CHAPTER 1
1. Introduction

1.1 Overview of Human Multiple Sclerosis

Demyelinating diseases although very difficult to define precisely, is a group of disorders that leads to the loss of myelin, by either direct myelin damage or oligodendrocyte depletion. But probably there is no disease in which the exclusive demyelination is the only feature. The idea of a demyelinating disease is, more or less, an abstraction that serves primarily to focus attention on one of the more striking and distinctive features of a group of pathological processes. Multiple sclerosis, called “M.S” by most physicians, is referred to by the British as disseminated sclerosis and by the French as “sclère rose en plaques”. It is among the most venerable of neurologic diseases and one of the most important by virtue of its frequency, chronicity and tendency to attack young adults. It is a chronic condition characterized clinically by episodes of focal disorder of the optic nerves, spinal cord and brain, which remit to a varying extent and recover over a period of years.

The diagnosis may be uncertain at the time of onset and in the early years of the disease, when the signs and symptoms point to a lesion present at only one locus of the nervous system (Barrihof et al., 1997). Later, as the disease recurs and disseminates throughout the cerebrospinal axis, the diagnostic accuracy approaches 100 percent. A long period of latency (1 to 10 years or longer) between a minor initial symptom, which may not even come to medical attention, and the subsequent development of more characteristic symptoms and signs may delay the diagnosis. In most cases there is a relapsing-remitting pattern, i.e., the initial manifestations improve partially or completely, to be followed after a variable interval by the recurrence of the same abnormalities or the appearance of new ones in other parts of the nervous system (Dalton et al., 2002). However, in more than half of patients, the disease takes the form of an intermittently progressive illness (relapsing remitting, the typical type of MS), and sometimes of a steadily progressive one, especially in patients more than 40 years of age.
The signs and symptoms of M.S are the consequence of the underlying neuropathologic changes. The primary mechanism of injury is the inflammatory demyelination and is associated with variable degree of axonal damage. Either of the mechanisms may produce clinical features. The role of axonal damage is clear cut, disrupting conduction (Howell et al., 2010) and accumulating neurological deficits. Demyelination may result in either slowing down of conduction or complete failure of transmission (Fig.1.1). The former will produce symptoms when the slowing becomes critical. As the pathologic damage may involve any area of the central nervous system (CNS), the spatial arrangement of the lesion(s) also plays a role in symptomatology.

M.S can produce any symptom or sign that might occur with damage to the CNS (Sastre-Garriga and Tintore, 2010), especially white matter tracks. The most common findings include optic neuritis, weakness, sensory loss, ataxia, nystagmus, bladder dysfunction, and cognitive impairment, but the full list is quite long. The progressive impairment or disability that occurs over time with M.S results from one of two mechanisms involving neuroinflammation induced direct myelin damage or the depletion of the oligodendrocytes which also leads to demyelination and axonal disturbances (Comabella and Khoury, 2011). There is either stepwise worsening due to accumulated deficits from residua of exacerbations or gradual, inexorable progressive disease. The relative
role of exacerbations and progressive disease in the accumulation of deficits has been
debated, but the data are clear that both impact the long-term course of the illness. Recent
data from a meta-analysis of several clinical trials in MS demonstrate that residual deficit
from exacerbations occurs after at least 50% of attacks. Later in the course of MS,
progressive disease seems to contribute more strongly to the disability (Confavreux et al.,
2000).

1.2 Clinical Subtypes of M.S

The clinical course of MS, although quite variable, tends to follow one of several specific
courses characterized by either a relapsing pattern or a progressive pattern. In relapsing
forms of MS, there are multiple acute exacerbations of neurologic dysfunction lasting days
to months, with a variable degree of recovery and then stability until the next exacerbation,
which can occur weeks to decades later. There are at present no reliable biologic makers
(either MRI or clinical laboratory) that distinguish the disease course patterns, so they were
decided by consensus, based on a survey of the international MS clinical research community
published in 1996 (Lublin and Reingold, 1996). The clinical course patterns can be divided
into four subtypes: relapsing-remitting, primary progressive, secondary progressive, and
progressive-relapsing, as outlined next.

1.2.1 Relapsing-remitting (RRMS)

Relapsing-remitting (RR) MS (Fig. 1.2A) is the commonest form at presentation and is
characterized by clearly defined disease relapses with full recovery or with sequelae and
residual deficit upon recovery. Periods between disease relapses are characterized by a lack
of disease progression. The defining elements of RR MS are episodes of acute worsening of
neurologic function followed by a variable degree of recovery, with a stable course between
attacks. Approximately 85 to 90% of patients with MS start with an RR course.

1.2.2 Primary progressive (PPMS)

Primary progressive (PP) MS (Fig. 1.2B) is characterized by disease progression from onset
with occasional plateaus and temporary minor improvements allowed. Approximately 10%
of patients have this form of MS. The essential element in PP MS is a gradual, nearly
continuously worsening baseline with minor fluctuations, but no distinct relapses. While near continuous progression is required in this definition, it was recognized that progression at a constant rate throughout disease was unlikely and that accommodation must be made for variations in the rate of progression over time. PP M.S is quite distinct from RR MS (especially the absence of any exacerbations), causing some to suggest that it may represent a different disease. However, current evidence suggests that PP is a subtype of typical MS.

1.2.3 Secondary progressive (SPMS)

Secondary progressive (SP) MS (Fig. 1.2C) is characterized by an initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions, and plateaus. SP MS may be seen as a long-term outcome of RR MS, in that almost all SP patients initially begin with RR disease as defined here. However, once the baseline between relapses begins to progressively worsen, the patient has switched from RR MS to SP MS. This transition from RR to SP occurs in up to 50% of RR MS patients, although it can take many years and is unpredictable.

Figure 1.2: Different clinical subtypes of human M.S as per disease progression. All the four subtypes have different patterns. RRMS-Relapsing Remitting Multiple sclerosis, PPMS-Primary Progressive Multiple sclerosis, SPMS-Secondary Progressive Multiple sclerosis and PRMS-Progressive Relapsing Multiple sclerosis. Source, Harrison’s Principles of Internal Medicine.
1.2.4 Progressive-relapsing (PRMS)

In the progressive-relapsing (PR) form of MS (Fig. 1.2D), there is progressive disease from onset, with clear acute relapses, with or without recovery, with periods between relapses characterized by continuing progression. Approximately 5 to 6% of patients have this form of MS, but there are data now accruing that PP MS patients may convert to PR at a rate of almost 1% per year. This will be better understood once a large clinical trial in PP MS has completed.

The term “chronic progressive M.S,” used frequently in the past has been discarded in favor of one of the more descriptive progressive forms just described. MS can also be categorized by outcome. At the extremes, MS can be designated as either benign or malignant. Benign MS has been defined as disease that allows patients to remain fully functional in all neurologic systems 15 years after disease onset. Although this form may comprise 10 to 15% of patients, diagnosis, and thus prognosis, is difficult and by definition requires 15 years. Even then, relapses or progression can occur, sometimes as late as 25 years later.

1.3 Prevalence of M.S

MS is the commonest of the demyelinating diseases and neurologic disability in young adults. MS affects 2.5 million people worldwide. The prevalence of MS in North America is about 100 per 100,000 and incidence of about 6 per 100,000, increasing with latitude. Approximately 350,000 persons in the United States have MS, and this number may be an underestimation.

In India according to data from mid eighties; the prevalence was 1.33 in 100000 in west coast (Singhal, 1985). In last 15 years, since the imaging has improved the prevalence has doubled mostly in north western India. Moreover, the Parsi community has been studied to be at a greater risk as compared to others where the prevalence is 26 in 100000 (Wadia and Bhatia, 1990). It is speculated that the numbers may increase with better diagnostic facilities.

MS is approximately threefold more common in women than men. The age of onset is typically between 20 and 40 years (slightly later in men than in women), but the disease can
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present across the lifespan. Approximately 10% of cases begin before 18 years of age and extremes with onset as early as 1–2 years of age or as late as the eighth decade have been described.

The average age at onset is 32, and patients tend to live in excess of 35 years from time of diagnosis. Therefore, although the actual number of individuals is not large compared to some other diseases, the longevity and the potential for serious disability produce considerable economic consequences. The cost of MS in the United States is $9.6 billion per year, around $34,000 per year for each patient, exclusive of the costs of disease-modifying agents.

1.4 Brief overview of etiopathogenesis of M.S

The epidemiologic data accumulated till date point to both, genetic susceptibility and some environmental factors that are encountered in childhood and, after years of latency, evoke the disease. In recent years, speculation has grown that this factor is an infection, most often viral. A body of indirect evidence has been marshaled in support of this idea, based largely on alterations in humoral and cell-mediated immunity to viral agents (Johnson et al., 1984; Lampert et al., 1978; McFarlin and McFarland, 1982). However, to this day no virus (including all known members of the human retrovirus family) has been seen in or isolated from the tissues of patients with MS despite innumerable attempts to do so. Moreover, no satisfactory viral model of MS has been produced experimentally.

Recently, the bacterial agents Chlamydia pneumoniae and Borrelia burgorferi (the agent of Lyme disease) and herpesvirus type 6 have been similarly implicated by the finding of their genomic material in MS plaques, but any evidence for their direct participation in the disease process is, at the moment, not compelling. If indeed some obscure infection is the initial event in the genesis of MS, then a secondary factor must be operative in later life to reactivate the neurologic disease and cause exacerbations. One popular view is that this secondary mechanism is an autoimmune reaction, attacking some component of myelin and, in its most intense form, destroying all tissue elements, including axons. Several lines of argument have been advanced in support of this view. One is inclined to draw an analogy
between the lesions of MS and those of acute disseminated encephalomyelitis, which is almost certainly an autoimmune disease of delayed hypersensitivity type. In support of this possibility is the finding of antibodies to specific myelin proteins—e.g., myelin basic protein (MBP)—in both the serum and cerebrospinal fluid (CSF) of MS patients, and these antibodies, along with T cells that are reactive to MBP and to other myelin proteolipids, increase with disease activity; moreover, MBP cross-reacts to some extent with measles virus antibodies. The arguments that a chronic viral infection reactivates and perpetuates the disease are, however, less convincing than those proposing a role for viruses in the initiation of the process in susceptible individuals. A possible way in which viral infections and autoimmune reactions in the nervous system might be linked to the abnormal expression of autoantigens on CNS cells has been suggested by Johnson et al., 1982. He found that several different viruses (rubeola, rubella and varicella) could cause the sensitization of T lymphocytes against myelin basic protein. This implies that the T lymphocyte recognizes an identical structure in both the virus and the myelin sheath. The hypothesis continues with the notion that once the autoimmune process is initiated by a virus in childhood, it can later be reactivated by any of the common viral infections to which the individual is exposed, particularly in the far higher northern and southern latitudes. This phenomenon of "molecular mimicry" (a shared antigen between the virus and CNS myelin, the oligodendrocyte, or cerebral vessels) has been invoked as a mechanism in several diseases, notably in rheumatic fever, certain paraneoplastic diseases, and Guillain-Barre’ syndrome, but remains speculative in MS.

The role of humoral and cellular factors in the production of MS plaques is not fully understood. The deposition of immunoglobulin in the plaques of patients with acute and relapsing-remitting disease, but not those with progressive MS, has been alluded to earlier. Humoral immune system is also involved in the etiopathogenesis which is evident from the presence of oligoclonal immune protein antibodies in the CSF of most patients, which are produced by B lymphocytes within the CNS. Sera from patients with MS (and some normal controls), when added to cultures of nervous system tissue from newborn mice in the presence of complement, can damage myelin, inhibit remyelination, and block axonal conduction. Antibodies to oligodendrocytes are present in the serum of up to 90 percent of
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patients in some studies but far less frequently in others. Of note also are the findings of Berger et al., 2003 that show a relationship between the clinical progression of early and monosymptomatic M.S and the presence of serum autoantibodies against oligodendrocyte glycoprotein and myelin basic protein. They have reported that the absence of antibodies, a finding in 38 percent of their patients, identified a group with more benign disease, at least for several years. Whether this further indicates, as they suggest, that humoral immune mechanisms are essential to the production of M.S is uncertain.

Autoantibodies have been found that are directed against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP). It has also been demonstrated that subsets of T cells (CD4, Th2 cells and CD8) and are activated by MBP and MOG to activate B cells, the production of oligoclonal bands and membrane attack complexes, and the release of cytokines (Fig. 1.3); tumour necrosis factor alpha (TNF-α), interleukins, interferon gamma (IFN-γ). The inflammatory process erodes the blood-brain barrier and ultimately destroys both oligodendroglia and axons. The eventual functional outcome reflects both the activity of this inflammatory cascade and the degree of axonal damage. In other cases, there may be a compromise of oligodendroglial function and axonal degeneration in the absence of prominent inflammation (French-Constant, 1994).
Nevertheless, most immunologists currently subscribe to the notion that MS is mediated by a T-cell sensitization to myelin. This idea is supported by numerous lines of evidence including the observation that T cells initiate the lesions of experimental allergic encephalomyelitis (EAE), which is assumed to be an animal model of MS, as suggested by Adams et al., 1952. Although the entry of autoreactive T cells into the CNS results in a perivascular inflammatory reaction, its relationship to MS is unclear. Conceivably, intense T-cell stimulation is in itself sufficient to induce demyelination, but it is also possible that the primary target of the immune reaction is the myelin sheath or some component thereof and that the T-cell infiltration is the result of the demyelination. Other investigators believe that an additional insult is required, as illustrated by the EAE animal model, in which myelin alone is not a sufficient factor but always requires an adjuvant immune stimulus. Also incorporated into most theories of the immune pathogenesis is an alteration of the blood-brain barrier, represented by adhesion of lymphocytes to endothelial cells. Whether this is an active interaction or a passive event triggered by antigenic attraction is not clear; nonetheless, these cell-vascular interactions have been incorporated into pathogenic theories and is the basis of newer treatments for MS.

As matters now stand, the focus of attention is on the pathogenic role of specific subsets of T lymphocytes, which regulate humoral immune responses either as potentiators (T-helper cells) or as inhibitors (T-suppressor cells) of immunoglobulin production by the B lymphocytes. So-called helper (CD4) T cells are found in abundance within MS plaques and surrounding venules (perivascular cuffing). It has been demonstrated that T-cell receptors respond to antigens presented by (major histocompatibility complex or MHC) class II molecules on macrophages and astrocytes. This interaction is thought to stimulate T-cell proliferation and a cascade of related cellular events, including the activation of B cells and macrophages and the secretion of cytokines (one of which is IFN-γ). These cellular events are accompanied by a breakdown of the blood-brain barrier and, if sufficiently intense, by destruction of myelin. A reduction in T cells, both helper and suppressor subsets, or an increase in the ratio of helper-suppressor cells, may precede an acute attack and appears to be associated with increasing disability in patients with MS. Always in the background is the
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element of genetic susceptibility, presumably making certain individuals prone to these immunologic events.

The foregoing data notwithstanding, it must be added that the immune mechanisms in MS are not fully specified and the autoimmune hypothesis is not beyond challenge. It is noteworthy that the prevalence of other diseases of presumed autoimmune origin is no higher in MS patients than in the general population (De Keyser, 1988). Moreover, the course of MS has been altered only inconsistently by the administration of any of the non-specific immunosuppressive therapies (Goodkin et al., 1992). Also worthy of comment is the relatively ineffective remyelination of the MS plaque. When remyelination of denuded axons occurs, thinly myelinated fibres are produced, creating areas of so called “shadow plaques”. Histological evidence suggests that some of the oligodendrocytes are destroyed in areas of active demyelination but also that some of the remaining ones have little ability to proliferate. Instead, there is an influx of oligodendroglial precursor cells, which mature into oligodendrocytes and provide the remaining axons with new myelin. Probably the astrocytic hyperplasia in regions of damage and the persistent inflammatory response account for some of the inadequacy of the reparative process (Prineas et al., 1993).

1.5 Rationale for conducting the Present Study

A perusal of literature reveals heterogeneity within the aetiology and pathophysiology of the human multiple sclerosis. Therefore study of the cellular changes following induction of autoimmune neuroinflammation in an animal model needs extensive understanding whether the described model fulfils the criteria of the human disease for extrapolation. The data generated would lead to a standardised animal model in our country where Wistar rats are used extensively and at the same time lead to the understanding some of the basic cellular changes following EAE. The present study has attempted to increase the literature regarding the human multiple sclerosis by following objectives
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Objectives

The present study was conducted to study the effect of multiple sclerosis on the cellular changes in Wistar rats with following main objectives

❖ To characterize animal model for human Multiple sclerosis (EAE) in Wistar rats
❖ To evaluate periventricular and cholinergic cell death after EAE
❖ To evaluate ventricular proliferation and role of CSPG3 in EAE.

The strategy adopted to achieve these objectives has been outlined in the Fig. 1.4.

1.6 Implications of the Present Study

The study of experimental autoimmune encephalomyelitis in Wistar rats will lend significant insight into the following

❖ The investigation will elaborate the disease patterns followed by two different myelin antigens in Wistar rats which can be helpful in studying the nature of the disease with dimensions of time and specificity.
❖ The mechanism of the cellular loss will enhance the present literate regarding the toxic metabolite generation in cellular phase of the disease and can be used for the pursuit of potential drug discovery.
❖ Proliferation of precursor cells following the disease and their migration to the injured structures and concomitant expression of extracellular matrix proteoglycans will uncover the key towards the endogenous cellular repair mechanisms.
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Figure 1.4: Schematic Representation of the Work Plan