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Reactive Glia and Proliferation of Ependyma in Guinea Pigs with Experimental Allergic Encephalomyelitis.

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Research Article

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ABSTRACT

Experimental allergic encephalomyelitis (EAE) is a cell mediated autoimmune disease in animals, comparable to some of the spontaneously occurring human demyelinating diseases like multiple sclerosis (MS), and disseminated perivascular encephalomyelitis, as a useful working model for investigating demyelinating processes and the associated neuropathogenesis. 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 in the ratio of 1:1 into the foot pad of the animal. The animals after injection were observed for clinical features of the disease. The clinical severity of the animals was assessed by the standard method of Keith and McDermott (1980). EAE is characterized by inflammation and demyelination. Onset of the disease symptoms appeared between day 9 and 14 after first injection. The extent of latent period was found to be 9 to 14 days after first injection. The clinical severity of the disease progressed on the succeeding days, marked by inflammatory lesions of different severity followed by perivascular demyelination in the neuraxis. The fibres in the demyelinating lesions were demyelinated with relatively well preserved axons and adjacent neurons in the grey matter. Different grades of astrocytosis were observed around the lesion sites. The astrocytes increase in number and some of them were hypertrophied. After the myelin break down, the demyelinated zones were studded with astrocytes, probably could provide only a supportive framework to the naked axons in the absence of oligodendrocytes. The ependyma lining the central canal of a few segments of the spinal cord and lining the fourth ventricle became stratified. Reactive astrogliosis, whereby astrocytes undergo varying morphological changes, is a ubiquitous but poorly understood hallmark of all central nervous system pathologies. The possible involvement of glial proliferation and their morphological aspects is discussed.

INTRODUCTION

Experimental models of autoimmune diseases help to elucidate the pathogenesis of autoimmune diseases in man. Experimental allergic encephalomyelitis (EAE) is one such model. EAE has attracted wide attention as a meaningful model for probing the basis of autoimmune reactivity and defining its role in neurologic disorders characterized by inflammation and demyelination as in multiple sclerosis (MS) in man. EAE has been induced in several genetically susceptible animals such as mice, rat, guinea pig, rabbit and monkey. The most suitable of the experimental animals being guinea pig and Lewis rat [1, 2].

EAE is mainly a primary demyelinating disease in which myelin and myelin forming cells (oligodendrocytes) are primarily damaged whereas the axons and the axonal elements are to a greater extent spared. It is evident that the crucial distinction between primary and secondary demyelination depends upon the preservation or initial sparing of the axons and neurons in the former group.

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Astrocyte proliferation and perivascular lymphocytic infiltration are conspicuous among the cellular changes in the lesions of EAE [3, 4, 5] and inactive lesions of MS [6].

Ependymal cells lining the central canal and fourth ventricle proliferate in response to damage. These cells have the potential to differentiate into neural support cells. However, there is controversy as to whether the response of the ependymal cells is a result of injury or repair. This study demonstrates using the guinea pig EAE model, a proliferative response of the ependymal cells occurred as a result of the disease; is suggestive of ependyma reacts towards replenishing glial cells (especially astrocytes) to fill up the damaged sites.

MATERIAL AND METHODS

EAE was induced in the adult healthy guinea pigs (Cavia porcellus) of both sexes 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.

Experimental animals

Adult healthy animals of both sexes weighing 400 to 500 grams were used for the study. All animal were acclimatized to the laboratory conditions prior to the experimentation. During acclimatization and throughout the experimental period the animals were given free access to water and standard diet for the animals.

Preparation of brain antigen

Fresh homologous whole brain and spinal cord of guinea pig were dissected out and washed in freshly prepared normal saline. 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.

Adjuvant

The homologous CNS tissue emulsion (homogenate) and Freund's complete adjuvant (Diffco Laboratories USA) were (at laboratory temperature) thoroughly mixed in the ratio of 1:1 just before injection.

Dose and route of injection

0.5 ml of homologous whole brain and spinal cord antigen (CNS emulsion + complete Freund's adjuvant) was injected intradermally with a fine syringe needle (22) 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) [7] and the following clinical symptoms were observed:

- 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 and
- Death

Histological

Experimental animals were grouped into three groups of seven 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 whether the animals were clinically ill or not 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 anaesthesia. 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:

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- Luxol Fast Blue and neutral red
- Holzer's stain
- Silver carbonate impregnation

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.

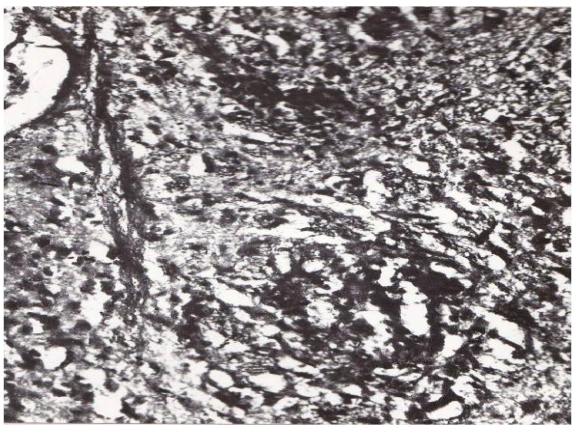
RESULTS

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. There was no damage to axis cylinders. The axons and neuronal elements were well preserved. On day 20 (day 6 after third injection), two to three animals showed inflammatory lesions in the cerebellum (Fig.1) and brainstem (Fig.2, 3).

Figure 1: Section of cerebellar white matter on day 6 after third injection showing perivascular cuffing with astrocytes in the surrounding white matter. Astrocytic processes are seen weaving basket pattern. Holzer's stain X 256

Figure 2: Section of brain stem on day 6 after third injection showing advanced inflammatory lesion. Microgliocytes on their way to transform into gitter cells and macrophages in and around the lesion could be seen engulfing the myelin debris. Luxol fast blue-neutral red X 200



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A few perivascular lesions with lymphocytic infiltration with astrocytes in the vicinity were seen in cerebellum. The brainstem lesions were marked by the phagocytic microgliocytes forming gitter cells and macrophages in and around the lesions. (Fig 2, 3)

Figure 3: Transverse section of brain stem on day 6 after third injection showing an inflammatory lesion with microgliocytes and macrophages in its vicinity, Luxol fast blue-neutral red X 256

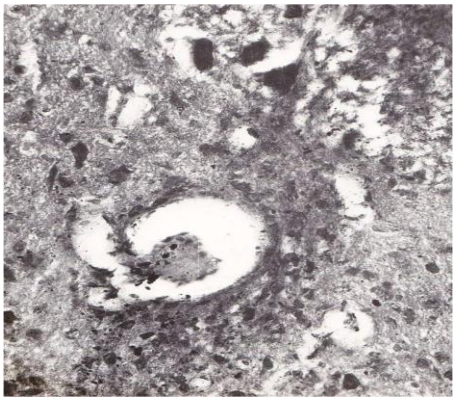
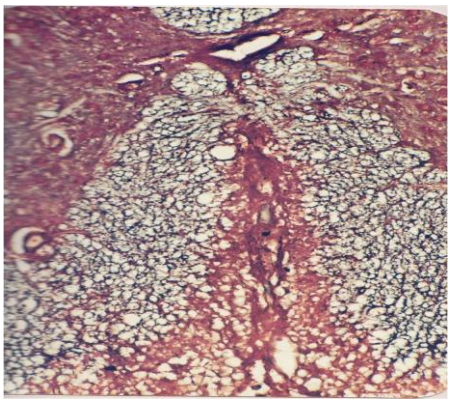


Figure 4: Transverse section of spinal cord on day 10 after third injection showing large plaques of demyelination in the ventral funiculus bordering anterior median fissure. Oligodendrocytes are scanty in their population. Astrocytes have increased in number and their processes appear to have woven a basket pattern. Luxol fast blue-neutral red X 107



On day 24 (day 10 after third injection), one animal died of the severity of disease showed demyelination in the spinal cord , brainstem and cerebellum with large plaques of demyelination in all the three funiculi of the spinal cord. There was no damage to axis cylinders. The axons and neuronal elements were well preserved. Astrocytosis was seen in and around the plaques. Astrocytes and their processes appeared to have a woven basket pattern (Fig.4, 5). Oligodendrocytes were scanty in the lesion site and have disappeared. Large plaques of demyelination were found in cerebellum mainly surrounding the blood vessels (Fig.6). Inflammatory lesions were not found in cerebral cortex.

Figure 5: Transverse section of spinal cord on day 10 after third injection showing large plaques of demyelination in the periphery of the lateral funiculus. Oligodendrocytes are scanty in their population. Astrocytes have increased in number and their processes appear to have woven a basket pattern. Luxol fast blue-neutral red X 107

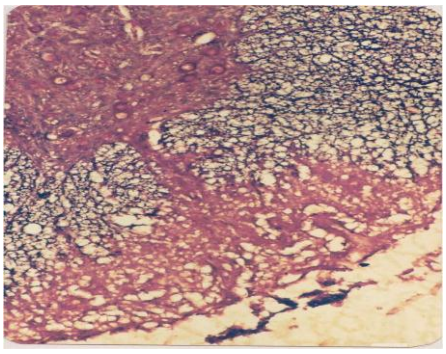


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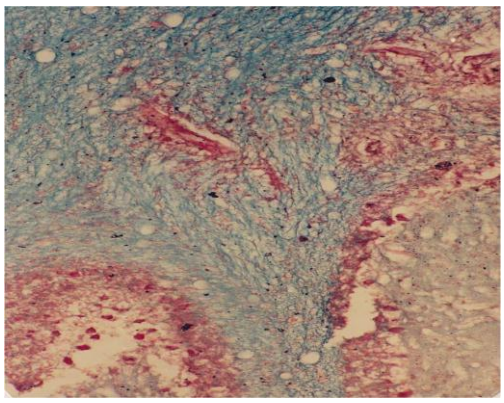
Figure 6: Section of cerebellum on day 10 after third injection showing perivascular demyelination in white matter. The fibres in the vicinity of the vessels are demyelinated.
Luxol fast blue-neutral red X 42.3



On day 26 after first injection (day 12 after 3rd injection) two animals showed more pronounced focal demyelination in spinal cord, brain stem and cerebellum. The plaques of demyelination in the spinal cord were large in the posterior funiculus (Fig. 7, 8, 9, 10). This plaque was continuous with dorsal horn and substantia gelatinosa. The neuronal elements however were well preserved. Oligodendrocytes were totally absent in the lesion sites and scanty in the neighbouring white matter.

Figure 7: Transverse section of spinal cord on day 12 after third injection showing large plaques of demyelination in the dorsal funiculus extending up to the dorsal grey horn. Luxol fast blue-neutral red X 42.3

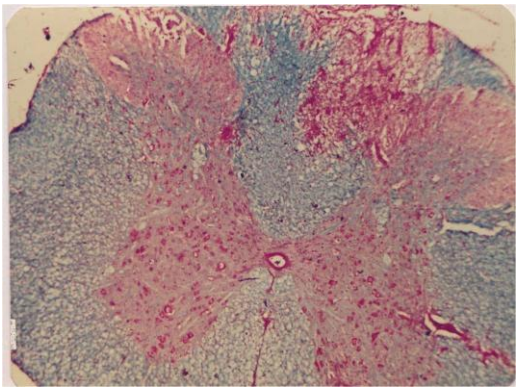


Figure 8: Transverse section of spinal cord on day 12 after third injection showing large plaques of demyelination in the dorsal funiculus and ventral funiculus.
Luxol fast blue-neutral red X 50.4

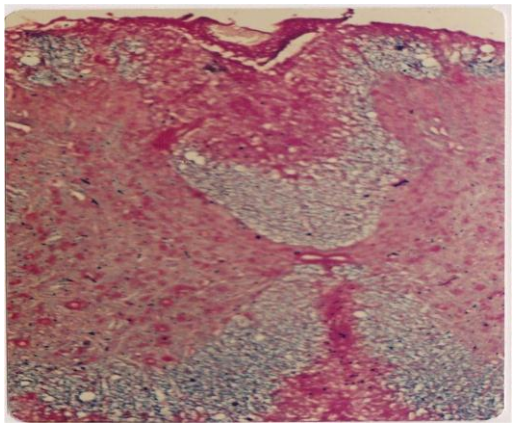


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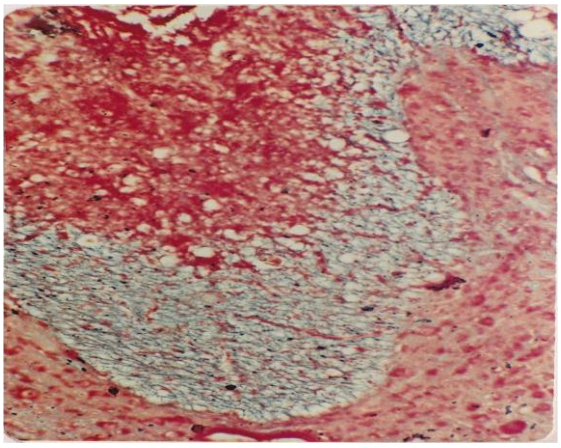
Figure 9: The demyelination plaques of the figure 8 are magnified showing astrocytosis Luxol fast blue-neutral red X 107



Astrocytes of various grades were seen in and around the lesion site (Fig.11, 12, 13.14).

Figure 10: Section of the brain stem (medulla oblongata) on day 12 after third injection showing severe demyelinated plaque in the inferior cerebellar peduncle. Note the degenerative changes of oligodendroglial cells in the vicinity of the lesion, Luxol fast blue-neutral red X 168

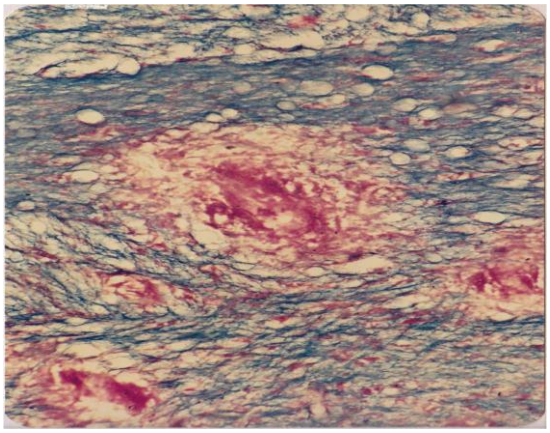


Figure 11: Section of the brain stem on day 12 after third injection showing astrocytosis in and around the lesion site. The astrocytes increased in population hypertrophied, their processes are seem to have woven a basket pattern. Holzer's stain X 200

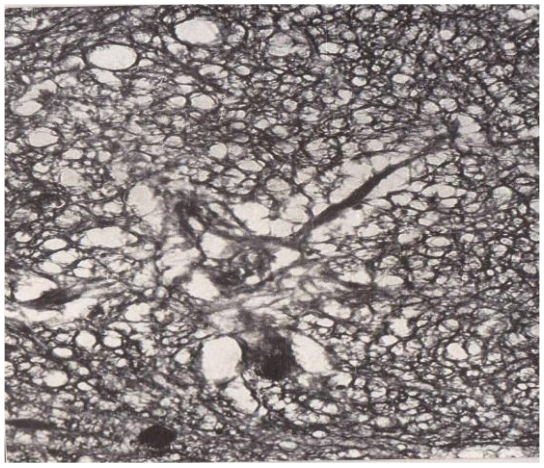


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Figure 12: Section of the spinal cord on day 12 after third injection showing vigorous astrocytosis in the white matter. Holzer's stain X 256

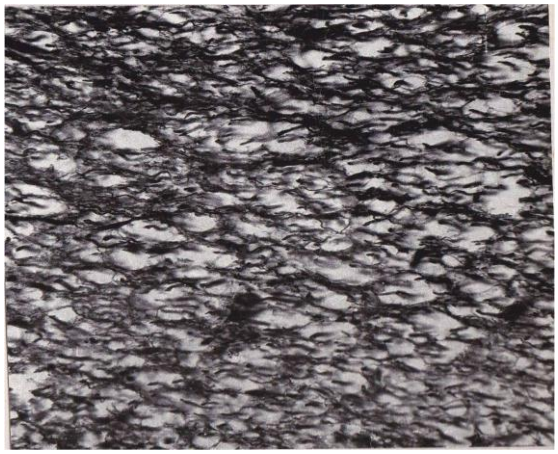

Figure 13: Section of the spinal cord on day 12 after third injection showing vigorous astrocytosis in the white matter neighbouring the lesion. Holzer's stain X 256

The ependyma lining the central canal of a few segments of the spinal cord, dorsal surface of the medulla oblongata and pons which forms the floor of the fourth ventricle showed changes characteristics of response to injury. The ependyma of the central canal (Fig.15, 16, 17) and floor of the fourth ventricle (Fig. 18, 19, 20, 21) became stratified.

Figure 14: Section of the spinal cord on day 12 after third injection showing vigorous astrocytosis with enlargement of extracellular spaces. Holzer's stain X 256

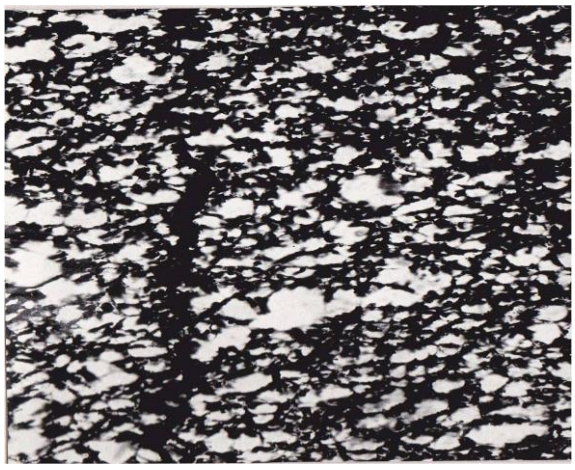

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Figure 15: Section of the spinal cord on day 12 after third injection showing central canal lined with more than one layer of ependymal cells. Silver carbonate impregnation. X 256

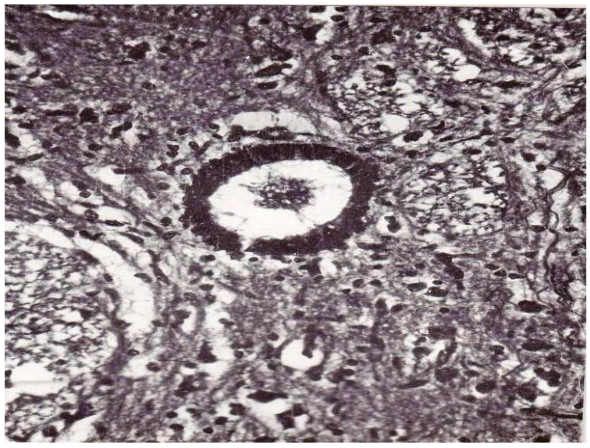

Figure 16: Central portion of the figure 15 magnified to show proliferation of ependymal cells. Arrow shows the dividing cell of the ependyma. Silver carbonate impregnation. X 1025

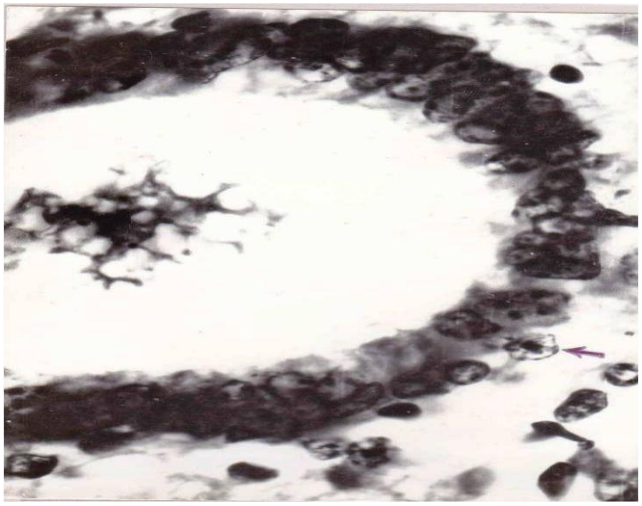

Figure 17: Section of spinal cord on day 12 after third injection, showing central canal lined by stratified ependyma. Silver carbonate impregnation. X 409

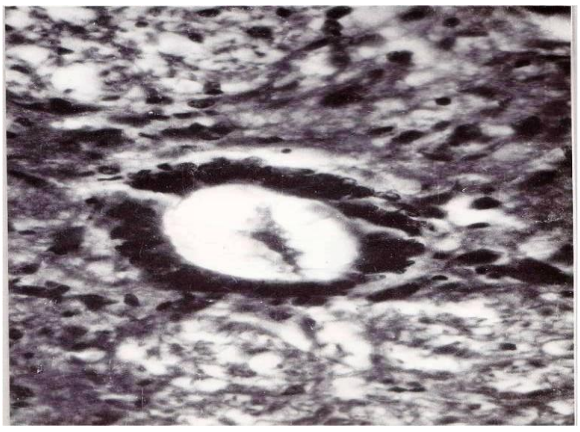

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Figure 18: Transverse section of the brain stem on day 12 after third injection showing the floor of the fourth ventricle with stratified ependyma and subependymal gliosis (mainly astrocytes).Silver carbonate impregnation. X 256, E—Ependyma, SEP—Subependymal region, VENT—4th Ventricle

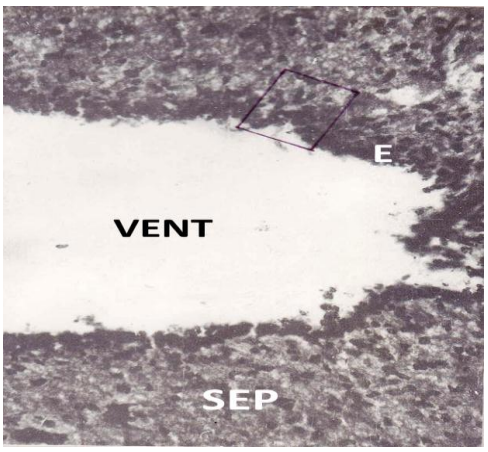

Figure 19: A portion of the Fig. 18 magnified to show proliferation of ependyma. The ependyma shows two to three layered lining. The subependymal glia (mainly astrocytes) are typical reactive and microglia are notable. Silver carbonate impregnation. X 1025

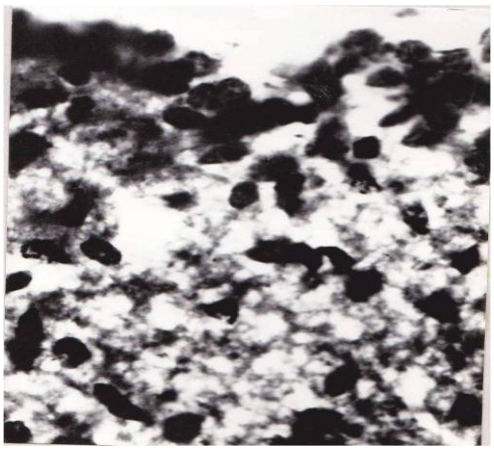

Figure 20: A portion of the ependymal wall (brain stem – 4th ventricle) showing stratification. The subependymal glia (mainly astrocytes) have appeared to be typical reactive and microglia are enlarged and increased in number. Silver carbonate impregnation. X 409

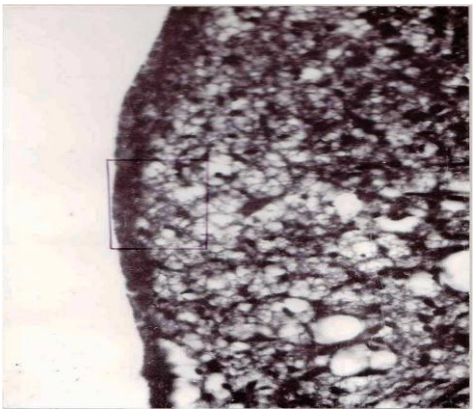

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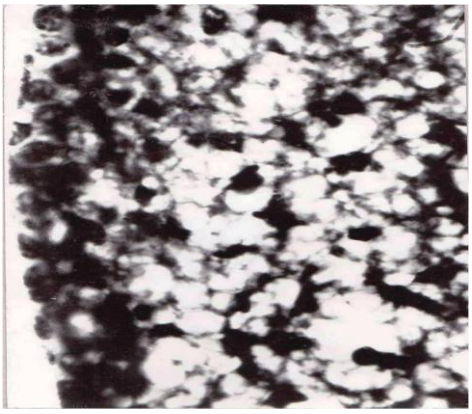
Figure 21: Higher magnification of Fig. 20 showing proliferation of ependymal cells and reactive subependymal glia. Silver carbonate impregnation. X 1025



The subependymal glial cells (mainly astrocytes) were found to be increased in population, hypertrophied with their processes appeared to have woven a basket with increased microgliocytes (Fig.20, 21)

DISCUSSION

Development of the lesion is the characteristic of a delayed type hypersensitivity reaction in which augmenting inflammatory cells comprise a major component of perivascular infiltrate [8]. The early lesions in EAE consisting of perivascular mononuclear cell infiltration seem to represent the first morphological expression of an autoimmune process of CNS. The primary event in allergic encephalomyelitis is infiltration by cells into the neighbourhood of veins in response to antigen. These cells are lymphocytes followed by dark staining mononuclear cells and histiocytes, probably of hematogenous origin [9]. In the present study lymphocytes and mononuclear cells were the main cell types observed to have infiltrated in the perivascular space and adjacent parenchyma. However a few polymorphonuclear cells were also seen. Majority of these lymphocytes are T-lymphocytes absolutely necessary for the induction of EAE [10].

It is the common property of astrocytes to show changes in response to almost every type of injury or disease in the CNS. Hypertrophy, hyperplasia and increase in number of astrocytes usually result after breakdown of myelin or demyelination [11]. It is well known that there is a wide spread astrocyte reaction in acute EAE as evidenced by increased staining for glial fibrillary acidic protein [12,13]. Adult oligodendrocyte precursors (OLPs), also known as “NG2 cells,” also generate a subset of protoplasmic astrocytes during perinatal development [14, 15]. Adult OLPs continue to divide and generate new myelinating OLs in the healthy adult rat CNS [16, 17, 18, 19, 20], though at a steadily decreasing rate with age [21, 22]. They do not appear to generate astrocytes during normal adulthood [19, 20].

In the present study it was noted that the hypertrophied astrocytes were seen in the vicinity of perivascular lesions. The hypertrophied nature of astrocytes in the inflammatory stage of EAE is probably secondary to the parenchymal changes. It is known that during the acute phase of multiple sclerosis and EAE [23] proliferating cells also belonging to perivascular inflammatory cells and parenchymal glial cells have entered the cell proliferation cycle [24]. Astrocytic proliferation and perivascular lymphocytic infiltration are conspicuous among the cellular changes in the active lesions of MS[6]. Raine [25] reported membrane specialization between naked CNS axons and fibrous astroglial cell processes, developing subsequently to demyelination. Ultrastructural changes of astrocytes in the organotypic cultures of mouse spinal cord tissue revealed sheet like astrocytic processes completely and closely surrounding the demyelinating fibres and frequently engulfing myelin fragments [26].

Moore and Raine [27] envisaged that the astroglial elements invariably were in close proximity to subpial region and chronically demyelinated lesions and were derived from subpial astrocytes. This phenomenon of subpial astrocytic response to inflammation and demyelination may be related to the protection of damaged white matter against subsequent inflammatory events. In the present investigation during the late stage of EAE, the demyelinated zones were studded with astrocytes which could provide a supportive framework to the naked axons after demyelination.

The reactive astrocytes play essential roles in preserving neural tissue and restricting inflammation after moderate focal brain injury [28]. The origin of reactive astrocytes has been controversial, some reports suggesting that they are generated from NG2 cells. [29,30,31,32] Reactive astrocytes originated solely from resident quiescent astrocytes, and that different mechanisms contributed to the formation of reactive astroglia in spinal gray and white matter, i.e. reactive astrocytes were derived by both hypertrophy and hyperplasia of fibrous astroglia in white matter, but solely by phenotypic transformation of protoplasmic astroglia in gray matter [33].

Reactive gliosis has been considered to be the major cause of regenerative failure of the mature mammalian central nervous system (CNS). It is classically defined by increased glial fibrillary acidic protein expression. However, the response to injury is not uniform throughout the CNS [34]. Loss or dysfunction of reactive astrocytes leads to a prolonged increase in leukocyte infiltration, failure of Blood-Brain Barrier (BBB) repair with vasogenic edema, neuronal degeneration, and increased outgrowth of nerve fibers in injured CNS parenchyma [35].

A prominent feature of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) is the accumulation of enlarged, multipolar glial fibrillary acidic protein (GFAP) and brain lipid binding protein (BLBP), immunoreactive astroglia within and at the margins of the inflammatory demyelinating lesions. Whether this astrogliosis is due to both astroglial hyperplasia and hypertrophy or solely to astroglial hypertrophy is controversial. Peter Bannerman et al. [36] reported the coincidence of the first appearance of inflammation and clinical deficits in mice with myelin oligodendrocyte glycoprotein peptide (MOG peptide)-induced EAE, the radially oriented, bipolar, GFAP, and BLBP positive cells (adult radial glia) present in normal spinal cord white matter undergo mitosis and phenotypic transformation to hypertrophic astroglia. Many hypertrophic “reactive” astrocytes appear in the demyelinating/remyelinating spinal cord. These $GFAP^+$ astrocytes tend to accumulate at the periphery of demyelinated lesions where they form a dense glial “scar.” This could be a barrier to inward migration of NG2 cells and thus might inhibit repair, especially after multiple episodes of demyelination at the same locus such as occurs in relapsing-remitting MS. The morphology, density and proliferation rate of astroglia can independently define the discrete cytoarchitecture of the adult mammalian CNS, and support the concept that regional astroglial heterogeneity reflects important molecular and functional differences between distinct classes of astroglia [37].

Perivascular cells are a heterogeneous population found in the central nervous system (CNS) and the peripheral nervous system (PNS). Several terms are used for these cells, including perivascular cells, perivascular macrophages, perivascular microglia, fluorescent granular perithelial cells (FGP). Perivascular cells, although a minor component of the CNS, are important immunoregulatory cells. Perivascular cells are bone marrow derived, continuously turn over in the CNS, and are found adjacent to CNS vessels. Thus, they are potential sensors of CNS and peripheral immune system perturbations; are activated in models of CNS inflammation, autoimmune disease, neuronal injury and death; and are implicated as phagocytic [38]. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system.

Microglia respond to virtually any, even minor pathological events in the CNS. There is increasing evidence that microglia play an active part in degenerative CNS diseases. In autoimmune diseases microglia probably have dual functions. Microglia present antigen to infiltrating T cells and exert effector functions thereby locally augmenting immune responses. On the other hand, microglia has the capacity to downregulate T cell responses [39].Microglial activation in MS and EAE is thought to contribute directly to CNS damage through several mechanisms, including production of proinflammatory cytokines, matrix metalloproteinases, and free radicals. In addition, activated microglia serve as the major antigen-presenting cells in the CNS, likely contributing to aberrant immune reactivity at this site. A mechanistic understanding of the way in which microglia are activated and ultimately inhibited is crucial for the formulation of therapeutic modalities to treat MS and other CNS autoimmune diseases [40]

Ponomarev ED et al. [41] found that microglial cell activation was evident before onset of disease symptoms and infiltration of peripheral myeloid cells into the CNS. Activated microglial cells underwent proliferation and upregulated the expression of CD45, MHC class II, CD40, CD86, and the dendritic cell marker CD11c. At the peak of EAE disease, activated microglial cells comprised 37% of the total macrophage and dendritic cell populations and co localized with infiltrating leukocytes in inflammatory lesions. The microglial cells become activated early in EAE disease and then differentiate into both macrophages and dendritic-like cells, suggesting they play an active role in the pathogenesis of EAE and MS [41].

In the present study the brainstem lesions were marked by the phagocytic microgliocytes forming gitter cells and macrophages in and around the lesions (Fig.2, 3) with a few inflammatory cells surrounding the blood vessels. The fibres in the immediate vicinity of the vessels were demyelinated. Activated microglia are mainly scavenger cells but also perform various other functions in tissue repair and neural regeneration. They form a network of immune alert resident macrophages with a capacity for immune surveillance and control. Activated microglia can destroy invading micro-organisms, remove potentially deleterious debris, promote tissue repair by secreting growth factors and thus facilitate the return to tissue homeostasis [42].

In multiple sclerosis, oligodendrocytes die, possibly through autoimmune attack, and demyelination results. During this and other demyelinating diseases there is spontaneous regeneration of lost OLs and myelin [43]. In the present study, degeneration and disappearance of oligodendrocytes in the demyelinating lesions were seen. Oligodendrocytes were found to be scanty in and around the lesion sites. Oligodendroglial damage and disintegration of myelin lead to functional disturbances in demyelinating diseases. The destruction of

oligodendrocytes by a virus may result in sensitization of the host to self oligodendroglial antigens [43] .The possible cause for the degeneration and disappearance of oligodendrocytes during demyelination may be due to humoral factors or interaction of mononuclear cells and oligodendrocytes. Sensharma and Singh. (1975) [11] attributed the degeneration of cells due to local anoxia. The presence of antibodies directed against galactocerebrocide, a surface antigen of oligodendrocytes has been described in patients of MS [44, 45] and in EAE animals [43]. Infection with vaccinia virus induces antimyelin and anti-oligodendrocyte antibodies [46] .Oligodendroglia in demyelinated lesions depleted during the period of active demyelination [47]. Invasion of apparently normal myelin sheaths by mononuclear cells is preceded by phagocytosis of degenerated oligodendroglial cells. This phagocytosis of oligodendroglial cells supports that the mononuclear cells are noxious to oligodendrocytes [48]. Later in the illness, hypertrophic astroglia found at the margins of the lesions, while the lesions themselves remained depleted of oligodendroglia, suggesting that migration of oligodendroglial lineage cells into the lesions was retarded by the intense perilesional gliosis [49].

Calza, et al. [50] have suggested that in EAE animals the breakdown of the BBB exposes precursors to unique environmental conditions, perhaps bringing into the CNS molecules able to alter the balance between quiescent and active progenitors in favour of the active one. On the other hand, Tourbah et al. [51] suggested that the inflammation itself promotes survival and migration of transplanted oligodendrocyte progenitors in the adult CNS. The adult mammalian brain and spinal cord contain glial precursors that express platelet-derived growth factor receptor α subunit (PDGFRA) and the NG2 proteoglycan. These “NG2 cells” descend from oligodendrocyte precursors in the perinatal CNS and continue to generate myelinating oligodendrocytes in the gray and white matter of the postnatal brain. It has been proposed that NG2 cells can also generate reactive astrocytes at sites of CNS injury or demyelination. In the demyelinating diseases, there is spontaneous regeneration of lost OLs and myelin.

The adult mouse Sub Ventricular Zone (SVZ) is a source of newly generated oligodendrocytes and thus may contribute, along with oligodendrocyte precursors, to the replacement of oligodendrocytes in inflammatory demyelinating diseases of the central nervous system such as multiple sclerosis. The adult SVZ represents a reservoir of cells that can be reactivated in response to inflammatory demyelination. Although their direct implication in myelin repair remains to be elucidated, these cells are likely to be involved in these repair mechanisms because they are recruited by lesioned areas and differentiate into the appropriate glial lineage [52].

Adult CNS neuroepithelial precursors (NPC) express nestin [53] both in vivo and in vitro [54]. During spinal cord development, nestin is expressed around the central canal and is progressively reduced with maturation [55]. These nestin-expressing precursors proliferate and migrate after spinal cord injury [56, 57, 58]. In immature [59,60] and adult mammals [61] ependymal cells proliferate in the normal and injured spinal cord [62,63,64,65,58,66] which is enhanced by growth factor infusion [67,68] . Mouse spinal cord NPCs reside near the central canal because multipotent, self-renewing neurospheres are generated only if the central canal is cultured [69]. The adult rat spinal cord periventricular region neurospheres are multipotent, producing neurons, astrocytes, and oligodendrocytes, whereas spheres derived from the parenchymal white matter are glial-restricted progenitors, producing only astrocytes and oligodendrocytes, but not neurons in vitro [70]. The native differentiation pattern of periventricular spinal cord NPCs is region specific because most of the progeny differentiate into oligodendrocytes [70].

Proliferation of ependymal cells has been more commonly reported in response to spinal cord injury [71, 72, 62, 59, 64, 73]. In the present investigation the ependyma was seen to become stratified lining the central canal of a few segments of the spinal cord and in the floor of the fourth ventricle. The proliferative activity of the ependyma in the central canal of the spinal cord in response to significant reactive events is triggered by local injury [60]. This study demonstrates using the guinea pig EAE model, a proliferative response of the ependymal cells occurred as a result of the disease. Interestingly, a more pronounced [74] ependymal proliferative effect was seen in animals being fed a phase 2 enzyme inducer. Injury to the neural elements may occur in animals with EAE and the ependyma proliferate in response to injury. The subependymal astrocytes observed were hypertrophied and reactive along with a number of microglial cells characteristic of an injury in the present investigation. The ependymal proliferation with in the damaged central nervous tissues might ameliorate symptoms and signs of EAE through stimulating myelin synthesis [74]. Adult rat spinal cord ependymal NPCs differentiate preferentially into oligodendrocytes and reactive glia which may support axonal regeneration in future trials of transplant therapy for spinal cord injury [70]. The ependymal lining also contains the so called neural stem cells. There is a recruitment of newly generated precursors in the active phase of EAE. The recruitment of progenitor cells from the SVZ into the areas of demyelination has been demonstrated in chemically induced demyelination [75].These precursors seem to find a migration pathway. Indeed, Ki67-positive nuclei are closely associated with NADPH diaphorase-reactive processes, which possibly correspond to radial astrocytes [76]. Processes from these cells span the white matter from the pial surface to the gray-white matter interface and could represent a migratory path [16]. The ependymal cells play a role in the post-inflammatory response of the brain and also may be involved in the remyelination process [74].

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CONCLUSION

The inflammatory lesions consisted of perivascular cuffing with lymphocytes and mononuclear cells in the perivascular space and surrounding parenchyma. The mononuclear cells were seen to be in close proximity with the myelin sheath and oligodendroglia. The oligodendrocytes showed signs of degeneration and finally disappeared from the lesion site and immediate vicinity. The perivascular demyelination was restricted to that part of the white matter which was infiltrated by mononuclear cells.

Different grades of astrocytes were observed in and around the lesion site. The astrocytes increased in number and some of them hypertrophied. After the myelin breakdown during the late stages of EAE, the demyelinated zones were studded with astrocytes, and could provide only a supportive framework to the naked axons in the absence of oligodendrocytes.

The macrophages increased in number and size, engulfing the myelin fragments in the lesion site. The microgliocytes becoming the gitter cells were found to increase in number, suggestive of the removal of debris.

Ependyma lining the central canal of a few segments of the spinal cord and lining the fourth ventricle became stratified. The subependymal astrocytes were found to be reactive together with microgliocytes increased in number and size. The proliferation of ependyma in EAE indicates that the ependyma reacts towards replenishing glial cells (especially astrocytes) to fill up the damaged sites. The ependymal proliferation with in the damaged central nervous tissues might ameliorate (improve) symptoms and signs of EAE through stimulating myelin synthesis.

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