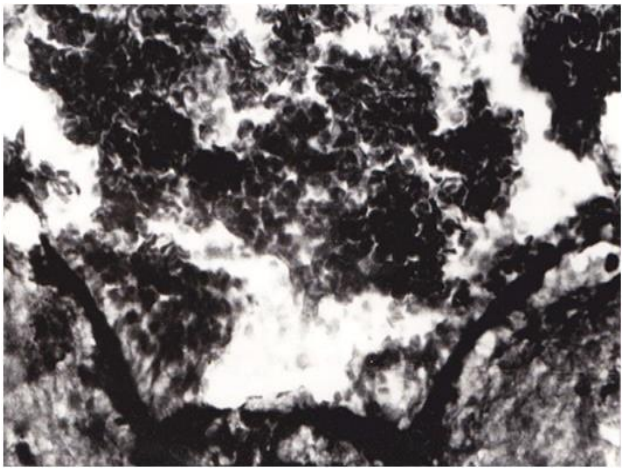

Figure 10: A portion of the cerebral aqueduct is magnified to show the inflammatory cells. Luxol fast blue and neutral red x268.

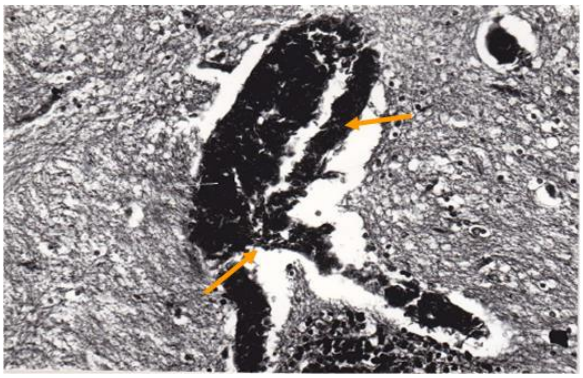

Figure 11: White matter of cerebellum showing large scale necrotic area with packed mass of rounded macrophages (arrows) and destruction of the surrounding parenchyma. Lymphocytes in its vicinity. Hematoxylin eosin x256.

On day 16 after first injection mid brain showed perivascular cuffings with fibrin deposition in the vessel wall and in its perivascular space and softening of the neuropil (Figure 12, 13). Cerebellum showed collections of lymphocytes in the grey matter (Figure 14).

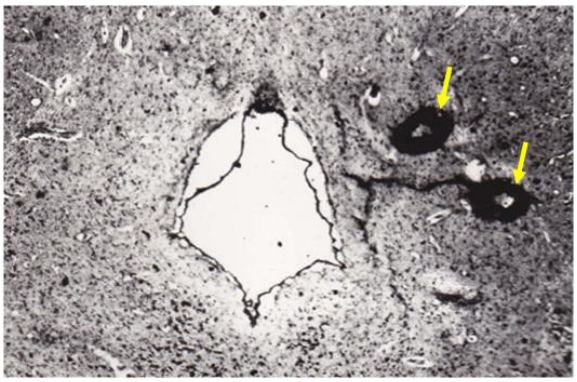

Figure 12: TS of mid brain showing perivascular cuffings with deposition of fibrin in and around the walls of the vessels (arrow) and softening of the neuropil. PTAH stain x42.3.

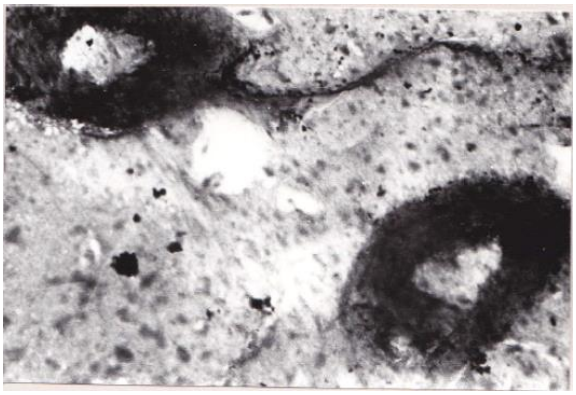

Figure 13: A portion of the Figure 12 is magnified to show the fibrin and surrounding softening area with vacuolated neuropil. PTAH stain x163.

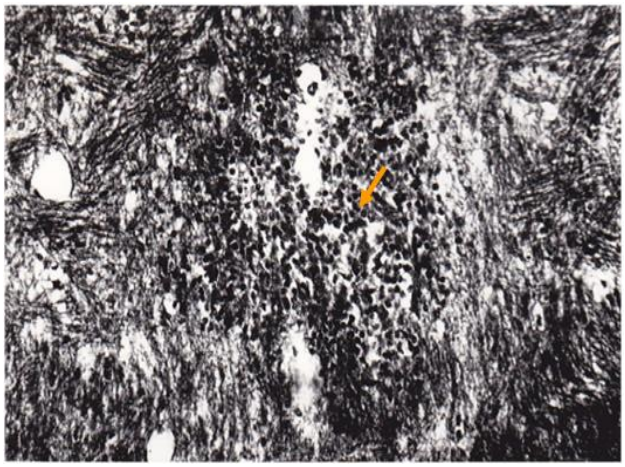

Figure 14: Section showing the white matter of cerebellum presenting a large group of lymphocytes (arrow). Luxol fast blue and neutral red x256.

On day 18 after first injection (day 4 after 3rd injection) two out of five animals showed severe inflammatory lesions in the cerebellum. The inflammatory lesions of the cerebellum consisted of perivascular infiltration by lymphocytes, in the vessel lumen, in the perivascular space and surrounding parenchyma. The vessel lumen was filled with thrombus. A few mononuclear and polymorphonuclear cells were also scattered in the surrounding parenchyma (Figure 15).

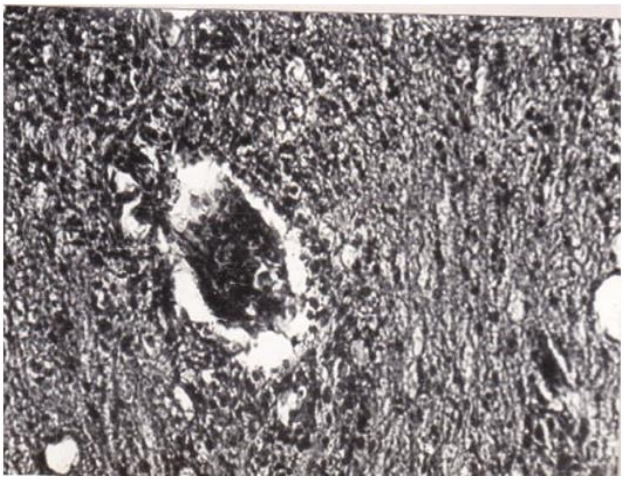

Figure 15: Cerebellar white matter showing severe inflammatory lesion with lymphocytes in lumen of the vessel, perivascular space and surrounding parenchyma; mononuclear cells in the surrounding parenchyma. Vessel lumen is filled with thrombus. Large phagocytic cells are seen surrounding the cuffing. L F B & neutral red x256.

On day 22 after first injection (day 8 after 3rd injection) perivascular demyelination was seen in medulla oblongata with vacuolated appearance of myelin debris and necrotic area surrounding the lesion. The vessel lumen was occluded (Figure 16, 17).

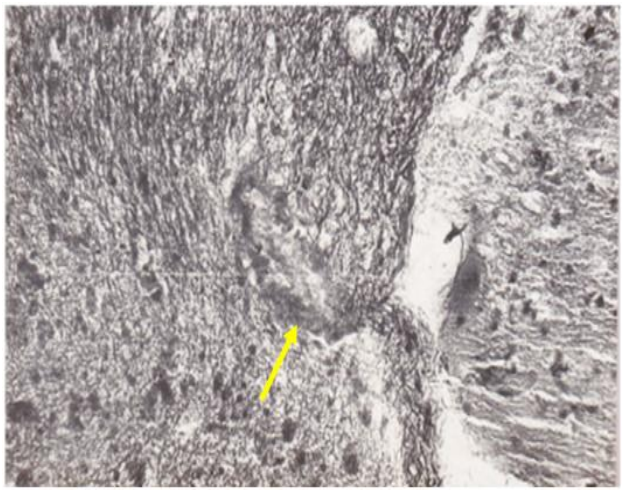

Figure 16: Section of the brainstem showing perivascular demyelination and necrosis. The vessel (centre field) is thrombosed and occluded (arrow). The fibres in the immediate vicinity of the lesion are demyelinated. Luxol fast blue and neutral red x256.

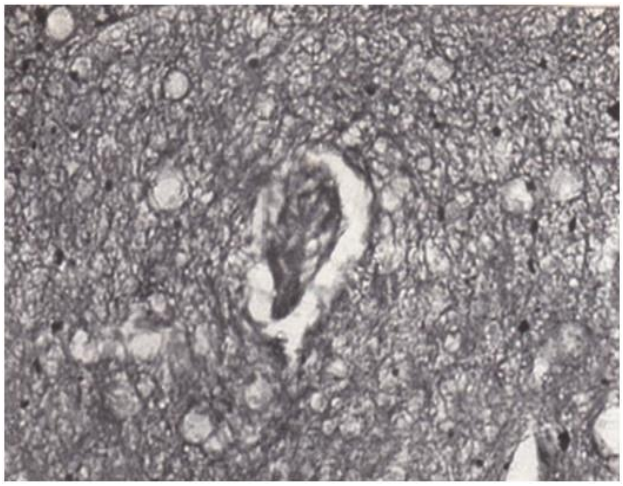

Figure 17: Brainstem showing vacuolated appearance of a small focal demyelinated site with vessel in the centre occluded and thrombosed. Luxol fast blue and neutral red x256.

On day 26 after first injection (day 12 after 3rd injection) three animals showed more pronounced focal demyelination in spinal cord, brainstem and cerebellum. Tissue disruption and necrosis in the white matter as well as grey matter was seen in the lumbar and thoracic segments of the spinal cord (Figure 18). The degeneration of grey and white matter resulted in the formation of big cavities resembling cavity of myelomalacia. Lymphocytes and mononuclear cells were found outside the cavity on their way to damage the surrounding parenchyma; small vacuolated spaces are evident (Figure 19, 20, 21).

A semilunar shaped cavity was found dorsal to the central canal in the grey matter and to certain extent involving the white matter of the dorsal funiculus (Figure 22). At this stage the brain stem and cerebellum were also in the stage of demyelination (Figure 23).

The lesions were found to be severe with damaged oligodendrocytes in the vicinity of the lesions. Astrocytosis of various grades were seen in and around the lesion sites. Two animals died on day 12 after 3rd injection. Inflammatory lesions occurred at all levels of the neuraxis but were more numerous in spinal cord and brain stem and they were not accompanied by necrosis at any level. It showed severe plaques of demyelination in all the funiculi of the lumbar segments of spinal cord with cavity formation in the ventral grey column and adjacent white matter (Figure 24).

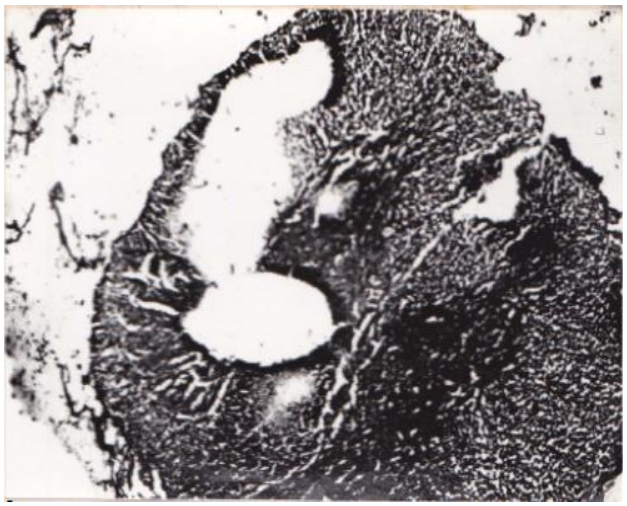

Figure 18: TS of spinal cord showing a large cavity resulting from tissue disruption involving white matter and grey matter, necrosis and neurodegeneration. Luxol fast blue and neutral red x50.3.

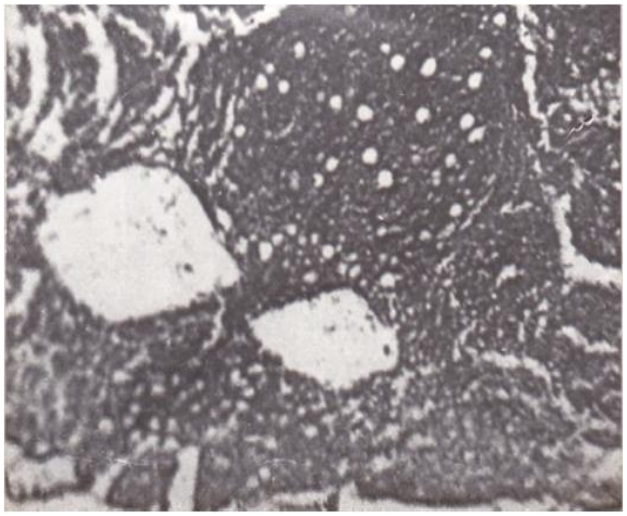

Figure 19: Section of spinal cord showing two cavities with adjacent vacuolated necrotic neuropil. Luxol fast blue and neutral red x64.5.

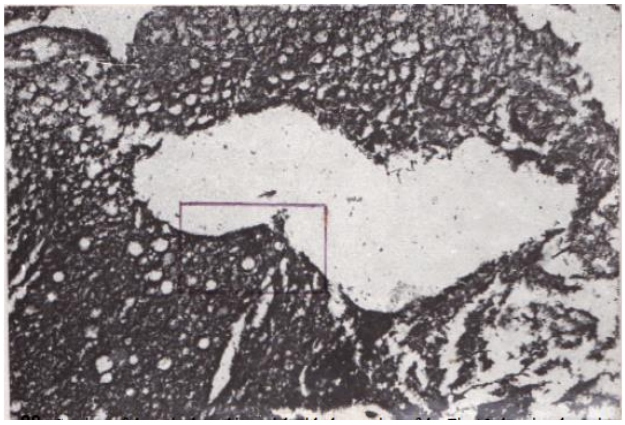

Figure 20: Section of the spinal cord in serial with the section of the Figure 19 showing the union of two cavities resulting in a single large cavity with the surrounding wide spread necrotic vacuolated neuropil. Luxol fast blue and neutral red x64.5.

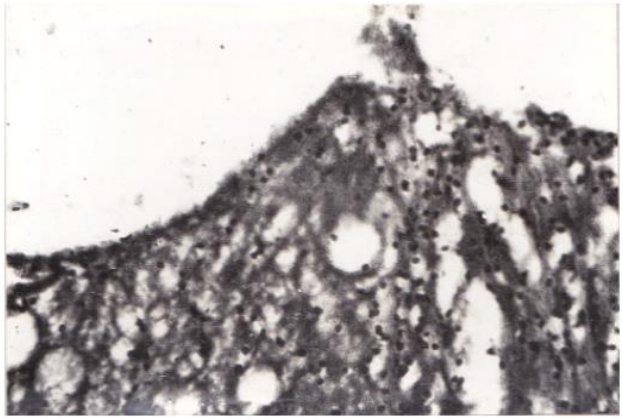

Figure 21: A portion of the wall of the cavity Figure 20 is magnified, showing lymphocytes and mononuclear cells outside the cavity on their way to damage the surrounding parenchyma. Vacuolated spaces are evident. Luxol fast blue and neutral red x256.

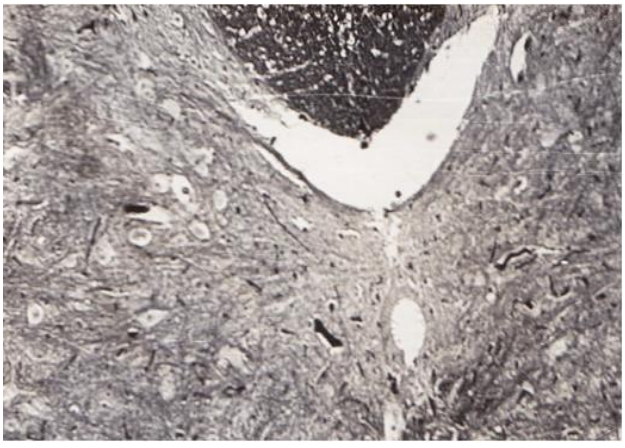

Figure 22: Section of spinal cord showing a semilunar shaped cavity in between the grey matter and dorsal funiculus. L F B and neutral red x128.

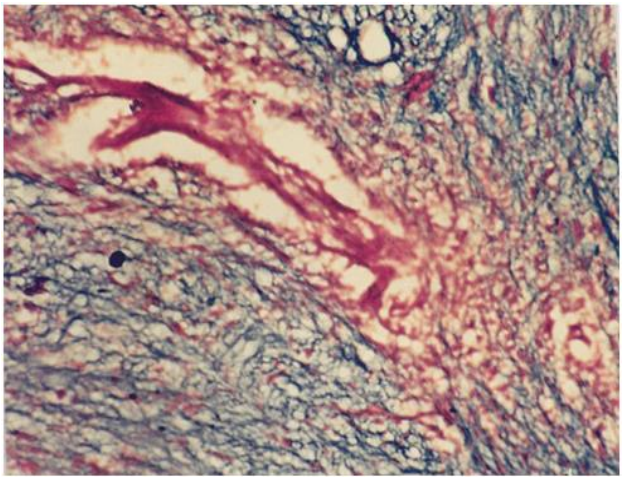

Figure 23: Cerebellum showing demyelination around a blood vessel with degenerating oligodendrocytes and astrocytosis. L F B and neutral red x168.

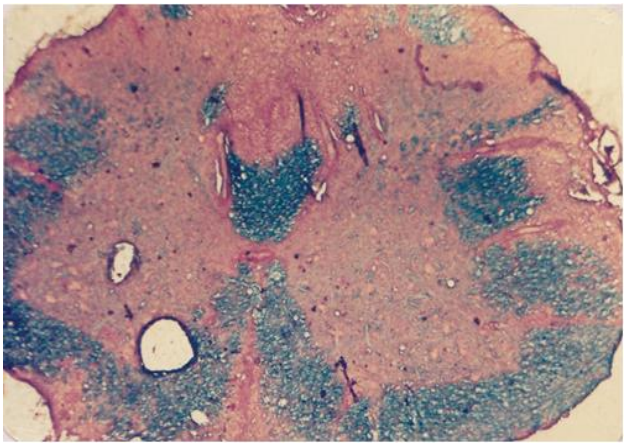

Figure 24: TS of spinal cord on day 12 after 3rd injection showing severe large demyelinated plaques in all the funiculi. The ventral horn with adjacent white matter showing cavity. Luxol fast blue and neutral red x42.3.

DISCUSSION

The development of the lesion is characteristic of a delayed type hypersensitivity reaction in which augmenting inflammatory cells (predominantly cells of the mononuclear series) comprise a major component of the perivascular infiltrate.[5] In the present study mild inflammatory lesions were seen in the lower segments of spinal cord on the day 13 and 14 after first injection. With the progress of clinical severity of the disease, the inflammatory lesions increased and extended into higher segments of the spinal cord and brain stem. Blood Brain Barrier (BBB) is related to the perivascular infiltration and the clinical signs and symptoms. Clinical signs have also been shown to depend on the involvement of these augmenting inflammatory cells.[6] The onset of the disease and the appearance of first clinical signs correlated with the maximum level of the BBB permeability. It need not

correlate with the presence of cellular infiltrates. Enhanced BBB permeability persisted on day 14 (peak of the disease) and started to decline upon recovery of the animals at day 17.[7] So far, it is unknown what causes the opening of the BBB early in the course of EAE. The increased permeability may be the result of the activation of the perivascular macrophages[8] observed an increased expression of ED2 (an activation marker) on perivascular and meningeal macrophages before the onset of clinical signs of EAE. Considering their early activation, it is likely that, by secreting inflammatory mediators[9-11] these phagocytic cells play an important role in the opening of the BBB. Significant increase in blood-brain barrier permeability to small molecules was found to precede clinical symptoms by one day in the lumbar spinal cord and to coincide with the onset of clinical disease in other regions. In all regions, increased blood-brain barrier permeability preceded the occurrence of histological lesions (perivascular cellular infiltrates). No permeability increase to large molecules could be demonstrated.[12]

The early lesions in EAE consisting of perivascular mononuclear cell infiltration. The fundamental types of lesions were found to be focal and invariably related to venules, veins and small arterioles. Older lesions show greater or lesser degree of parenchymal invasion by mononuclear cells from vessels possessing cuffs. The notable changes which occur during EAE include fibrin deposition and accumulation of inflammatory cells around blood vessels.[2] Immunohistochemical analysis confirmed the presence of blood-borne compounds such as fibrinogen in the brain parenchyma at the start of the disease.[7] Inflammatory lesions of localized hyperacute EAE are rich in fibrin, neutrophils and polymorphonuclear leukocytes.[13] In the present study a few animals which died early showed perivascular cuffings with variable amount of fibrin deposition in the acute phase of EAE. But the fibrin deposition represents nothing more than a morphologic testimony of increased permeability of vessels in the areas involving immunologic injury in the neuraxis. However fibrin deposition appears to have no active role in the constellation of clinico-histopathologic features of EAE.[2]

EAE has been considered an experimental model for demyelinating disease in man, especially for acute disseminated encephalomyelitis.[1] Hyperacute EAE has been described as a close replica of acute hemorrhagic necrotizing leukoencephalopathy in man.[14,15] The putative similarities with EAE have provided evidence for an autoimmune etiology for the entire spectrum.[3] Multiple Sclerosis (MS) and its model Experimental Autoimmune Encephalomyelitis (EAE) are debilitating paralytic diseases caused by inflammation, demyelination and axonal degeneration of the Central Nervous System (CNS). Whilst the autoimmune nature of MS is strongly suggested by evidence of myelin specific autoreactive T cells and antibodies, EAE is an experimentally induced CNS specific autoimmune disease.[16]

The histopathology of EAE is characterized by perivascular infiltrates of mononuclear inflammatory cells. Invasion of the neural parenchyma by inflammatory cells is accompanied by demyelination but not usually by necrosis. However, animals with very severe EAE may suffer axonal destruction, hemorrhage, exudation of fibrin, necrosis and thrombosis of vessels, and focal infarction. Very severe EAE usually ends fatally before liquefactive necrosis appears, but the full development of encephalomalacia or myelomalacia is observed occasionally.[3] The myelomalacia is attended by a change in the character and distribution of the EAE lesions but not by vascular occlusions.[17] The present study demonstrates a few severe inflammatory lesions with lymphocytes in the lumen of the vessel, perivascular space and a few mononuclear cells in the surrounding parenchyma. The vessel lumen was filled with thrombus. Large phagocytic cells were also seen surrounding the cuffing.

MS is a chronic inflammatory demyelinating disease of the central nervous system, in which the myelin sheath has been considered to be the primary target for many years. However, an increasing number of reports have focused on neurodegenerative aspects of the disease pathogenesis. Recent studies in post-mortem MS biopsies and in the animal model EAE have shown that key features of neurodegeneration, i.e. axonal transection; neuronal cell atrophy and neuronal death already occur in early disease phases.[18] However the cause of neuronal damage still remains elusive, since both demyelination-dependent and direct immune cell-mediated mechanisms have been suggested so far.

Necrosis was not found in ordinary EAE, but it does occur occasionally as a sequel to unusually severe inflammation. It would probably occur in every animal with the exceptionally severe hyperacute form of EAE except for the fact that these animals usually die too quickly. It is of interest that spinal cord necrosis in hyperacute EAE has been accompanied by necrosis and thrombosis of small vessels.[17] So far no report of necrosis, tissue degeneration, and cavity formation has been described in the acute and sub-acute stage of the disease EAE. In the present study necrosis, tissue degeneration and cavity formation was seen in those animals which died in the early stages of the disease and late stage after the progressive clinical signs of paralysis and attainment of moribund state of the disease. It is obvious that these animals were suffering from the severity of the disease. There were no signs of hyperacute phase of the disease yet these detrimental changes occurred. Animals which died early in the course of the disease also showed similar changes. What might be the possible cause of these drastic changes? Large areas of necrosis and tissue destruction may result from interference with blood supply in areas where most of the larger vessels are affected. Whether any change in BBB permeability was a cause for these changes? Acute Hemorrhagic Leukoencephalitis (AHL), also known as

acute necrotizing encephalopathy, acute hemorrhagic encephalomyelitis, acute necrotizing hemorrhagic leukoencephalitis is a hyperacute and frequently fatal form of acute disseminated encephalomyelitis. AHL is relatively rare; it is seen in about 2% of ADEM cases[19] and is characterized by necrotizing vasculitis of venules and hemorrhage and edema.[20] Death is common in the first week[21] and overall mortality is about 70%[22] but increasing evidence points to favorable outcomes after aggressive treatment with corticosteroids, immunoglobulins, cyclophosphamide, and plasma exchange.[23] In the present study two animals died in the early stages of the disease and two animals died in the late stages of the disease. All the four animals showed lesions resembling severe hyperacute form of EAE. But these animals were not injected with pertusis vaccine with the adjuvant and died in the acute phase of the disease.

Axonal degeneration has been identified as the major determinant of irreversible neurological disability in patients with MS. During secondary progressive MS, chronically demyelinated axons may degenerate due to lack of myelin-derived trophic support. The authors hypothesize that reduced trophic support from damaged targets or degeneration of efferent fibers may trigger preprogrammed neurodegenerative mechanisms.[24] However, the close proximity of areas with prominent axonal loss and areas containing inflammatory infiltrates (e.g., T cells, macrophages) suggest that axonal damage is closely associated with inflammation. Different soluble or cellular mediators of the immune response have been shown to damage axons in experimental systems, and these may be responsible for neurodegeneration in human disease.[25]

Based on electron microscopic observation, Field and Raine (1966)[26] described that the axonal changes precede alteration of myelin sheath and hence EAE is, therefore, not primarily a demyelinating disease. Post-mortem MS biopsies and in the EAE have shown that key features of neurodegeneration, i.e. axonal transection, neuronal cell atrophy and neuronal death already occur in early disease phases [18]. The concept of MS as an inflammatory neurodegenerative disease has important clinical implications regarding therapeutic approaches, monitoring of patients, and the development of neuroprotective treatment strategies.[27]

The question arises whether axon pathology is a critical event in the formation of multiple sclerosis lesions, or just a consequence of demyelination in later plaque development. Studies on tissue destruction in multiple sclerosis hitherto have focused predominantly on demyelination, although the damage to axons had been described from the beginning of multiple sclerosis research.[28] The destruction of axons was thought to be a feature of chronic multiple sclerosis in its later stages, when the disease has entered a progressive course. Recently, a study on autopsy tissue revealed that axonal transection was even a feature of the early stages of the disease and also appeared early during lesion evolution.[29] In the present study necrosis and softening of neuropil surrounding the perivascular inflammatory lesions occurred early in the inflammatory stage of the disease (acute phase). The studies of Bitsch et al.[30] have implications for the current understanding of multiple sclerosis pathology, pathogenesis and treatment strategies. Demyelination and axonal injury are at least partially distinct events with different mechanisms involved. Both occur early in the disease course, even in chronic multiple sclerosis. This has major implications for therapeutic strategies, which aim at preventing both demyelination and axonal loss in MS.[29]

Mononucleosis was often accompanied by myelomalacia; this was probably responsible for the permanence of the paralysis and lethal outcome.[3] Levine (1970)[31] proposed that the nonspecific reactive lymphocytes in the cuffs are protective to the neural parenchyma, perhaps because they prevent the passage of inflammatory cells into parenchyma (vascular blockade). Removal of the protective influence of the perivascular lymphocytes has been associated with unique and detrimental modifications of the histopathological changes of EAE. This is commonly seen in hyperacute phase of the disease. These unusual drastic changes observed in the present study cannot be considered hyperacute EAE changes because the encephalitogen does not contain pertusis vaccine. At the same time the animals did not enter into chronic relapsing phase, because the animals died within 26 days post-inoculation. Fibrin deposition bordering the walls of the vessels predetermines the hyperacute phase of the disease. But mild deposition of fibrin may also be seen in acute phase. Exaggeration of mononuclear cells may be responsible for these changes. Levine and Sowinski (1976)[3] termed this exaggerated condition of mononuclear cells as monocytic EAE. Hyperacute EAE might represent a "vasculonecrotic" reaction superimposed upon the "Perivascular Island" and "invasive-destructive" lesions that are typical of the delayed hypersensitivities.[32] Hyperacute EAE is a reproducible laboratory model for human acute necrotizing hemorrhagic encephalopathy.

The findings of the present study indicate that the severity of inflammation leads to drastic histopathological changes of necrosis, cavity formation, and neurodegeneration and axis cylinder damage. The fibrin deposition in the walls of the vessels, thrombosis and occlusion are the features of hyperacute phase of the disease but may occur in acute phase also. Whether these features of the axonal and neuronal damage and necrosis are a regular event in the severity of the acute phase of EAE? In the present study, pertusis vaccine was not used with the adjuvant, hence the question of hyperacute phase is ruled out. Still the lesions are closely similar to acute disseminated encephalomyelitis or necrotizing hemorrhagic leukoencephalitis of a rare kind in humans.

CONCLUSION

The findings from this study have implications for the current understanding of EAE pathology, pathogenesis and are comparable with the MS. Demyelination and axonal injury occur early in the disease course. Axonal degeneration has been identified as the major determinant of irreversible neurological disability in patients with multiple sclerosis.

Necrosis, tissue degeneration/destruction and cavity formation may occur in acute and sub-acute phase of the disease as well. This has major implications for therapeutic strategies, which aim at preventing both demyelination and axonal loss in MS. Necrosis was not found in ordinary EAE, but it does occur occasionally as a sequel to unusually severe inflammation.

The findings of the current study closely resemble the acute disseminated encephalomyelitis or acute necrotizing hemorrhagic leukoencephalitis of a rare kind in humans with a high mortality rate. EAE and MS are no more primary inflammatory and demyelinating diseases as axonal damage and neurodegeneration may occur in the acute and sub-acute phase of the disease.

DEDICATION

This research paper is dedicated to my supervisor late Professor Dr. G. C. Sensharma, Professor of Neuro Anatomy and Head of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

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