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Review Article

NEUROINFLAMMATORY MECHANISMS OF CYTOKINES IN MULTIPLE SCLEROSIS

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Abstract

Multiple Sclerosis (MS) is an autoimmune disease characterized by the inflammatory demyelination of neurons in the central nervous system (CNS). There are various cytokines involves in the pathogenesis of Multiple Sclerosis such as interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1β), osteopontin, interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-18 (IL-108) and transforming growth factor-β (TGF- β). Mostly used animal models for Multiple Sclerosis is experimental autoimmune encephalomyelitis (EAE). A model induced in Dark Agouti rats by immunization with the N-terminal fragment of myelin oligodendrocyte glycoprotein. Moreover, CD^{4+} or T helper cells also play an important role in the initiation of antigen-specific immune responses. It is possible to regulate the immune response and induce tolerance to disease through manipulation of the T helper response. In the present study a detailed review on role of cytokines in Multiple Sclerosis shell carried out.

Keywords: - : Multiple Sclerosis; Cytokines; Demyelination; Plaques; Rat; EAE

Introduction

Multiple Sclerosis is an autoimmune disease of the brain and spinal cord characterized by the inflammatory demyelination of neurons in the central nervous system (CNS) [1],[2]. As in the case with many other autoimmune diseases, Multiple Sclerosis (MS) primarily occurs in women, with the onset of clinical symptoms occurring between 15 and 50 years of age. The clinical manifestations of Multiple Sclerosis are the result of an immune reaction consisting of the penetration of the blood-brain barrier (BBB), entrance into the CNS and recognition of the myelin basic protein (MBP) and proteolipid (PLP) as foreign. The immune system attack on these proteins induces the stripping of the protective coating of myelin and the eventual formation of plaques. These plaques or lesions can be found throughout the central nervous system but are most prominently found in the white matter, optic nerve ,brainstem, spinal cord and cerebellum. The formation of these plaques causes the conduction of action potentials along the axon to be reduced, resulting in neurocognitive or neuromuscular impairment. A clinical symptom of Multiple Sclerosis includes optic nerve dysfunction, internuclear ophthalmoplegia, upper motor neuron weakness, tremors, ataxia, sensory disturbances and autonomic dysfunction (Bansil). In fact, autopsy studies indicate that 20% of Multiple Sclerosis cases are clinically silent during life. Furthermore both sexes are affected, women more frequently and at an earlier age. The mean duration of the disease is over 20 years. Moreover, attacks of Multiple Sclerosis are associated with changes in peripheral blood monocyte and lymphocyte properties. Besides, this viral infection of brain remains a possible cause of multiple sclerosis, despite the fact that all attempts to isolate, rescue, or "passage" a virus from Multiple Sclerosis brains or to visualize a virus within them has failed. Spinal cord lesions produce a myriad of motor and sensory problems. Corticospinal tract interruption results in the classical features of upper motor neuron dysfunction (weakness, spasticity, hyperreflexia, clonus, babinski response, loss of abdominal skin reflexes). Severe spinal cord lesions can result in loss of function, sometimes total, below the level of the lesion and less complete lesions can result in the hemi cord syndrome of Brown-sequard. Moreover, cytokines plays an important role in the

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pathogenesis of Multiple Sclerosis as evidenced by altered cytokine profiles in the CNS and peripheral mononuclear cells of Multiple Sclerosis patients. Oligodendrocytes and microglia constitutively express in brain of Multiple Sclerosis patients. There are various cytokines involves in the pathogenesis of Multiple Sclerosis such as interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1β), osteopontin, interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-6 (IL-6),

The brain is the command center of the body. In addition to thinking and feeling, it receives information and sends order to different parts of the body. Orders from the brain travel through the spinal cord. From the spinal cord, orders travel to the rest of the body through peripheral nerves. Together, the brain and spinal cord are called the "Central Nervous System." The nerves in the rest of the body are called the "peripheral nervous system." Different areas of the brain control different functions. Like other tissue in the body, the brain is made of cells. The cells of the brain and nerves are called neurons. Each neuron has a body and an axon. Axons are long fibers that are similar to electrical wires. A special material called myelin covers axons [1]. Myelin, which provides a covering or insulation for nerves, improves the conduction of impulses along the nerves and also is important for maintaining the health of the nerves. In multiple sclerosis, the myelin in certain parts of the brain, spinal cord, or CNS is destroyed [3]. In multiple sclerosis, the myelin that covers nerve cells becomes inflamed, swollen, and detached. It is then destroyed, forming a scar over the axons. Sclerosis means scar. When myelin is destroyed, the neurons communicate less effectively, causing the symptoms of MS. For instance, if the myelin of vision neurons is destroyed, the vision is affected. If the myelin of muscle neurons is destroyed, the muscle becomes weak [1]. In multiple sclerosis, inflammation causes the myelin to disappear. Consequently, the electrical impulses that travel along the nerves decelerate, that is, become slower. In addition, the nerves themselves are damaged. As more and more nerves are affected, a person experiences a progressive interference with functions that are controlled by the nervous system such as vision, speech, walking, writing, and memory [3]. Multiple Sclerosis (MS) is an important disease. The World Health Organization (WHO) has placed MS on its `Top 100' list of diseases of great importance, i.e., diseases that cause considerable `loss of health' in a global perspective [4]. There are two types of MS. The most common type is "relapsing-remitting (RR) MS"; which affects 90% of patients with MS [{1}]. In "primary-progressive (PP) MS", there is a continuous, gradual decline in a person's physical abilities from the outset rather than relapses. About 10%-20% of individuals begin with PP-MS. Those beginning with RR-MS can then enter a phase where relapses are rare but more disability accumulates, and are said to have "secondary-progressive (SP) MS". About 50% of RR-MS individuals will develop SP-MS within 10 years [3]. The cause of MS is elusive and there is still no cure for MS. Several lines of evidence suggest that an immune mediated mechanism plays a role in MS. The long-established intense inflammatory reaction seen in the MS lesion indicates the involvement of the immune system, probably in disease pathogenesis. An association between immune response genes and MS is also well documented. As further support, alterations of the immune system and the involvement of a number of immune variables in the disease have frequently been reported in MS. A certain class of these immune variables namely, the cytokines incontestably influences the course of MS. Treatment of MS patients with recombinant IFN-γ induced clinical exacerbations; making it plausible that endogenously produced IFN-γ might also have detrimental effects in MS. The related cytokine IFN-β has, on the other hand, beneficial effects in both relapsing remitting and secondary progressive4 MS. Although the involvement of cytokines in the disease development is beyond question, the levels of cytokines in MS and their interplay are still only partly known [4]. Cytokines are critical components of the immune inflammatory process and are implicated in oligodendrocyte cell death, axonal degeneration and neuronal dysfunction, which are key features in MS pathology and the substrate of irreversible deficits [5].

MS is a common case of chronic disability in young adults. Disease is characterised by episodes separated in time and space, in which areas of the central nervous system become demyelinated (plaques). These plaques often are centred around venules. Around half of all patients with the disease are unable to walk unaided within 15 years of diagnosis [5]. As in the case with many other autoimmune diseases, Multiple Sclerosis (MS) primarily occurs in women [2]. Usually, a person is diagnosed with Multiple Sclerosis between 20 and 50 years of age, but Multiple Sclerosis has been diagnosed in children and in the elderly. Both sexes are affected; Women are twice as likely as men to be affected by Multiple Sclerosis earlier in life [3]. The mean duration of the disease is over 20 years [6].

Discovery of MS

Until the early years of the 19th century, physicians relied on

superstition, hearsay, and the wisdom of the ancients to care for the sick. Medical ideas were not scientifically tested. Even so, physicians were sometimes good observers and we can identify people who undoubtedly had MS from descriptions written as long ago as the Middle Ages. MS has always been with us.

MS was among the first diseases to be described scientifically. The 19th-century doctors did not understand what they saw and recorded, but drawings from autopsies done as early as 1838 clearly show what we today recognize as MS. Then, in 1868, Jean-Martin Charcot, a professor of neurology at the University of Paris, who has been called "the father of neurology", carefully, examined a young woman with a tremor of a sort he had never seen before. He noted her other neurological problems including slurred speech and abnormal eye movements, and compared them to those of other patients he had seen. When she died, he examined her brain and found the characteristic scars or "plaques" of MS.

Dr. Charcot wrote a complete description of the disease and the changes in the brain which accompany it. However, he was baffled by its cause and frustrated by its resistance to all of his treatments. They included electrical stimulation and strychnine-because this poison is a nerve stimulant. He also tried injections of gold and silver, as they were somewhat helpful in the other major nerve disorder common at that time-syphilis [7]. It was Dr. Jean Martin Charcot (1825 - 1893) who first scientifically described, documented, and named the disease process, we still call Multiple Sclerosis. So named from the many scars found widely dispersed throughout the central nervous system (CNS), but are usually found to be arrayed in a symmetrical pattern near the Cerebrum's Lateral Ventricles. The first patient Dr. Freud ever treated was his former Nanny, who had Multiple Sclerosis.

Historical Facts:

1400:- The earliest written record of someone with MS was Lydwina of Schieden, dutch patron Saint of Ice Skaters.

1838:- Medical drawings clearly show what we today recognize as MS, but 19th century doctors did not understand what they saw and recorded.

1868:- Jean-Martin Charcot, professor of neurology at the University of Paris, wrote the first complete description of MS and the changes in the brain which accompany it.

1878:- Myelin was discovered by Dr. Louis Ranvier.

1916:- Detailed microscopic description made by James Dawson revealed the basic damage done in MS.

1919:- Abnormalities in the spinal fluid were discovered in MS, but their significance remained puzzling for decades.

1925:- Lord Edgar Douglas Adrian recorded the first electrical nerve transmissions, which helped prove demyelinated nerve cannot sustain electrical impulses.

1928:- The oligodendrocyte cell that makes myelin was discovered.

1935:- Dr. Thomas Rivers demonstrated that nerve tissue, not viruses, produced a MS-like illness. This animal form of MS, called EAE or experimental allergic encephalomyelitis, paved the way to our present theories of auto- immunity, for it demonstrated the body can generate an immunologic attack against itself.

1946:- National Multiple Sclerosis Society founded by Sylvia Lawry.

1948:- Under an early NMSS grant, oligoclonal bands discovered in the spinal fluid by Elvin Kabat and others provided a diagnostic test suggestive of MS and linking MS to immune system problems.

1965:- Definite criteria for MS diagnosis developed by NMSS expert committee and White blood cells that react against a protein in nerve insulating myelin were discovered in MS [8].

1969-1970:- ACTH used to treat MS exacerbations. This was the first controlled trial of a successful treatment for MS: it used newly standardized diagnostic criteria and rating scales to evaluate the efficacy of treatment.

1981:- MRI first used to examine a person with MS. MRI revolutionized diagnosis and provided evidence that MS is a constantly active disease even when symptoms abate.

1993:- Beta-interferon 1b (Betaseron) approved as the first drug to alter the course of MS [7].

Epidemiology

Epidemiological studies have been used to help identify possible exogenous factors which may be associated with multiple sclerosis. These studies have shown that the epidemiology of MS is very different from other autoimmune diseases such as rheumatoid arthritis and lupus, and further support the role of exogenous factors in the development of MS. The most prolific research of the epidemiology of MS has come from John F. Kurtzke. In his article, "Epidemiologic Evidence for Multiple Sclerosis as an Infection", Kurtzke describes the geographic and time distribution of MS cases throughout the world. In the United States, the pattern of MS appears to be residence based with a higher annual rate of MS associated mortality occurring in states above the 37˚ North latitude. This data also indicates that there is little difference between MS death rates in

rural areas compared to urban areas; however when also categorized according to race, whites living in urban areas generally have higher rates of age-adjusted MS mortality. In Europe, the high prevalence zone of MS cases has been shown to be between 44˚ and 64˚ North latitude.

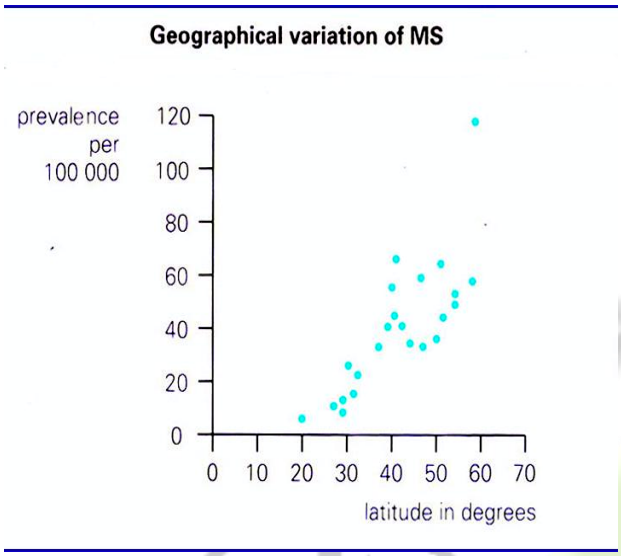


Figure- 1 Geographical Variation of MS

Some of Kurtzke's most extensive work on the epidemiology of MS has investigated the epidemics of Multiple Sclerosis on the Faroe Islands. Kurtzke continued to investigate the epidemiology of MS on the Faroe Islands and discovered that there were almost no reported cases of Multiple Sclerosis among residents of the Faroe Islands before 1943. In 1974, Kurtzke began a retrospective epidemiologic study to determine the nature of the association between clinical onset of Multiple Sclerosis and infection by a MS-associated pathogen. The study included all MS patients who were residents the Faroe Islands and had not lived off the islands for longer than 2 years [1]. It is estimated that in the United States ~350,000 individuals suffer from MS [3].

Pathogenesis

Histological assessment is only occasionally necessary. This is usually in patients with atypical presentations. Studies comparing the clinical, radiological and post-mortem neuropathological features of MS are continuing to provide insights into this disease

Severe demyelination prevents conduction of nerve impulses through myelinated axons; partial myelin injury results in delayed conduction, inability to transmit fast trains of impulses.

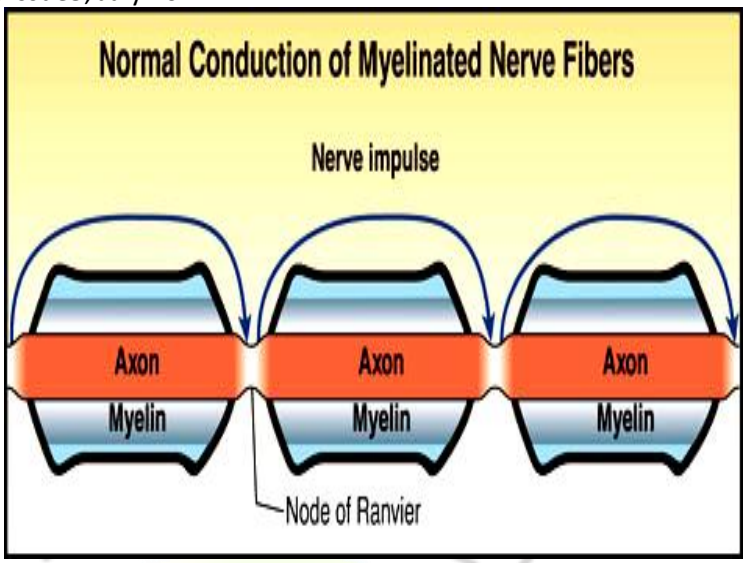


Figure- 2 Normal Conduction of Myelinated Nerve Fibers

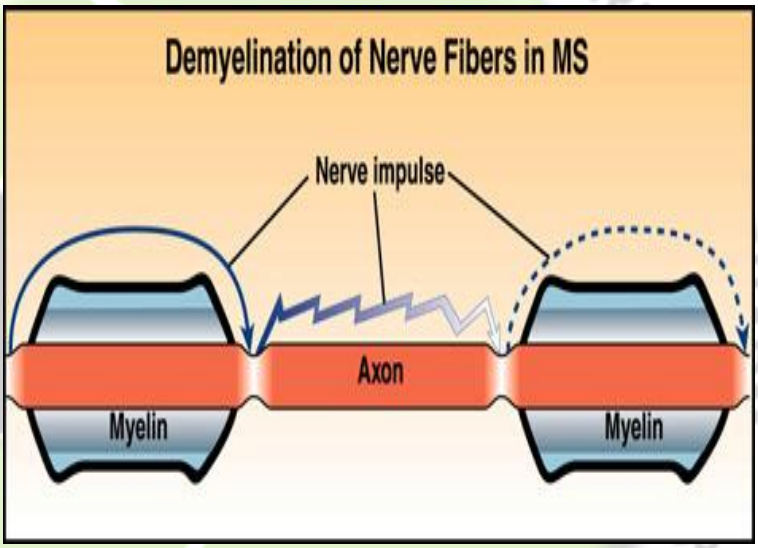


Figure- 3 Demyelination of Nerve Fibers in MS

Autoimmune response - damage caused by inflammatory cells – T cells and macrophages. Structure of plaques are-
1.Evidence for T cell responses against myelin basic protein

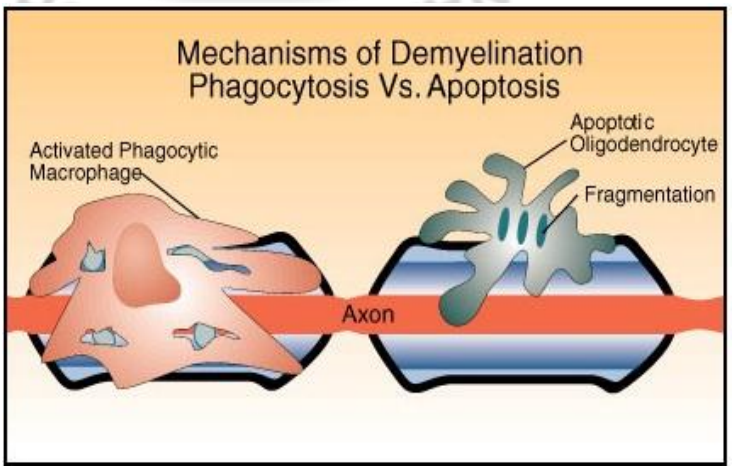


Figure- 4 Mechanism of Demyelination

2. Pathogenesis mediated mainly by T cells and macrophages;-

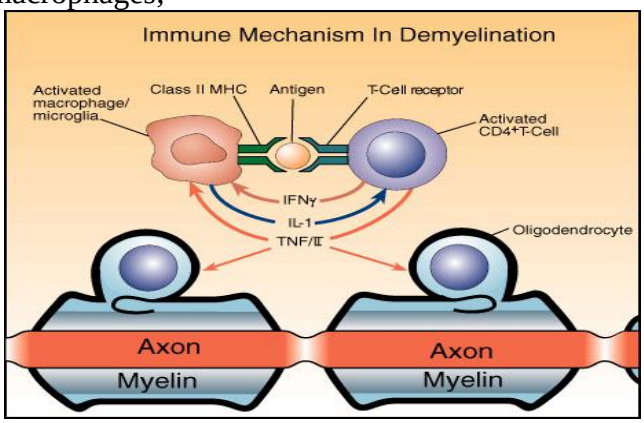


Figure- 5 Immune Mechanism in Demyelination

Figure- 6 Effector Phase of the Immune Response in MS

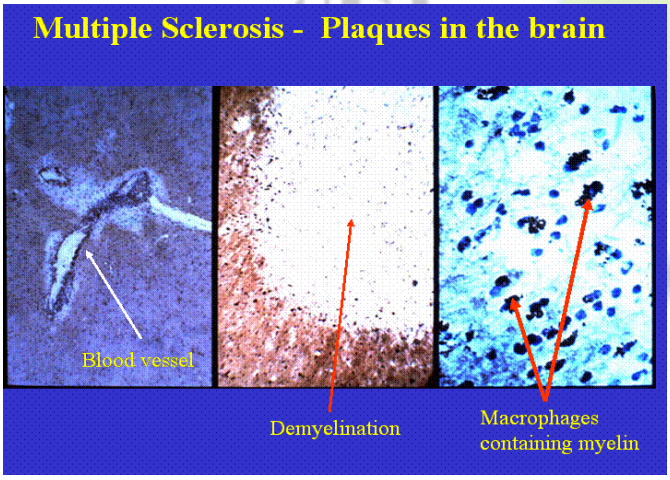


Figure- 7 Plaques in the Brain

Role of infectious agents in etiopathogenesis:-

Attempts to identify an environmental and presumably infective factor have been unsuccessful. However, 40% of new clinical events are associated with a presumed virus infection and 10% of all infections occurring in MS patients are followed by a new relapse strongly suggestive of a viral aetiology [4].

Causes of Multiple Sclerosis

Scientists do not know what causes the destruction of myelin in MS. Some scientists believe that cells of the immune system attack the myelin in the CNS. The immune system usually attacks germs and foreign bodies. Cells of the immune system may be attacking myelin in MS because they mistake it for a foreign, harmful material. This type of disease is called an autoimmune disease. Some researchers think that after certain types of viral infections, the immune system starts attacking the myelin of the CNS as if it were the virus [1]. Clinical manifestations of Multiple Sclerosis are the result of an immune reaction consisting of the penetration of the blood-brain barrier (BBB), entrance into the CNS and recognition of the myelin basic protein (MBP) and proteolipid (PLP) as foreign. The immune system attack on these proteins induces the stripping of the protective coating of myelin and the eventual formation of plaques. These plaques or lesions can be found throughout the central nervous system but are most prominently found in the white matter, optic nerve, brainstem, spinal cord and cerebellum. The formation of these plaques causes the conduction of action potentials along the axon to be reduced, resulting in neurocognitive or neuromuscular impairment [2].

The cause of Multiple Sclerosis is still unknown. In the last 20 years, researchers have focused on disorders of the immune system and genetics for explanations. The immune system is the body's defender and is highly organized and regulated. If triggered by an aggressor or foreign object, the immune system mounts a defensive action which identifies and attacks the invader and then withdraws. This process depends upon rapid communication among the immune cells and the production of cells that can destroy the intruder. In multiple sclerosis, researchers suspect that a foreign agent such as a virus alters the immune system so that the immune system perceives myelin as an intruder and attacks it. The attack by the immune system on the tissues that it is supposed to protect is called autoimmunity, and Multiple Sclerosis is believed to be a disease of autoimmunity. While some of the myelin may be repaired after the assault, some of the nerves are stripped of their myelin covering (become demyelinated). Scarring also occurs, and material is deposited into the scars and forms plaques [3].

Is Multiple Sclerosis inherited?

Although its role is unclear, genetics may play a role in multiple sclerosis. European gypsies, Eskimos and African Bantu essentially do not develop multiple sclerosis, while Native Indians of North and South America, Japanese and other Asian groups have a low incidence. The general population has less than a one-percent chance of developing multiple sclerosis. The chance increases in families where a first-degree relative has the disease. Thus, a brother, sister, parent, or child of a person with Multiple Sclerosis stands a

1% to 4% chance of developing multiple sclerosis.Similarly, an identical twin runs a nearly 30% chance of acquiring Multiple Sclerosis whereas a non-identical twin has only a 4% chance if the other twin has the disease. These statistics suggest that genetic factors play a major role in multiple sclerosis. However, other data suggest that environmental factors also play an important role [3].

Neuroinflammatory Mechanism Of Cytokines

Severe spinal cord lesions can result in loss of function, sometimes total, below the level of the lesion and less complete lesions can result in the hemi cord syndrome of Brown-sequard [9]. Moreover, cytokines plays an important role in the pathogenesis of Multiple Sclerosis as evidenced by altered cytokine profiles in the CNS and peripheral mononuclear cells of Multiple Sclerosis patients. A recent study demonstrated that oligodendrocytes and microglia constitutively express cytokine receptors, particularly of the Th2 type, and that these are up-regulated in MS. There are various cytokines involves in the pathogenesis of Multiple Sclerosis such as:-

1. Interferon-γ (INF-γ)
2. Tumor necrosis factor-α (TNF-α)
3. Interleukin-1 (IL-1β)
4. Interleukin-3 (IL-3)
5. Interleukin-4 (IL-4)
6. Interleukin-6 (IL-6)
7. Interleukin-10 (IL-10)
8. Interleukin-12 (IL-12)
9. Interleukin-18 (IL-18)
10. Osteopontin
11. Transforming growth factor-β (TGF- β)

Interferon-γ (INF-γ)

IFN-γ is produced by T cells and NK cells. Its functions include activation of mononuclear cells. It is typically produced by Th1 cells and is the principal marker of a Th1 response. IFN-γ is expressed in the CNS at the onset of EAE, its expression increases during the peak of disease and decreases during disease remission. Over-expression of IFN-γ in the CNS of mice results in a progressive demyelinating disease. In MS patients, a progressive course of disease was significantly more frequent in carriers of the IFN-γ receptor-2 (IFNGR2). In addition, T cell receptor-mediated IFN-γ and IL-10 secretion are increased in RR and SP patients. Clinical attacks correlate with increased IFN-γ production in vitro and administration of IFN-γ to MS patients precipitated clinical attacks. MS patients experiencing relapse have significantly increased peripheral blood mononuclear cell (PBMC) interferon-γ (IFN-γ) production after PHA stimulation compared with patients in remission, but this production was reduced after treatment with interferon-β. Similar results were found in patients with primary progressive MS. These results suggest that IFN-γ has an activating role in sustaining inflammation in EAE and MS, although it may have regulatory functions that become apparent in its complete absence.

Tumor necrosis factor-α (TNF-α)

TNF-α is a soluble 17-kDa protein composed of identical subunits. It is released in response to bacterial infections and is an endogenous pyrogen producing cachexia and inducing acute phase reactants. Intraperitoneal injections of anti-TNF-α antibody abrogated clinical EAE in an SJL/J adoptive transfer model, and CNS tissue from these mice showed no pathological infiltrates or demyelination. Mice transgenic for TNF-α expression in the CNS develop a spontaneous chronic inflammatory demyelinating disease, characterized by infiltration of CD4+, CD8+ T cells, astrogliosis, and demyelination, which was completely reversed by peripheral administration of a neutralizing murine anti-TNF-α antibody. These results point to an inflammatory role for TNF and suggest that absence of TNF should ameliorate EAE. TNF-α has a direct effect on the induction of oligodendrocyte apoptosis and demyelination.

In humans, expression of TNF-α in the CNS is significantly up-regulated in MS lesions and is expressed by macrophages, microglia, and astrocytes in chronic active lesion. Several Studies have found a positive correlation between TNF-α levels and clinical course of MS; increased production of TNF-α after in vitro stimulation was reported to precede the onset of the relapse by 2 weeks. TNF- α level in serum and CSF are associated with disease activity by MRI. It has been found that levels of soluble TNFR1 and soluble TNFR2 are increased in patients with chronic progressive MS; these increments correlated with increases in the Expanded Disability Status Scale (EDSS) in chronic progressive disease and the appearance of new gadolinium enhancing lesion. Furthermore, lymphotoxin and TNF-α level were reported to be increased in MS patients compared to normal controls.

Interleukin-1 (IL-1β)

IL-1 is a 17-kDa protein that is mostly produced by monocytes and macrophages but is also produced by endothelial cells, B cells, and activated T cells and is upregulated in the CNS during the induction of EAE. IL-1β injected into the rat brain at the time of experimental ischemia or traumatic injury causes increased neuronal cell death and edema, and over-expression of IL-1 receptor antagonists in the CNS blocks these effects. Addition of

IL-1 in vitro results in neuronal apoptosis and its neurotoxic effects appear to be dependent on the expression of iNOS. Moreover, IL-1β causes oligodendrocyte death in cocultur with astrocytes and microglia, but not in pure culture of oligodendrocytes alone. The mechanism appears to be related to impairment in the uptake and metabolism of glutamate by astrocytes, since glutamate receptor antagonists blocked the toxicity. Thus, IL-1, alone or in combination with other factors, may be important in neuronal and axonal damage in the CNS in MS. However, IL-1 has also been shown to induce the production of nerve growth factor (NGF) in vitro, suggesting that it may also have some neuroprotective effects

Interleukin-3 (IL-3)

IL-3 is a cytokine growth factor produced by CD4+ T cells and microglia. IL-3 mRNA is up-regulated in the CNS of MS patients compared with controls by microarray analysis. IL-3 is present in the CNS during the acute phase of EAE. Systemic over-expression of IL-3 in the CNS of mice results in severe neurological dysfunction characterized by degenerated, vacuolated neurons, predominantly motorneurons. Interestingly, in this model, there was no demyelination in the CNS. In contrast, transgenic expression of IL-3 under the astrocytic glial fibrillary acid protein (GFAP) promoter resulted in a predominantly demyelinating disease with minimal axonal pathology.

Interleukin-4 (IL-4)

IL-4 is produced by CD4+ Th2 cells and participates in the differentiation and growth of B cells. In vitro, IL-4 inhibits the activation of Th1 cells, and this, in turn, decreases the production of IL-1 and TNF-α. IL-4 amplifies the Th2 response through activation of its receptor on T cells, resulting in activation of the intracellular signaling factor STAT6, which induces transcription of Th2-related genes. Studies in IL-4 knockout mice have led to unexpected and often conflicting results. IL-4-deficient mice on both the PLJ and C57BL/6 background had a similar incidence, mean maximal score or fatality rate as wild-type mice. There was a slight prolongation of disease in PLJ IL-4-deficient mice, suggesting that IL-4 may play a role in the termination of disease. There have been a number of therapeutic strategies that increase IL-4 production including protection from EAE via induction of oral tolerance using myelin proteins or by blocking T cell costimulatory signals with CTLA4Ig. The overall conclusion appears to be that up-regulation of IL-4 may reduce the severity of EAE, while its absence generally does not alter the course of disease, possibly because in the absence of IL- 4 other Th2 cytokines may substitute for its function and contribute to the induction of tolerance in EAE. Studies of IL-4 in MS are limited. IL-4 was expressed in high levels in both acute and chronic active MS lesions. Increased expression of IL-4 secretion by CD3 stimulated PBMCs was demonstrated in secondary progressive MS patients treated with cyclophosphamide/ methylprednisolone compared with untreated patients

Interleukin-6 (IL-6)

Mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells synthesize the Th1 cytokine IL-6 in response to IL-1 and to a lesser extent, TNF-α. IL-6 is also synthesized by some activated T cells and by astrocytes and microglia in the CNS.

It is important for the growth and differentiation of B cells. IL-6 is up-regulated in the CNS during the induction phase of disease in both murine MBP and MOG induced models of EAE and in a Lewis rat model. Over-expression of IL-6 in the CNS results in a neurodegenerative pathology. Administration of an anti-IL-6-neutralizing antibody reduces the incidence of actively induced and adoptively transferred EAE. In MS, IL-6-positive cells were identified as macrophages and astrocytes by morphological criteria during neuropathological examination. Approximately 10–17% of the astrocytes and up to 2% of the macrophages within the lesion expressed IL-6. But the highest numbers of IL-6 expressing cells were found in inactive demyelinating lesions. There was a significant increase of IL-6-positive cells in lesions with oligodendrocyte preservation, whereas absence of IL-6 expression correlated with oligodendrocyte loss. In addition T cells from MS patients had significantly more IL-6 receptor.

Interleukin-10 (IL-10)

IL-10 is produced by monocytes, macrophages, B cells and Th2 cells. It inhibits the production of several cytokines, including IL-1, TNF-α and the proliferation of T cells in vitro. Its primary function is to inhibit cytokine production by macrophages. It also reduces MHC II and costimulatory molecule expression. IL-10 mRNA was continuously expressed throughout the course of EAE in SJL mice immunized with PLP. Systemic administration of human recombinant IL-10 suppressed IFN-γ induced MHC class II up-regulation in rat peritoneal macrophages and reduced the incidence and severity of EAE in Lewis rats. Mice over-expressing human IL-10, but not murine IL-10 under the MHC class II promoter were resistant to the development of EAE. In humans, studies using PCR show that PBMCs have a decreased level of IL-10 before the onset of an exacerbation in RR MS patients. Levels of IL-10 were significantly lower in SP patients compared with RR patient

4 weeks before the occurrence of MRI activity and 6 weeks before a clinical relapse. PLP-reactive T cells clones isolated from patients during acute disease demonstrated a bias towards a Th1 phenotype with high production of IFN-γ and TNF-α. During remission the isolated clones showed increased production of IL-10 and TGF-β compared with controls.0

Interleukin-12 (IL-12)

IL-12 is produced principally by monocytes and dendritic cells and is critical for the differentiation of Th1 cells. IL-12 is a disulfide-linked heterodimer p70 complex composed of 1 p40 and 1 p35 subunit. The p35 component is synthesized by most cell types, while the p40 component is synthesized only by mononuclear phagocytes and dendritic cells. IL-12 mRNA was found to be elevated immediately prior to the onset of disease in a monophasic EAE rat model as well as in a relapsing murine model. In a Lewis rat EAE model, IL-12 p40 mRNA levels as measured by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) increased a few days after the peak of T cell infiltration into the CNS as did IFN-γ synthesis. In contrast, IL-12 p35 expression remained unchanged during the course of disease. Systemic administration of recombinant IL-12 to rats exacerbated the clinical symptoms of EAE and induced relapses in animals that had recovered from the initial clinical attack. Administration of IL-12 worsened disease even in the presence of anti-IFN-γ, suggesting that IL-12 may regulate disease through an IFN-γ independent mechanism. In humans, IL-12 was found to be up-regulated in patients with MS; IL-12 p40 is expressed in acute MS plaques but not in infarcts from the same brain. IL-12 p40 mRNA levels in unstimulated PBMCs were increased in SP and RR patients compared with controls and correlated with the development of active lesions on MRI. In contrast, IL-12 p35 were decreased in both groups compared with controls. Serum levels of IL-12 p70 were reported to be increased in CP patients, and production of IL-12 p70 by stimulated PBMCs was higher in CP patients than controls or patients with acute MS. More importantly, therapies such as cyclophosphamide normalize the high levels of IL-12 expressed by monocytes in CP MS. Furthermore, IFN-β, one of the approved MS therapies, inhibits IL-12 and induces reciprocal changes in IL-10. In vivo administration of salbutamol (Albuterol) down-regulates the expression of IL-12 by monocytes in MS patients. A clinical trial of Albuterol in combination with Copaxone is currently underway. Clinical trials with anti-IL-12 antibodies have been initiated for treatment of MS.

Interleukin-18 (IL-18)

IL-18 is synthesized as an inactive precursor protein that exhibits structural homology to IL-1 and must be processed by caspase 1 for functional activation. IL-18 mRNA expression as determined by semiquantitative RT-PCR correlates with disease course and lymphocytic infiltration of the CNS in EAE. Patients with MS exhibit increased serum concentrations of IL-18. Specifically, IL-18 is higher in the serum of SP MS compared with RR MS and in patients during exacerbations compared with patients with stable disease. Anti-CD3/CD28-induced IL-18 production by PBMCs is increased in both RR and SP MS and correlates with disease duration in SP MS.

Osteopontin

Osteopontin (OPN) is a pleotrophic cytokine that activates macrophage chemotaxis, promotes Th1 responses, and activates B-cells. OPN was increased in cDNA libraries derived from plaques dissected from brains of patients with MS and absent from control brains and brains from rats with EAE. Osteopontin-deficient mice are resistant to progressive EAE. In RR MS patients OPN protein levels in plasma are increased, while levels in PP and SP MS patients were similar to healthy control levels. Interestingly, active RR patients had higher OPN protein levels than patients without relapses.

Transforming growth factor-β (TGF- β)

The TGF family of molecules has 2 principal members; transforming growth factor-α (TGF-α) is a polypeptide growth factor for epithelial and mesenchymal cells, and TGF-β a factor required for cell survival. The TGF-β family of molecules consists of TGF-β1, TGF-β2, and TGF-β3. TGF-β is produced by T cells, predominantly of the Th3 type, activated monocytes, astrocytes, and microglia. The actions of TGF-β are highly pleiotropic; it inhibits proliferation of T cells, inhibits maturation of cytotoxic lymphocytes and NK cells and inhibits activation of macrophages.. It also acts on other cells to counteract the activities of pro-inflammatory cytokines. TGF-β2 decreases migration of lymphocytes in vitro and homing of cells into the CNS in vivo. TGF-β1 also inhibits TNF-α production. Mice deficient for TGF-β1 develop an uncontrolled multi-focal inflammatory disorder. In MS, TGF-β expression was decreased in the CNS, associated with endothelial cells, and TGF-β mRNA in PBMCs of RR MS patients is decreased. In the Lewis rat model of EAE, expression of TGF-β mRNA in the CNS increased at the peak of disease and shortly preceding recovery. TGF-β protein is expressed during recovery of EAE in Lewis rats. However, in the DA rat model of EAE, expression of TGF-β was almost absent.

TGF-β1, TGF-β2, and TGF-β3 were shown to be present in perivascular inflammatory CNS lesions of SJL mice with EAE Most importantly; TGF-β levels were increased after 6 months of therapy with IFN-β in RR MS, suggesting a normalization of the levels that may decrease ongoing inflammation. MS patients orally polarized with daily treatments of bovine myelin were found to have increased frequencies of TGF-β secreting T cell lines in response to MBP or PLP [10].

Clinical Signs & Symptoms of Multiple Sclerosis

Symptoms of Multiple Sclerosis may be single or multiple and may range from mild to severe in intensity and short to long in duration. Complete or partial remission from
symptoms occurs early in about 70% of individuals with multiple sclerosis.

Visual disturbances may be the first symptoms of multiple sclerosis, but they usually subside. A person may notice a patch of blurred vision, red-to-orange or red-to-gray distortions (color desaturation), or monocular visual loss (loss of vision in one eye). Visual symptoms due to optic nerve inflammation (optic neuritis) in Multiple Sclerosis usually are accompanied or preceded by eye pain [3] and double vision (diplopia), involuntary eye movement (nystagmus) [11].

Limb weakness with or without difficulties with coordination and balance may occur early.

Muscle spasms, fatigue, numbness, and prickling pain are common symptoms.

There may be a loss of sensation, speech impediment (typically a problem articulating words), tremors, or dizziness.

Fifty-percent of people experience mental changes such as:

1. Decreased concentration,
2. Attention deficits,
3. Some degree of memory loss.
4. Inability to perform sequential tasks.
5. Impairment in judgment.
6. Depression or
7. An uncontrollable urge to laugh and weep [3].

Balance and equilibrium abnormalities (e.g., dizziness, vertigo, uncoordinated movements, tremor)

Bladder and bowel dysfunction (e.g., urgency, incontinence, nocturia, constipation)

Behavioral changes (e.g., mood swings, depression)

Cognitive dysfunction (e.g., impaired memory, reasoning, concentration)

Facial numbness

Sexual dysfunction (e.g., erectile dysfunction, sexual inactivity) [11].

As the disease worsens, individuals may experience sexual dysfunction or reduced bowel and bladder control. Heat appears to intensify Multiple Sclerosis symptoms for about 60% of those with the disease. Pregnancy seems to reduce the number of attacks, especially during the third trimester [3].

Common	**Uncommon**
Sensory problems (numbness or Tingling of a body part)	Bladder
.	Bowel problems
Weakness	
.	Sexual dysfunction
Difficulty walking	
.	Cognitive difficulties
Monocular decreased vision	
.	Pain
Poor coordination [12]	

Symptoms of the disease vary, depending on where the damage occurs, and range from minor physical annoyances to major disabilities [11].

Diagnosis of Multiple Sclerosis

Due to the broad range and subtleties of symptoms, Multiple Sclerosis may not be diagnosed for months to years after the onset of symptoms. Physicians, particularly neurologists, take detailed histories and perform complete physical and neurological examinations.

MRI (Magnetic Resonance Imaging) scans with intravenous gadolinium helps to identify, describe, and in some instances date lesions in the brain (plaques).

An electro-physiological test, evoked potentials, examines the impulses traveling through the nerves to determine if the impulses are moving normally or too slowly.

Finally, examining the cerebro-spinal fluid that surrounds the brain and spinal cord may identify abnormal chemicals (antibodies) or cells that suggest the presence of multiple sclerosis.

Collectively, these three tests help the physician in confirming the diagnosis of multiple sclerosis. For a definite diagnosis of multiple sclerosis, dissemination in time (at least two separate symptomatic events or changes on MRI over time) and in anatomical space (at least two separate locations within the central nervous system, which can be demonstrated by MRI or neurological exam) must be demonstrated [3].

Clinical features:

The clinical features are central to the diagnosis. The distribution of lesions in time and space are characteristic of the disease

Diagnosis requires occurrence of at least two lesions in the central nervous system, separated in time and space

Signs and symptoms that may occur include: optic atrophy, cerebellar dysarthia, ataxia, urinary symptoms, spastic paraparesis, dementia, and mood disturbance.

MRI of brain/spinal cord (plaques)

Visual evoked potential (delayed central conduction in the visual pathways)

Cerebrospinal fluid examination findings are non-specific:

Increased lymphocytes in active phase, raised protein. Oligoclonal bands on electrophoresis (local synthesis of immunoglobulins)

Any of these tests can produce false positives and false negatives [5].

Plaques detected by MRI scan in the brain

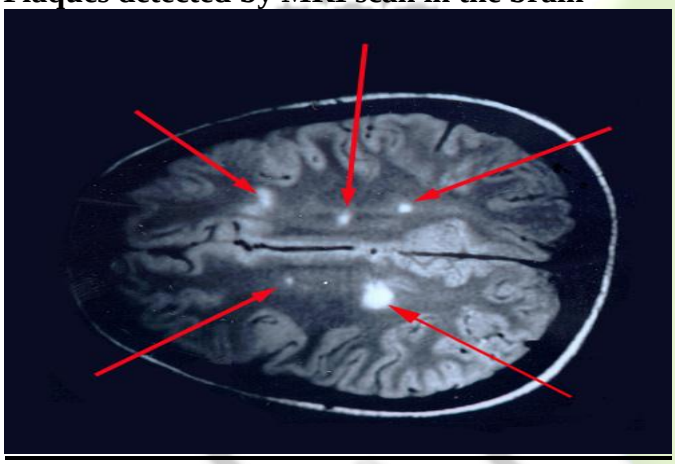


Figure-8 Plaques detected by MRI scan in the brain

Plaque detected by MRI scan in the spinal cord

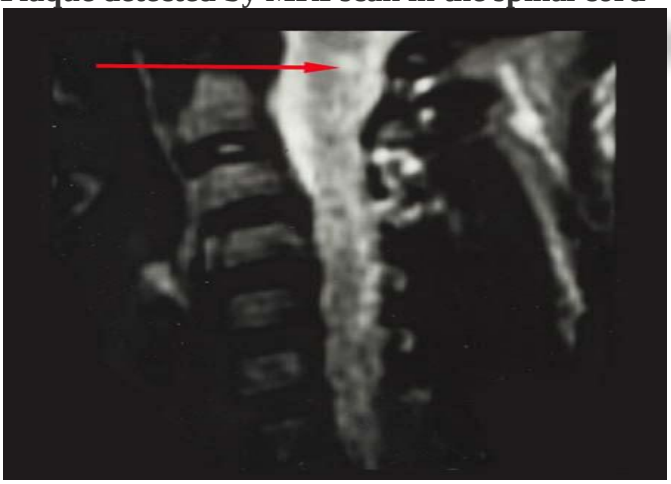


Figure-9 Plaque detected by MRI scan in the spinal cord

monitor. Brain stem auditory evoked potentials are

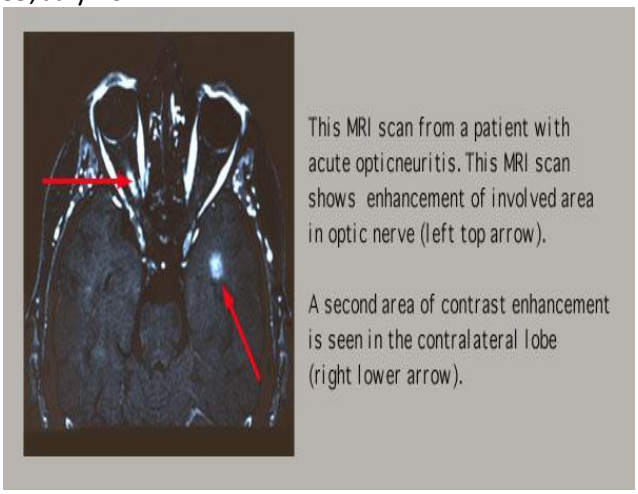
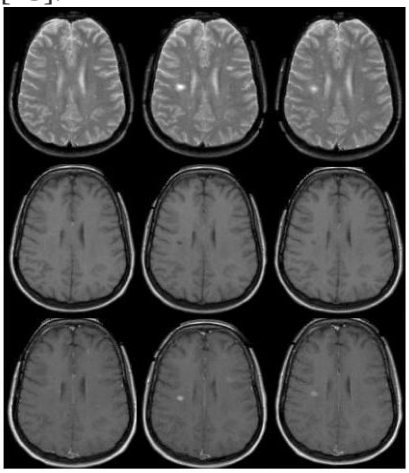


Figure-10 Lesions seen in the area of optic nerve [5]

Tests can facilitate the diagnosis of MS, particularly when there are fewer than two abnormal signs on the neurological examination. In this instance, an abnormal test can be used to document a second sign.

Magnetic resonance imaging (MRI):

The brain MRI is the most sensitive test for detecting structural abnormalities due to MS-related disease activity. MRI scans show focal brain abnormalities in more than 90% of patients with clinically definite MS. The MRI scan can also distinguish between new or old lesions, and thus provides a measure of disease activity. The MRI is also useful for excluding other neurological conditions that might be confused with MS. Because the imaging abnormalities seen in MS patients can also be seen in other medical conditions, a diagnosis of definite MS cannot be based solely upon the MRI [13].



Each column contains three identical MRI slices obtained the same day with different image sequences. From top to bottom T2-weighted, T1-weighted and T1-weighted with gadolinium injection. The three sets of images were made one month apart.

Figure-10 MRI scans of brain

Evoked Potentials:

Evoked potentials reflect changes in the electrical activity that occurs within the CNS due to sensory input (a stimulus). The electrical response to the stimulus is measured by electrodes applied to the scalp. Visual evoked potentials are obtained by stimulating the eye with a checkerboard pattern of light and dark squares that are alternated on a television

produced by click sounds applied through earphones. Somatosensory evoked potentials are produced by electrically stimulating nerves in the hands or feet. The time between application of the stimulus and occurrence of the evoked potential provides a measure of the nerve's ability to conduct electrical impulses from one point to another. If the response time is slowed, this suggests that the nerve pathway is not functioning properly as a result of demyelination. These tests are abnormal in 70-90% of patients with clinically definite MS and often detect abnormalities that are not apparent on neurological examination. Because these tests measure function within the brain or spinal cord, they complement the information about brain structure provided by the MRI.

Lumbar Puncture (Spinal Tap):

Cerebrospinal fluid abnormalities are detected in 80-90% of patients with clinically definite MS. These abnormalities include an increase in the number of cells and immunoglobulin proteins suggesting an inflammation or a heightened immune response. This test may be used to establish a diagnosis in patients who have experienced a slowly progressive decline in function without exacerbations (i.e., patients with so-called primary progressive MS) and who have no abnormalities seen on the brain MRI scan. In such instances, a diagnosis of definite MS cannot be made without an abnormality in the spinal fluid. The spinal fluid analysis may also be useful in excluding an infection that may be difficult to distinguish from MS [13].

Animal Models Used In Multiple Sclerosis

EAE has been induced in a number of different animal species including mice, rats, guinea pigs, rabbits, macaques, rhesus monkeys and marmosets. Immunization of mice, rats, or primates with myelin proteins (MBP, MOG, PLP) or other autoantigens (PLP) [12]. For various reasons including the number of immunological tools, the availability, lifespan and fecundity of the animals and the resemblance of the induced disease to MS, mice and rats are the most commonly used species [14].

Here, we present a model induced in Dark Agouti rats by immunization with the N-terminal fragment of myelin oligodendrocyte glycoprotein. This specific model shows several similarities to MS, such as a relapsing-remitting disease course, demyelination and axonal degeneration. By immune histochemical characterization, lesions could be detected mostly in the spinal cord, but also in the optic nerve, brainstem, and cerebellum and in different areas of the forebrain. The mimicking of particular features of MS and the occurrence of special disease entities like optic neuritis, Devic's disease and the acute MS form of Marburg's type makes this EAE type an excellent model for investigating certain aspects of the pathophysiology seen in MS [15].

Treatment of Multiple Sclerosis

CD4+ or T helper cells play an important role in the initiation of antigen-specific immune responses. Therefore, it is possible to regulate the immune response and induce tolerance to disease through manipulation of the T helper response. Skewing of the T cell response towards a Th2 cytokine profile may be most efficient during antigen priming. A Th2 response may have beneficial effects on the disease course; the importance of a bias towards the Th2 pathways is emphasized by the effects on cytokines pathways of 2 approved drugs for MS: Copaxone and IFN-β [10].

There are many issues for the patient and physician to consider in treating multiple sclerosis. Goals may include:

improving the speed of recovery from attacks (treatment with steroid drugs);

reducing the number of attacks or the number of MRI lesions; or

Attempting to slow progression of the disease (treatment with disease modifying drugs or DMDs).

An additional goal is relief from complications due to the loss of function of affected organs (treatment with drugs aimed at specific symptoms). Most neurologists will consider treatment with DMDs once the diagnosis of relapsing remitting Multiple Sclerosis is established. Many will begin treatment at the time of the first Multiple Sclerosis attack, since clinical trials have suggested that patients in whom treatment is delayed may not benefit as much as patients who are treated early.

It is important for patients to talk to their doctor before deciding to go on therapy since DMDs differ in their uses (for example, one DMD may be used for slowing progressing disability but not for treatment of the first attack of MS; another DMD may be used for reducing relapses but not for slowing progressing disability). Finally, utilizing support groups or counseling may be helpful for patients and their families whose lives may be affected directly by multiple sclerosis.

Once goals have been set, initial therapy may include medications to manage attacks, symptoms, or both. An understanding of the potential side effects of drugs is critical for the patient because sometimes side effects alone deter patients from drug therapy. Patients may choose to avoid drugs altogether or choose an alternative drug that may offer relief with fewer side effects. A continuous dialogue

between the patient and physician about the medications is important in determining the needs for treatment. Drugs known to affect the immune system have become the primary focus for managing multiple sclerosis. Initially, corticosteroids, such as prednisone (Deltasone, Liquid Pred, Deltasone, Orasone, Prednicen-M) or methylprednisolone (Medrol, Depo-Medrol), were widely used. However, since their effect on the immune system is non-specific (general) and they may use may cause numerous side effects, corticosteroids now tend to be used to manage only severe Multiple Sclerosis attacks (that is, attacks leading to physical disability or causing pain).

Interferons for relapsing multiple sclerosis

Since 1993, medications that alter the immune system, particularly interferons, have been used to manage multiple sclerosis. Interferons are protein messengers that cells of the immune system manufacture and use to communicate with one another. There are different types of interferons, such as alpha, beta, and gamma. All interferons have the ability to regulate the immune system and play an important role in protecting against intruders including viruses. Each interferon functions differently, but the functions overlap. The beta interferons have been found useful in managing multiple sclerosis.

Interferon beta-1β (Betaseron®) was the first interferon approved in the U. S. to manage RR-MS in 1993.

In 1996, intramuscular interferon beta-1α (Avonex®) gained FDA approval for RR-MS.

Subcutaneous Interferon beta-1α (Rebif®) was approved in the U. S. in 2002.

The FDA also approved the marketing of Interferon beta-1β under the brand name Extavia® in 2009. Overall, patients treated with interferons experience fewer relapses or a longer interval between relapses. Avonex® and Rebif® are used to slow progressing disability. The most common side effect is a flu-like syndrome that includes fever, tiredness, weakness, chills, and muscle aches. This syndrome tends to occur less frequently as therapy continues. Other common side effects are injection site reactions, changes in blood cell counts, and abnormalities of liver tests. Regular liver tests and blood counts are recommended for patients receiving beta-interferons. Periodic thyroid function testing also is recommended because of the effects of beta-interferons on the thyroid gland. With the concomitant use of analgesics and evolving nursing experience with managing local skin reactions, the tolerability to interferons seems to have improved over the years.

Clinical trials of beta-interferon in patients with the first attack of Multiple Sclerosis showed that in this early patient population, the second attack was delayed. Interferons approved by the FDA for treatment at the first attack of Multiple Sclerosis include Avonex®, which is administered intramuscularly once a week, and Betaseron® or Extavia®, which are administered subcutaneously every other day.

Available beta-interferons include:

Interferon beta-1α (Rebif®) is used for the treatment of patients with relapsing forms of Multiple Sclerosis to decrease the frequency of clinical relapses and delay the accumulation of physical disability. Efficacy of Rebif® in chronic progressive Multiple Sclerosis has not been established.

Interferon beta-1α (Avonex®) is used for the treatment of patients with relapsing forms of Multiple Sclerosis to slow the accumulation of physical disability and decrease the frequency of clinical relapses. Patients with multiple scleros1is in whom efficacy has been demonstrated include patients who have experienced a first clinical episode and have MRI features consistent with multiple sclerosis. Safety and efficacy in patients with progressive Multiple Sclerosis has not been established.

Interferon beta-1β (Betaseron® and Extavia®) are used for the treatment of relapsing forms of multiple sclerosis, to reduce the frequency of clinical relapses. Patients with Multiple Sclerosis in whom efficacy has been demonstrated include patients who have experienced a first clinical episode and have MRI features consistent with multiple sclerosis.

Other medications approved for relapsing multiple sclerosis

Glatiramer acetate (Copaxone):- It is another DMD that is approved for reducing the frequency of relapses in RR-MS. Glatiramer acetate is a synthetic (man-made) amino acid mixture that may resemble a protein component of myelin. It is thought that the immune system reaction against myelin in Multiple Sclerosis may be blocked or diminished by glatiramer acetate. A reaction occurring immediately after the injection of glatiramer acetate is common, affecting one out of 10 patients. The reaction may involve flushing, chest pain or tightness, palpitations, anxiety, shortness of breath, tightness in the throat, or hives. The reaction usually resolves within 30 minutes and requires no treatment. Some patients may be at risk of developing lipoatrophy, inflammation and destruction of fat tissue beneath the skin at the site of injection.

Natalizumab (Tysabri®)**:-** It is a drug approved by the FDA to treat relapsing multiple sclerosis. Natalizumab is a monoclonal antibody against VLA-4, a molecule required for

immune cells to adhere to other cells, and penetrate into the brain. It is administered via monthly intravenous infusions. Natalizumab is used alone for the treatment of patients with relapsing forms of Multiple Sclerosis to delay the progression of physical disability and reduce the frequency of clinical relapses. The safety and efficacy of natalizumab beyond two years are unknown.

Mitoxantrone (Novantrone®):- It is approved by the FDA for the treatment of Multiple Sclerosis (SP-MS, PR-MS, and worsening RR-MS). Mitoxantrone is a chemotherapy drug that carries the risk of serious cardiac side effects or cancer (leukemia). Because of these serious side effects, physicians tend to reserve its use for more advanced or worsening cases of multiple sclerosis, and there is a limit to the total amount of mitoxantrone that can be administered. Cardiac monitoring prior to each dose and yearly following the last dose of mitoxantrone also is necessary.

Mitoxantrone is used for reducing neurologic disability and/or the frequency of clinical relapses in patients with SP-MS, PR-MS, or worsening RR-MS (for example, patients whose neurologic status is significantly abnormal between relapses). Mitoxantrone is not used in the treatment of patients with PP-MS.

Fingolimod (Gilenya®):- It is a daily oral medication to treat MS that was approved by the US FDA in September 2010 as the first oral medication to treat MS. Although the exact mechanism of action of fingolimod is unclear, it appears to work by reducing the number of lymphocytes (a type of white blood cell that is important for immunity and the inflammation process) in the blood. Fingolimod is taken daily in capsule form. It is not a cure for MS, but it has been shown to decrease the number of MS flares and slow down the development of physical disability caused by MS. Like many injectable therapies for MS, the long-term safety of fingolimod is unknown. The most common side effects of fingolimod are headache, flu, diarrhea, back pain, elevations of liver enzymes in the blood, and cough. Other side effects are also possible including eye problems, so those taking this drug should have regular ophthalmologic evaluations [3].

The Future Directions for Managing Multiple Sclerosis

There is a great deal of ongoing research in multiple sclerosis, and there continues to be a focus on the immune system in investigational therapies. In addition, scientists are trying to develop techniques that allow brain cells to generate new myelin or that prevent the death of nerves. Other promising approaches include the use of precursor (neuronal stem or progenitor) cells that could be implanted into the brain or spinal cord to repopulate areas of missing cells. Future therapy may involve methods designed to improve impulses traveling over the damaged nerves. Scientists also are exploring the effects of diet and other environmental factors on multiple sclerosis.

Discussion

Multiple Sclerosis (MS) is a disease which progressively injures the nerves of the brain and spinal cord. Injury to the nerves in Multiple Sclerosis may be reflected by alterations of virtually any sensory or motor (muscular) function in the body. MS can be treated with medication. Some cases of MS are benign and only need to be observed without any treatment. The cause of Multiple Sclerosis is unknown, but it has become widely accepted that genetic, immunological, and environmental factors play a role. Although the cause of MS still eludes us, our improved understanding of the pathogenesis of the disease has opened the door to new therapies. By targeting molecules that are directly involved in the pathogenesis of MS, these therapies may be more efficacious and specific, and less toxic. Alterations in cytokine balance may be part of autoimmune disease pathogenesis. However, these alterations may be epiphenomena of a more complex disease process rather than the causal effect in the pathological cascade. Experimental models such as EAE are critical in developing novel therapeutic targets, now in use in patients. Novel treatment strategies should modulate both innate and adaptive immunity and promote a deviation to a predominant Th2 response, which has the potential of immune modulation as well as neuroprotection. Such strategies may include statins and blockade of costimulatory pathways that have been associated with Th1 to Th2 cytokine shift in animals with EAE. Keeping healthy life habits and staying connected with friends and family are great ways to cope with Multiple Sclerosis and limit the fatigue and stress it may place on the body. It can be debilitating, however, most people with MS are able to lead normal, active lives and pursue their hobbies.

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