

# Multiple Sclerosis

*Etiology, Diagnosis,  
and New Treatment Strategies*

Edited by

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## *Dedication*

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*To my parents, Loretta and Francis, whose selfless love and paramount guidance have conferred upon me a life graced with wisdom, respect, and compassion.*

“I am ready to concede that the different disguises assumed by disseminated sclerosis are coarse masks, and that today, when recent works have illuminated the field of diagnosis, it is scarcely permissible to be caught in the snare.”<sup>1</sup>

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<sup>1</sup>Lectures on the Diseases of the Nervous System Delivered at La Salpetriere, JM Charcot, translated by George Sigerson, London: The New Sydenham Society, MDCCCLXXVII, Volume LXXII, p. 185.

## Series Editor's Introduction

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The diagnosis and treatment of multiple sclerosis (MS) used to be very simple. Diagnosis was based on the presence of multiple lesions scattered in space and time with some support from lumbar puncture. Specific treatments were nonexistent. Corticosteroids were the only available treatment and, in desperation, were sometimes even administered intrathecally without much evidence for efficacy. The belief that multiple sclerosis is caused by a combination of host genetic susceptibility and environmental triggers is a very old one but remains the leading hypothesis. The explosion of new information concerning pathology, pathophysiology, clinical classification, imaging, and treatment during the past decade or so is truly impressive and is beginning to provide hope that this disease will come under better control in the near future. Yet, at the present time, we still remain at a waystation in its understanding and management. We may understand the enemy a little bit better, but are still very limited in our therapeutic options.

*Multiple Sclerosis: Etiology, Diagnosis, and New Treatment Strategies* provides a comprehensive and state of the art overview of MS. The chapters emphasize current criteria for diagnosis and subtype classification and incorporate the rapidly developing body of pathologic information, much of which can now be accessed during life with a dazzling array of new and emerging magnetic resonance imaging (MRI) techniques. As discussed by several of the authors in this volume, clinical and MRI criteria have become intertwined in the very influential McDonald criteria, which allow for earlier diagnosis. This appears to be extremely important since emerging data suggest that early treatment with disease-modifying drugs during the more acute inflammatory phase may impact progression of the disease. Despite modest gains in the treatment of early and relapsing MS, chronic progressive MS remains a major therapeutic challenge. The discovery of axonal pathology, imaging techniques able to identify and quantify brain atrophy, and greater awareness of the cumulative effects of MS on cognitive function receive much deserved attention in this volume.

Before these new developments, it was recognized that the identifiable clinical manifestations of MS were only the tip of the iceberg. One has to

feel that the discoveries made in the past decade also represent only the tip of the iceberg. As described by Drs. Dangond and Olek in the last two chapters of this book, we are at the threshold of new discoveries and knowledge which should finally control this common and dreaded disease.

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## Foreword

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The understanding of pathology and treatment in multiple sclerosis (MS) has progressed exponentially since the 1990s as a result of increased basic and clinical research. To date, five medications are now approved by the Food and Drug Administration and available for use in patients with MS. In addition to the elucidation of the basic mechanisms involved in the pathogenesis of the immune system, the approval of these medications can be attributed to the diligence of the pharmaceutical companies.

The advent and evolution of magnetic resonance imaging (MRI) has revolutionized the diagnosis and treatment of MS. Not only has image quality improved with the advent of higher Tesla magnets and fast fluid-attenuated inversion recovery techniques, but expanded areas of utilization such as three- and four-dimensional techniques, magnetic resonance spectroscopy, functional MRI, atrophy measures, and magnetization transfer ratio imaging have also arisen. Formal guidelines for the use of MRI in MS have been recently published and incorporated into clinical trials. In addition, MRI is now included in the current McDonald criteria, which allows for earlier diagnosis of MS which will result in earlier treatment, ultimately benefiting patient care.

New treatment strategies are being developed based on a detailed understanding of the immune process as well as pathological findings showing specific patterns of disease in terms of myelin destruction and axonal loss. The current knowledge of the immune system is presented in terms of the relevance to clinical application. From broad immunosuppression to specific antibody therapies, we have been able to broaden our clinical trial capability and consider combination therapy based on the mechanisms of actions of various medications on the immune system. In addition, new therapies on the horizon, such as bone marrow transplant and myelin repair, are presented.

Also included are state-of-the-art presentations on neuropsychological aspects and pregnancy-related issues facing patients with MS. Inclusion of these issues in designing clinical trials is crucial in the development of treatments that will cover all aspects of this long-term degenerating neurological disease.

*Multiple Sclerosis: Etiology, Diagnosis, and New Treatment Strategies* addresses all the current immunological, imaging techniques, neuropsychological, and pregnancy issues and shows their relationship to the development of new and improved therapies to combat this devastating neurological disorder.

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## Preface

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*Multiple Sclerosis: Etiology, Diagnosis, and New Treatment Strategies* synthesizes current concepts about the evaluation, treatment, and future directions in multiple sclerosis (MS). In addition to discussing the current medications for treating relapsing-remitting MS (Avonex, Betaseron, Copaxone), this book focuses on recently approved medications (Novantrone and Rebif), new indications for medications (CHAMPS Trial), and medications in development (Oral Interferon Tau, Oral Copaxone, Oral Cellcept). Immunosuppressive therapy for progressive disease as well as symptomatic therapy is also discussed.

The evaluation of MS has been greatly enhanced by magnetic resonance imaging (MRI) and newer imaging techniques, such as magnetic resonance spectroscopy, functional MRI, and three-dimensional MRI, which was pioneered at Harvard University. These modalities have also aided in clinical trials leading to the first Food and Drug Administration approved medication to treat MS. The role of MRI is reviewed in Chapter 3.

The role of immunological research in MS is paramount. Special attention is given to the immunological mechanisms involved in the autoimmune process and in developing treatment strategies based on these concepts. We present the current thinking and latest discoveries in immunology as it relates to MS. Groundbreaking B-cell research has been pioneered at the University of California, Irvine. The basic understanding of the autoimmune mechanism and its application to specific immunotherapies is explored in Chapters 5 and 6. Immune markers are crucial for understanding and following the disease and are covered in detail.

*Multiple Sclerosis: Etiology, Diagnosis, and New Treatment Strategies* provides insight into the future of MS treatment. The mechanism and application of myelin repair is presented in detail in Chapter 11. Future therapies such as bone marrow transplant, combination therapy, and specific immunomodulatory treatment are discussed in Chapter 12. These therapies are based largely on basic immunologic research.

This book should prove valuable to general neurologists and primary care physicians who treat many patients with MS. We hope they find it useful.

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# Signs and Symptoms of Multiple Sclerosis

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Stanley van den Noort, MD

## INTRODUCTION

The signs and symptoms of multiple sclerosis (MS) range from those that command the immediate attention of patient and doctor to those that escape the attention of patient and family over the course a lifetime. For nearly a century, we have discovered some examples of MS found at autopsy that were never recognized by the family. In the modern era, we find examples of MS on magnetic resonance imaging (MRI) scans done for other reasons, such as a motor vehicle accident.

The author believes that the best classification of the symptoms begins with those that involve the mind, sensation, and movement, those that result from brainstem lesions and involve autonomic dysfunction.

## HIGHER CORTICAL SYMPTOMS

We are increasingly cognizant that axonal destruction, as well as demyelination, proceeds together even early in the disease process (1). The responsible lesions that affect the mind are usually subcortical and pericallosal. Individually, these lesions are almost always asymptomatic. Over time, they may collectively degrade the highest intellectual functions, with special deficits in the ability to make complex decisions using multiple variables and to remember complex networks that tie together information. The personality may change subtly. Lesions that separate and disrupt the integration of neural systems can reproduce almost any psychiatric disorder. Depression, anxiety, paranoia, obsessions, loss of inhibition, and variants of neurosis may be encountered (2). The author has seen frank psychosis consistent with schizophrenia.

In the last year, the author saw a young woman for whom the first symptom of MS was visual hallucinations and a dream in which she saw herself get up, go downstairs, and kill her husband. This same dream would recur with visual effects of great clarity in full color, and the sequence and details were always the same. She also saw large animals in great detail and in full color. It is probable that these

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were examples of peduncular hallucinations caused by lesions in the posterior hypothalamus. Vivid visual hallucinations are almost always organic. Separating these phenomena from psychiatric disorders is often difficult. When they are acute, one can sometimes surmise that the sudden change in personality or behavior is so out of context that an organic process can be suspected. However, when these symptoms are subtle, it is usual to find a psychiatric illness that can delay the diagnosis of MS for years or even decades (3,4).

A disproportionate percentage of patients with MS have migraine headaches. This can involve visual phenomena, hemiparesis, transient alterations of sensation and speech, and even transient global amnesia. These can complicate the diagnostic process. The author once treated a medical student who experienced a classic migraine in the physiology laboratory and then developed persistent ataxia, which was the first manifestation of his MS. Between attacks, migraineurs are photophobic, sonophobic, and unable to tolerate smells.

If one asks the average patient with MS to list his or her major problems, the patient usually lists fatigue (5), impaired sleep, and impaired memory as highly significant. If one asks the average middle-aged or young adult what bothers him or her the most, it is common for this same list to be identified. This is particularly true in American lifestyles, which are often more frantic and complex than those in other societies and cultures (6). The number of things that I am required to remember in the eighth decade of my life is far greater than that required of the average individual a century ago. Often these things are not really important, and if we are slightly depressed or distracted, these failures of recall are misinterpreted as signs of decreased brain function. The separation of these relatively normal or average symptoms from those that trouble the patient with MS is often difficult to achieve. Neuropsychological testing in good hands can be extremely helpful (7). It is also expensive and can be misleading. The author has had several instances of patients with MS who had been extremely successful in their careers and were recognized by others or by themselves as not functioning at that same level of performance. In some cases, neuropsychological testing may be approximately normal, when, in fact, the intellect has declined from a high level to a more average level (8).

In summary, some slowing of performance, some impairment of memory, and some degradation of ability to handle a checkbook or other average task are commonly encountered in MS. When recognized by the patient, this creates anxiety. When recognized by a spouse, it often becomes a rift in a marriage, and divorce is common. When recognized by an employer, it often results in dismissal, with drastic consequences for the patient. It is not rare for a person who has been regarded as depressed and ineffective for several decades to finally be diagnosed with MS. A majority of patients with MS are able to manage their work, their families, and their responsibilities without impairment for the duration of their lifespan, which is no different than that of the average person.

Other cortical disturbances, such as stuttering, may be seen. Aphasia is rare in MS. Distortions of sensation of cortical or subcortical nature on one side of the

body are not uncommon. Often, these result in a slight increase in threshold for pain accompanied by an excessive response to painful stimuli above the threshold for pain. The normal individual has mechanisms to suppress perception of a shoe, a piece of clothing, or some minor disturbance of sensation that could be uncomfortable. Loss of suppressor systems leads to preoccupation with a modest discomfort in a hand or shoulder. This obsession, in turn, may result in surgical procedures, which are not usually helpful. Psychoses may occur and cause various higher cortical symptoms, but they are rare in MS.

In speech, the inability to find a word or to occasionally say a wrong word is endemic in normal individuals in our society and usually does not indicate a disease process. Rarely, patients with MS may have seizures of a focal or general nature. There is a slightly higher percentage of epilepsy in patients with MS than in the general population. There is a recent report of compulsive scratching of a body part or similar compulsions in patients with left frontal atrophy. The author had a patient with MS who was bright and was an executive. He had become obsessed with a minor lesion on his chin, which he compulsively scratches, producing an ulcer (9). He had not responded to high doses of a serotonin reuptake inhibitor. It was necessary to restrict his hands at night with gloves and to bandage the area to prevent scratching. He had not previously shown features of obsessive-compulsive disorder.

There are new computerized techniques for psychometric assessment that may be used in an MS clinic setting, and these will accelerate the diagnostic process. In complex circumstances, particularly those that involve divorce settlements, changes in employment status, and the assessment of intellect in professionals, the old approach of neuropsychological testing by an individual who is familiar with the types of deficits found in patients who have MS is helpful.

The separation of symptoms resulting from MS from those symptoms related to emotional responses and psychiatric pathology in the average person without MS is frequently difficult if not impossible. A neurologist who is trained and experienced in psychiatric problems is probably best equipped to cope with these issues. Having a psychologist and a consulting psychiatrist as part of an MS clinic is highly desirable but almost never affordable. A social worker who can examine the effects of the illness on the patient's human environment and recommend practical steps to solve problems is of great value.

The current American economy and its structure are unkind to many of these patients who are quickly fired for nonperformance or inadequate performance after many years of service without adequate analysis (10). The no-fault divorce procedures can produce similar unfair solutions.

## **SENSORY SYMPTOMS**

Distortions of sensation on one side of the body are common in MS and can lead to many diagnostic errors and therapeutic misadventures. Nearly 15% or more of patients diagnosed with MS have had inappropriate surgery for their MS symptoms in the decade before diagnosis. Perhaps most common among these is podiatric

surgery on toes and feet. The hand with presumed carpal tunnel syndrome or a presumed tardy ulnar palsy is another common site. Surgery on the cervical or lumbar discs mistakenly believed to cause the patient's symptoms is common. Dental procedures to deal with facial discomforts are also seen.

In disorders of sensation involving the opposite side from head to toe arise from demyelinating lesions in the cerebral hemisphere, thalamus, and adjacent areas. In other instances, the impaired sensation only involves the arm, leg, and trunk on one side. In some patients, for reasons unknown, one can have impaired perception of pin on the entire side of the body, except for the area of the scalp innervated by C2–3, which is spared. These sensation impairments often lead to distortions of normal sensation or phantom sensations, which present diagnostic challenges. The author has had patients who, when touched on the toe with a pin, will complain of a sharp pain in the shoulder on the ipsilateral side. Dysesthesias, crawling sensations, and electrical shocks are often reported by the patient. In some instances, there is a replica of a thalamic syndrome on the ipsilateral or contralateral side. It is likely that this occurs with lesions well below the thalamus.

One of the most common distortions of sensation involves proprioception. This is often more severe in the hand than in the foot, presumably because of cervical cord lesions in the dorsal root entry zone. This finding is more common in MS than in any other illness. Some patients will present with a hand that has no position sense (the useless hand of Oppenheim). With this severe deficit, motor performance is impaired as well and probably results from the inability to organize a movement in the absence of appropriate sensation. The most common example of this occurs when one goes to the dentist and receives local anesthesia that numbs the tongue and lip on one side. No motor nerves have been blocked, but for 1 or 2 hours after this procedure, one is often modestly dysarthric. Movement requires some degree of sensation to proceed normally. Patients with lesions in the dorsal columns of the spinal cord usually have proprioceptive defects on both sides. This results in the inability to stand with eyes closed (Romberg's sign) and is often associated with band-like constricting sensations about a limb or the trunk. These patients often complain of inability to take a deep breath, and truncal discomforts leading to the diagnostic pursuit of problems in the various organs commonly results in therapeutic misadventures. A central cord syndrome in which pain perception is lost while touch is preserved is rare in MS but can occur. Focal itching is common and can be a distressing symptom. Meralgia paresthetica is common and may be related to a normally subclinical meralgia paresthetica exaggerated by inability to self-regulate. Neuralgic pains of a sudden lancinating unpleasant character are usually seen in a trigeminal distribution. However, glossopharyngeal neuralgia may also be seen in MS. Shooting pains in other parts of the body are also seen. The author has several patients who have paroxysmal or steady otalgia on one side, which can be quite distressing for them. Disturbances of sensation in the tongue, cheek, and pharynx are rare. Classic trigeminal neuralgia is quite common in MS and is the most common cause of trigeminal neuralgia in patients younger than 50 years. It is often

triggered by talking, chewing, tooth brushing, or touching the face as in classic tic douloureux.

Babinski described electrical sensations from head to toe on neck flexion in soldiers with gunshot wounds of the neck. Lhermitte described this phenomenon as common in patients with MS. It still may be seen in neck injuries and cervical cord damage from other causes. These paroxysms are severe and distracting. Partial sensations of a more restricted range are also seen.

Transverse myelitis on one side often causes a loss of sensation, which involves precisely half of the perineum. It may also cause loss of sensation involving both sides of the perineum. MS is a common cause of numbness of the perineum. Bilateral loss of sensation in the perineum resulting from MS is the one sensory symptom that the author treats with high-dose IV Solu-Medrol for 4 days.

Although MS is believed to be a painless disorder, neuropathic pain of great severity, which is the most difficult to manage, is common. This often represents a form of phantom pain that responds poorly to analgesics and most other measures.

Sensation is often difficult to examine and requires repeated examinations over short intervals of time. Hysteria and malingering can produce diagnostic dilemmas. It is common for patients with neurological diseases, including MS, to present impairments of sensation, which appear to be hysterical. This often represents an attempt to convince the examiner of the severity of the problem and that there is some loss of sensation. This is also often seen in the eye. Many patients will describe monocular diplopia and multiple images, leading to a hysterical diagnosis when, in fact, they are describing competing images that are often overlapping and may be compounded by astigmatism. There is no rule that patients with MS cannot have hysterical personalities.

Sensory examinations are usually best done for only a short time and, if necessary, repeated at a later time. The author once had a visual field examination at the end of a busy day and produced a chaotic visual field finding, which caused great concern. A repeat examination after a sound night's sleep produced a normal visual field. In testing cutaneous sensation, the author prefers to use a sharp corsage pin (safety pins are too dull and needles are too sharp). The pinwheels produce accommodation and may be misleading. The author also uses a 128 tuning fork to test vibratory sensation. For position sense, it is best to grasp the finger or toe by the sides and use an up-and-down technique while the patient closes his or her eyes. It often takes some time for patients to learn, assess, and report the direction of movement as opposed to whether the final position of the digit is up or down. The author also likes to test sensation of movement in the second toe, which is much more difficult for patients and much more sensitive, as a test of proprioception. The Romberg sign is also of great value and should be done with the feet bare and close together. With eyes open, the normal individual, and most patients with MS, can sustain that posture without a problem. However, when the eyes are closed, they may fall in almost any direction. If that direction is always in the arms of the examiner, it may raise other questions. The author likes to watch the tendons on the



dorsum of the foot and the dance of those tendons when the eyes are closed. This was part of the description of the sign by Dr. Romberg.

## MOTOR SYMPTOMS

The motor problems in MS are, again, various and complicated. The most common motor manifestation is a paraparesis or quadriparesis primarily resulting from cord lesions. This often begins with a slowing of gait, a tendency for one of the legs to give way, and, when coupled with spasticity, may produce calf discomfort after walking some distance, closely resembling claudication. If the anterior tibial muscles are weak, tripping will be the predominant presentation. When spasticity is present, extensor spasms and spontaneous ankle clonus are often present. With a severe paraparesis, there may be flexor spasms that represent a form of the triple flexion response. These are triggered by discomfort of the skin, a full bladder, or a full rectum. These cause the legs to pull up with contraction of the psoas and hamstring muscles. In early mild cases, these signs are frequently difficult to elicit, but the symptom should be taken seriously. Based on the author's experience, flexor spasms are best controlled with pramipexole, a nonergot dopamine agonist.

The extensor plantar response first described by Babinski is part of a triple flexion response, with dorsiflexion of the great toe after noxious stimulation of the lateral aspect of the sole. It is said that it was Babinski's nurse who told him that when she took off patients' shoes, their great toes went up. The author can often avoid the necessity of testing for this sign by watching the shoes being removed.

The extensor spasms of the legs are often most prominent on awakening. In the hand and foot there is a slowness of repetitive movements. In the upper limbs it is important to try and establish the motor level of the lesion by sequentially testing the strength of the arm muscles. If the lesion is above C5, one may see impairment of the shoulder shrug on that side. Tendon reflexes may be reduced or exaggerated, depending on the level of the lesion and the integrity of the tendon reflex arc. It is often found that the arm is a little spastic, but the tendon reflexes are absent because of lesions in the dorsal root entry zone that interrupt the reflex circuit. For the author, spasticity in the arm is best elicited by a rapid passive movement of the hand from pronation to supination, which will usually produce a catch and then a give. One can also demonstrate the catch and give in the biceps. With higher motor lesions, one often encounters a dystonic hemiparesis marked by extension of the arm as opposed to the more common spastic flexion of the arm. With dystonia, there is no giveaway on further stretching, and the affected muscles are the antagonists of those that have spasticity. With dystonia, the fist is often flat. One can have segmental weakness of a single nerve root owing to MS, which is often misinterpreted as resulting from a disc compression of a nerve root. In MS, weakness often seems most prominent in the finger extensors and intrinsic hand muscles; in the legs, the weakest muscles are usually the iliopsoas and the peroneal muscles.

## **SECONDARY SYMPTOMS OF WEAKNESS**

Any limb paresis causes a reduction in spontaneous movements, resulting in edema. This edema results from only a lack of movement and can only be reduced by vigorous exercise or by placing the limb in a position above the heart so water can run downhill. A sling, recliner chair, and footstool do little for this edema. Even diuretics are not smart enough to tell water to run uphill and are usually ineffective. Elastic stockings are usually useless because the patient cannot put them on. Raising the foot of the bed 3 inches on a block while the head is flat with a thin pillow works best.

## **CRANIAL NERVE/BRAINSTEM SYMPTOMS**

The cranial nerves and brainstem present an array of complex symptoms and signs. Distortions of smell are described in MS, but they are rarely a symptom and are usually not tested. Retrochiasmatic lesions in the visual pathway can occasionally cause symptoms, but that is quite uncommon. Hemianopsia is rare in MS. A decline in vision in one eye with a central loss of vision is the classic optic neuritis (ON) that often introduces or occurs in the context of MS. It is usually unilateral. Bilateral ON occurring at the same time may be seen in Devic's syndrome (neuro-myelitis optica). This is a variation of MS more common in Asian and South American people. The central loss of vision impairs reading. The loss is usually greater for red than for other colors and allows one to find a scotoma (using a red pinhead will find scotomas not recognized by a white pinhead). This can be demonstrated with simple confrontation using a corsage pin with a red top. Sometimes one will find some degree of central scotoma and visual acuity decline in the other eye. This may result from a previous ON that was not recognized by the patient. This loss of vision results in some instability of the light reflex in that it contracts to light, but the contraction is not sustained. If one is dexterous and can use the swinging light sign (Marcus-Gunn), it shows that the pupil will dilate with light when the light is moved from the eye that has contracted normally (afferent pupillary defect). The author does not have those motor skills, and if he did have them, he would not spend much time on this sign. What the author sees is a pupil that contracts to light and then dilates again quickly and may oscillate. This is called hippus and provides an objective confirmation of visual fatigue in that eye. ON is regularly attended by pain on eye movement and a degree of photophobia in the affected eye. The impairment of central vision may be minor, moving from 20/20 to 20/30 or may be near total blindness with a preserved rim of visual function at the periphery. There is often color blindness in the affected eye that is easily tested with Ishihara plates, if available. ON usually begins rather quickly over hours or a day, may get worse for a few days, then stabilize and gradually recover over weeks to months. Recovery is usually fairly good, and residual defects are usually not severe (11). If the other eye is normal, then the residual incapacity is of little consequence. However, if the normal or unaffected eye is damaged by some previous illness or by amblyopia

exanopsia, the functional deficit may be major. Early treatment with high-dose IV methylprednisolone or dexamethasone for 3–5 days is often helpful (12,13). ON that is near the disk may be attended by papilledema with hemorrhages. Most cases of ON are retrobulbar, and the disk at first is normal. Over time, the residual atrophy can be seen as pallor of the optic disk. When patients with past ON have fever or temperature rise from exertion, the central scotoma may reappear until the body temperature declines (Uhthoff's phenomenon). ON can occur as a disease *sui generis*, which is not followed by any further attacks on the nervous system. In some cases, ON attacks may move from one eye to the other and then back and forth with increasing residual visual deficits. In some cases, the ON precedes or follows an episode of transverse myelitis, which again may be mild or severe. However, in an eventual majority of cases, ON is an early manifestation of MS attended by several or many lesions on the MRI, which are typical of MS (14).

Ischemic ON can be a problem in the differential diagnosis. It usually develops more quickly and in an older age group and blindness is usually more severe; and return of vision is not to be expected. In some cases, vasculitis may respond to vigorous IV immunosuppression with steroids and/or cyclophosphamide. It is necessary to treat quickly. Leber's optic atrophy is a hereditary disorder that produces rapid loss of vision in childhood, almost always in boys. However, in women with Leber's optic atrophy, there may be white matter lesions in the brain and/or spinal cord as well. Embolic occlusion of the ophthalmic artery and thrombosis of the ophthalmic veins can produce confusion in diagnosis. Vitamin B<sub>12</sub> deficiency is believed to produce ON. Methanol poisoning usually produces bilateral blindness. Tobacco/alcohol amblyopia has become much less frequent but still can occur.

The third, fourth, and sixth cranial nerves may all be affected in MS. Lesions in the brainstem may produce gaze deficits, nystagmus, oscillopsia, oculomotor dysmetria, abnormal saccadic eye movements, and a common disorder called internuclear ophthalmoplegia (INO). This results from a lesion in the brainstem that interrupts the coordination of the third and sixth cranial nerves essential to effect conjugate lateral gaze. Typically, there is failure to adduct one eye when the other eye is abducted. This is usually attended by nystagmus in the abducted eye. This is one of the most common deficits seen in MS. It is easily recognized when it is severe but somewhat more difficult to recognize in milder cases. New neurophysiologic recording techniques can demonstrate INO and other complex gaze defects in a large percentage of patients with MS. This may be key in adding a second lesion disseminated in time and space. Although unilateral INO may be seen in vascular disease, bilateral INO almost always results from MS. The functional result of these deficits is diplopia. Diplopia is often subtle and represents an unstable competition between the eyes to display a clear image. The patient interprets this as blurred vision. Unstable eye movements are sometimes seen as oscillopsia in which images oscillate, producing a severe functional deficit in visual function. Many of these problems with vision and its coordination improve with time, with or without treatment. Some patients are left with severe diplopia, blurred vision, or "dizzi-

ness.” Vertical nystagmus is an indication of involvement of cerebellar pathways. Alcohol, benzodiazepines, and hypnotics will cause horizontal nystagmus, but a brainstem lesion usually causes vertical nystagmus. Many patients will have better functional vision if they occlude one eye with a patch, an opaque contact, or glasses with one lens covered with a plastic clip. In some instances, symptomatic nystagmus may be improved with memantine. The alleged disadvantage of monocular vision has to relent to acknowledge that approximately 10% of people passing you on the freeway have monocular vision. The author finds it helpful to look for vertical saccades on rapid upgaze. Another useful test is to examine the ability to stop the eyes in the midline when bringing them back from lateral or vertical gaze. Many normal individuals will fail to stop and go through the stop sign, but they quickly learn to correct this. Patients with MS often do not improve and may get worse on further testing. The oculomotor disorders that can occur in MS are worthy of an entire textbook. Various forms of nystagmus and difficulties with up or down gaze, lateral gaze, and oscillopsia are described. Vertical nystagmus is common and regarded as a sign of intrinsic disease associated with the cerebellum and its pathways. Isolated fourth nerve lesions are rare, but they do occur.

The trigeminal nerve and its connections are a common source of symptoms in MS. Facial dysesthesias, tingling, numbness, steady pain, and typical trigeminal neuralgia are seen. Bilateral facial numbness is a troublesome sign and reminds one of Sjögren’s syndrome. Trigeminal neuralgia in persons under the age of 50 years usually results from MS but obviously can have other causes as well.

Disturbance of the seventh cranial nerve is sometimes the first symptom of MS. The nerve has a fairly long course inside the brain before it emerges. A demyelinating lesion can affect the nerve, producing a Bell’s palsy. It is often difficult to confidently say that this particular Bell’s palsy results from an acute viral illness, whereas another one is more typical of MS. Accompanying symptoms, such as ataxia and sensory complaints, may provide a clue. As with classic Bell’s palsy, regeneration may be misdirected. This may result in fibers intended for the orbicularis oculi to penetrate the orbicularis oris or mentalis, producing a dimple on that side that appears with each blink of the eye on that side. This is a reliable sign of previous injury to the seventh nerve. Occasionally, regeneration may cause fibers bound for the lachrymal gland to find a salivary gland and vice versa, producing some interesting symptoms sometimes called crocodile tear syndrome. Lesions in the brainstem itself often cause a Horner’s syndrome. Bilateral lesions of the seventh nerve should cause some concern about another diagnosis, particularly forms of basal meningitis owing to syphilis, sarcoid, tuberculosis, or meningeal malignancies.

The vestibulocochlear nerve and its connections commonly cause quite violent symptoms of vertigo in MS. It is commonly misdiagnosed as an otological entity, such as vestibular neuronitis. The vertigo can be quite disabling and requires the same treatment that any acute attack would require. Dizziness with movement in any direction is common, and specific positional vertigo in a single position may

also be seen. It can last for a long time and be the major cause of disability. Careful testing may be required to distinguish between central and peripheral pathology. Vertigo is commonly associated with nausea and vomiting, regardless of the cause. Tinnitus is rare in MS and is common in otologic disease. Abrupt deafness can be an attack of MS. Obviously, other pathology must be excluded. It is rare for the deafness to be total and bilateral deafness resulting from MS is outside of the author's experience. This deafness will be of a central type so that air conduction would be greater than bone conduction and the Rinne test will lateralize to the side of best hearing.

Lesions in the medulla itself may cause severe singultus (hiccup), which can be a troublesome and persistent symptom for as long as 3 years. This usually responds fairly dramatically to baclofen (Lioresal) in modest doses. Adjacent lesions in the medulla and at higher levels produce difficulty swallowing and speaking. Speech in MS is commonly disturbed and usually presents as slurred speech with irregularities of cadence and emphasis. It is both cerebellar and spastic. Aphonia is rare. The speech tends to remain quite rapid and is often difficult to understand. Difficulties with swallowing are commonly present and usually affect water drinking more than solid food. The pseudobulbar affect introduces an emotional quality to the speech, which is not necessarily intended by the speaker. The affect projected is usually a sad one, so that one senses the patient is going to cry (15) at any moment. Alternatively, an effort to smile may produce giggling, which must not be misconstrued.

Other lesions in the brainstem will rarely cause some difficulties with ventilation. More commonly, spinal lesions may affect chest wall movement and even the diaphragm. Lesions at many levels of the brainstem may produce orthostatic hypotension. Cardiac arrhythmias are described in MS. The author has seen patients with migraine whose aura consisted of bouts of atrial flutter and occasionally atrial fibrillation. The author has two patients with MS who, after high-dose IV steroids, developed what was interpreted as a classic Wolf-Parkinson-White syndrome. This persisted for days, but in both cases, it disappeared before electrical correction of the defect could be arranged.

## CEREBELLAR SYMPTOMS

A common disability in MS comes from lesions in the cerebellar pathways. Normal cerebellar function modulates movements so that the approach to a target is direct and accurate. It may be likened to an automatic pilot, which adjusts the plane from one position to another to keep it in a straight line. In the absence of this modulation, the movements are from side to side, perpendicular to the direction of movement, and most prominent in the proximal muscles. Over time, many patients learn to regulate this by holding the shoulder close to their side and making the movements from finger to nose rapid to hide the dysmetria. They also tend to hit the side of the finger rather than approach the tip. One can often tell that a finger-nose test is abnormal when the strike of the finger against your finger is harder on

one side than the other. In the trunk, cerebellar defects cause a truncal ataxia, making standing in a steady posture without support difficult. This produces titubation, which is a vertical oscillation of the body. This moves the head in a direction of one trying to nod "yes." Rapid alternating movements in the hand and foot are slowed and irregular. The heel-shin test must be done when the patient is supine and will demonstrate this perpendicular deviation from the direction sought determined by proximal muscles. It is sometimes difficult to differentiate this from the weakness seen in limb-girdle muscular dystrophy, which produces a similar instability of proximal muscles by a different mechanism. The gait is broad based, and tandem walking is not feasible. Lurching from one side to the other is usual. There is no fundamental difference between cerebellar ataxia and the motor performance of someone who is intoxicated with alcohol.

## **SPINAL CORD SYMPTOMS**

Central and spinal lesions commonly affect bladder and bowel function, sexual function (16), and blood pressure. Frequency of urination is a ubiquitous symptom in MS (17). Occasionally, it is paradoxically associated or replaced by hesitancy. Urgency may demand access to facilities faster than the patient can achieve, resulting in incontinence. Severe depletion of sensory input from the bladder, usually with position sense loss in the limbs, may produce an atonic bladder, which fills and overflows. Many patients with MS are chronically dehydrated in their attempt to minimize voiding (18). Stubborn constipation is also a nearly universal symptom. At the same time these patients are reluctant to use laxatives for fear of incontinence. Usually the best approach is to affect some means of emptying the bowel and then neglect it during most of the day. Miralax and pyridostigmine are the author's major treatment mainstays in this area.

Sexual dysfunction is usually erectile failure in the male, which is often alleviated by Viagra and similar agents (19). In women, it takes the form of insensitivity resulting in poor libido. Clitoral vibrators may be helpful. Segmental disorders of sweating may be seen. Localized piloerection may be seen. Spinal cord lesions may produce dysautonomia with enormous spikes of high blood pressure from a full bladder, full rectum, or a decubitus ulcer.

## **APPROACHES TO THE NEUROLOGICAL EXAMINATION AND DIAGNOSIS**

The neurological examination has to be comprehensive while also dealing with the rigors of modern medicine. Serial 7s or alternating letters and numbers, the eyes, speech, effect of neck flexion, finger-nose test, finger and second toe position sense, plantar responses, and tandem plus Romberg with bare feet are the author's critical elements.

The diagnosis of MS rests on the dissemination of signs and symptoms in time and in anatomic space. Often, one finds that the patient history provides that information. Colleagues in the United Kingdom and Europe believe the criteria used for

first and second attacks must be confirmed by examination. That is often difficult to achieve but is included in the current diagnostic criteria (20). The newly adopted criteria are a bit rigid, but they provide good guidelines to highly accurate diagnosis. If one sees a young woman who had difficulties with bladder control for 3 months with tingling feet, which then clears, and a year later she presents with loss of central vision in one eye, the diagnosis is fairly clear without any further diagnostic procedures. Today, it is essential in almost all cases to get a good MRI following the guidelines set by the National Multiple Sclerosis Society, not only for a diagnosis but also to guide treatment. A family history of MS or other autoimmune disease is often helpful (21,22).

Diagnostic errors go in both directions, but they are infrequent in the hands of any experienced neurologist. It is important to share with the patient the working diagnosis of MS before it is fully confirmed. Many career decisions are affected by the diagnosis. The diagnosis should never be withheld. One should always remember never to use the words “always” and “never” in medicine. The author suggests withholding the diagnosis of MS from a patient with psychosis or who is suicidal (23) for some time. At the same time, a certain diagnosis of MS that turns out to be wrong can lead to anger and lawsuits. Almost without exception, honesty with patients is imperative but should not be simplified by not making a firm diagnosis when the criteria have not been fully met. Most medical care proceeds on a working diagnosis, and MS is no exception.

Some patients with MS, perhaps 5% with a higher proportion of men, have a slowly progressive course from the onset. It is likely that this group will have limited memory of the early course of their illness and a sizable proportion will believe that “denial” is a river in Egypt. Over time, it is occasionally possible to get acknowledgment that the first stumbling went away for a month and then returned. A few years ago, the author saw a 70-year-old man who had developed tingling in his right hand and thought he might have had a stroke. His MRI showed advanced and rather severe MS. He was referred to the author because the author was the only neurologist in his age group. After detailed questioning, the patient acknowledged that friends had told him he walked like John Wayne for 30 years, that he found it necessary to take a Valium to control his emotions when he went to a wedding or a funeral, and that his mother had suffered from MS.

The large proportion of men with primary progressive MS is consistent with male tendencies to deny and neglect illness for as long as possible. It also reflects that there are more men than women in hospitals, that women see doctors more often, and that those women live longer than men.

Many patients with MS have paroxysmal symptoms, which do not represent a relapse but rather some brief disturbance of circuitry in affected areas of the brain. The most obvious are trigeminal neuralgia and Lhermitte phenomenon. Extensor and flexor spasms are paroxysmal in many patients. Oscillopsia and vertigo are commonly paroxysmal. If these phenomena clear, at least for a time in seconds or hours, they are probably in this category. If they last for more than 24 hours, one



needs to presume that this may be a relapse. In some instances, heat or fever can produce dramatic increases in symptoms that will subside with the body temperature's return to normal. Paroxysmal inability to walk, inability to use a limb, inability to write, and paroxysmal dysarthria are examples of the phenomena. Paroxysmal vision disturbances occur but, with a higher proportion of migraineurs, these symptoms may be an aura with or without a headache.

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## Differential Diagnosis, Clinical Features, and Prognosis of Multiple Sclerosis

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### INTRODUCTION

The diagnosis and prognosis of multiple sclerosis (MS) has changed dramatically over the years from the first descriptions from St. Lidwina of Schiedam (1380–1433) and Augustus D’Este (grandson of George III) between 1822 and 1848 to the pathological descriptions of Cruveilhier (1829–1842) and Carswell (1838). Serious study and synthesis of clinical and pathological human MS began with the work of Jean Martin Charcot at the Salpêtrière in Paris in the last three decades of the 19th century. Recently, there has been a trend to classify MS as an immune-mediated demyelinating disease of the central nervous system (CNS). This classification is useful as a diagnostic tool, as demonstrated by Schumacher (1962) and Poser (1983). The new diagnostic criteria (1), which is discussed in detail in the remainder of this chapter, has changed the diagnosis, prognosis, and treatment of MS.

### DIAGNOSIS

The cornerstone of the MS diagnosis remains the neurological history and physical examination. There are no clinical findings that are unique to this disorder, but some are highly characteristic (Table 1). Common presenting MS symptoms are listed in Table 2. The typical patient presents as a young Caucasian adult female with two or more clinically distinct episodes of CNS dysfunction, with at least partial resolution. The history and physical examination are most important for diagnostic purposes, although numerous laboratory tests support the diagnosis (Table 3). To improve the homogeneity of MS patient groups being studied, the Schumacher Committee on Diagnostic Criteria for MS (2) elaborated six items that are required to diagnose clinically definite MS: objective CNS dysfunction, involvement of white-matter structures, two or more sites of CNS involvement, relapsing-remitting or chronic (more than 6 months) progressive course, age 10–50 years at onset, and no better explanation of symptoms as assessed by a competent

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**Table 1**  
**Common Clinical Features of Multiple Sclerosis**

Clinical features suggestive of multiple sclerosis	Clinical features not suggestive of multiple sclerosis
Onset between ages 15 and 50	Onset before age 10 or after 60
Relapses and remissions	Steady progression
Optic neuritis	Early dementia
Lhermitte sign	Rigidity, sustained dystonia
Internuclear ophthalmoplegia	Cortical deficits, such as aphasia, apraxia, alexia, and neglect
Fatigue	Deficit developing within minutes
Worsening with elevated body temperature	

**Table 2**  
**Presenting Symptoms in Multiple Sclerosis Patients**

Symptom	Males (%)	Females (%)	Total (%)
Sensory disturbance—limbs	25.1	33.2	30.7
Visual loss	15.1	16.3	15.9
Motor (subacute)	10.4	8.3	8.9
Diplopia	8.5	6.0	6.8
Gait disturbance	8.3	3.2	4.8
Motor (acute)	4.2	4.4	4.3
Balance problems	4.0	2.5	2.9
Sensory disturbance—face	2.5	2.9	2.8
Lhermitte's phenomenon	2.3	1.6	1.8
Vertigo	1.5	1.8	1.7
Bladder disturbance	1.1	0.9	1.0
Limb ataxia	1.3	0.9	1.0
Acute transverse myelopathy	0.6	0.8	0.7
Pain	0.8	0.3	0.5
Unclassified	2.5	2.6	2.5
Polysymptomatic onset	11.9	14.5	13.7

Adapted from refs. 68 and 69.

**Table 3**  
**Comparison of Sensitivity of Laboratory Testing in Multiple Sclerosis**

	Visual evoked response (%)	Brainstem auditory evoked response (%)	Somato-sensory evoked potentials (%)	Oligo-clonal bands (%)	Magnetic resonance imaging (%)
Clinically definite multiple sclerosis*	80–85	50–65	65–80	85–95	90–97

\*Numbers show the percentage of patients with abnormal study results.

neurologist. These criteria made no use of laboratory studies. Such stringent criteria would exclude some patients with MS; for example, they were fulfilled in only 95% of a group of patients who came to autopsy study. The criteria were modified for diagnosis in 1983 by Poser et al. (3), expanding the age at onset to 59 years and using data derived from laboratory studies, including analysis of cerebrospinal fluid (CSF), evoked potentials (EP), and neuroimaging. These criteria were developed to ensure that only patients with MS were included in research studies. Recently, McDonald et al. (1) have proposed new diagnostic criteria, which include stringent guidelines for magnetic resonance imaging (MRI) and timing intervals to determine possible or definite multiple sclerosis. The outcomes are classified as:

- The diagnosis of MS is given if diagnostic criteria are fulfilled.
- The diagnosis of possible MS is given if the criteria are not completely met.
- The diagnosis of not MS is given if the criteria are fully explored and not met.

Another significant change with the newer criteria (sometimes referred to as the McDonald criteria) is the incorporation of MRI findings. The new diagnostic criteria are shown in Table 4 and in the next section. The new criteria consider CSF analysis, EPs, and neuroimaging, as well as creating a category for patients with clinically isolated syndromes. This information allows patients to be treated at an earlier phase in their disease and will provide the greatest effect on the long-term prognosis of the disease for most patients.

### ***Magnetic Resonance Imaging Demonstration of Space Dissemination for McDonald Diagnostic Criteria for Multiple Sclerosis (4,5)***

Three out of four of the following:

1. One gadolinium-enhancing lesion OR nine T2-hyperintense lesions if there is no gadolinium-enhancing lesion;
2. At least one infratentorial lesion;
3. At least one juxtacortical lesion; and/or
4. At least three periventricular lesions.

If the first scan is performed less than 3 months after the onset of the clinical event, a second scan done 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan, not less than 3 months after the first scan that shows a new T2 lesion or an enhancing lesion, will suffice.

## **RADIOLOGICAL STUDIES**

MRI is the test of choice to support the clinical diagnosis of MS. The characteristic lesion demonstrated on MRI is the cerebral or spinal plaque. Pathologically, plaques consist of a discrete region of demyelination with relative preservation of axons, although spectroscopic and pathological studies suggest axonal loss may be an integral part of the demyelinating process (6). Histological examination of active

**Table 4**  
**McDonald Diagnostic Criteria for Multiple Sclerosis (1)**

Clinical presentation	Additional data needed for multiple sclerosis (MS) diagnosis
Two or more attacks; objective clinical evidence of two or more lesions	None
Two or more attacks; objective clinical evidence of one lesion	Dissemination in space, demonstrated by MRI OR Two or more MRI-detected lesions consistent with MS plus positive CSF OR Await further clinical attack implicating a different site
One attack; objective clinical evidence of two or more lesions	Dissemination in time, demonstrated by MRI OR Second clinical attack
One attack; objective clinical evidence of one lesion (monosymptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by MRI OR Second clinical attack OR Two or more MRI-detected lesions consistent with MS plus positive CSF and dissemination in time, demonstrated by MRI
Insidious neurological progression suggestive of MS	Positive CSF and dissemination in space, demonstrated by: (1) Nine or more T2 lesions in: brain, (2) Two or more lesions in spinal cord, or, (3) Four to eight plus one spinal cord lesion OR Abnormal visual evoked response (VER) associated with four to eight brain lesions, or with fewer than four brain lesions plus one spinal cord lesion demonstrated by MRI AND Dissemination in time, demonstrated by MRI OR Continued progression for 1 year

*Notes:* Positive CSF-oligoclonal bands (detected preferably by isoelectric focusing) or raised immunoglobulin-G index.

plaques reveals perivascular infiltration of lymphocytes (predominantly T-cells) and macrophages with occasional plasma cells. Perivascular and interstitial edema may be prominent.

Plaques suggestive of MS are typically found on MRI in the periventricular region, corpus callosum, centrum semiovale, and, to a lesser extent, deep white-

matter structures and basal ganglia. MS plaques usually have an ovoid appearance, and lesions are arranged at right angles to the corpus callosum as if radiating from this area. The plaques appear hyperintense on proton density and T2-weighted studies and are hypointense (if visible at all) on T1-weighted images.

MRI detects many more MS lesions than computed tomography (CT) and is able to detect plaques in regions that are rarely abnormal on CT, such as the brain stem, cerebellum, and spinal cord. Most lesions seen on MRI correlate with pathologic lesions (7). However, some lesions that are extensive on MRI show only small plaques on pathological examination, suggesting that much of the abnormal MRI signal may be a result of increased water content of the brain around such plaques resulting from presumed disruption of the blood–brain barrier.

Patients with clinically definite MS have typical white-matter lesions on MRI in more than 90% of cases. However, CNS lesions resulting from other disorders (e.g., ischemia, systemic lupus erythematosus [SLE], Behçet's disease, other vasculitides, human T-cell lymphotropic virus [HTLV]-1, and sarcoidosis) may appear similar to MS lesions on MRI. This is particularly true for ischemic lesions, which make MRI criteria much less reliable for the diagnosis of MS in patients older than 50 years (8).

In contrast, the frequency of abnormal signals on spinal cord MRI in normal individuals is only 3%, because the non-MS hyperintense signal seen in older patients on cranial MRI does not occur in the spinal cord. Newer technology MRI detects lesions in the spinal cord in 75% of patients with definite MS (9).

The overall sensitivity and specificity of MRI depend on the diagnostic criteria employed. In one study of 1500 brain MRI scans that included 134 scans of patients with a clinical diagnosis of MS, using the criteria of three or four areas of increased signal intensity resulted in a high sensitivity for the diagnosis of MS (90 and 87%, respectively) but a low specificity (71 and 74%, respectively) and positive predictive value (23 and 25%, respectively) (8). Accuracy was improved with criteria that included at least three areas of increased signal intensity plus two of the following features: lesions abutting body of lateral ventricles, infratentorial lesion location, and size >5 mm. Using these criteria, specificity improved to 96%, positive predictive value increased to 65%, and sensitivity decreased slightly to 81%.

MRI scanning is more sensitive and specific for predicting evolution to clinically definite MS than other studies, such as CT scans, CSF parameters, or EPs (10). This was illustrated in a 2-year follow-up of 200 patients referred for suspected MS that found 30% (50% of those under age 50 years) had developed clinically definite MS, of whom 84% had initial MRI scans that were strongly suggestive of MS (11). In contrast, the number of patients who had CSF oligoclonal bands, abnormal visual EPs, or an abnormal CT when initially studied were 69, 69, and 38%, respectively. In a second, 5-year study of 89 patients, progression to clinically definite MS occurred in 37 out of 57 (65%) with an initially abnormal MRI, and only 1 of 32 (3%) with a normal MRI (12). Again, MRI was a better predictor of progression to clinically definite MS than CSF analysis.

Patients who progress to clinically definite MS have a higher lesion load at presentation than those who do not progress (13). Increasing initial lesion load also correlates with a decreasing time to development of MS clinically. Lesion load may also have implications for long-term prognosis.

However, the extent of cranial MRI abnormalities does not necessarily correlate with the degree of clinical disability. Patients with small numbers of lesions may be quite disabled, whereas others can function well despite a large burden of disease detected by MRI. There are several possible explanations for this observation: lesions may occur in areas that are clinically silent, small lesions in the spinal cord can cause major disability in the absence of cerebral lesions, MRI may miss lesions that are clinically relevant (1), such as those in cortex, basal ganglia, and brain stem, and large plaques detected by MRI may not have functional correlates but reflect increased tissue water without impairment of neural function.

The amount of ongoing MRI activity (new or enlarging lesions and/or gadolinium-enhancing lesions) exceeds the observed clinical activity by a factor of 2 to 10 (14). This not only may reflect the factors discussed above but also may result, in part, from underreporting of minor symptoms and underrecognition of minor signs in patients with MS. However, it does suggest that MS is a much more dynamic and active disease than is clinically apparent and that MRI is essential to studies of therapy in MS.

Efforts continue to delineate differences in the MRI appearance of acute or active lesions and chronic lesions. Acute lesions tend to be larger with somewhat ill-defined margins and become smaller with sharper margins as resolution occurs. This presumably reflects resolution of edema and inflammation present at the time of acute plaque formation, leaving only residual areas of demyelination, gliosis, and enlarged extracellular space with remission. The MRI appearance of primary progressive MS shows a smaller total disease burden, a greater preponderance of small lesions, fewer gadolinium-enhancing new lesions, and acquisition of fewer lesions per unit time than the secondary progressive form of MS.

Gadolinium-DTPA, a paramagnetic contrast agent that can cross only disrupted blood–brain barrier, has been used to assess plaque activity (15). Gadolinium increases signal intensity on T1-weighted images. The accumulation of gadolinium in plaques is associated with new or newly active plaques and with pathologically confirmed acute inflammation in MS. Gadolinium enhancement usually remains for <1 month but may persist up to 8 weeks in acute plaques. Gadolinium enhancement diminishes or disappears after treatment with corticosteroids, a therapy believed to restore integrity of the blood–brain barrier permeability.

It is difficult to distinguish the edema of an acute plaque from the gliosis and demyelination of a chronic plaque with conventional MRI technology. Phosphorus MR spectroscopy can provide information on phospholipid metabolism, and proton spectroscopy can generate information about other metabolic components, such as *N*-acetylaspartate (NAA), an exclusively neuronal marker, creatine phosphate (Cr) (energy), choline (membrane component), and lactic acid (LA). Chronic MS brains

have a reduced amount of NAA in comparison to C and Cr; a reduced NAA/Cr ratio is the common means of expressing such reduction. This reduced ratio implies loss of neurons or axons, which is consistent with pathological studies and appears to parallel disability in MS (16).

## **CEREBROSPINAL FLUID**

CSF findings alone cannot make or exclude the diagnosis of MS, but they can be useful adjuncts to clinical criteria. The CSF is grossly normal in MS; it is clear, colorless, and under normal pressure. Total leukocyte count is normal in two-thirds of patients, exceeding 15 cells/ $\mu$ L in fewer than 5% of patients and only rarely exceeding 50 cells/ $\mu$ L (a finding that should raise suspicion of another etiology). The predominant cell type is the lymphocyte, the majority of which are T-cells. CSF protein (or albumin) level is normal in the majority of patients with MS. Albumin determinations are preferable because albumin is not synthesized in the CNS and thus gives a better indication of blood–brain barrier disruption than does total protein, some of which may be synthesized within the CNS (i.e., immunoglobulin [Ig]). Albumin levels are elevated in 20–30% of patients, although less than 1% of patients have a level twice that of normal (Table 5). A common finding in MS is an elevation of CSF immunoglobulin level relative to other protein components, implying intrathecal synthesis. The immunoglobulin increase is predominantly IgG, but the synthesis of IgM and IgA is increased also. The IgG shows an excess of IgG  $\lambda$  and  $\kappa$  light chains. The IgG level may be expressed as a percentage of total protein (normal <11%), as a percentage of albumin (normal <27%), by use of the IgG index (normal value <0.66), or by use of a formula for intra-blood–brain barrier synthesis of IgG. An abnormality of CSF IgG production as measured by the IgG index or IgG synthesis rate is found in more than 90% of patients with clinically definite MS, and different formulas have differing sensitivity and specificity. The sensitivity of IgG as a percentage of protein or albumin is slightly lower (Table 5) (17).

Linked to the elevation of IgG is the finding of oligoclonal bands (OCBs) in the cathodal region of an electrophoretic analysis of CSF. When normal CSF is electrophoresed, the cathodal region shows only a homogeneous blur of immunoglobulin. In MS and other conditions usually associated with inflammation, electrophoretic analysis reveals numerous discrete bands distinct from the background; these bands represent excess antibody produced by one or more clones of plasma cells. In subacute sclerosing panencephalitis, the majority of these OCBs represent antibody directed against the causative agent, measles virus. However, in MS, there is no disease-specific antigen yet identified against which the majority of bands are directed. The pattern of banding remains relatively consistent in individual patients during the disease course, although bands may be added over time. Occasionally, patients with definite autopsy-proved MS do not have OCBs.

A common method for electrophoresis uses agarose gels, but a more sensitive assay is the use of isoelectric focusing on polyacrylamide gels. OCBs are found in 85–95% of patients with clinically definite MS (Table 5). Up to 8% of CSF samples



**Table 5**  
**Cerebrospinal Fluid Abnormalities in Multiple Sclerosis**

	Albumin (%)	IgG/TP (%)	IgG/ Albumin (%)	IgG Index (%)	Oligoclonal Banding of Ig (%)
Clinically definite multiple sclerosis (MS)	23	67	60–73	70–90	85–95
Normal controls	3		36	3	7*

*Abbr:* IgG/TP, immunoglobulin (Ig) G value/total protein.

\*Other neurological diseases.

from patients without MS show OCBs and most are from cases of chronic CNS infections, viral syndromes, and autoimmune neuropathies. The presence of OCBs in patients who are monosymptomatic predicts a significantly higher rate of progression to MS than the absence of bands: 25 vs 9% at a 3 year follow-up (18). However, one must not assume that the presence of OCBs is equivalent to a diagnosis of MS, given the number of false-positive results that can occur and the variability in technique and interpretation in different laboratories.

The presence of myelin components and antimyelin antibodies in CSF and other body fluids has been used to a limited extent as a measure of CNS myelin destruction and presumed demyelinating activity in the CNS (19).

## EVOKED POTENTIALS

Evoked potentials (EPs) are the CNS electrical events generated by peripheral stimulation of a sensory organ. The use of EPs is the detection of a CNS abnormality of function that may be clinically undetectable. In the case of MS, detection of a subclinical lesion in a site remote from the region of clinical dysfunction supports a diagnosis of multifocal disease. The EPs also may help define the anatomical site of the lesion in tracts not easily visualized by imaging (optic nerves and dorsal columns). The three most frequently used EPs are somatosensory EP (SSEP) both upper and lower extremities, visual (VER), and brainstem auditory-evoked responses (BAER). MRI technology has largely eliminated the use of EPs, given the much greater anatomical information obtained and the much higher sensitivity of MRI in the diagnosis of MS (Table 3).

Patients with clinically definite MS have abnormal VERs in 85% of cases. The VER is particularly useful in patients who lack clear clinical evidence of dysfunction above the level of the foramen magnum, such as those with a chronic progressive myelopathy. Ocular or retinal disorders must be excluded before attributing abnormal VERs to demyelination in the optic pathways.

**Table 6**  
**Summary of Ancillary Testing in Multiple Sclerosis**

Test	Percentage abnormal with definite multiple sclerosis (%)
Brainstem auditory evoked response	50–65
Somatosensory evoked potentials	65–80
Visual evoked response	80–85
Cerebrospinal fluid (CSF) Immunoglobulin (Ig) G Index	70–90
CSF oligoclonal bands	85–95
Brain magnetic resonance imaging	90–97

SSEPs are abnormal in 77% of patients with MS, including approximately one-half of those who do not have sensory signs or symptoms. Some patients with clinical evidence of posterior column dysfunction may have abnormal SSEPs.

BAER abnormalities are less frequent in MS than VER or SSEP abnormalities, being present in 67% of patients with MS.

Consistent with these findings, guidelines from the American Academy of Neurology state that VERs are probably useful to identify patients with clinically definite MS, SSEPs are possibly useful, and there is insufficient evidence at this time to recommend BAER as a useful test for diagnostic purposes (20).

Synthesis of the previous data on ancillary testing is presented in Table 6.

**AGE OF ONSET**

Most studies agree that the median age of onset is 23.5 years of age. The peak age of onset is approximately 5 years earlier for women than for men. The mean age of onset is 30. Relapsing-remitting MS tends to have an earlier onset, averaging 25–29 years, compared with the relapsing-remitting progressive type with an average of onset of 25–29 years, and a mean age of conversion to progressive MS of 40–44 years. Primary progressive MS has a mean age of onset of 35–39 years. The onset of MS can occur as late as the seventh decade, although rarely. Mean age of onset is 30.6 years, median is 27 years, and peak incidence is 25 years.

**SEX DISTRIBUTION**

Autoimmune diseases in general and MS in particular affect more women than men. In a summary of 30 incidence and prevalence studies, a cumulative ratio of female to male subjects was 1.77:1.00.

**MORTALITY**

Mortality caused by MS is difficult to ascertain because of poor data collection and reporting. The US Department of Health and Human Services report of deaths

in 1992 indicates that 1900 US citizens died of MS in that year, giving MS a US mortality of 0.7 per 100,000. The mean age of death of all patients with MS was 58.1 years, compared with a national average of 70.5 for all causes of death. The life expectancy of patients with MS was therefore calculated to be 82.5% of the normal life span. In Denmark, in an exceptionally complete survey of the country, median survival after diagnosis for men was 28 years and for women 33 years, compared with matched population death rates of 37 and 42 years, respectively. In another study, MS mortality figures were calculated for England and Wales from 1963 to 1990. During this time, there was a steady and consistent decline in the death rate attributable to MS compared with the overall death rate. Patients with MS tended to live longer, and other diseases were more likely to be the cause of death. Current estimates indicate that about half of the deaths in MS patients directly result from their disease, slightly more than half if accidents, and suicide are included as indirect causes.

## **ROLE OF IMMUNE SYSTEM STIMULI**

Because the pathogenesis of MS is believed to involve the immune system, it has been hypothesized that a stimulus of the immune system (e.g., a vaccine) may trigger the disease. However, two well-designed studies have refuted this theory, one finding no association between hepatitis B vaccination and the development of MS (21), and the other finding no association between several different vaccines and disease relapse in patients with MS (22).

On the other hand, a possible infectious stimulus of the immune system has received more support in the literature. Many viruses have been associated with MS, although none has been conclusively linked to the disease (23). A role for Epstein-Barr virus (EBV), which causes infectious mononucleosis, is supported by observations that there is an increased risk of MS after infectious mononucleosis (24) and that MS is rare among people without serum anti-EBV antibodies (25). Furthermore, a prospective serologic study of women in the Nurses' Health Study found significant elevations in anti-EBV antibody titers before the onset of MS, particularly antibody to the EBV nuclear antigen 2 (EBNA-2) (26). Although these findings do not confirm that EBV is an etiologic agent, they are suggestive and warrant further study. For further information on the immune response and the role of B-cells in MS, please refer to Chapter 6.

## **GEOGRAPHIC AND RACIAL DISTRIBUTION**

More than 250 prevalence surveys have been conducted, serving as the basis for the delineation of geographic risk for MS. High-frequency areas of the world, with current prevalence of 60 per 100,000 or more, include all of Europe, including Russia, southern Canada, the northern United States, New Zealand, and the southeastern portion of Australia. In many of these areas, the prevalence is more than 100 per 100,000, with the highest reported rate of 300 per 100,000 occurring in the Orkney Islands. In the United States, the prevalence is 0.1%, or a total of 250,000 persons with MS.

Low-risk areas include most of South America, Mexico, most of Asia, and all of Africa. One possible conclusion is that MS is a place-related illness, with a latitude gradient. However, notable exceptions then need to be explained. Japan, situated at the same latitude as areas of high prevalence in Europe, is a low-risk area. Second-generation Japanese in the United States retain their parents' low risk of MS. The white population of South Africa, of medium MS prevalence, is surrounded by a black population in which the disease is uncommon. Native North Americans, especially of pure Amerindian background, have a low prevalence, but they are surrounded by a white population with a medium or high MS risk.

It seems plausible then that race is a determinant of MS risk, with populations of white extraction, especially from Northern Europe being the most susceptible. People of Asian, African, or Amerindian origin have the lowest risk, whereas other groups are variably intermediate. Migration data have often been used to support the view that a transmissible agent is involved in the pathogenesis of MS. The data indicate that persons migrating from an area of high risk to an area of low risk after the age of puberty carry their former high risk with them. With migration during childhood, the risk seems to be that of the new area to which the person has migrated. The data are not always clear-cut. Japanese in Japan are at low risk for MS. People of Japanese extraction who are living in the United States have a higher risk, although this risk is less than their neighbors of Northern European extraction. However, those Japanese who migrate to this country do not acquire the risk of their new area. Comparable data are available for persons moving to Israel from Europe (high risk).

The frequency of familial occurrence of MS has varied from 3 to 23% in different studies. The studies with the higher percentages are those in which ascertainment was more intense; that is, the more one looks, the more one finds. An overall risk in first-, second-, and third-degree relatives of at least 15% seems a reasonable estimate. The risk is highest for siblings and decreases progressively for children, aunts, uncles, and cousins (Table 7). For genetic counseling purposes, it may be stated that the sibling risk is 3–5%, approximately 30–50 times the background risk for this same population. In some studies, unaffected family members have had abnormalities on MRI, implying that the risk may be even higher. The risk applies to blood relatives; only a few studies of adopted children have been done, but they show no increased risk. One unexplained finding is the marked deficiency of transmission from father to son.

Twin studies have shown the familial nature of MS in dramatic fashion. The risk for dizygotic twin pairs is the same as that for siblings; that is, 3–5%. The risk for monozygotic twins is at least 20%, and if the subjects are followed for long periods of time and if various nonclinical data are included, the risk may reach 38.5% (27). Because the highest rates for the genetic basis of MS are less than 50%, there must be a contribution by nongenetic factors. There are several candidate genes for MS, including human leukocyte antigen, T-cell receptor, MBP, portions of the immunoglobulin chain, and mitochondrial genes. Three entire genomic scans for MS susceptibility genes have been reported, without an identifiable region of major interest (26–28). The data argue for nonmendelian polygenic inheritance.

**Table 7**  
**Risk of Developing Multiple Sclerosis in Family Members**

A. Parent with multiple sclerosis (MS)	Son	Daughter
Mother	3.8%	3.7%
Father	0.8%	2.0%
B. Sibling with MS sister brother		
Female	5.6%	2.2%
Male	3.5%	4.1%
C. Twin with MS either sex		
Identical	25–40%	
Nonidentical	4%	

*Source:* Modified from ref. 70.

## CLINICAL SYMPTOMS AND PHYSICAL FINDINGS

Although the clinical syndrome of MS is classically described as a relapsing-remitting disorder that affects multiple white-matter tracts within the CNS, with usual onset in young adults, the disorder displays marked clinical heterogeneity. This variability includes age of onset, mode of initial manifestation, frequency, severity and sequelae of relapses, extent of progression, and cumulative deficit over the course of time. The varied clinical features reflect the multifocal areas of CNS myelin destruction (MS plaques), although discrepancies occur between the extent of clinical and pathological findings.

There are no clinical findings that are unique to MS, but some are highly characteristic of the disease. Common presenting symptoms of MS are listed in Table 2. The typical patient presents as a young adult with two or more clinically distinct episodes of CNS dysfunction with at least partial resolution.

## CRANIAL NERVE DYSFUNCTION

### *Impairment of the Visual Pathways*

Optic neuritis (ON) is the most frequent type of involvement of the visual pathways, usually presenting as an acute or subacute unilateral syndrome characterized commonly by pain in the eye accentuated by ocular movements, which is then followed by a variable degree of vision loss (scotoma) affecting mainly central vision. Bilateral ON does occur, but one needs to distinguish whether it is truly simultaneous or sequential. Bilateral simultaneous ON is rare in MS, and its occurrence in isolation may suggest another diagnosis, such as Leber’s hereditary optic atrophy or toxic optic neuropathy. In bilateral ON in MS cases, the impairment begins asym-

metrically and is usually more severe in one eye. Recurrence is highly variable. In a large ON treatment trial, 15% of placebo-treated patients developed recurrent (ipsilateral or contralateral eye) ON within 6 to 24 months after the initial bout of ON (31). Mapping of visual fields reveals a central or cecocentral scotoma (central scotoma involving the physiological blind spot). The finding of a bitemporal hemianopia is rare in MS; if present, it should raise the suspicion of a mass lesion compressing the optic chiasm. Although uncommon, homonymous field defects can be seen in MS caused by involvement of the optic radiations.

Patients with ON have a relative afferent pupillary defect (Marcus Gunn pupil). The afferent pupillary defect is tested by shining a bright light alternately in each eye (the swinging flashlight test), and in the case of unilateral optic nerve dysfunction the abnormal pupil paradoxically dilates when the light is shifted from the normal to the affected eye. The interpretation of this sign becomes difficult when the degree of optic nerve impairment is similar in the two eyes. When the acute ON lesion involves the head of the optic nerve, one observes disc edema (papillitis), a finding more commonly seen in children than in adults. More often, the lesion of the optic nerve is retrobulbar, and funduscopic examination is normal in the acute stage. Later, the optic disc becomes pale as a result of axonal loss and resultant gliosis. This pallor predominates in the temporal segment of the disc (temporal pallor). After an attack of acute ON, 90% of patients regain normal vision, typically during a 2- to 6-month period. Desaturation of bright colors, particularly red, is often reported by patients who have recovered from ON; some also report a mild nonspecific dimming of vision in the affected eye.

Uhthoff's phenomenon refers to a decrease in visual acuity after an increase in body temperature. This can occur after exercise, a hot bath, or fever. This phenomenon, which reflects subclinical demyelination or preexistent injury to the optic nerve, may occur without a history of clinical involvement of the optic nerve. A similar phenomenon can occur at other sites of CNS dysfunction with an increase in body temperature.

Bitemporal hemianopia is rare in MS and, if present, should raise the suspicion of a mass lesion compressing the visual pathways. Homonymous field defects are uncommon but can be seen in MS resulting from involvement of the optic radiations.

Because many patients with MS present with ON as their first neurologic event, it is interesting to consider how many patients who have ON go on to develop MS. The reported risk of progression to clinically diagnosed MS ranges from 15 to 75%. In one population-based study, 39% of 95 patients with isolated ON progressed to clinically definite MS by 10 years of follow-up, 49% by 20 years, 54% by 30 years, and 60% by 40 years (32). There was no difference in the risk of developing MS between men and women. The presence of oligoclonal bands in the CSF of such patients has been associated with an increased risk of developing MS.

MRI can help differentiate groups of patients with ON who are likely or unlikely to develop MS (31). Between 50 and 72% of patients with ON have cranial MRI

appearances consistent with MS. Of those with lesions on MRI, there is a 55 to 70% risk of developing clinically definite MS or laboratory-supported definite MS within 5 years (12). In contrast, patients with isolated ON and no evidence of disseminated lesions on MRI have only a 6 to 16% risk of developing MS after 4 years or more of follow-up. The incidence of MRI abnormalities in children with ON is less than in adults; this observation, coupled with clinical experience, suggests that the rate of progression to MS in children with isolated ON may be less than in adults.

## **IMPAIRMENT OF THE OCULAR MOTOR PATHWAYS**

Impairment of individual ocular motor nerves is infrequent in MS. When present, the involved nerves are, in decreasing order of frequency, Cranial Nerves VI, III, and, rarely, IV. More frequent findings are those that reflect lesions of vestibulo-ocular connections and internuclear connections. Nystagmus is a common finding in MS. One form of nystagmus particularly characteristic of MS is acquired pendular nystagmus, in which there are rapid, small amplitude pendular oscillations of the eyes in the primary position resembling quivering jelly. Patients frequently complain of oscillopsia (subjective oscillation of objects in the field of vision). This type of nystagmus usually is seen in the presence of marked loss of visual acuity. Internuclear ophthalmoplegia, defined as abnormal horizontal ocular movements with lost or delayed adduction and horizontal nystagmus of the abducting eye, is secondary to a lesion of the medial longitudinal fasciculus on the side of diminished adduction. Convergence is preserved. When present bilaterally, it is usually coupled with vertical nystagmus on upward gaze. Although most suggestive of MS, a bilateral internuclear ophthalmoplegia can be observed with other intraaxial brainstem lesions, including brainstem glioma, vascular lesions, Arnold-Chiari malformations, and Wernicke's encephalopathy. Ocular pursuit movements are frequently saccadic rather than smooth. Ocular dysmetria may coexist with other signs of cerebellar dysfunction and other ocular oscillations, such as intrusive saccadic movements (square wave jerks).

## **IMPAIRMENT OF OTHER CRANIAL NERVES**

Impairment of facial sensation, subjective or objective, is a relatively common finding in MS. The occurrence of trigeminal neuralgia in a young adult is frequently an early sign of MS. Facial myokymia, a fine undulating wavelike facial twitching, and hemifacial spasm can be caused by MS, but other causes of a focal brainstem lesion must be excluded. Unilateral facial paresis can occur, but taste sensation is almost never affected. In these syndromes, as with acute oculomotor palsy, the nerve is affected in its course within the neuraxis, rather than peripherally. Vertigo is a reported symptom in 30 to 50% of patients with MS and is commonly associated with dysfunction of adjacent cranial nerves. Resulting symptoms include hyperacusis or hypoacusis, facial numbness, and diplopia. Complete hearing loss,



usually unilateral, is an infrequent complaint. Malfunction of the lower cranial nerves is usually of the upper motor neuron type (pseudobulbar syndrome).

## **IMPAIRMENT OF THE SENSORY PATHWAYS**

Sensory manifestations are a frequent initial feature of MS and are present in almost every patient at some time during the course of disease. The sensory features can reflect spinothalamic, posterior column, or dorsal root entry zone lesions. The sensory symptoms are commonly described as numbness, tingling, pins and needles, tightness, coldness, or swelling of limbs or trunk. Radicular pains, unilateral or bilateral, can be present, particularly in the low thoracic and abdominal regions, or a band-like abdominal sensation may be described. An intensely itching sensation, especially in the cervical dermatomes, usually unilateral, suggests MS.

The most frequent sensory abnormalities on clinical examination are the following: varying degrees of impairment of vibration and joint position sense, decrease of pain and light touch in a distal distribution in the four extremities, and patchy areas of reduced pain and light touch perception in the limbs and trunk. A bilateral sensory level is a more frequent finding than a hemisensory (Brown-Séquard) syndrome. Patients commonly report that the feeling of pinprick is increased or feels like a mild electric shock or that the stimulus spreads in a ripple fashion from the point at which it is applied. The sensory useless hand is a characteristic but uncommon feature, consisting of an impairment of function secondary to a pronounced alteration of proprioception, without loss of power. A lesion of the relevant root entry zones in the spinal cord is postulated in such cases.

## **IMPAIRMENT OF MOTOR PATHWAYS**

Corticospinal tract dysfunction is common in MS. Paraparesis, or paraplegia, is a much more common occurrence than is significant weakness in the upper extremities. With severe spasticity, extensor or flexor spasms of the legs and sometimes the trunk may be provoked by active or passive attempts to rise from a bed or wheelchair. The physical findings include spasticity, usually more marked in the legs than in the arms. The deep tendon reflexes are exaggerated, sustained clonus may be elicited, and extensor plantar responses are observed. All of these manifestations are commonly asymmetrical. Occasionally, deep tendon reflexes may be decreased because of lesions interrupting the reflex arc at a segmental level, and one may observe an inverted reflex wherein one reflex, such as the triceps, is lost and the efferent component is represented by a contraction of a muscle below the lesion, such as the triceps muscle. The Achilles' reflex can be absent in lesions of the sacral segments of the spinal cord with or without concomitant sphincter and sexual problems. Occasionally, reduced reflexes reflect hypotonia resulting from cerebellar pathway lesions. Amyotrophy, when observed, most frequently affects the small muscles of the hand; lesions of the motor root exit zones may produce muscle denervation caused by axon loss. Secondary entrapment neuropathies are



also a cause of muscle atrophy in patients with MS. A common pattern of disease evolution seen in the spinal form of MS is an ascending pattern of weakness that begins with involvement of the lower extremities and spreads to involve first one upper extremity and then the other, beginning in the intrinsic hand muscles. Frequently, there is an associated weakness of the trunk muscles with abnormal posture and involvement of respiratory muscles.

## **IMPAIRMENT OF CEREBELLAR PATHWAYS**

Cerebellar pathway impairment results in gait imbalance, difficulty in performing coordinated actions with the arms, and slurred speech. Examination reveals the usual features of cerebellar dysfunction, such as dysmetria, decomposition of complex movements, and hypotonia, most often observed in the upper extremities. An intention tremor may be noted in the limbs and head. Walking is impaired by truncal ataxia. Ocular findings of nystagmus, ocular dysmetria, and frequent refixation saccades suggest cerebellar or cerebellovestibular connection dysfunction. Speech can be scanning or explosive. In severe cases of MS, there is complete astasia (inability to stand), inability to use the arms because of a violent intention tremor, and virtually incomprehensible speech. Cerebellar signs are usually mixed with pyramidal (corticospinal) tract signs.

## **IMPAIRMENT OF BLADDER, BOWEL, AND SEXUAL FUNCTIONS**

The extent of sphincter and sexual dysfunction often parallels the degree of motor impairment in the lower extremities. The most common complaint related to urinary bladder dysfunction is urgency, usually the result of uninhibited detrusor contraction, reflecting a suprasegmental lesion. As the disease progresses, urinary incontinence becomes more frequent. With involvement of sacral segments of the spinal cord, symptoms of bladder hypoactivity may evolve, such as decreased urinary flow, interrupted micturition, and incomplete bladder emptying. An atonic dilated bladder that empties by overflow results from loss of perception of bladder fullness and is usually associated with urethral, as well as anal and genital hypoesthesia, and sensory deficits in the sacral dermatomes. A dysynergic voluntary sphincter, interrupting bladder emptying, will lead to frequent small volume urinations, combined with a large postvoiding residual. When evaluating bladder incontinence or urgency in patients with MS, one must exclude other causes, particularly in multiparous women. Urinary tract infections are common in MS, especially in women. These infections usually do not cause fever and back pain and may increase the extent of bladder dysfunction.

Constipation is more common than fecal incontinence and can reflect both upper and lower motor neuron impairment in addition to decreased general mobility. Almost all patients with paraplegia require special measures to maintain regular bowel movements.

Sexual dysfunction, although frequently overlooked, is a common occurrence in MS. Approximately 50% of patients become completely sexually inactive second-

ary to their disease, and an additional 20% become sexually less active. Men experience various degrees of erectile dysfunction, often with rapid loss of erection at attempted intercourse, whereas loss of ejaculation is less common. Most women preserve their orgasmic capabilities, sometimes even in the presence of complete loss of bladder and bowel function. Sexual dysfunction can be the result of multiple problems, including the direct effects of lesions of the motor and sensory pathways within the spinal cord in addition to psychological factors involved with self-image, self-esteem, and fear of rejection from the sexual partner. Mechanical problems created by spasticity, paraparesis, and incontinence further aggravate the problem.

## **COGNITIVE IMPAIRMENT**

Data from formal neuropsychological studies indicate that cognitive involvement has been underreported in MS. Neuropsychological test results have shown that 34–65% of patients with MS have cognitive impairment. The most frequent abnormalities are with abstract conceptualization, recent memory, attention, and speed of information processing. Patients refer to memory loss or frustration. The abnormalities are usually not apparent during a routine office visit. In a fast-paced environment with multiple stimuli, the cognitive deficit of the MS patient is most obvious. Aphasia, neglect syndrome, cortical blindness, or marked behavioral problems are rare.

Two kinds of recent data have added urgency to the need to assess cognitive deficits: the demonstration by Trapp and colleagues (6) of ongoing axon loss in central white matter beginning at the earliest stages of MS and the demonstration that thinning of the corpus callosum, enlargement of the ventricular system, and other evidences of brain atrophy can be measured accurately by MRI and also begin earlier than previously believed.

Cross-sectional studies have shown some degree of affective disturbance in up to two-thirds of patients with MS (33). Depression is the most common manifestation and is, in part, secondary to the burden of having to cope with a chronic, incurable disease. Some data suggest that depression is more common in patients with MS than in others with chronic medical conditions, in whom the lifetime risk of depression was 12.9% in one study. This contrasts with a study of 221 patients with MS, in whom the risk for depression was 34% (32). Some data indicate a comorbid association, presumably genetic, between bipolar illness and MS. Frontal or subcortical white-matter disease may also be a contributory causative factor. Euphoria is usually associated with moderate or severe mental impairment. Patients may manifest a dysphoric state with swings from depression to elation. On occasion, acute cerebral lesions can manifest as a confusional state.

## **EPILEPSY**

Epilepsy is more common in patients with MS than in the general population, occurring in 2 to 3% of patients (33). Convulsions may be either tonic-clonic or partial complex. They generally are benign and transient and respond well to antiepileptic drug therapy or require no therapy. The prevalence of cortical syn-

dromes, such as aphasia, apraxia, and agnosia, is low. As an example, in a study of 5715 patients with MS, 51 (0.89%) experienced seizure activity (36). Generalized tonic-clonic seizures were most common (35 patients, 69%), followed by simple or complex partial seizures (11 patients, 22%). Of the 45 patients who received antiepileptic drug therapy, 35 (78%) became seizure free, whereas 5 (11%) had intractable seizures.

## CLINICAL FEATURES DISTINCTIVE OF MULTIPLE SCLEROSIS

Although there are no clinical phenomena that are unique to MS, some are highly characteristic of the disease (Table 1). Bilateral internuclear ophthalmoplegia has been mentioned. Lhermitte's phenomenon is a transient sensory symptom described as an electric shock radiating down the spine or into the limbs on flexion of the neck. It may be infrequent or occur with the least movement of the head or neck. Although most frequently encountered in MS, this symptom can be seen with other lesions of the cervical cord, including tumors, cervical disc herniation, postradiation myelopathy, and after trauma.

Paroxysmal attacks of motor or sensory phenomena may arise as a manifestation of demyelinating lesions. Within the brainstem, lesions can cause paroxysmal diplopia, facial paresthesia, trigeminal neuralgia, ataxia, and dysarthria. Motor system involvement results in painful tonic contractions of muscles of one or two (homolateral) limbs, trunk, and occasionally the face, but these rarely occur in all four limbs or the trunk. These paroxysmal attacks usually respond to low doses of carbamazepine and frequently remit after several weeks to months, usually without recurrence.

Heat sensitivity is a well-known occurrence in MS (Uhtoff's phenomenon); small increases in the body temperature can temporarily worsen current or preexisting signs and symptoms. This phenomenon is encountered in other neurological diseases but to a lesser extent and is presumably the result of conduction block developing in nerves as the body temperature increases. Normally, the nerve conduction safety factor decreases with increasing temperature until a point is reached at which conduction block occurs; this point of conduction block is reached at a much lower temperature in demyelinated nerves.

Fatigue is a characteristic finding in MS, usually described as physical exhaustion that is unrelated to the amount of activity performed. Many patients complain of feeling exhausted on waking, even if they have slept soundly. Fatigue can appear also during the day but may be partially or completely relieved by rest. There is a poor correlation between fatigue and the overall severity of disease or with the presence of any particular symptom or sign. Unlike cognitive deficit, no MRI findings correlate with fatigue, or with depression (37–40). Fatigue is often seen in association with an acute attack and may precede the focal neurological features of the attack and persist long after the attack has subsided.

## DIAGNOSTIC CRITERIA

The new McDonald criteria (Table 4) has several advantages. The former categories of possible, probable, and definite MS have become obsolete. The MRI criteria are based on extensive data of Barkof and Tintore and are designed to retain sensitivity while enhancing specificity. They will have little usefulness in patients with clear-cut demyelinating syndromes, such as ON or a brainstem syndrome; in such cases, many clinicians will be satisfied with less stringent MRI criteria. In patients with obscure symptoms, the criteria will help avoid premature diagnosis and treatment. In addition, criteria for primary progressive MS are proposed. Revisions to the McDonald criteria have been proposed. For example, the role of spinal cord lesions must be evaluated and included. It may take some time before the final criteria are decided on and accepted as the standard for diagnosis.

There remains the clinical problem, distinct from research criteria, of the patient early in the course who does not meet such diagnostic criteria. In the setting of a monophasic neurological illness that is clinically consistent with MS and in the presence of multifocal white-matter lesions on MRI consistent with demyelinating plaques, the diagnosis of MS is almost certain. In Brex et al.'s long-term study (41), which followed patients with initial demyelinating episodes for up to 14 years, in practical terms, no diagnoses were encountered other than suspected MS or definite MS. In addition, follow-up studies have shown that a significant percentage of patients with MRI lesions detected at onset do not progress to clinically symptomatic MS, after many years of follow-up. The issue of the monophasic demyelinating disease is discussed in the section on Clinically Isolated Syndromes. Such patients may be classed as suspected MS; they may in fact represent particularly benign forms of the disease.

A common error is to overinterpret multiple hyperintense lesions on MRI as equivalent to MS. Clinical symptoms must be consistent with MS. A few white-matter lesions in T2-weighted MRI scans are not infrequent, particularly in the elderly, and do not indicate a diagnosis of MS. CNS vasculitides, such as SLE, Sjögren's disease, polyarteritis nodosa, syphilis, retroviral diseases, and Behçet's disease, may all produce multifocal lesions with or without a relapsing-remitting course. SLE can present as a recurrent neurological syndrome before the systemic manifestations of this disease declare themselves. Behçet's syndrome is characterized by buccogenital ulcerations in addition to the multifocal neurological findings. Although rare, acute disseminated encephalomyelitis (ADEM) must be considered in the differential diagnosis. An MS-like phenotype associated with mitochondrial gene defects has been described, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL); it is of note that when there are multiple MS cases in a family, maternal transmission is more frequent than paternal transmission.

More important than features characteristic for MS are features that should prompt the clinician to reconsider the diagnosis of MS. Many physicians fail to

pursue further diagnostic steps when a patient is diagnosed with MS. Features that should alert the clinician to the possibility of other diseases include:

- Family history of neurological disease.
- A well-demarcated spinal level in the absence of disease above the foramen magnum.
- Prominent back pain that persists.
- Symptoms and signs that can be attributed to one anatomical site.
- Patients who are over 60 years of age or younger than 15 years at the onset of disease.
- Progressive disease.

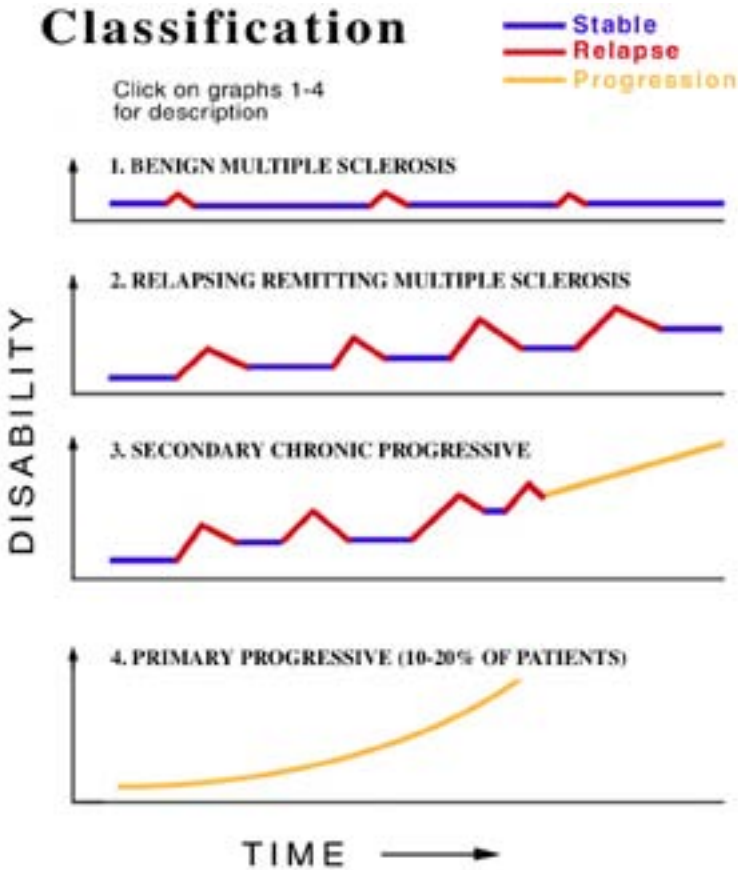
None of these features excludes the diagnosis of MS, but in these situations, one should explore the possibility of other etiologies before accepting the diagnosis.

The differential diagnosis of MS is limited in the setting of a young adult who has had two or more clinically distinct episodes of CNS dysfunction with at least partial resolution. Diagnostic difficulties arise in patients who have atypical presentations, monophasic episodes, or progressive illness.

- The unusual nature of some sensory symptoms and the difficulty patients experience in describing such symptoms may result in a misdiagnosis of hysteria.
- A monophasic illness with symptoms attributable to one site in the CNS creates a large differential that includes neoplasms, vascular events, or infections.
- The most trouble arises with progressive CNS dysfunction; great care must be taken in these patients to exclude treatable etiologies (compressive spinal cord lesions, arteriovenous malformations, cavernous angiomas, and Arnold-Chiari malformation), infection (HTLV-1), HIV, or hereditary disorders (adult metachromatic leukodystrophy, adrenomyelo-leukodystrophy, and spinocerebellar disorders).

## COURSE

The most characteristic clinical course of MS is the occurrence of relapses (Fig. 1), which can be defined as the acute or subacute onset of clinical dysfunction that usually reaches its peak from days to several weeks, followed by a remission during which the symptoms and signs resolve partially or completely. The minimum duration for a relapse has been arbitrarily established at 24 hours. Clinical symptoms of shorter duration are less likely to represent what is considered as a true relapse (i.e., new lesion formation or extension of previous lesion size). Worsening of previous clinical dysfunction can occur concurrently with fever, physical activity, or metabolic upset and last for hours to a day or more. Such worsening is believed to reflect conduction block in previously demyelinated axons. Relapses of MS vary markedly regarding CNS site involved, the frequency of attacks (the free interval between relapses ranges from weeks to years), the mode of onset (from quite sudden to subacute), and the duration, severity, and quality of remission. The frequency of relapses is highly variable and depends on the population studied and the closeness of observation and recording by patients and physicians. Summaries of many studies provide an average figure of 0.4–0.6 relapses per year. Patients followed closely in clinical trials have higher relapse rates, probably reflecting self-selection and closer reporting and examinations in such studies. The attack rate in the placebo group in clinical studies ranges from 0.8 to 1.2 attacks per year. In



**Fig. 1.** Relapsing-remitting MS: Clearly defined relapses with full recovery or with sequelae and residual deficit on recovery. The periods between disease relapses are characterized by a lack of disease progression. Primary-progressive MS: Disease progression from onset with occasional plateaus and temporary minor improvements allowed. Secondary-progressive MS: Initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions, and plateaus. Two severity outcomes are also described.

general, relapses are more frequent during the first years of the disease and tend to wane in later years. A course marked by relapses, interspersed by periods during which the disease seems relatively dormant, is termed relapsing-remitting.

The course of MS can be expressed in patients as follows:

1. Severe relapses, increasing disability, and early death.
2. Many short attacks, tending to increase in duration and severity.
3. Slow progression from onset, superimposed relapses, and increasing disability.

4. Slow progression from onset without relapses.
5. Abrupt onset with good remission followed by long latent phase.
6. Relapses of diminishing frequency and severity, slight residual disability only.
7. Abrupt onset, few if any relapses after first year, no residual disability (42).

Approximately 15% of patients never experience a second relapse. The exact frequency of such benign MS is unknown, however, because many such individuals never come to medical attention. Autopsy studies found significant numbers of cases with CNS pathology consistent with MS and yet no documented clinical evidence of such disease. Similarly, MRI studies have shown MS-like plaques in T2-weighted scans in patients who have never had a neurological episode. Asymptomatic relatives of patients with MS have MRI lesions consistent with demyelination in up to 15% of these relatives (43). The use of MRI may expand the spectrum of MS by detecting milder cases that previously were not included in prognosis studies.

A standardization of terms has been agreed on to determine the pattern and course of the illness (44). Four categories of disease are described:

- *Relapsing-remitting MS*: Clearly defined relapses with full recovery or with sequelae and residual deficit on recovery. The periods between disease relapses are characterized by a lack of disease progression.
- *Primary-progressive MS*: Disease progression from onset with occasional plateaus and temporary minor improvements allowed.
- *Secondary-progressive MS*: Initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions, and plateaus.
- *Progressive-relapsing MS*: Progressive disease from onset, with clear acute relapses, with or without full recovery. The periods between relapses are characterized by continuing progression.

Two severity outcomes are also described:

1. Benign MS is a disease in which the patient remains fully functional in all neurological systems 15 years after the disease onset.
2. Malignant MS is a disease with a rapid progressive course, leading to significant disability in multiple neurological systems or death in a relatively short time after disease onset.

Data from a clinic-based study of 1100 patients (45) who represented the population of the region found that 66% of patients at onset had relapsing and remitting disease, 15% had relapsing-progressive, and 19% had progressive disease from the onset. Patients evolved from a relapsing-remitting course to a progressive course; 85% of patients began with a relapsing course, but the proportion continuing as relapsing disease decreased steadily, so that by 9 years from onset, only 50% were still relapsing. Likewise, the probability of reaching 6 on the Kurtzke disability score was 50% 16 to 17 years after onset. The course of MS with onset after the age of 40 was progressive in more than 60% of patients.

The rate of clinical progression of MS is variable. The commonly used index of clinical disability, the Kurtzke disability status score (DSS), or the expanded version called the expanded disability status score (EDSS), uses numbers ranging from



0, for normal examination and function, to 10, for death caused by MS. This scale is nonlinear, with great emphasis on ambulation capabilities with scores above 4.

Most MS populations have bimodal distributions of EDSS scores, with peaks at values of 1 and 6 (ambulation with unilateral assistance). The time spent by a patient at a given level of disability varies with the score. Thus, for patients with DSS scores of 4 or 5, median time spent at these levels was 1.2 years, whereas for those at DSS 1, median time to stay at that level was 4 years, and at DSS 6, 3 years. These results have powerful implications for the conduct of clinical studies with respect to patient selection, stratification, and duration of follow-up: if many patients of DSS 1 or 6 are included, little movement is seen in a group followed for 1 or 2 years. The rate of progression with chronic progressive disease in the placebo groups of three clinical trials ranged from 0.5 to 0.7 points per year on the DSS scale.

In a cohort of 308 patients followed for 25 years, the following data emerged (46):

- 80% of the patients had reached the progressive phase by 25 years.
- 15% of the patients had died.
- 65% of the patients had reached EDSS 6 (requiring aids for walking).
- 50% of the patients reached EDSS 6 within 16 years of onset.

The EDSS, although universally used in clinical trials, has numerous serious limitations. Even with special training and examiner blinding, interrater and intrarater variations in scoring are common. EDSS scores of 4 and higher depend almost entirely on the ability to walk. Developing dementia, vision loss, or weakness of hands may pass undetected by the scoring. An obvious implication of these facts is that other outcome measures should be used as well, and that minor changes in EDSS alone should not be overinterpreted.

The Multiple Sclerosis Functional Composite Scale (MSFC) is a more recent clinical tool designed to avoid the problems encountered with the EDSS. The MSFC consists of three parts: (1) Paced Auditory Serial Addition Test (PASAT), (2) 9-Hole Peg Test (9HPT), and (3) Timed 25-Foot Walk (T25FW). These three measures take into account cognition, upper extremity, and lower extremity functions. A z-score is obtained for each measure, and a combined z-score is then derived. The MSFC has been validated in several clinical trials. The tests can be performed by a nonphysician and are highly reproducible and predictable (47–49).

## EFFECT OF EXOGENOUS FACTORS ON THE COURSE

The role of several exogenous factors either influencing the development of MS or inducing disease exacerbations has been examined using epidemiological techniques. A disproportionately high number of relapses occur in patients with MS who have suffered recently from viral infections, and a high number of infections are followed by acute attacks. Increased interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  produced by cells of the immune system during viral infections may play a role in this increased relapse rate by increasing expression of major histocompatibility complex class II antigens and adhesion molecules on cells



of the immune system and CNS, with a resultant increase in the number of activated T-cells being attracted to the CNS. Controversy exists about a link between occurrence of stressful events and exacerbation of MS. Trauma is not implicated in disease induction or relapse, although in the experimental animal model EAE, lesions are most prominent at sites of preexistent traumatic lesions. Performance of neurological diagnostic procedures, such as myelography and lumbar puncture, has not been linked with aggravation of the MS disease course, neither has administration of local or general anesthetics. Recent data do not establish a link between vaccination and disease exacerbations, and few clinicians withhold immunization programs, for example, for influenza or hepatitis.

## **EFFECT OF PREGNANCY ON THE COURSE**

MS is a disease that predominantly affects women and has a maximum incidence during childbearing years. The influence of pregnancy on MS has been repeatedly examined, with evidence that relapses are reduced late in pregnancy and are more frequent than expected in the 3-month postpartum period. However, this is not the finding in all studies. There is general agreement that the overall prognosis is no different in women who have been pregnant compared with those who have not. Studies of women with MS reveal no increase in stillbirths, ectopic pregnancies, or spontaneous abortions. These data would suggest that pregnancy has no ill effect on MS and that MS has no negative effect on the fetus or the course of pregnancy. In a study of postmenopausal women, there (50). An important issue in the pregnant woman with MS is to avoid exposing the fetus to toxic drugs (Table 8).

## **PROGNOSIS**

Although great individual variability exists regarding disease prognosis, several factors have been identified as possible prognostic indicators. The rate of clinical progression of MS is variable. The commonly used index of clinical disability, the DSS, or the expanded version (EDSS), uses numbers ranging from 0 for normal examination and function to 10 for death caused by MS. This scale is nonlinear, with great emphasis on ambulation capabilities with scores higher than 4. Most MS populations have bimodal distributions of EDSS scores, with peaks at values of 1 and 6 (ambulation with unilateral assistance). The time spent by a patient at a given level of disability varies with the score. Thus, for patients with DSS scores of 4 or 5, median time spent at these levels was 1.2 years, whereas for those at DSS 1, median time to stay at that level was 4 years, and at DSS 6, 3 years. These results have powerful implications for the conduct of clinical studies with respect to patient selection, stratification, and duration of follow-up: if many patients of DSS 1 or 6 are included, little movement is seen in a group followed for 1 or 2 years. The rate of progression with chronic progressive disease in the placebo groups of three clinical trials ranged from 0.5 to 0.7 points per year on the DSS scale.

A large database of 1844 MS patients was analyzed to determine predictors of disability. This study concluded that it takes longer to reach landmarks of irreversible disability in younger female patients with relapsing disease, patients present-

**Table 8**  
**Safety in Pregnancy of Drugs Used in the Treatment of Multiple Sclerosis**

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Category B: Animal data showing no harm to the fetus; no human data available  
 Glatiramer acetate (Copaxone)  
 Pemoline  
 Oxybutynin  
 Fluoxetine (and other selective serotonin reuptake inhibitors)  
 Desmopressin

Category C: Animal data shows harm to the fetus; no human data available  
 Corticosteroids  
 Interferon (IFN)- $\beta$ -1a (Avonex/Rebif)  
 IFN- $\beta$ -1b (Betaseron)  
 Baclofen  
 Amantadine  
 Tizanidine  
 Carbamazepine

Category D: Known to cause fetal harm when administered to pregnant women  
 Azathioprine  
 Cladribine  
 Cyclophosphamide  
 Mitoxantrone (Novantrone)

Category X: Contraindicated for use during pregnancy  
 Methotrexate

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Source: Modified from ref. 50.

ing with ON, and patients with fewer relapses in the first years of the disease. The study also showed that these good prognostic clinical variables held true for patients up to an EDSS of 4 but did not seem to remain predictive of the time course of disability past 4 to landmarks 6 and 7 (51).

Another MS study between 1976 and 1987 in Norway verified these results and also evaluated primary progressive (PP) patients. The probability of being alive after 15 years was 94.8%. The probability of managing without a wheelchair was 75.8%, of walking without assistance was 60.3%, and of not being awarded a disability pension was 46%. The probability of still having a relapsing remitting (RR) course after 15 years was 62%. Analysis of the total MS population showed that patients with PPMS had more than 7.5 times higher risk of reaching EDSS = 6 than patients with RRMS (52).

Between 1990 and 1998, 98 newly diagnosed patients were evaluated for prognosis using six risk factors:

1. Age at onset (<40 vs >40)
2. Symptoms at onset (isolated sensory or cranial nerve vs motor or sensory plus motor)
3. MRI (at first attack vs CDMS)

4. Interval between first and second attack (>2.5 or <2.5 years)
5. Attack frequency in first 2 years (<2 or >2)
6. Completeness of recovery from initial attack (good vs poor)

Analysis showed 17% with low risk of progression (0–1 risk factors) and 24% with high of progression (4–6 risk factors). The high-risk group did significantly worse in terms of final EDSS and progression to higher EDSS. At the time of diagnosis of CDMS, MRI findings suggestive of MS were seen in 84%, suspicious in 13%, and negative in 3% (53).

*Sex:* MS appears to follow a more benign course in women than in men.

*Age at onset:* The average age at onset of MS is 29 years. Onset at an early age is seemingly a favorable factor, whereas onset at a later age carries a less favorable prognosis. As previously stated, the pattern of disease varies in different age groups, with the relapsing-remitting form being more common in younger patients and the progressive form being more common in the older age group. Data are lacking regarding whether prognosis differs as a function of age in patients with similar patterns of disease.

*Initial disease course:* The relapsing form of the disease is associated with a better prognosis than progressive disease. A high rate of relapses early in the course of illness may correlate with shorter time to reach EDSS 6, as does a short first interval between attacks.

*Initial complaints:* Among initial symptoms, impairment of sensory pathways or cranial nerve dysfunction, particularly ON, are found in several studies to be favorable prognostic features, whereas pyramidal and particularly brainstem and cerebellar symptoms carry a poor prognosis. Both benign and fulminant forms of MS are recognized. There is no agreement among workers in the field as to the meaning of these terms. It is the general experience that a patient whose disease has had a benign course for 15 years only rarely develops a more severe course. Patients with mild disease (EDSS score 0–3) 5 years after diagnosis only uncommonly progress to severe disease (EDSS score 6) by 10 years (7.5% of patients) and 15 years (11.5% of patients) (46). The term malignant MS is variably used by different workers; some use it to imply a rapid course, others to a clinical course in which there are frequent severe relapses with little recovery. Clues to etiology, susceptibility, and resistance factors must be present in such extremes of the clinical spectrum, but they remain elusive at present. Entities such as Devic's disease, Baló's concentric sclerosis, and particularly Marburg's disease are more fulminant variants of MS with early disability and even death.

## OPTIC NEURITIS

The incidence of MRI abnormalities in children with ON is less than that in adults, which, when coupled with clinical experience, suggests that the rate of progression to MS in children with isolated ON may well be less than that in adults. Five-year data from the original Optic Neuritis Treatment Trial revealed that the 5-year cumulative probability of developing clinically definite MS was 30% and

did not differ by treatment group (oral prednisone, IV methylprednisolone, and placebo). However, MRI was a strong predictor; the 5-year risk of developing clinically definite MS was 16% in patients with no brain MRI lesions and 51% in patients with three or more lesions (31).

## **MYELOPATHIC SYNDROMES**

### *Acute Myelopathy*

Patients presenting with acute complete transverse myelitis have a cited risk of MS of only 5–10%. However, partial or incomplete myelitis is a much more common clinical entity and bears more relevance to MS. Studies examining the issue of acute partial myelitis as an initial presentation of MS found that 57–72% of such patients had cranial MRI abnormalities consistent with MS. Follow-up from 3 to 5 years found that 60–90% of these patients developed MS, whereas 10–30% of those with normal MRI developed MS (12). CSF studies suggest that patients with monosymptomatic disease with positive OCBs have a higher risk of evolution to MS than those without OCBs, although CSF results do not help further in prognosis when compared with MRI alone. CSF analysis would be most useful in a situation in which MRI is not available.

### *Chronic Myelopathy*

In patients with chronic progressive myelopathy, 60–70% have cranial MRI abnormalities consistent with MS in the absence of clinical evidence of disease above the level of the spinal cord. What remains unclear is whether the remaining 30% have a disease other than MS or whether MS can manifest as a purely spinal disorder. Probably both situations apply; improved spinal neuroimaging should help resolve this issue.

## **VARIANTS OF MULTIPLE SCLEROSIS**

Diseases affecting CNS myelin can be classified on the basis of whether a primary biochemical abnormality of myelin exists (dysmyelinating) or whether some other process damages the myelin or oligodendroglial cell (demyelinating). Demyelinating diseases in which normal myelin is disrupted include autoimmune, infectious, toxic and metabolic, and vascular processes (Table 9). Dysmyelinating diseases in which a primary abnormality of the formation of myelin exists include several hereditary disorders (Table 9), infectious demyelinating disease (progressive multifocal leukoencephalopathy), toxic and metabolic demyelinating diseases, and vascular demyelinating disease (Binswanger's disease). A list of differential diagnoses can be found in Table 10.

MS is a condition with many variable forms, but in most cases the common signs and symptoms described are readily apparent, and with proper laboratory confirmation, the diagnosis is not difficult. Some patients have their entire clinical illness confined to the optic nerves. One optic nerve may be affected sequentially

**Table 9**  
**Diseases of Myelin**

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*Autoimmune*

Acute disseminated encephalomyelitis  
Acute hemorrhagic leukoencephalopathy  
Multiple sclerosis

*Infectious*

Progressive multifocal leukoencephalopathy

*Toxic/metabolic*

Carbon monoxide  
Vitamin B<sub>12</sub> deficiency  
Mercury intoxication (Minamata disease)  
Alcohol/tobacco amblyopia  
Central pontine myelinolysis  
Marchiafava-Bignami syndrome  
Hypoxia  
Radiation

*Vascular*

Binswanger's disease

*Hereditary disorders of myelin metabolism*

Adrenoleukodystrophy  
Metachromatic leukodystrophy  
Krabbe's disease  
Alexander's disease  
Canavan-van Bogaert disease  
Pelizaeus-Merzbacher disease  
Phenylketonuria

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after another, or there can be simultaneous bilateral visual loss, a state that is uncommon in classic MS. In some instances, a head MRI will show scattered intracerebral lesions in addition to lesions of the optic nerves or CSF examination will show OCB, attesting to some degree of dissemination of the lesions. Children and preadolescent patients are more likely than adults to have recurrent or simultaneous optic neuropathy. The distinction from an MS variant can be challenging. Sarcoidosis is commonly a diagnostic consideration in patients with bilateral ON. However, there are several inflammatory demyelinating disorders that bear an unknown relationship to MS. They are listed here as variants of MS, rather than as separate illnesses, because it is often found, after long follow-up, that the disease has reverted to a more standard variety of MS.

### ***Recurrent Optic Neuropathy***

There are patients whose entire clinical illness is confined to the optic nerves. They may have sequential affection of one nerve, then the other, or they may have

**Table 10**  
**Differential Diagnosis in Multiple Sclerosis**

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*Inflammatory diseases*

Granulomatous angiitis  
 Systemic lupus erythematosus  
 Sjögren’s disease  
 Behçet’s disease  
 Polyarteritis nodosa  
 Paraneoplastic encephalomyelopathies  
 Acute disseminated encephalomyelitis/postinfectious encephalomyelitis

*Infectious diseases*

Lyme neuroborreliosis  
 Human T-cell lymphotropic virus type 1 infection\*  
 Human immunodeficiency virus infection  
 Progressive multifocal leukoencephalopathy\*  
 Neurosyphilis\*

*Granulomatous diseases*

Sarcoidosis  
 Wegener’s granulomatosis  
 Lymphomatoid granulomatosis

*Diseases of myelin*

Metachromatic leukodystrophy (juvenile and adult)\*  
 Adrenomyeloleukodystrophy\*

*Miscellaneous*

Spinocerebellar disorders\*  
 Arnold-Chiari malformation  
 Vitamin B<sub>12</sub> deficiency\*

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\*Indicates disorders that are predominantly important to differentiate in the setting of progressive disease.

simultaneous bilateral vision loss, a state that is quite uncommon in classic MS. In some instances, MRI of the head shows (in addition to lesions of the optic nerves) scattered intracerebral lesions, or a CSF examination shows OCB, attesting to some degree of dissemination of the lesions. Children and preadolescent patients are more likely than adults to have recurrent or simultaneous optic neuropathy. Rarely there is slowly progressive optic neuropathy, similar to that seen with optic nerve sheath tumors, such as meningioma. The distinction from an MS variant can be challenging. In bilateral ON, sarcoidosis is commonly a diagnostic consideration.

***Devic’s Disease (Neuromyelitis Optica)***

A combination of bilateral optic neuropathy and cervical myelopathy comprise this condition, which most authorities now classify as a variant of MS. Reported cases indicate that the myelopathy tends to be more severe, with less likelihood of

recovery, and that the neuropathological features at autopsy are those of a much more severe necrotic lesion of the cord rather than incomplete demyelination (54). In some patients, the optic neuropathy and the myelopathy occur at the same time; in others, one or the other component is delayed. The longer the interval, the more like typical MS is the pathology. Because the optic nerve and the cervical spinal cord are two of the locations in the nervous system in which MS lesions are typically found, many patients could be classified as having Devic's disease or syndrome. Little is to be gained by this nomenclature, because Devic's syndrome can be a manifestation of acute disseminated encephalomyelitis (ADEM) (*see* Acute Disseminated Encephalomyelitis), or rarely of other autoimmune disease, such as SLE. This is especially true of patients with relapsing Devic's syndrome, comprising approximately one-half of the patients. In a few patients, the distinction between an MS variant and SLE (so-called lupoid sclerosis) is essentially impossible to make, and some of these are patients with neuromyelitis optica (NMO). A study of 80 patients with NMO revealed that predictors of a relapsing course were longer interattack interval between the first two clinical events, older age at onset, female sex, and less severe motor impairment with the sentinel myelitis event. A history of other autoimmune disease, higher attack frequency during the first 2 years of disease, and better motor recovery after the index myelitis event were associated with mortality resulting from relapsing NMO with almost one-third of the deaths secondary to recurrent myelitis with respiratory failure and concomitant medical complications (55).

### *Slowly Progressive Myelopathy*

A syndrome of slowly progressive spinal cord dysfunction can present a major diagnostic challenge. If there are no sensory signs or symptoms, the entity known as primary lateral sclerosis, one of the group of motor neuron disease, may be the cause. HTLV-1 infection, vitamin B<sub>12</sub> deficiency, and human immunodeficiency virus infection all can be excluded by appropriate testing. Spinal dural arteriovenous fistula can cause a steadily or stepwise progressive myelopathy, usually in the lower spinal segments. Adrenomyeloneuropathy should be considered. Numerous patients remain who do not fit into these categories and whose spinal MRI results are repeatedly negative. VERs, CSF OCBs, and MRI of the head show no sign of demyelination elsewhere. No firm diagnosis is possible. Minor clues that MS is present may be furnished by a Lhermitte's sign that has come and gone or by undue sensitivity to elevated temperature. The degree of compression of the cervical cord by intervertebral disc disease is often an issue in the middle-aged patient, because a majority of persons have some degree of disc disease. There is little doubt that some laminectomies have been carried out for cervical spondylosis where MS was the final correct diagnosis. Progressive myelopathy caused by MS is part of the primary progressive MS group and carries the poor prognosis typical of that group. The choice of therapy is difficult. Some patients do better for a time with monthly IV corticosteroid therapy.

### ***Acute Tumor-Like Multiple Sclerosis (Marburg Variant)***

Some patients with demyelinating disease present with a large acute lesion of one hemisphere or rarely other locations, such as the spinal cord. Mass effect may occur, with compression of the lateral ventricle and shift across the midline. The clinical abnormalities in such patients are variable: they may be slight even in a patient with a massive lesion, whereas confusion, hemiparesis, or neglect syndrome may be seen in another patient with a lesion that appears no different. Much of the T2 bright lesion volume is often caused by edema and may be rapidly responsive to corticosteroids. (This change with corticosteroids also may occur with glioma or CNS lymphoma and is, therefore, not a useful diagnostic criterion.) Biopsy is often required.

In a series of 31 patients with Marburg variant, the prognosis was good, most patients recovered well clinically, and their lesion volume rapidly cleared (56). In 24 of the patients, the demyelinating lesion was solitary, whereas in the others there were one or more satellite nodules. Six of the patients were older than 57 years. At follow-up, 28 of the patients did not develop additional evidence of demyelinating activity during a 9-month to 12-year period. Others have reported a higher rate of recurrent disease, particularly a conversion to more ordinary types of MS, both clinically and by scan criteria (57).

### ***Acute Disseminated Encephalomyelitis (ADEM)***

This variant is classically described as a uniphasic syndrome occurring in association with an immunization or vaccination (postvaccination encephalomyelitis) or systemic viral infection (parainfectious encephalomyelitis). Pathologically, perivascular inflammation, edema, and demyelination within the CNS are present. Clinically, patients present with the rapid development of focal or multifocal neurological dysfunction. Prototypical illness arises after acute measles infection or rabies vaccine administration. Uncertainty regarding the diagnosis occurs when patients with clinical features of ADEM occur on the background of viral infections or vaccine administration not significantly linked with the syndrome by epidemiological criteria.

Neurological sequelae complicate 1 in 400 to 1 in 1000 cases of measles infection (58). Multiple subgroups of patients have been described, including those with diffuse cerebral features, focal or multifocal cerebral findings, cerebellar dysfunction, and spinal cord abnormalities; patients do not develop peripheral nerve damage or relapses of disease.

In addition to measles, an array of other viral and bacterial infections have tentatively been associated with ADEM, including rubella, mumps, herpes zoster, herpes simplex, influenza, EBV, coxsackievirus, *Borrelia burgdorferi*, mycoplasma, and leptospira. Acute encephalomyelitis occurring in the background of nonspecific viral illness is difficult to diagnose with certainty and to distinguish from episodes of MS.



The occurrence of neuroparalytic accidents as a consequence of the Pasteur rabies vaccine prepared from spinal cords of rabbits inoculated with fixed rabies virus was recorded soon after introduction of the treatment: the incidence of encephalomyelitis associated with the original Pasteur rabies vaccine prepared in rabbit brain has been estimated at 1 per 3000 to 35,000 vaccinations. Similar neurological complications were observed as a consequence of the Jenner vaccine used for the prevention of smallpox. Postvaccination ADEM does not result from the direct cytopathic effects of the virus but rather to immune-mediated mechanisms directed against specific components of the CNS (59).

ADEM also has been associated with other vaccines, including pertussis, rubella, diphtheria, and measles. The association between influenza vaccination, particularly the swine flu vaccine, and ADEM has been the subject of medicolegal controversy.

ADEM has been reported after the administration of some drugs. These drugs include sulfonamides and paraaminosalicylic acid (PAS)/streptomycin.

All of these associations can only be substantiated by strong epidemiological evidence or by the development of a pathognomonic laboratory finding for ADEM. However, neither of these circumstances currently exists.

Clinical features of the postvaccination and parainfectious syndromes are similar, except that the postrabies vaccination complications frequently involve the peripheral nervous system as well as the CNS. Many patients with postrabies immunization illness have only mild clinical features of fever, headache, or myalgia without CSF pleocytosis.

The hallmark clinical feature of the disorder is the development of a focal or multifocal neurological disorder after exposure to virus or receipt of vaccine. In some, but not all cases, a prodromal phase of several days of fever, malaise, and myalgias occurs. The onset of the CNS disorder is usually rapid (abrupt or up to several hours), reaching peak dysfunction within several days. Initial features include encephalopathy ranging from lethargy to coma, seizures, and focal and multifocal signs reflecting cerebral (hemiparesis), brain stem (cranial nerve palsies), and spinal cord (paraparesis) involvement. Other reported findings include movement disorders and ataxia. Each of these findings may occur as isolated features or in various combinations.

Features deemed characteristic of ADEM include simultaneous bilateral ON, loss of consciousness, meningismus, loss of deep tendon reflexes and retained abdominal reflexes in the presence of Babinski's reflexes, central body temperature of  $>100^{\circ}\text{F}$  ( $37.8^{\circ}\text{C}$ ), and severe shooting limb pains. By comparison, features characteristic of MS are unilateral ON, diplopia, hyperactive reflexes, and preserved awareness. Headache is an equivocal feature.

Recovery can begin within days, with complete resolution noted on occasion within a few days but more often over the course of weeks or months. Relapses are rare. Recovery from ADEM is more rapid compared to MS and usually more complete.

The mortality rate varies among reported series but is usually estimated at 10 to 30%, with complete recovery rates of 50% cited. Poor prognosis is correlated with severity and abruptness of onset of the clinical syndrome. Measles virus-associated ADEM may carry a worse prognosis than vaccine-associated disease. In earlier series, the occurrence of acute hemiplegias, which were interpreted as vascular occlusions and akin to the syndrome of acute hemiplegia of childhood, carried a particularly unfavorable prognosis regarding recovery.

Multifocal CNS lesions are generally evident on MRI that are initially indistinguishable from those observed in MS (60). Pathologically, ADEM produces scattered small perivenous lesions, often uniform in size, but this feature is not reliably detected by MRI. After several weeks, ADEM lesions show at least partial resolution without the appearance of new lesions, unlike MS. In some cases, lesions can persist. MRI in ADEM, as with MS, is more sensitive than CT scanning, which may, in some cases, reveal enhancing lesions.

The usual CSF formula is normal pressure, little or no (<100 cells/ $\mu$ L) increase in cell count, and a modest increase in protein. Well-documented cases exist with totally normal CSF pressure, cell counts, and protein content. Cases with high cell counts, including some polymorphonuclear cells and high protein values, represent a more necrotizing disease process. The high counts usually return to normal within a few days. The CSF Ig content is not usually increased, and OCB patterns are not usually observed. The content of myelin basic protein (MBP) in the CSF may be increased, as it can be in many conditions in which myelin destruction occurs, as in MS, or as part of more widespread tissue destructive process, such as cerebral infarction.

In many patients with postrabies vaccination and postmeasles ADEM, systemic blood lymphocyte sensitivity to MBP can be demonstrated in vitro, even though generalized cellular reactivity is depressed in patients with systemic measles virus infection. Although technically difficult to assess, CSF lymphocyte sensitivity to MBP may be even more marked than is systemic lymphocyte sensitivity. The occurrence of cases without MBP sensitivity indicates that this assay is insufficiently sensitive to establish or exclude the diagnosis of ADEM.

The diagnosis of ADEM can usually be made with confidence in the setting of a clear-cut antecedent event strongly associated with the disorder, such as measles infection or vaccination. The occurrence of an acute focal or multifocal CNS syndrome subsequent to a more nonspecific viral illness or vaccination in which the epidemiological link with ADEM is weak creates a wider differential diagnosis:

- An initial episode of what will prove to be MS: The presence of increased CSF IgG levels may favor MS. Follow-up MRI may be needed to distinguish the two disorders because the initial MRI scans can appear similar (60). The occurrence of a nonspecific viral illness before the onset of the clinical neurological syndrome does not distinguish between MS and ADEM because the incidence of exacerbations of MS is increased after such infections.
- CNS vasculitis with or without systemic features (such as disseminated intravascular coagulation or serum sickness)

- Multiple cerebral infarcts, particularly embolic from infected cardiac valves
- Chronic meningitis or granulomatous disease (sarcoidosis).

In addition, encephalitis, abscess, or tumor needs to be excluded if the main clinical feature is unifocal.

## ACUTE HEMORRHAGIC LEUKOENCEPHALITIS

Acute hemorrhagic leukoencephalitis is a rare entity that represents a hyperacute form of ADEM (61). The most frequent antecedent history is that of an upper respiratory infection. Given the nonspecific nature of the antecedent event and the lack of a specific diagnostic clinical laboratory test, the exact incidence and full clinical spectrum of the disorder can only be estimated and is based largely on descriptions of autopsy-proved cases.

The clinical manifestations, including focal or multifocal signs, seizures, and obtundation, mimic ADEM but develop more abruptly and are more severe. Relapse after initial recovery has been described. Fever is common.

The CSF usually demonstrates increased pressure, protein, and both white and red cells. The peripheral white blood cell count also is usually increased.

CT scans in suspected clinical cases show an initially normal scan followed by low-density white-matter lesions developing within 72 hours of the first symptoms. With improvement, the lesions on CT may largely resolve. MRI may yield additional information on lesion evolution.

The differential diagnosis of this syndrome includes entities that present as rapidly evolving focal cerebral disorders with fever and obtundation. These include brain abscess and encephalitis, particularly resulting from herpes simplex, in addition to those syndromes considered in the section on ADEM (*see* Acute Disseminated Encephalomyelitis).

## ACUTE AND SUBACUTE TRANSVERSE MYELITIS

Acute and subacute transverse myelitis is defined as the development of isolated spinal cord dysfunction over hours or days in patients in whom there is no evidence of a compressive lesion. In the combined experience of several series reviewing complete transverse myelitis, 37% of patients reported a preceding febrile illness. The initial symptoms are paresthesias, back pain, or leg weakness; 37% of patients had the maximal deficit within 1 day, 45% in 1 to 10 days, and 18% in more than 10 days (62).

Patients presenting with acute complete transverse myelitis have a cited risk of MS of only 5–10%. However, partial or incomplete myelitis is a much more common clinical entity and bears more relevance to MS; 57–72% of patients with acute partial myelitis as an initial presentation have cranial MRI abnormalities consistent with MS (12,63). Over 3 to 5 years, 60 to 90% of these patients develop MS, whereas 10–30% of those with a normal MRI developed MS. CSF studies suggest that patients with monosymptomatic disease and positive OCBs have a higher risk of evolution to MS than those without OCBs, although CSF results do not help

further in prognosis when compared to MRI alone. CSF analysis is most useful in the situation where MRI is not available.

## **CEREBELLITIS**

Acute, isolated ataxia has been observed after many different viral illnesses, but most frequently in association with varicella infections. Cerebellar ataxia accounts for 50% of the postvaricella neurological syndromes, which overall occur in 1 in 1000 cases of childhood varicella (64).

The prognosis for recovery is excellent, although the duration of symptoms varies from a few days up to 3 to 4 weeks. That most cases remit spontaneously and the etiology (direct invasion vs autoimmune) is unresolved leaves the issue of corticosteroid therapy unsettled.

## **CLINICALLY ISOLATED SYNDROMES (CIS)**

Clinically isolated syndromes (CISs) are single, monosymptomatic attacks compatible with MS (e.g., ON) that can create a diagnostic and therefore therapeutic, dilemma. More than 80% of patients with a CIS and MRI lesions go on to develop MS, whereas approximately 20% have a self-limited process (41). Identifying those 80% may have particular importance because some studies suggest that starting disease modifying therapies early in the course of MS improves outcomes.

The newer McDonald criteria incorporate MRI findings, potentially allowing for earlier diagnosis of patients with clinically isolated syndromes because two clinical events are not necessary. However, it is not clear that these criteria are sufficiently accurate in patients with CIS to make decisions regarding disease modifying therapy.

At least two studies have evaluated the ability of the McDonald criteria to predict which patients with clinically isolated syndromes will go on to develop MS (65,66). One study prospectively evaluated 50 patients with CIS by clinical and MRI examinations at 3 months, 1 year, and 3 years of follow-up. At 1 year, fulfillment of the newer criteria had a sensitivity, specificity, and accuracy of 83% (65). The second study was an analysis of 139 patients that is limited by a retrospective design and that MRI was performed at 1 year rather than 3 months and inconsistently used contrast (66). Nevertheless, the sensitivity, specificity, and accuracy in predicting conversion to MS were similar to the previous report (74%, 86%, and 80%, respectively).

The conversion rate from a clinically isolated syndrome to clinically definite multiple sclerosis, defined as the patient's development of a second clinical attack, was evaluated by several investigators (Table 11). The conversion rate with an initially normal brain MRI was 6% after 5 years, 11% after 10 years, and 19% after 14.1 years. Only 4% of the patients with an initially normal brain MRI followed for 10 years reached an EDSS greater than 5.5, whereas patients with > 10 MRI lesions at onset had a conversion rate from 80 to 88% and up to 73% reached a score >5.5 on the EDSS.

**Table 11**  
**Risk of Multiple Sclerosis After Monosymptomatic Episodes\***

Investigator (Ref)	Follow-up	Patients	MRI lesions (initial)	Conversion rate to			
				CDMS	EDSS > 3	EDSS > 5.5	
Morrissey 1993 (12)	5 years	32	0	6%	0		
		6	1	17%	0		
		18	2–3	67%	17%		
		13	4–10	92%	30%		
		16	>10	80%	56%		
O’Riordan 1998 (71)	10 years	27	0	11%	0	4%	
		3	1	33%	0	0	
		16	2–3	87%	31%	27%	13%
		15	4–10	87%	75%		20%
		20	>10	85%			35%
Brex 2002 (41)	14.1 years	21	0	19%	0	0	
		18	1–3	89%	31%	53%	12.5%
		15	4–10	87%	80%		38%
		17	>10	88%			73%

\* The conversion rate to clinically definite MS (CDMS) indicates that the patient had a second clinical episode.

Adapted from refs. 12, 41, and 71.

Recently, patients with CIS were tested for serum antibodies to myelin oligodendrocyte protein (MOG) and MBP to predict time to definite MS. A second relapse occurred in 95% seropositive for both antibodies, 83% seropositive for MOG antibodies, and only 23% of seronegative patients (67). Thus, additional prospective studies demonstrating greater accuracy in patients with clinically isolated syndromes are necessary before diagnosis based on the new criteria alone can be used to begin disease-modifying therapy. Assessment of B-cell measurements in the CSF may be more accurate as discussed in Chapter 6. In either case, early detection can lead to early treatment which has been shown to slow the disease progression.

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# Role of Magnetic Resonance Imaging in the Diagnosis and Prognosis of Multiple Sclerosis

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Robert Zivadinov, PhD, MD, and Rohit Bakshi, MD

## INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by demyelination and axonal loss for which the exact immunopathogenic mechanisms underlying disease initiation and progression are unknown. In the last two decades, magnetic resonance imaging (MRI) has become the most important laboratory diagnostic and monitoring tool in MS (1). Moreover, MRI is 5 to 10 times more sensitive than clinical data in the assessment of disease activity (2). The sensitivity of T2-weighted images (T2-WI) in detection of MS lesions, together with the ability of gadolinium (Gd)-enhanced T1-WI to reflect increased blood–brain barrier (BBB) permeability associated with active inflammatory activity, allows the demonstration of spatial and temporal dissemination of MS lesions earlier than is possible from clinical assessments. Therefore, in the last decade, metrics derived from conventional MRI have been widely employed in therapeutic clinical trials (3–6). Several conventional MRI protocols, in conjunction with clinical assessment, are now routinely used to detect therapeutic effects and extend clinical observations (7).

## USE OF MRI IN MULTIPLE SCLEROSIS

Recently, several cross-sectional and longitudinal studies (8,9) have shown that conventional and nonconventional MRI techniques can be used as surrogate markers in monitoring the destructive pathological processes that most likely are related to disease activity and clinical progression. These MRI techniques are able to reveal a range of pathological substrates of MS lesions that include edema, inflammation, demyelination, and axonal loss (10). Therefore, in a disease with a high degree of longitudinal variability of clinical signs and symptoms within and between patients

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and with no current adequate biological markers of disease progression, MRI techniques provide a powerful tool to noninvasively study disease pathology (11). However, although conventional MRI has substantially contributed to the diagnosis and prognosis of MS, the sensitivity and specificity of nonconventional MRI is limited (12). For example, hyperintense lesions on T2-WI show pathological non-specificity, are not sensitive to disease affecting normal-appearing gray and white matter, show an unreliable correlation with clinical measures of disability, and provide incomplete assessments of therapeutic outcomes. Hyperintensity on T2-WI of MS lesions is related primarily to increased water content and thus cannot distinguish inflammation, edema, demyelination, Wallerian degeneration, and axonal loss (13). Although the presence of Gd-enhancing lesions on T1-WI indicates disruption of the BBB, it does not provide sufficient information about the extent and severity of the inflammatory phase, the constitution of its cellular components, or resultant tissue damage (12). In addition, conventional MRI is unable to detect and quantify the extent and severity of tissue damage occurring in so called normal appearing brain tissue, including normal appearing gray and white matter, which probably contributes to short-term and long-term clinical impairments (14–18).

In the past few years, a host of nonconventional MRI techniques, which are able to monitor disease evolution, have been introduced for the assessment of MS. Measurement of brain and spinal cord atrophy, hypointense lesions on T1-WI (“black holes”), hypointensity on T2-WI (T2 hypointensity), magnetization transfer imaging (MTI), diffusion-weighted imaging (DWI), proton MR spectroscopy (MRS), functional MRI (fMRI), cell-specific MRI, perfusion MRI, and ultra-high-field MRI are emerging as promising tools for improving our understanding of the pathophysiology of MS.

The aim of this chapter is to review the role of conventional and nonconventional MRI techniques in the diagnosis and prognosis of MS.

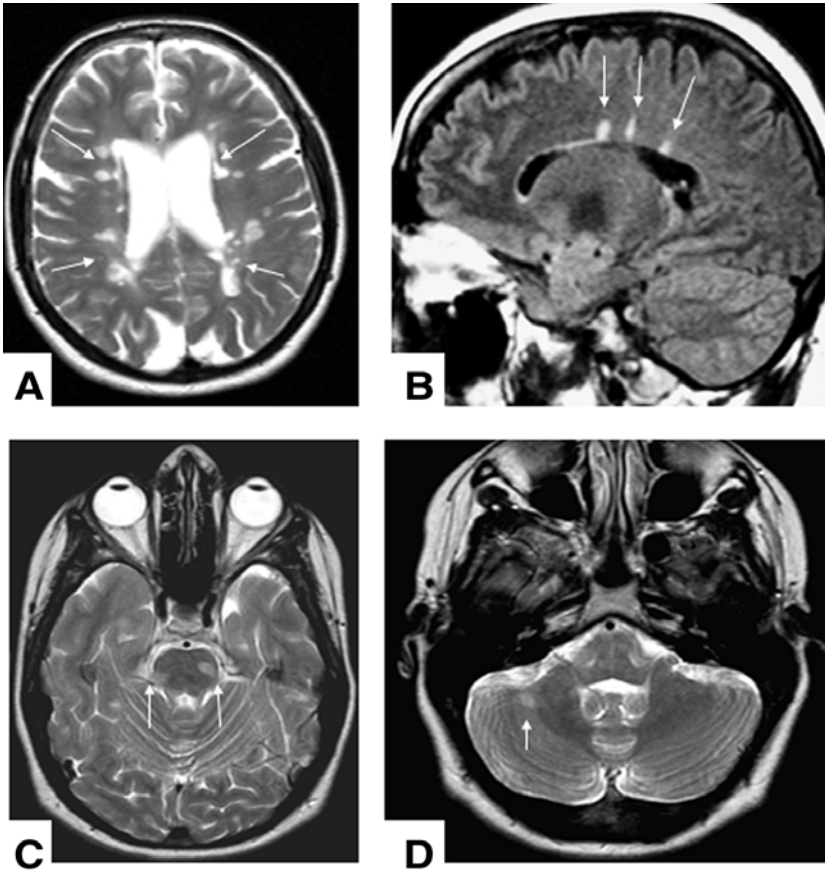
## **ROLE OF MAGNETIC RESONANCE IMAGING IN THE DIAGNOSIS OF MULTIPLE SCLEROSIS**

### ***Conventional Magnetic Resonance Imaging in the Diagnosis of Multiple Sclerosis***

#### ***Involvement of the Brain***

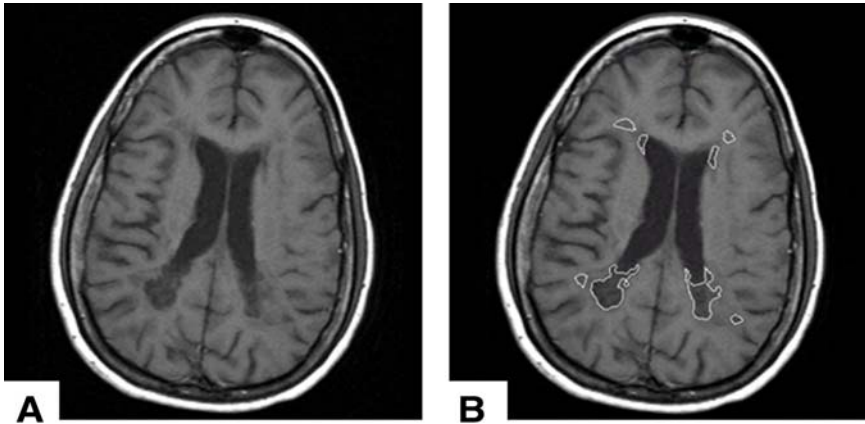
In clinical practice, the diagnosis of MS is usually based on the principle of dissemination in time and space of a neurologic illness compatible with MS in the absence of other causes (19).

MS lesions (“plaques”) may be seen practically anywhere in the brain, including white matter or gray matter. However, the most typical sites are in white matter such as the periventricular region (Fig. 1A), corpus callosum (Fig. 1B), and posterior fossa (Fig. 1C). When involving the periventricular white matter, lesions typically make contact with the ependymal surface of the ventricles. Corpus callosum involvement is characteristically seen on the inner surface adjacent to the lateral ventricles. Typical sites of posterior fossa involvement include the brain stem,



**Fig. 1.** (A) Axial T2-weighted image (WI) in a 33-year-old man with relapsing-remitting MS (RRMS) showing multiple hyperintense lesions in the periventricular white matter (*arrows*). Note the classic oval/ovoid appearance of lesions, the size of each is generally  $\pm 5$  mm and many directly contact the ventricular ependyma. (B) Sagittal fluid-attenuated inversion recovery (FLAIR) in a 44-year-old man with relapsing-remitting MS (RRMS) showing multiple pericallosal lesions (*arrows*) with a classic perivenular orientation (Dawson’s fingers); (C,D) Axial T2-WI of a 26-year-old woman with RRMS demonstrating typical hyperintense lesions in the pons (C, *arrows*) and right cerebellum (D, *arrow*).

middle cerebellar peduncles, and cerebellar white matter (Figs. 1C,D). Although axial images are more sensitive for posterior fossa lesions (Fig. 1C), sagittal images are clearly superior in identifying callosal and pericallosal lesions (Fig. 1B). The long-axis of periventricular lesions is frequently perpendicular to the long-axis of the lateral ventricles resulting from perivenular demyelination (Dawson’s fingers) (Fig. 1B). MS lesions are commonly oval or ovoid (Fig. 1), isointense to hypointense on T1-WI (Fig. 2), and hyperintense on T2-WI and proton density-

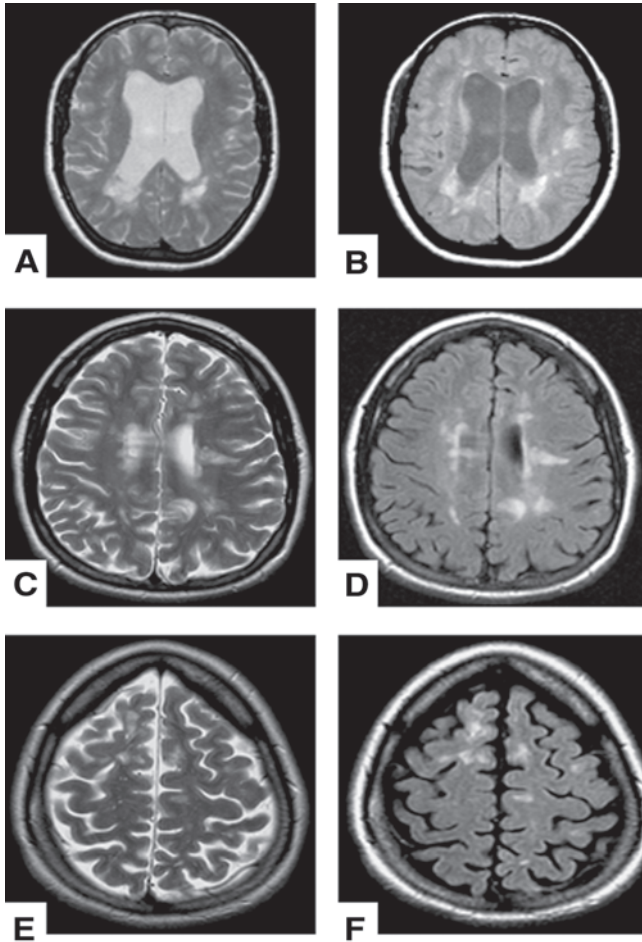


**Fig. 2.** (A) Axial T1-weighted image (WI) in a 39-year-old man with secondary progressive MS (SPMS) with severe disability (Expanded Disability Status Scale [EDSS] score of 6.5) and multiple hypointense lesions (black holes) in the periventricular white matter. (B) Hypointense T1 lesions are segmented for volumetric analysis at the edges of the anterior and posterior horns of the lateral ventricles using an edge finding computerized analysis technique.

weighted images (PD-WI) (Figs. 1 and 3) and fluid-attenuated inversion recovery (FLAIR) images (Figs. 3 and 4), and are usually  $>5$  mm in diameter. These hyperintensities become confluent as the disease progresses (Fig. 5).

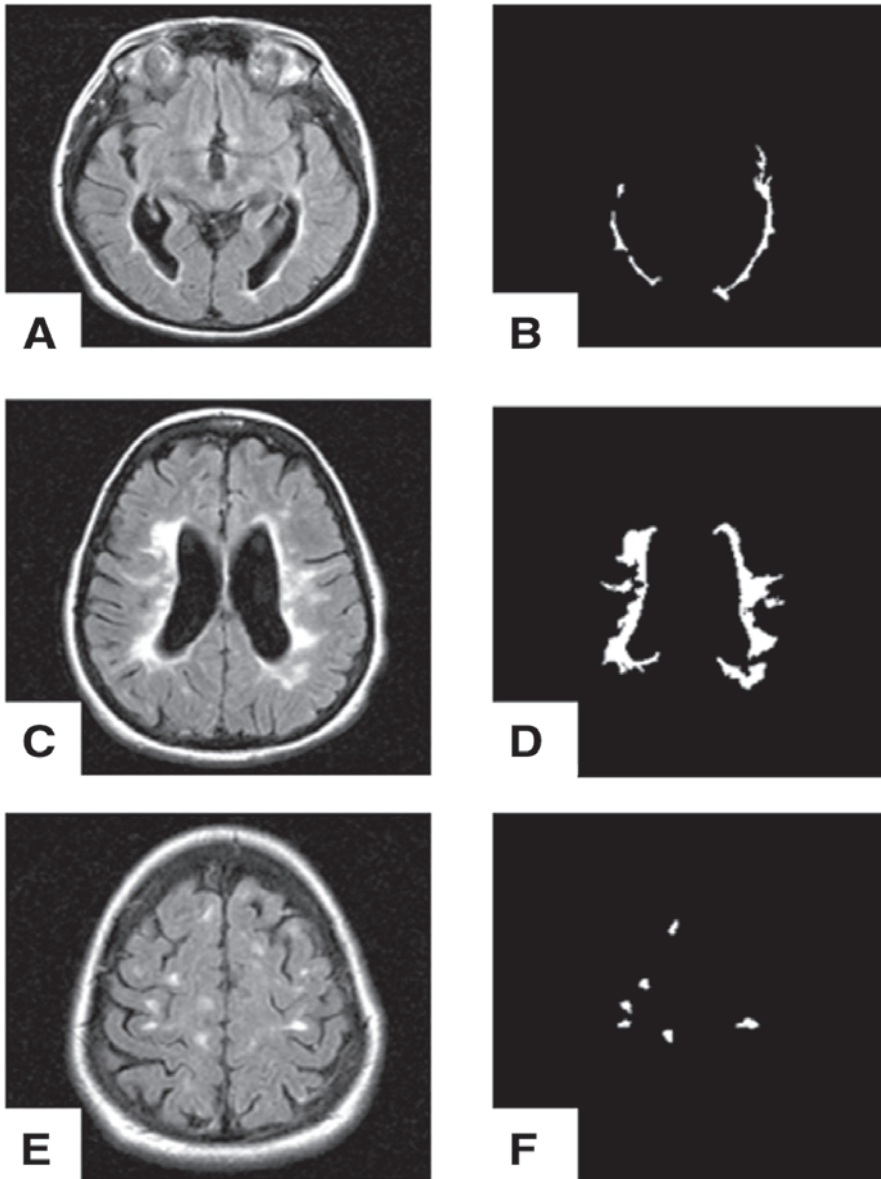
Conventional spin-echo (CSE) images of the brain are routinely used for MS diagnosis because of their high sensitivity in the detection of MS lesions (11). CSE T2-WI are also used to monitor short-term MS activity (the number of new and enlarging lesions on serial scans) (20) and to monitor long-term disease evolution by assessing the changes of total T2 lesion volume (T2LV) (21) over time. Changes in lesion size and T2LV are believed to be related to disease activity (22). However, small periventricular lesions may be indistinguishable from the adjacent high signal cerebrospinal fluid (CSF) on T2-WI. Better lesion to CSF contrast is achieved with PD-WI because of the relatively lower signal intensity of CSF on this sequence and improved lesion to tissue contrast (Fig. 3). T2-WI and PD-WI can be acquired in a single sequence by using either a conventional dual spin-echo technique or a rapid acquisition relaxation enhanced (RARE) dual spin-echo sequence (23).

In the last several years, continuous technical improvements of MRI hardware and software have led to the development of new pulse sequences with more efficiency and sensitivity. Among them, turbo or fast spin-echo (TSE or FSE) (24) and fast-FLAIR (25) (Figs. 3 and 4) have already demonstrated their use in a variety of neurologic diseases, including MS. FSE showed greater sensitivity than CSE in the detection of areas of T2 prolongation in MS (24). Fast FLAIR sequences are especially helpful in evaluating periventricular and cortical lesions where CSF signal may mask these plaques on T2-WI (20,25) (Fig. 3). In a study of 84 patients with



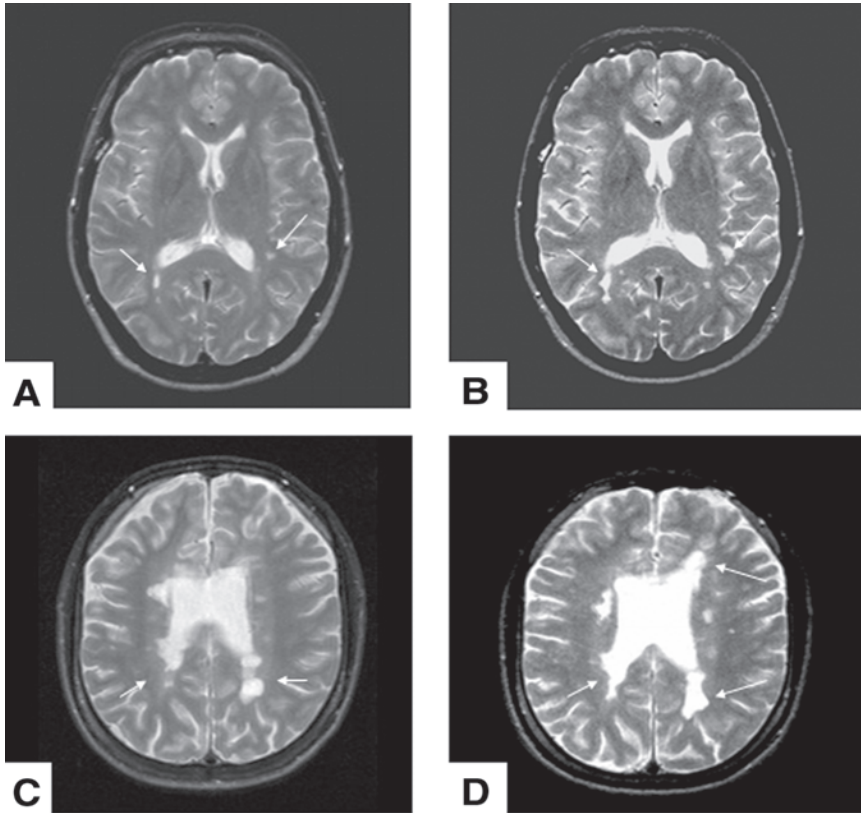
**Fig. 3.** (A) Axial fast spin-echo (FSE) T2-weighted image (WI) and (B) proton density-WI (PD-WI) in a 42-year-old woman with relapsing-remitting multiple sclerosis (RRMS) show typical hyperintense confluent periventricular lesions. Note the improved lesion contrast achieved with PD-WI because of the relatively lower signal intensity of cerebrospinal fluid (CSF) vs lesions. Axial images of 28-year-old woman with RRMS show clear evidence how fluid-attenuated inversion recovery (FLAIR) (D,F) is superior to FSE T2-WI (C,E) in the detection of periventricular (C,D) and cortical/juxtacortical (E,F) lesions.

MS, 810 cortical or juxtacortical lesions were identified by fast FLAIR while only 26% of these lesions were seen on T2-WI (25). However, in the posterior fossa (Fig. 1C) and spinal cord, FLAIR detects significantly fewer lesions than T2-WI (26). Areas of T2 prolongation can also be detected using short tau inversion recovery (STIR) sequences. This sequence may be superior to T2-WI in detecting spinal cord lesions in MS when using certain scanning platforms (27). Because of fat



**Fig. 4.** Axial fast fluid-attenuated inversion recovery (FLAIR) images in a 32-year-old man with relapsing-remitting multiple sclerosis (RRMS) showing hyperintense lesions (A,C,E) and the output resulting from computer-assisted segmentation showing lesions only (B,D,F). FLAIR sequences are especially helpful in evaluating supratentorial periventricular (A–D) and juxtacortical (E,F) lesions where cerebrospinal fluid (CSF) signal may mask these plaques on T2-WI (*see also* Fig. 3).

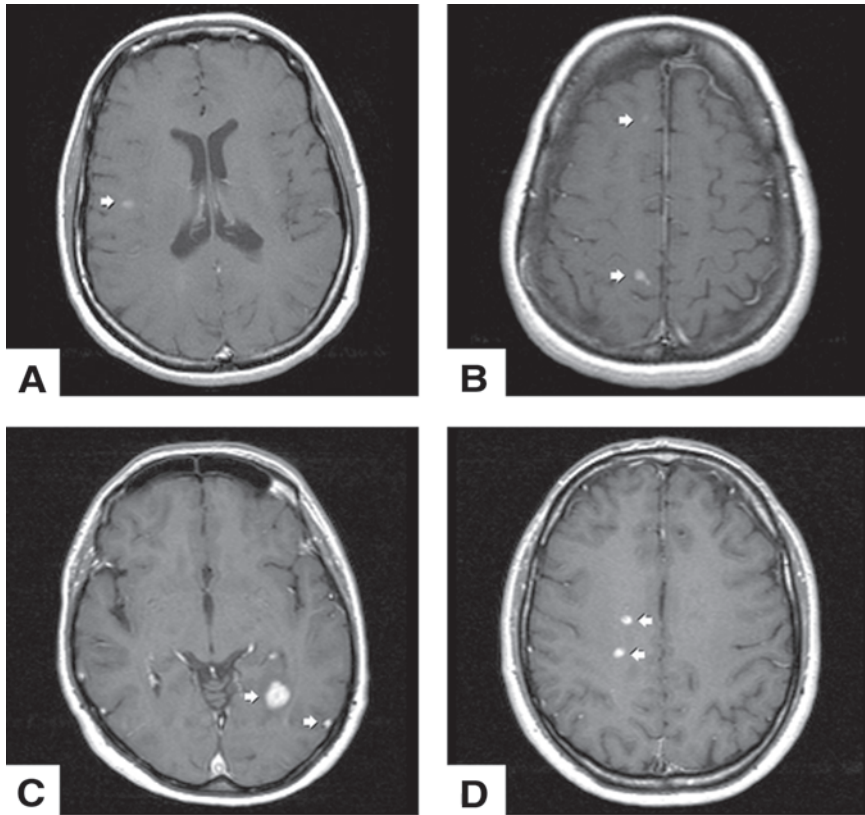




**Fig. 5.** Natural history data: evolution of confluent T2 lesions over 5 years in untreated patients. (A) Baseline scan and (B) 5-year follow-up scan show the enlargement and development of confluence of T2 lesions (*white arrows*) in a 31-year-old man with relapsing-remitting multiple sclerosis (RRMS). (C) Baseline scan and (D) 5-year follow-up scan show increasing confluence of T2 lesions (*white arrows*) in a 35-year-old man with RRMS.

suppression, the STIR sequence is an added advantage when imaging the optic nerves because the contrast between lesions and the surrounding retrobulbar fat is increased (28).

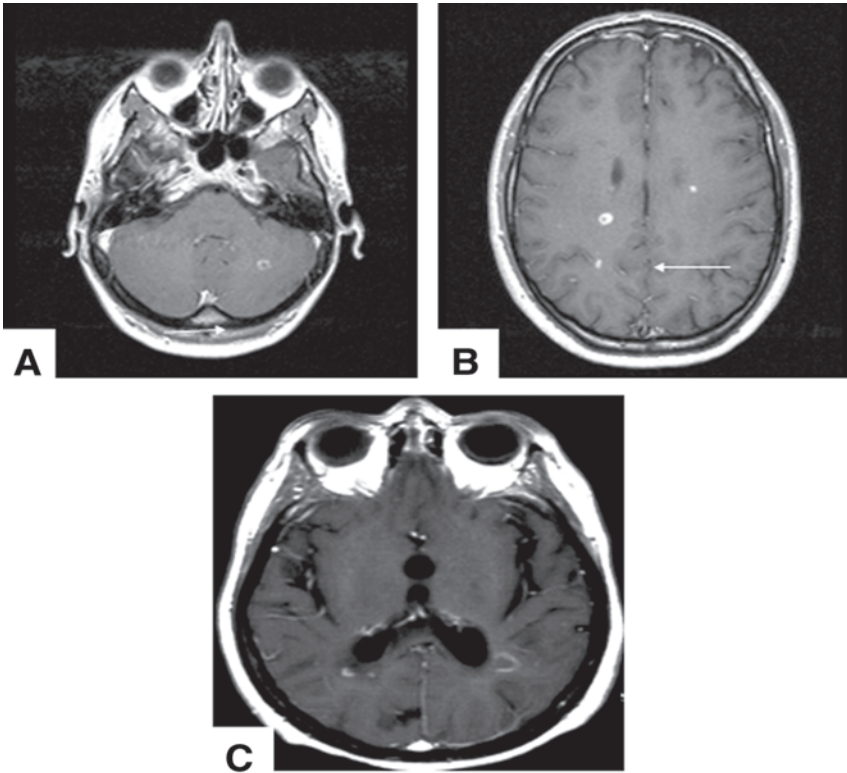
Gd-enhancing lesions on T1-WI (Figs. 6A,B) often correspond to areas of high signal on T2-WI and low-signal intensity on unenhanced T1-WI, probably owing to edema. Approximately 10–20% of lesions on T2-WI will show contrast enhancement at any one time. Gd enhancement is a transient phenomenon in MS and usually disappears after 30 to 40 days; persistence of enhancement should caution against the diagnosis of MS (29). It is not completely clear if inflammation is the triggering event that causes demyelination and axonal degeneration, although previous longitudinal studies demonstrated that the presence of active lesions on serial



**Fig. 6.** Axial gadolinium postcontrast T1-weighted images (WI) (A,B) show homogeneously enhancing (hyperintense) lesions, indicating disruption of the blood–brain barrier and acute inflammation in a 33-year-old woman with relapsing–remitting multiple sclerosis (RRMS). Axial postcontrast T1-WI in a 28-year-old man shows how a double dose of contrast is useful to increase the sensitivity in detecting active areas of inflammation (C,D).

scans carries a high risk of continuous disease activity (30,31). A meta-analysis by Kappos et al. (32) demonstrated that Gd enhancement predicts the occurrence of relapses, but it is not a strong predictor of the development of cumulative impairment or disability. There are several methods that can be used to increase the sensitivity of Gd-enhanced MRI for the detection of active MS lesions, which leads to increased statistical power of patient samples and the follow-up duration needed to show a treatment effect (33). This increased sensitivity may be obtained with higher doses of Gd (Fig. 6C,D) (34,35) (e.g., a triple dose instead of a standard 0.1 mmol/kg dose) (36), MTI (37), thinner slices, or delayed imaging (38). Wolansky et al. (36) were the first to show that triple-dose Gd is more sensitive than single-dose Gd in detecting MS lesions. At the authors' centers, they use a 5-minute delay after





**Fig. 7.** Axial postcontrast T1-weighted image (WI) demonstrates concentric ring-enhancing lesions (*arrows*) in the infratentorial (**A**) and periventricular (**B**) regions in a 27-year-old woman with relapsing-remitting multiple sclerosis (RRMS). Characteristic open-ring-enhancing lesion with increased specificity for MS (*arrow*) in 52-year-old women scanned during an MS relapse (**C**).

single-dose Gd infusion to increase the sensitivity, which they believed is cost effective and practical. The majority of enhancing plaques are clinically asymptomatic, although the presence of ongoing enhancement suggests continuing disease activity, which likely contributes to cumulative pathophysiology.

Gd-enhancement patterns may provide some specificity of the underlying pathology of lesions (Fig. 7). Concentric ring-enhancing lesions with central contrast pallor (Fig. 7) arise in previously damaged areas or in areas of accelerated local inflammation (39–45). They are larger and of longer duration than homogeneously enhancing lesions (39,40,42). Magnetization transfer ratios (MTRs) (41,43) and apparent diffusion coefficients (ADCs) (44) are lower in ring-enhancing than in homogeneously enhancing lesions. Moreover, ring-enhancing lesions weakly predict the development of persisting hypointense lesions on T1-W1 (46). Therefore,

ring-enhancing lesions are believed to be related to accelerated disease activity and extensive tissue damage (39,45,46) and may mark a type of inflammation in more aggressive forms of disease. Ring enhancement may also occur in an incomplete (open) ring pattern, which is somewhat more specific for MS than infections or neoplastic diseases (Fig. 7).

On noncontrast T1-WI, the authors have observed that plaques frequently possess an outer ring of hyperintensity (T1 shortening), which may represent paramagnetic effects of free radicals, lipid-containing macrophages, or proteinaceous accumulation (unpublished data). This phenomenon has been poorly characterized in the literature and only mentioned anecdotally. The authors are in the process of completing a formal study on this phenomenon.

MRI findings in patients with MS are not disease specific. A number of conditions can cause multiple T2 hyperintense white-matter abnormalities appearing similar to MS. The MRI pattern of MS is usually relatively specific when age, clinical information and the full range of MRI abnormalities (including lesion number, topography, size, and shape) are considered. However, clinical correlation remains the cornerstone of diagnosing MS. Table 1 lists the most common conditions to be considered in the differential diagnosis when confronted with multifocal lesions on MRI scans suggestive of MS or clinical entities suggesting MS (11,19,47,48).

In particular, lesions of ADEM (49), a monophasic illness, may resemble those of early MS. ADEM is typically triggered by a viral infection or vaccination, although idiopathic cases may also occur. MRI typically shows multifocal white-matter lesions involving the cerebral hemispheres, cerebellum, and brain stem that may or may not be distinguishable from MS; however, involvement of the subcortical gray-matter nuclei and large size and early confluence of lesions favor ADEM (Fig. 8) (49). Nonetheless, ADEM is a clinical diagnosis that is only supported by imaging findings and a follow-up scan after 3 months, which has been suggested for identifying new lesions suggestive of MS (50).

### *Involvement of the Spinal Cord*

Spinal cord hyperintensities on T2-WI have been detected in 50 to 90% of patients with MS (51–53) (Fig. 9). Several MRI studies demonstrated that the frequency of Gd-enhancing lesions is higher in the spinal cord than in the brain (54,55). The enhancement may become more apparent when scanning the entire cord (55) or using a triple dose of Gd (56). The presence of characteristic cord lesions may increase the confidence in diagnosing MS (51). In addition, cord lesions may be seen in approximately 5–15% of patients with a clinical picture suggestive of MS but a normal brain MRI scan (57), and in 30% of those presenting with clinically isolated syndromes (CIS) of the brain or optic nerve suggestive of MS (58). Moreover, spinal cord lesions appear to be symptomatic more often than brain lesions (59) and correlate better with the degree of physical disability (60). Proper sensitivity for the detection of cord involvement requires optimization of MRI hardware and pulse sequences. We prefer 1.5 T closed bore systems with fast-spin echo sag-

**Table 1**  
**Principal Conditions Mimicking Multiple Sclerosis on Magnetic Resonance Imaging Scans or Considered in the Clinical Differential Diagnosis of Multiple Sclerosis**

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*Multiple sclerosis (MS) variants*

Acute malignant MS (Marburg variant)  
 Charcot type  
 Clinically isolated syndromes  
 Neuromyelitis optica (Devic's disease)  
 Schilder's disease  
 Solitary inflammatory masses (leukoencephalitis)

*Normal aging*

*Migraine*

*Cerebrovascular diseases*

Collagen vascular disease  
 Diabetes  
 Hypertension  
 Periventricular leukomalacia  
 Primary central nervous system vasculitis  
 Subcortical atherosclerotic encephalopathy (Binswanger's disease)  
 Susac syndrome

*Infectious and inflammatory diseases*

Abscesses  
 Acute disseminated encephalomyelitis  
 Human immunodeficiency virus encephalitis  
 Lyme disease  
 Progressive multifocal leukoencephalopathy  
 Sarcoidosis  
 Subacute sclerosing panencephalitis  
 Syphilis  
 Tuberculosis

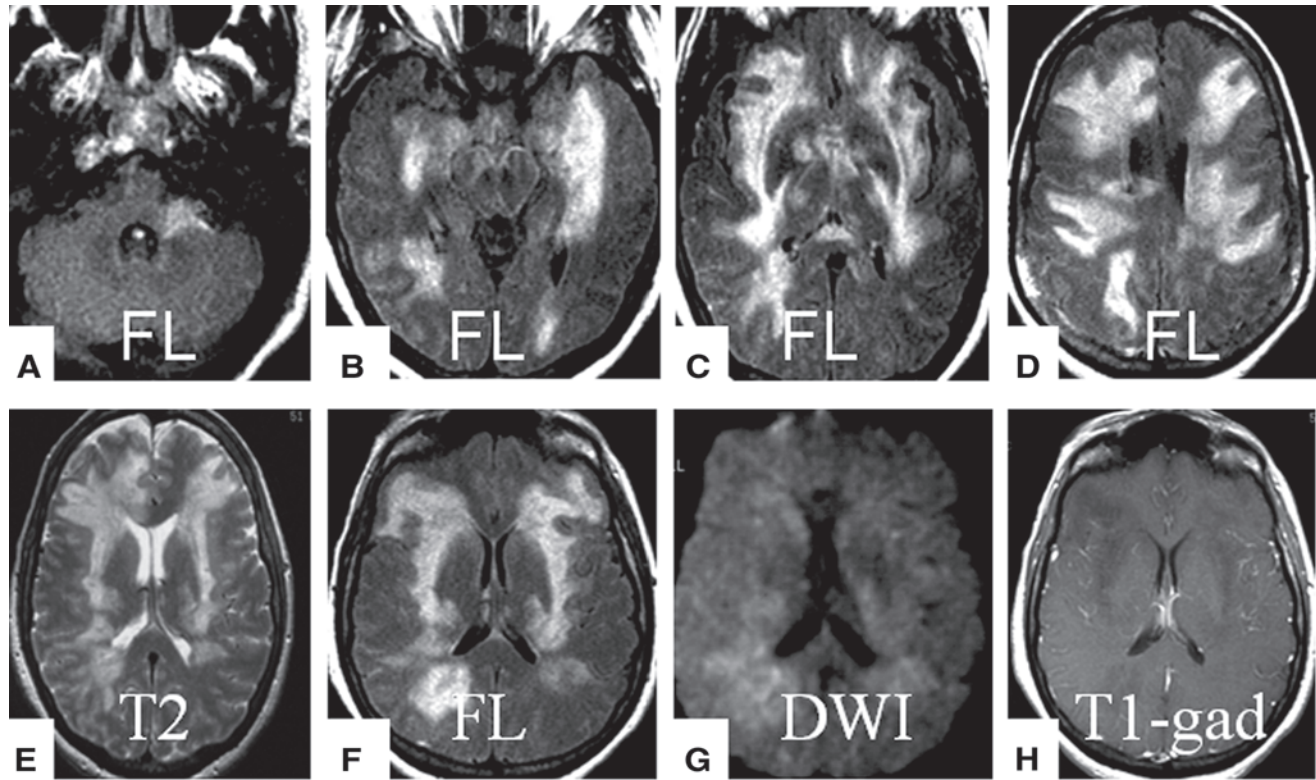
*Toxic/metabolic diseases*

Chemotherapy or radiotherapy effects  
 Leukodystrophies  
 Mitochondrial diseases  
 Osmotic myelinolysis  
 Toluene toxicity  
 Vitamin B<sub>12</sub> deficiency

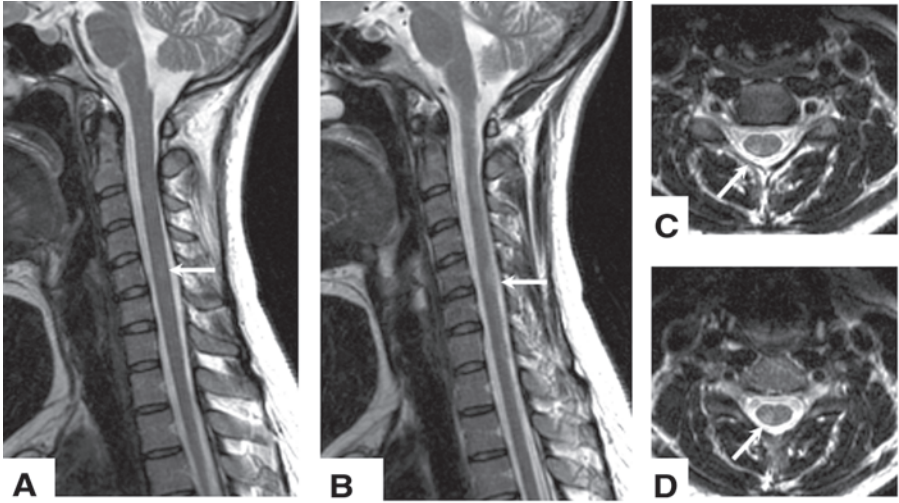
*Neoplastic disease*

Metastases  
 Primary brain or intravascular lymphoma

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**Fig. 8.** Fatal acute disseminated encephalomyelitis (ADEM) in a 51-year-old woman after an upper respiratory infection. (A–F) Note the multiple hyperintensities in the infratentorial and supratentorial region on fluid-attenuated inversion recovery (FLAIR) (FL) and T2-weighted image (WI). (G) Diffused WI (DWI) shows slight hyperintensity of the lesions resulting from T2 shinerthrough. (H) The lesions are isointense to mildly hypointense on T1WI and nonenhancing after gadolinium infusion (T1-gad). Minimal or no mass effect is noted. The large size of lesions, confluence, and involvement of the thalamus and globus pallidus favors ADEM instead of multiple sclerosis.



**Fig. 9.** Spinal fast-spin echo (FSE) T2-weighted image (WI) of a 30-year-old man with RRMS. (A,B) Contiguous sagittal T2-WI show hyperintense lesions (*arrows*) in cervical spinal cord. (C,D) Contiguous axial T2-WI demonstrate a hyperintense lesion (*arrows*) in the cervical cord (C5-6 level). The lesion is classic for MS, involving less than one spinal level and less than one-half of the cord diameter.

ittal and axial T2-WI with 3–4 mm slice thickness for detection of hyperintense intramedullary lesions (Fig. 9). On some scanning platforms, PD-WI or STIR may also be useful.

The diagnosis of MS can be easily made in subjects with acute spinal cord symptoms when MRI of the brain shows typical white-matter lesions and CSF demonstrates intrathecal immunoglobulin (Ig) G synthesis and oligoclonal bands. However, when MRI of the brain is normal or atypical, the CSF is normal, or other concomitant factors are present (e.g., cord-compressive lesion), alternative causes must be considered. MRI is sensitive in detecting focal demyelinating syndromes, such as myelitis (61), leukoencephalitis (62), and optic neuritis (ON) (63). Myelitis, an inflammatory syndrome associated with intramedullary dysfunction of the spinal cord, is often associated with an identifiable etiology, such as infectious, postinfectious, postvaccination, collagen vascular disease, toxic, paraneoplastic, sarcoidosis, or MS-related myelitis (Fig. 9) (61). Diagnosing myelitis promptly and accurately and distinguishing these various etiologies has therapeutic and prognostic value but may be difficult with routine clinical and laboratory testing. MRI plays a central role in the differential diagnosis of myelitis.

Acute transverse myelitis (ATM) is a focal inflammatory disorder of the spinal cord, resulting in motor, sensory, and autonomic dysfunction. A set of uniform diagnostic criteria and nosology for ATM has been proposed to avoid the confusion



that inevitably results when investigators use differing criteria (64). This has ensured a common language of classification, reduced diagnostic confusion, and laid the groundwork for multicenter clinical trials. In addition, a framework is suggested for evaluation of individuals presenting with ATM signs and symptoms. The best treatment often depends on a timely and accurate diagnosis. Because ATM is relatively rare, delayed and incomplete workups often occur. Identification of etiologies may suggest medical treatment, whereas no clearly established medical treatment currently exists for idiopathic ATM. Bakshi et al. (61) studied brain and spinal MRI findings in 22 consecutive patients with various forms of myelitis, including non-MS-related ATM and myelitis associated with preexisting or subsequent MS (MS-myelitis). They compared non-MS-related ATM ( $n = 9$ ), to myelitis associated with MS (MS-myelitis,  $n = 13$ ). Several major differences between ATM and MS-myelitis patients were found:

1. The patients with ATM were significantly older than patients with MS at the time of the myelitis diagnosis (mean age 46 vs 35,  $p > 0.05$ ).
2. ATM appeared as a “longitudinal myelitis,” with fusiform cord expansion on T1-WI and intramedullary increased signal on T2-WI, each involving multiple spinal levels (mean = 7–8). However, MS-myelitis lesions appeared focal, involving significantly fewer spinal levels (mean = 1–2,  $p > 0.001$ ).
3. Brain MRI was more likely to be normal in ATM (78%) than in MS-myelitis (15%,  $p < 0.001$ ).

The most striking MRI finding was that ATM appeared tumor-like as a “longitudinal” lesion that included fusiform spinal cord swelling on T1-WI and hyperintense intramedullary lesions on T2-WI. Thus, MRI findings in ATM, although suspicious for a primary spinal cord neoplasm and suggesting the need for a biopsy, may reflect an inflammatory disorder. The MRI features of ATM in the Bakshi et al. series were readily distinguishable from MS-myelitis. However, MRI findings were similar in ATM because of vaccination, infection, sarcoidosis, and collagen vascular disease. The differential diagnosis of these lesions on MRI studies also includes infarction or vascular malformation of the spinal cord, which may require exclusion by clinical and laboratory findings. MS-myelitis is most common in the cervical cord and appears focal (involving one to two levels or less), well circumscribed, and hyperintense on T2-WI (61). Spinal cord expansion associated with MS may reflect a more acute or subacute myelopathy rather than clinically silent or chronic myelopathies. Axial MRI may also be helpful in distinguishing ATM and MS-myelitis. ATM is typically located in the central aspect of the cord and occupies more than one-half of the cord diameter, whereas MS-myelitis is typically more peripheral and involves less than one-half of the cross-sectional cord area.

#### *Role of MRI in New Diagnostic Criteria for Multiple Sclerosis*

No single clinical feature or diagnostic test is sufficient for the diagnosis of MS. A formal review of diagnostic criteria occurred in 1983, at which time degrees of diagnostic certainty were identified by categories ranging from clinically definite to laboratory-supported definite, clinically probable, and laboratory-supported

**Table 2**  
**Magnetic Resonance Imaging Diagnostic Criteria for Multiple Sclerosis**

Authors (Ref.)	Features
Paty et al. (1988) (66)	Either four lesions or three lesions present with one being in periventricular region
Fazekas et al. (1988) (67)	Three or more lesions with two of them presenting at least one of the following lesion characteristics: <ul style="list-style-type: none"> <li>• Size of a lesion no. 6 mm</li> <li>• A lumpy-bumpy ventricular interface</li> <li>• An infratentorial lesion</li> </ul>
Barkhof et al. (1997) (68)	At least one lesion with gadolinium-enhancement At least one lesion in juxtacortical region At least one lesion in infratentorial region At least three lesions in periventricular region

probable MS (65). An international panel has recently revised the diagnostic criteria (McDonald criteria) (50). The panel aimed to create criteria that could be used by a practicing physician and adapted for clinical trials and would integrate MRI, include the diagnosis of primary progressive (PP) disease, clarify definitions used in the diagnosis of MS, and simplify the diagnostic classification and descriptions. The McDonald criteria reaffirmed the concept of “dissemination in time and space.”

To use MRI to support the diagnosis of MS, multiple characteristic lesions should be present. Several authors have proposed MRI criteria to help differentiate between MS and other causes of hyperintense lesions in the brain (66–68) (Table 2). In 1997, Barkhof et al. (68) suggested that contrast-enhanced and juxtacortical and infratentorial T2 lesions are more specific for MS than the more commonly seen periventricular lesions. In a prospective comparison with earlier sets of criteria proposed by Paty et al. (66) and Fazekas et al. (67), Barkhof et al. demonstrated that their criteria achieved the highest accuracy in predicting conversion to MS in patients presenting with CIS (Table 3) (68). Based on these findings, the McDonald panel maintained that stringent MRI criteria should be followed in making a diagnosis of MS. The panel chose the guidelines of Barkhof et al. (68) and Tintore et al. (69) for the definition of dissemination in space, which requires at least three of the following:

1. At least one Gd-enhancing lesion or nine T2 hyperintense lesions if no Gd-enhancing lesion.
2. At least one or more infratentorial lesion.
3. At least one or more juxtacortical lesion.
4. At least three or more periventricular lesions.
5. Note: one cord lesion can substitute for one brain lesion.

The following MRI criteria may be used to demonstrate dissemination in time (Table 4):

1. Gd-enhancing lesion demonstrated in a scan done at least 3 months after the initial clinical attack at an anatomic site different from the first attack;

**Table 3**  
**Predictive Value of Magnetic Resonance Imaging Developing**  
**a Second Attack Indicative of Clinically Definite Multiple Sclerosis**  
**in Patients with Clinically Isolated Syndromes (Barkhof et al.) (68)**

Magnetic resonance imaging criteria	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Accuracy (%)
Paty et al. (1988) (66)	88	54	86	60	69
Fazekas et al. (1988) (67)	88	54	86	60	69
Barkhof et al. (1997) (68)	82	78	85	75	80

*Abbr:* NPV, negative predictive value; PPV, positive predictive value.

**Table 4**  
**Magnetic Resonance Imaging Criteria**  
**for Dissemination of Lesions in Time (50)**

- If a first scan occurs 3 months after the onset of clinical event, the presence of a gadolinium-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site implicated in the original clinical event. If there is no enhancing lesion at this time, a follow-up scan is required. The timing of this follow-up scan is not crucial, but 3 months is recommended (70). A new T2- or gadolinium-enhancing lesion at this time then fulfills the criterion of dissemination in time.
- If the first scan is performed <3 months after the onset of the clinical event, a second scan done 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not <3 months after the first scan that shows a new T2 lesion or an enhancing lesion will suffice.

2. In the absence of Gd-enhancing lesions at the 3-month scan, a follow-up scan after an additional 3 months showing a new Gd or T2-lesion.

Although the McDonald MRI criteria were generally regarded as difficult to apply, they allow MRI to be used to define dissemination in space and (with a repeat MRI) dissemination in time. The McDonald criteria accept the occurrence of new T2 lesions or new Gd-enhancing lesions appearing after a minimum interval of 3 months as acceptable evidence for dissemination in time. This minimum interval should be further validated. In addition, the optimal interval that provides the maximum yield in the most cost-effective manner needs to be defined. Emerging data from serial MRI studies that have been done might provide additional information to make such recommendations (71–75).

The McDonald criteria included new diagnostic criteria for PP MS (76). However, the diagnosis of PP MS remains difficult. The McDonald MRI criteria for the diagnosis of PP MS are probably insensitive, and it is clear that some clinically



typical cases will not meet the formal MRI criteria (77). Additional information is likely to become available as results of current PP MS clinical trials are published (77).

As mentioned, MS has many mimics. The McDonald criteria consider the differential diagnosis. Although it is possible to diagnose MS without MRI, the number of errors in diagnosis is reduced markedly when both clinical and MRI studies are available to the physician.

The authors believe that formal diagnostic criteria have a role in selecting patients for research trials to ensure uniformity but that for routine clinical practice, these criteria may be too restrictive and require long-term validation (*see Validation of New Diagnostic Criteria*).

#### VALIDATION OF NEW DIAGNOSTIC CRITERIA

Data on the sensitivity, specificity, and reliability of MRI in supporting a diagnosis of MS are limited. The McDonald guidelines cite the Barkhof et al. (68) and Tintore et al. (69) MRI criteria, even though they have not been validated longitudinally in MS populations. In the Barkhof et al. study (68), MRI scans of 33 patients who converted from clinically isolated syndromes (CIS) to clinically definite MS were evaluated. In the Tintore et al. study (69), 22 converting patients were studied for an average of 28 months. These numbers may not be sufficient to establish standards, and, therefore, such criteria (and those modified by the McDonald panel) should be validated for accuracy in the diagnosis of MS and not only for the predictive value of short-term conversion to clinically definite MS in CIS.

Four studies (71,73–75) have recently evaluated the ability of the new McDonald criteria to predict which clinically isolated syndrome (CIS) patients will develop clinically definite MS (65). In a recently published study of 50 patients with CIS, Dalton et al. (73) examined the value of MRI in predicting conversion to clinically definite MS within 3 years. At 3 months, 20 of 95 (21%) patients had developed MS according to the McDonald criteria, whereas only 7 of 95 (7%) had developed clinically definite MS. After 1 year, the corresponding figures were 38 of 79 (48%) and 16 of 79 (20%), and after 3 years, they were 29 of 50 (58%) and 19 of 50 (38%). The development of MS with the McDonald criteria after 1 year had a high sensitivity (83%), specificity (83%), positive predictive value (75%), negative predictive value (89%), and accuracy (83%) for clinically definite MS at 3 years (Table 5). The authors concluded that the use of the McDonald criteria more than doubled the rate of diagnosis of MS within a year of presentation with a CIS and that therefore the high specificity, positive predictive value, and accuracy of the new criteria for predicting clinically definite MS support their clinical relevance. In another study, Tintore et al. (71) reported the predictive value of the McDonald criteria in 139 patients who presented with CIS and who were followed for a median of 3 years. At 12 months, 11% had clinically definite MS according to the Poser criteria, compared to 37% with the McDonald criteria. Eighty percent of patients fulfilling these new criteria developed a second clinical episode within a mean follow-up of 49 months. The new criteria showed a sensitivity of 74%, specificity

**Table 5**  
**Ability to Predict the Conversion of Clinically Isolated**  
**Syndromes to Clinically Definite Multiple Sclerosis:**  
**Validation of the McDonald Criteria (50)**

Magnetic resonance imaging criteria	Sensitivity (%)	Specificity (%)	Accuracy (%)
Dalton et al. (2002) (73)	83	83	83
Tintore et al. (2003) (71)	74	86	80

of 86%, and accuracy of 80% in predicting conversion to clinically definite MS (Table 5). The authors emphasized that 1 year after symptom onset, more than three times as many patients with CIS were diagnosed with MS using McDonald criteria vs previous criteria. These two studies (71,73) retrospectively examined data collected for other reasons and used them to evaluate the McDonald criteria. In particular, the Tintore et al. study (71) is limited by the study design, the timing of the scans, and the inconsistent use of Gd. In both studies, the McDonald criteria were not strictly applied and they failed to show that these criteria can guide routine clinical practice (72). The results of these two studies are consistent with earlier studies suggesting that MRI has important limitations as a surrogate marker in clinical trials, especially in patients with CIS.

In CIS, the McDonald criteria require new Gd-enhancing lesions for dissemination in time at a 3-month follow-up scan. The diagnosis of MS in patients with a new lesion on T2-WI that appears at the 3-month follow-up scan is not permitted by the current criteria. However, it has been demonstrated that new T2 lesions are detected more often than new Gd-enhancement lesions after 3 months (70). Dalton et al. (74) addressed the accuracy of new T2 lesions at a 3-month follow-up scan in making the diagnosis of MS. In a cohort of 56 patients, these criteria were highly specific (95%) but poorly sensitive (58%) for clinically definite MS at 3 years. If new T2 lesions were allowed as an alternative for dissemination in time, sensitivity increased (74%) with a high specificity preserved (92%), enabling an accurate diagnosis of MS in more patients. The authors proposed that in adults aged 16 to 50 years presenting with CIS, the McDonald MRI criteria for dissemination in time should be expanded to include new T2 lesions seen on a 3-month follow-up scan when the first scan is obtained within 3 months of symptom onset.

Disease-modifying therapies, such as interferon (IFN)- $\beta$ , often show a discordant effect on clinical vs MRI disease effects, reducing MRI activity by greater than 70% (3–6) and the relapse rate by approximately 30%. In a recently published study (68), the predictive value of the modified Barkhof criteria for progression to clinically definite MS, as evidenced by the occurrence of a new clinical episode, has been examined (75). In addition, the predictive response to treatment with once-weekly subcutaneous IFN- $\beta$ -1a has been evaluated. Conversion to clinically defi-

nite MS within 2 years of follow-up, as evidenced by a new clinical episode, occurred (independent of treatment) in 41% of patients with Gd enhancement or nine or more T2 lesions vs 11% of those without either finding ( $p = 0.017$ ); similarly, proportions converting were 44 vs 31% for infratentorial lesions ( $p = 0.026$ ), 40 vs 35% for juxtacortical lesions ( $p = 0.413$ ), and 41 vs 17% for three or more periventricular lesions ( $p = 0.034$ ). The rate of conversion to clinically definite MS based on the modified Barkhof criteria (50) was 22% for two or fewer positive criteria, increasing to 47% with four positive criteria. For a cutoff of three positive criteria, the hazard ratio for time to clinically definite MS was 2.3 (95% confidence interval, 1.17–4.55;  $p = 0.016$ ). The results of validation of the modified McDonald criteria in the Early Treatment of MS (ETOMS) study (75) suggest that meeting the criteria increases the odds of conversion to clinically definite MS, and thus the number of patients needing to be treated to prevent one patient converting to clinically definite MS within 2 years decreases from 50 in patients with two or fewer positive criteria to 5.6 with four positive criteria. On the other hand, the effect of once-weekly IFN- $\beta$ -1a therapy seems to be stronger in the patients with a high number of positive criteria.

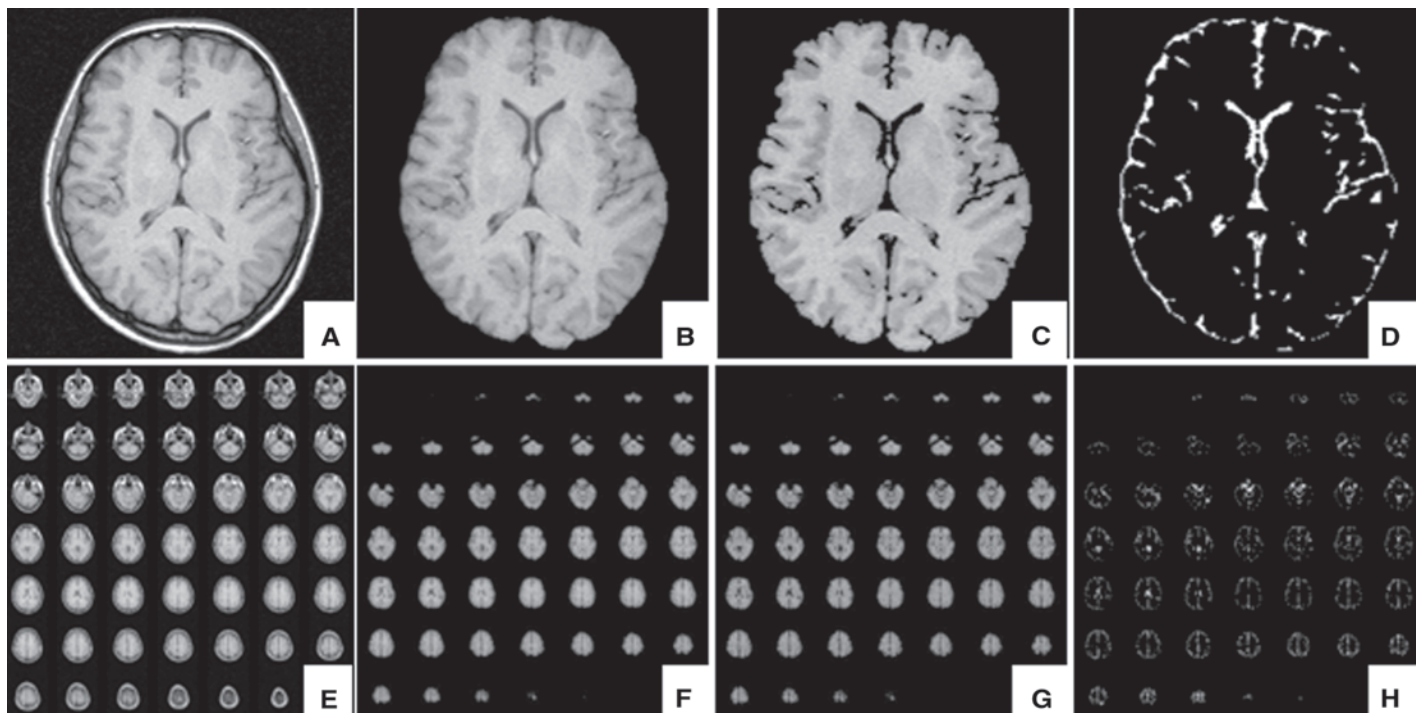
### *Nonconventional Magnetic Resonance Imaging in the Diagnosis of Multiple Sclerosis*

On T1-WI, most MS lesions are isointense to white matter, but some are hypointense or appear as “black holes” (BH) (8,78–80), particularly in the supratentorial region (Fig. 2). These hypointense lesions are nonspecific at a given time because nearly half will revert to normal in a few months, most likely resulting from remyelination and resolution of edema (81). Persistent BH, however, are markers of severe demyelination and axonal loss (8). Therefore, the pathological substrate of the accumulation of persisting BH is predominantly axonal damage (8,15,78,81), as shown in a postmortem histopathology-MRI correlation study (8). Such focal axonal loss most likely contributes to Wallerian degeneration. The correlation between BH volume and clinical disability (Expanded Disability Status Scale-[EDSS]) suggests that these may be clinically relevant for measuring disease progression (15,78–80). However, studies comparing various MRI metrics for their association with physical disability or cognitive dysfunction have shown that markers of global tissue degeneration, such as brain atrophy, have higher clinical predictive value than BH and other lesion assessment measurements (17,82,83). Thus, the utility of BH is not entirely clear and further longitudinal studies are necessary for validation of BH as therapeutic outcome measures. The role of BH in the setting of CIS is not well understood and will await extended subanalysis of the Controlled High-Risk Subjects Avonex MS Prevention Study (CHAMPS) (84,85) and ETOMS trial (75,86). For unknown reasons, BH are rarely seen in the posterior fossa or spinal cord, regardless of disease severity or lesion burden on T2-WI.

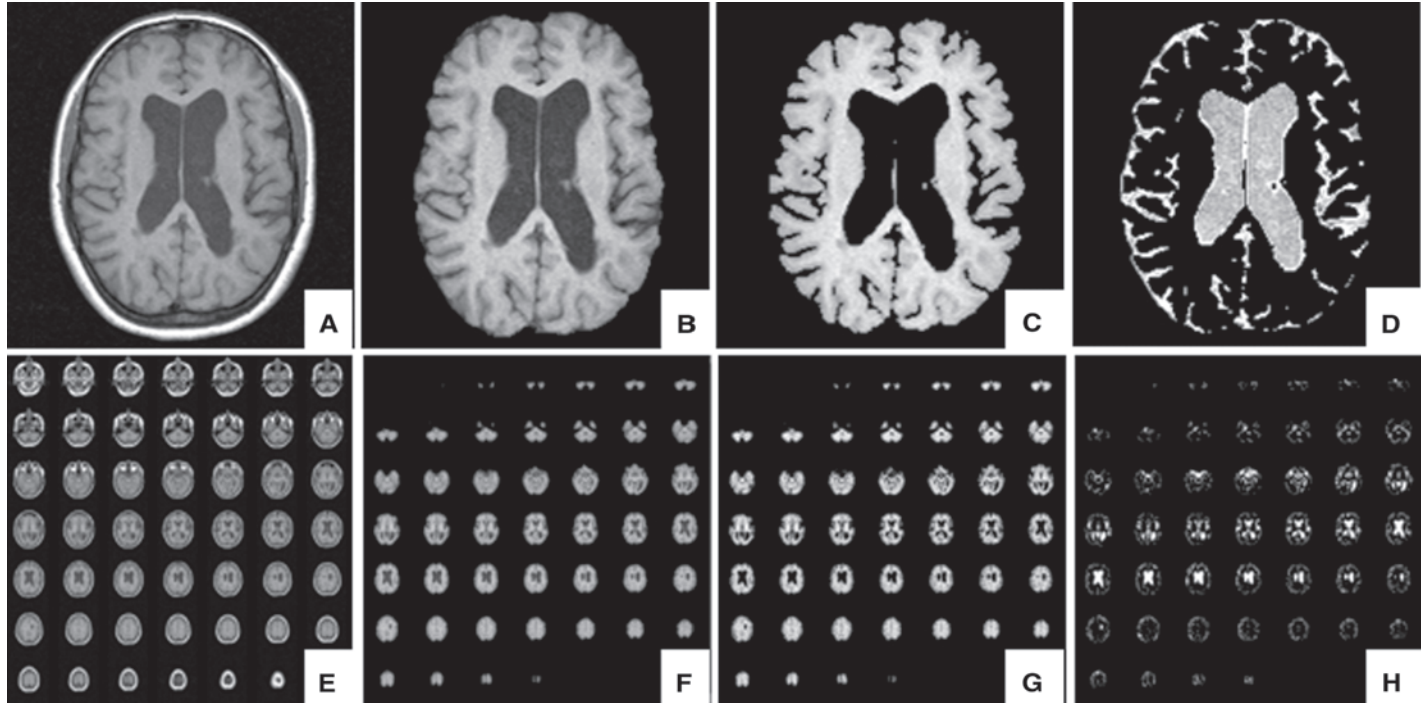
The presence of brain atrophy in MS has been recognized for many years. The measurement of brain atrophy seems to be of growing clinical relevance as a

biomarker of the disease process (16,17,82,83,87–91) (Figs. 10 and 11). There is a consensus developing that the assessment of brain atrophy by serial MRI represents a potentially powerful tool for monitoring disease progression and therapeutic efficacy in MS (16,17,82,83,88,89). When compared to conventional MRI lesion measurements, brain atrophy is a better predictor of clinical impairments, such as physical disability (17,79,83,90,92,93), cognitive dysfunction (82,89,93), depression (94), and quality of life (95). The pathophysiology of brain atrophy in MS is unknown but likely represents an epiphenomenon consisting of a combination of the accumulation of focally destructive plaques, axonal injury, neuronal loss, and distal effects of Wallerian degeneration (16,17,30,78,79). Another factor proposed to be related to brain atrophy is hypointensity of gray-matter structures on T2-WI (T2 hypointensity) (96,97). T2 hypointensity, suggestive of iron deposition, has been described early in the course of MS and is related to physical disability, disease course, and brain atrophy (96,97). It is not clear to what extent abnormal iron deposition in the brain contributes to disease pathophysiology or if it is purely an epiphenomenon of neurodegeneration. Although several studies have recognized the presence of brain atrophy in the earliest phases of MS (17,89,98–102), it is unclear whether measurable brain atrophy is present at the time of the first clinical episode and how rapidly it progresses at this early stage (Fig. 10). Several studies have addressed this issue (100–102). Brex et al. (100) followed 17 patients with CIS for 1 year, of whom nine developed MS and eight did not. Significant ventricular enlargement occurred after 1 year in the group that developed MS but not in the other group. The authors showed that atrophy, albeit mild, can be detected early in the course of MS. Dalton et al. (102) performed MRI of the brain on 55 consecutive patients with CIS, within 3 months of symptom onset and again 1 year later. Clinically definite MS had developed after 1 year in 16 of 40 patients with lesions on baseline T2-WI and 2 of 15 with a normal scan. Significant ventricular enlargement was seen in 27 of 55 patients who fulfilled the McDonald MRI criteria for MS at clinical follow-up. A significant increase in ventricular volume was also seen in 18 of 55 patients who developed clinical MS during the follow-up period. This study indicates that brain atrophy measures have a complementary predictive role in monitoring the course of MS, even in the earliest clinical stages. There is also evidence that spinal cord atrophy (101) is detectable in patients at the time of presentation with CIS in those at high-risk for developing MS.

MTI is an advanced MRI technique based on the interactions between protons that are unbound in a free water pool and those where motion is restricted because of binding with macromolecules (Fig. 12). Tissue damage in MS is usually reflected by a reduction in magnetization transfer ability and thus a decreased MTR. These decreases in MTR most likely reflect a reduction in the size of the tissue matrix and an increase in size of the free water pool. MTR is a quantitative measure. Although MTR decreases are not specific to any of the various MS pathologic substrates, a relationship has been shown between MTR and the percentage of residual axons and the degree of demyelination (103). MTR analysis can provide information about

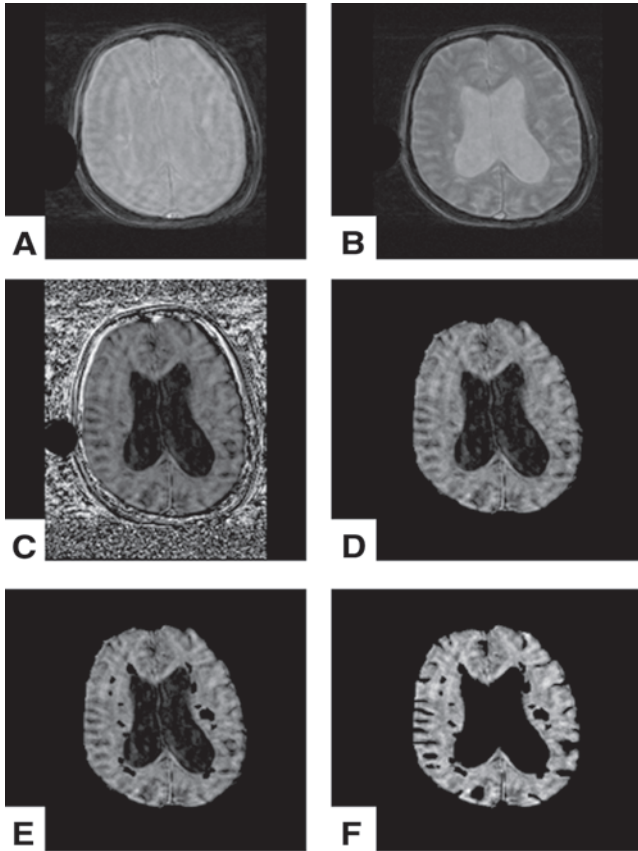


**Fig. 10.** Fully automated method of determining brain parenchymal fraction, a normalized measure of whole-brain atrophy in multiple sclerosis (MS), at the Buffalo Neuroimaging Analysis Center. (A–D) A single axial slice, spin-echo T1-weighted noncontrast, from a 27-year-old woman with clinically isolated syndromes (CIS) showing the raw image (A) after masking (removal) of extracranial tissue has been performed to isolate the outer brain contour (B) and after thresholding to separate the intracranial volume into brain parenchyma (C) and cerebrospinal fluid (CSF) (D). (E–H) Show that the algorithm is applied to all images from the midcerebellum to the vertex.



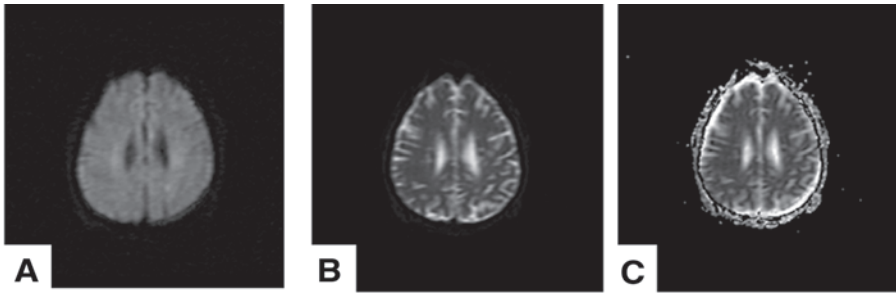
**Fig. 11.** Fully automated method of determining brain parenchymal fraction, a normalized measure of whole-brain atrophy at the Buffalo Neuroimaging Analysis Center, in a 44-year-old man with secondary progressive MS (SPMS). There is clear evidence of advanced atrophy, including prominence of cortical sulci (suggesting cortical atrophy) and ventriculomegaly (indicating central atrophy) as compared to a patient in the earliest phases of disease (Fig. 10). (A–D) A single axial slice, spin-echo T1-weighted noncontrast showing the raw image (A) after masking (removal) of extracranial tissue has been performed to isolate the outer brain contour (B) and after thresholding to separate the intracranial volume into brain parenchyma (C) and cerebrospinal fluid (CSF) (D). (E–H) Show that the algorithm is applied to all images from the midcerebellum to the vertex.





**Fig. 12.** Axial magnetization transfer (MT) images of the brain obtained by 3 D gradient-echo (GE) pulse PD-weighted images (PD-WI). (A,B) The image is shown with (A) and without (B) the on-resonance saturation radio frequency (RF) pulse. (C–E) The GE images are coregistered and MT ratio (MTR) maps are created (C). The MTR maps are coregistered with the corresponding masked (deskulled) PD-WI (D), and the lesion tracings on PD-WI are superimposed onto the MTR maps leading to nulling of lesions (E). An automated segmentation algorithm is applied to null the cerebrospinal fluid obtain MTR of the normal-appearing brain tissue (F).

tissue injury occurring in the whole brain (global) and in specific brain structures (regional) (18,19,104). MTI metrics have been correlated with the degree of disability (18,19,104). Recent MTI studies (105–108) have revealed clinically relevant pathologic changes in areas of white matter that appear normal on conventional images; such changes in normal appearing white matter (NAWM) occur early in the disease process, including at the time of presentation with CIS and provide prognostic information pertaining to the risk of developing MS.



**Fig. 13.** Example of magnetic resonance imaging (MRI) diffusion weighted images (DWI) in a 35-year-old woman with relapsing-remitting multiple sclerosis (RRMS). Echoplanar DWI is performed generating trace images with a b-factor of 1000 (A) and 0 (B)  $s/mm^2$  applied in three orthogonal directions. On the b-1000 DWI map (A) lesions appear as hyperintense areas compared with the surrounding tissue most likely resulting from T2 shiethrough rather than restricted diffusion. To calculate apparent diffusion coefficients (ADCs), an ADC map (C) is generated from images A and B. The diffusivity was computed separately in the  $x$ ,  $y$ , and  $z$  orthogonal directions, and the results averaged to form the mean ADC map (C).

MRS provides a quantitative assessment of disease involvement related primarily to two major pathologic aspects of MS, active inflammatory demyelination and axonal/neuronal injury (109). Inflammation and demyelination are represented by increases in choline, lactate, and lipids. Axonal and neuronal injury can be quantified through decreases in *N*-acetylaspartate (NAA). Decreases of NAA in the white matter of patients with MS reflect changes in density, size, or metabolism of axons (109). Decreased NAA levels have been correlated with disability (110,111). Moreover, axonal damage can be detected using MRS in patients with CIS (112–114).

Diffusion is the random translational motion of molecules in a fluid system. In the brain, diffusion is influenced by the microscopic architecture of tissue, including axons, cell membranes, and organelles. Water molecular diffusion can be measured *in vivo* using MRI, and consequently, DWI is sensitive to pathologic processes that modify tissue integrity and result in changes in the permeability or density of barriers restricting water molecular motion, thereby affecting tissue anisotropy. Thus, measures derived from DWI reflect changes in the size, shape, geometry, and orientation of tissues (Fig. 13) (115,116). DWI also provides quantitative data. MS lesions commonly show hyperintensity on DWI scans resulting from T2 shiethrough, which can be differentiated from true restriction of diffusion using ADC maps (Fig. 13). Tissue damage related to MS is usually reflected on DWI scans as increased diffusivity and increased ADC. Significant cross-sectional correlations between DWI and clinical findings in MS are emerging (116,117). However, the stage at which DWI can detect abnormalities in NAWM is not clear. There is only one study (118) demonstrating that DWI did not detect alterations in NAWM of patients with CIS at baseline but that such abnormalities became apparent 1 year later.



Functional MRI (fMRI) is a unique MRI technique that can noninvasively detect activation of brain areas during the performance of tasks. To investigate how early cortical reorganization occurs in the course of MS, fMRI has been used to study the visual and motor systems of patients with CIS (113,119–122). Recently, Pantano et al. (121) studied a group of 20 patients with early MS. In 10 patients, the CIS was hemiparesis, and in the other 10 patients, the CIS was ON. The group with hemiparesis showed functional adaptive changes that involved both the symptomatic and the asymptomatic hemisphere during a simple motor task. Moreover, the group with hemiparesis showed a significantly higher EDSS score and T1LV in the corticospinal tract than the group with ON. More severe specific damage to motor pathways in patients with a previous hemiparesis may explain the significantly higher involvement of ipsilateral motor areas observed in the group with hemiparesis than in the group with ON. Filippi et al. (120) studied cortical activation related to motor tasks in patients at presentation with CIS and a high risk for subsequently developing MS. Compared with healthy controls, the CIS group had an altered pattern of cortical activation, characterized by activation of the contralateral primary sensorimotor cortex. These data suggest that functional cortical reorganization occurs in response to injury at the earliest stages of MS.

## **ROLE OF MAGNETIC RESONANCE IMAGING IN THE PROGNOSIS OF MULTIPLE SCLEROSIS**

MRI plays a role in determining the risk for developing MS in patients who present with CIS. However, the direct relationship between early MRI findings and subsequent long-term disability is not clear. Usually the first manifestation of MS is CIS, involving the optic nerves, brainstem, or spinal cord. The time between the first clinical attack and subsequent relapses, as well as the rate of accumulation of sustained neurological disability, is highly variable among patients. Baseline clinical and MRI findings are only moderately predictive of these subsequent occurrences. Brain MRI at the time of presentation in patients with CIS is useful for excluding other neurologic conditions and to stratify the risk of developing MS (a high risk is indicated by subclinical evidence of spatial dissemination of lesions) (50). This is important in selecting patients for early disease-modifying treatments to reduce the likelihood of developing further clinical relapses and long-term disability (84,86,123).

### ***Use of Conventional Magnetic Resonance Imaging in the Prognosis of Multiple Sclerosis***

Focal areas of high signal, identical to those seen in patients with established MS, have been found on T2-WI in 50 to 80% of the patients at presentation with CIS (124–126). Several longitudinal studies using conventional MRI (127–132) showed that the number and volume of T2 lesions at presentation with CIS are associated with an increased risk of developing MS and lead to a higher level of disability at long-term follow-up.

Filippi et al. (129) studied 84 patients presenting with acute CIS of the optic nerves, brainstem, or spinal cord. At 5-year follow-up, 38 (45%) had developed probable or definite MS. Patients who developed MS during follow-up had a higher T2LV at presentation than those who did not. There was a strong correlation between baseline T2LV and both the increase in T2LV and accumulation of disability during the next 5 years. Ten year follow-up of this subgroup showed that 45 (83%) developed MS, of whom 11 (20%) had RR disease with at least moderate disability (EDSS > 3), 13 (24%) secondary progressive (SP) and 21 (39%) RR disease but mild disability (130). For those with a normal baseline MRI, progression to MS occurred in only three out of 27 (11%), and all had RR disease with mild disability. The number and volume of baseline T2 lesions correlated moderately with the degree of disability 10 years later ( $r = 0.45$ ,  $p = 0.001$ ) (131). After 14 years, MS developed in 44 of the 50 patients (88%) with abnormal MRI at presentation and in 4 of 21 patients (19%) with normal baseline MRI (132). The EDSS disability score at 14 years correlated moderately with T2LV on MRI at 5 years ( $r = 0.60$ ,  $p > 0.001$ ) and with the increase in T2LV over the first 5 years ( $r = 0.61$ ,  $p > 0.001$ ). Thus, early MRI findings have prognostic value in patients with CIS, in terms of both conversion to MS and the long-term development of disability. However, the relationships were only moderate, indicating that T2LV alone may not be adequate for decisions about the use of disease-modifying treatment.

Kappos et al. (32) investigated the prognostic value of Gd-enhanced MRI in a meta-analysis of data from five natural-course studies and four placebo groups of clinical trials of 307 patients, 237 with RRMS, and 70 with SPMS. Neither the initial scan nor monthly scans for 6 months were predictive of change in the EDSS in the subsequent 12 or 24 months. The mean number of Gd-enhancing-lesions in the first 6 monthly scans was weakly predictive of EDSS change after 1 year (odds ratio = 1.34,  $p = 0.082$ ) and 2 years (odds ratio = 1.65,  $p = 0.049$ ). The authors concluded that although Gd enhancement in MRI is a predictor of subsequent relapses, it is not a strong predictor of the development of cumulative impairment or disability. This discrepancy supports the idea that discordant mechanisms are operative in the occurrence of relapses and in the development of sustained disability in MS. Several groups investigated the predictive value of homogeneously- and ring-enhancing lesions vs disability progression (133–137). The findings suggested that the presence and frequency of Gd enhancement and changes in disability during a short period are predictive of future long-term deterioration. In one study (39), the frequency of ring-enhancement did not predict sustained disability 3 years later. However, in a subgroup of patients treated with IFN- $\beta$ -1b, ring-enhancing lesions were predictive of EDSS worsening after 3 years ( $p = 0.01$ ). Further studies are warranted to test whether the presence of ring-enhancing lesions predicts long-term disability progression.

### ***Use of Nonconventional Magnetic Resonance Imaging in the Prognosis of Multiple Sclerosis***

Several studies have used brain atrophy, T1-hypointense LV, MTI, DWI, and MRS to test whether the extent and severity of tissue loss in lesions and in normal-

appearing brain tissue at the time of CIS or in the early phase of RR MS may have value in predicting disability progression in the short- and long-term.

Several studies have shown that the development of brain atrophy in the early phase of RR MS is a good predictor of subsequent sustained neurological impairment in the long-term ( 5 years) (17,79,90). These findings indicate that once brain atrophy is present, irreversible damage has already occurred (Fig. 11). The ability of the CNS to compensate for the loss of axons and neurons likely depends on the location of atrophy and the available brain reserve capacity. The authors believe that once the level of tissue loss reaches a critical threshold, patients are likely to begin to suffer clinical impairments and disease progression.

Several studies showed moderate but significant correlations between the accumulation of BH and long-term disability progression from the earliest stages of RR MS (78–80,81,89,138–142). Results from the Multiple Sclerosis Collaborative Research Group trial (80) in placebo vs 80 IFN- $\beta$ -1a RRMS treated patients with mild to moderate disability showed a modest but significant correlation between T1-hypointense LV and disability at baseline and during the trial. In patients who received the placebo, there was a 29.2% increase in the mean volume of T1-hypointense LV over 2 years, as compared to an 11.8% increase in the patients treated with IFN- $\beta$ -1a. The development of T1 BH is influenced by prior inflammatory disease activity, as indicated by enhancing lesions, and can be partially prevented by disease-modifying therapy. The role of BH in the longitudinal monitoring of patients with MS is not clear and requires further validation studies.

Recently, four prospective MTI studies (105–108) demonstrated that subtle changes occurring in normal-appearing brain tissue are associated with an increased risk of developing MS in patients with CIS. The ability of MTI to predict the clinical evolution of MS has been investigated in a 5-year longitudinal study (143). The authors found significant differences in baseline MTR values in NAWM between clinically stable and worsening patients with MS. A strong correlation was found between baseline MTR and subsequent 5-year change in EDSS disability score. MTR of the NAWM at baseline correctly predicted clinical evolution in 15 out of 18 patients (1 false-positive and 2 false-negatives), yielding a positive predictive value of 77%, a negative predictive value of 88%, and an odds ratio of 28. These data support the belief that MTR abnormalities in NAWM can predict the clinical evolution of MS.

MRS has been used by several groups to predict clinical evolution in RRMS (14,144–147). In a 30-month longitudinal study of 29 patients with RR MS, De Stefano et al. (146) assessed NAA from a large central brain volume to evaluate the relationship between axonal/neuronal integrity and the accumulation of clinical disability. Changes in the NAA to creatine ratio correlated strongly with clinical disability suggesting that axonal injury contributes to long-term functional impairment of patients with MS.

DWI has been used to predict the clinical evolution of MS. In a recently published study, water diffusion changes in NAWM of 19 patients with CIS and in 12 healthy controls were compared (118). The MRI scans obtained at baseline and at

12 months were reviewed according to the McDonald criteria for the diagnosis of MS. T2- and T1-LVs in the whole brain and ADCs in NAWM were evaluated. Thirteen patients developed MS during the study and three other patients remained as possible MS. ADCs in NAWM were significantly higher in patients than in controls at the 12-month follow-up but not at baseline. This study suggested that DWI cannot detect alterations in NAWM of patients with CIS. After 1 year, when most patients develop MS, DWI abnormalities in NAWM become apparent. These abnormalities are correlated with T2LV and may contribute to neurological impairment.

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## Cognitive Dysfunction in Multiple Sclerosis

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### INTRODUCTION

Cognitive dysfunction is one of the more common symptoms of multiple sclerosis (MS), with an estimated point prevalence of 30 to 60%. The lifetime prevalence may be closer to 50 to 75%, as cognitive symptoms tend to progress with disease progression. Unfortunately, cognitive dysfunction can also be one of the more disabling symptoms of MS (1–3). This chapter reviews the prevalence, diagnosis, possible etiologies, assessment, and treatment of cognitive disorders in MS.

### DIAGNOSIS, CHARACTERIZATION, AND PREVALENCE

Cognitive dysfunction is diagnosed when patients experience a change in their cognitive functioning that produces functional impairment. The *Diagnostic and Statistical Manual of Psychiatric Disorders*, the primary diagnostic system used in American psychiatry, lists two cognitive disorders caused directly by MS: Dementia due to Multiple Sclerosis and Cognitive Disorder Not Otherwise Specified (NOS) due to Multiple Sclerosis (4).

Dementia resulting from MS is characterized by memory impairment and impairment in at least one other cognitive domain, which may manifest as aphasia, apraxia, agnosia, or disturbed executive functioning. This impairment must produce significant decline in social and occupational functioning. Frequently, this level of impairment precludes participation in most basic daily activities, including driving, shopping independently, and managing household finances, and may require that the patient be conserved.

Cognitive disorder NOS as a result of MS is a much milder condition, which may include memory dysfunction, and although producing some disturbance in social and/or occupational functioning, does not result in the broad functional impairments seen in dementia resulting from MS. Patients with cognitive disorder NOS resulting from MS may complain of difficulty with memory, attention, multitasking, wordfinding, and problems with organization and scheduling. These

complaints tend to be persistent, but they can wax and wane with fatigue and disease activity. These symptoms may reach a level where they result in occupational disability or unemployment, particularly in patients with intellectually challenging occupations. However, many patients with cognitive disorder NOS resulting from MS are able to continue working with some accommodations. Symptoms of cognitive disorder NOS resulting from MS can produce discord and difficulties in the patient's social and recreational functioning, but this discord is frequently a result of others in the patient's life attributing the patient's symptoms to willful ignoring rather than acknowledging the presence of cognitive change. Patients themselves may misattribute cognitive symptoms to depression, stress, aging, or "losing their minds."

Historically, researchers considered MS to be a subcortical dementia, consisting of recall failure with intact encoding and storage, impaired conceptualization reasoning, slowed information processing, and personality disturbance in the context of normal or near-normal intellectual and language functioning (5,6). However, this characterization does not adequately describe the presentation of many patients with MS and does not address recent imaging and immunological work that demonstrates that MS is truly a whole-brain disease rather than a disease affecting the white-matter tracts alone.

Numerous studies illustrate the depth and breadth of cognitive impairments that can be seen in MS. Thorton and Raz (7) and Zakzanis (8) in their meta-analyses found that impairments in all domains of memory, including problems with encoding and storage, were common in MS. Problems with processing speed, attention, and concentration may be among the most common problems (9–12). Wordfinding deficits and problems with fluency are often seen, particularly with timed generative naming or listing tasks (8,13,14). Difficulties with visual-spatial learning and memory, visuoperceptive organization, and visual-spatial construction tasks are found (8,15), and difficulties with executive functioning, including problems reasoning, sequencing, problem-solving, and shifting sets, can be seen (8,16,17). Executive dysfunction may be more common in primary-progressive MS (PPMS) than in relapsing-remitting MS (RRMS) or secondary-progressive (SPMS) (8). Cognitive symptoms can occur at any point in the disease course and have been confirmed in patients who are in the early stage of the disease, when their diagnosis remains probable rather than definite (18,19). These symptoms, even when individually fairly mild, have a negative cumulative effect on the patient's social and occupational functioning and level of independence. The seminal studies documenting this effect were by Rao, who found difficulties with memory and speed of processing to be the most common cognitive symptoms in MS (2). Patients who have these cognitive impairments are significantly more likely to be unemployed, report social difficulties, and need more personal assistance than patients who are not cognitively impaired, regardless of their level of physical disability (3,20). More recent data developed by the National Multiple Sclerosis Society found that 30 to 40% of Americans living with MS work full- or part-time outside the home, com-

pared to 64% of Americans in the general population (21). These data, although suggesting that many patients with MS are able to continue working in some capacity, do not address several important issues. Many people with MS are able to continue working but only by accepting accommodations, including reductions in hours, reductions in pay, and changing positions to easier or less personally fulfilling roles.

## **ETIOLOGY: IMAGING FINDINGS**

Numerous factors contribute to cognitive dysfunction in patients with MS. Of these, neuropathological changes in the white matter are best understood, although promising lines of research are developing our understanding of the roles of neuropathology in the gray matter, changes in neurochemistry, and neuroimmunology.

Techniques for imaging the in vivo brain have greatly expanded our understanding of cognitive dysfunction in MS. Although earlier research focused on white-matter pathology in MS, newer imaging techniques are allowing exploration of gray-matter pathology, axonal damage, and the subsequent atrophy seen in these patients. All these factors play a role in the cognitive dysfunction seen in patients with MS. Imaging studies find modest correlations between T1 and T2 lesion load, proton density (PD)-magnetic resonance imaging (MRI) lesion load, various measures of brain atrophy, various magnetic transfer ratio (MTR) histogram parameters, and cognitive dysfunction. Newer imaging techniques, such as spectroscopy and diffusion tensor imaging, are also likely to provide interesting insights about the causes of cognitive dysfunction in patients with MS.

## **LONGITUDINAL STUDIES OF CONVENTIONAL IMAGING**

The earliest MRI measures found to correlate with cognition in MS were T2 and T1 lesion burden. There is a well-established moderate correlation between these conventional imaging parameters and cognitive dysfunction (14,17,22–24). Although total T2 and T1 lesion burden acts as an adequate predictor of cognitive dysfunction, these measures alone do not account for all the variance in cognitive functioning. Several other factors are crucial, including disease duration, atrophy, axonal degeneration, and other changes in the normal-appearing white matter (NAWM).

Some studies have examined the relationships between cognitive dysfunction and lesion burden longitudinally. Sperling and colleagues (25) found that T2 lesion burden in the frontal and parietal white matter correlated with performance on measures of attention, processing speed, and verbal learning in their group of patients with RRMS and SPMS who were cognitively stable for a 4-year period. Hohol and colleagues (24) found significant correlations between total T2 lesion volume and ventricular enlargement and nonverbal memory, attention, and processing speed in their mixed group (RRMS, SPMS, and PPMS) of patients who were cognitively stable for 1 year and who demonstrated a statistically significant increase in ventricle size. Neither of these samples demonstrated a statistically significant increase

in lesion load, although both groups evidenced this trend. These studies suggest that the rate of change in both lesion volume and cognitive dysfunction is fairly slow, while establishing the significant effect of cortical atrophy. However, in both studies, some patients were receiving interferon (IFN)- $\beta$  or glatiramer acetate while enrolled, and some were not. Thus, it is possible that the relative stability in both cognitive and MRI measures of these groups reflect the benefits of treatment with immunomodulating agents.

A study providing 10-year follow-up data on the cognitive performance of early RRMS patients further supports the idea that cognitive progression is fairly slow for most patients, regardless of treatment (20). Even though no imaging data were available for these subjects, Amato and colleagues found significant increases in the number of patients considered to be mildly cognitively impaired 4 years after baseline assessment and the number of patients considered to be moderately cognitively impaired 10 years after the initial assessment. Of their 45 patients sample, only 7 received IFN medications, so this data may provide a more accurate view of the natural history of cognitive dysfunction in MS.

## NONCONVENTIONAL IMAGING

Although most studies have examined the relationship between T1 and T2 lesion volumes and neuropsychological test scores, there have also been promising studies correlating MTR histograms with neuropsychological functioning (14,18,26). Rovaris and colleagues, in two separate studies, found significant correlations between MTR mean histograms and the measures of verbal fluency, verbal learning and memory, and processing speed and cognitive flexibility. They also found that the average height of the MTR histogram and the peak location of this histogram accounts for 68% of the variance in cognitive functioning in their sample (27,28). In their sample of patients with RRMS and CPMS, Van Buchem and colleagues (26) found correlations between MTR histograms and numerous neuropsychological measures, including processing speed, memory, and conceptual processing. Zinadinov and colleagues (18) found significant relationships between MTR histogram parameters and cognitive dysfunction quite early in the disease course, further supporting the finding that cognitive symptoms are often seen in the initial presentation.

Studies have attempted to correlate protein magnetic resonance spectroscopy HMRS metabolite ratios and neuropsychological functioning. In their examination of the relationship between spectroscopy and neuropsychological functioning, Foong and colleagues (29) found no significant correlations between NAA/Cr ratios and neuropsychological measures. They split their sample into thirds, and the third with the lowest NAA/Cr ratio differed significantly from the other groups on spatial working memory test scores. The use of spectroscopy to develop a better understanding of cognitive dysfunction in MS is likely to lead to additional interesting findings. Krupp and colleagues found significant relationships between verbal learning and memory and problem solving and *N*-acetylaspartate (NAA) concentrations in the left periventricular regions (30–33).



## ATROPHY

Atrophy plays a significant role in the development of cognitive difficulties in MS, perhaps particularly in patients with frontal syndromes and more severe behavioral difficulties. Atrophy can begin early in the disease course. Zivadinov and colleagues found evidence of atrophy in the first 5 years after diagnosis in RRMS patients with low levels of physical disability. Following these patients for 5 years, they found that decreased brain parenchymal volume during this time predicts cognitive dysfunction and that patients who experienced greater levels of physical disability also demonstrated more atrophy (34). Frontal atrophy, supratentorial atrophy, and atrophy of the corpus callosum correlate with numerous cognitive problems, including problems with learning and memory, reasoning and attention, as well measures of global cognitive impairment and dementia (34–36). These findings suggest that frontal atrophy may predispose MS patients to mood and behavioral disturbance (37).

## FUNCTIONAL IMAGING

Functional MRI is also likely to provide valuable information about how cognitive dysfunction in MS develops. Staffen and colleagues found different activation patterns between controls and patients with MS performing a visual analog of the Paced Auditory Serial Addition Test (PASAT) (38). This line of work is particularly exciting because it may help us better understand both the neuropathology that causes cognitive dysfunction in MS and neuroprotective processes in the brain that contribute to cognitive reserve in these patients.

In summary, cognitive dysfunction is an early symptom of the disease for many patients and tends to be the result of several pathological changes in the brain, including discrete lesions, atrophy, and changes in the NAWM and likely in the gray matter as well.

## OTHER FACTORS IMPLICATED IN COGNITIVE DYSFUNCTION

### *Depression*

In addition to neuropathology, several other factors are implicated in disturbed cognitive functioning in patients with MS. Depression is another common symptom of MS, and it frequently has a negative effect on cognition (particularly memory), attention, and concentration. Depression, lack of social support, and cognitive impairment correlate with each other, independent of level of physical disability (39). Depression is also clearly correlated with the use of less effective coping styles, such as emotion-focused coping (40). These relationships are likely to be complex and interactive. The ability of severe clinical depression to reduce performance on neuropsychological measures is well established in healthy controls and in patients with known risk factors for cognitive dysfunction, such as traumatic brain injury (TBI). In patients with MS, Arnett and colleagues found that 25% of the variance in measures of depression is predicted by difficulties with

attention/working memory and that these difficulties are particularly apparent on timed tasks of reasoning and problem solving (41). This suggests that cognitive dysfunction may lead to impaired problem solving and poor coping in real-world situations, where people frequently must make flexible and instantaneous coping choices.

A cognitive domain that is especially vulnerable to depression in MS includes working memory and other aspects of executive functioning. Using the working memory system conceptualized by Baddeley and Hitch (42), deficits have been observed in the domains of central executive functioning. Furthermore, patients with MS who are depressed perform poorly when measures of central executive function include a speeded component. Using a capacity-demanding reading-span task and a less demanding word-span task, Arnett et al. (43) demonstrated significantly worse performance by patients with MS who were depressed when compared to patients with MS who were not depressed. Although differences in working memory have been noted in patients with MS regardless of depressive status, Arnett and colleagues have demonstrated that depression accounts for a significant proportion of the variance in central executive processes of working memory in MS. These findings are consistent with those of other researchers (44) and suggest that depression may exacerbate and/or contribute to existing deficits in central executive processing.

In another investigation, Arnett and colleagues (41) also demonstrated decreased functioning in planning abilities among patients with MS who were depressed compared to non-patients with MS who were not depressed using the Tower of London task. In addition to planning abilities, deficits were noted among other measures conceptualized as speeded central executive tasks, namely, the Symbol Digit Modalities Test (SDMT), the PASAT, a reading-span test, and the Visual Elevator subtest of the Test of Everyday Attention. In summary, among other cognitive domains, speeded attentional processing and nonspeeded central executive functioning are specifically susceptible to depression in MS (41,45,46).

## **Pain**

Pain is another frequent symptom of MS that has a well-established negative effect on cognitive functioning. People with MS can experience pain related to spasticity, paroxysmal symptoms, and headache, as well as neuropathic pain. The experience of MS-related pain can range from mild to fairly severe and may or may not be easily treated through medication, physical therapy, or other relief strategies. Pain produces both a direct and an indirect effect on cognitive functioning. People experiencing chronic or acute pain demonstrate decreased attention, processing speed, and difficulties with encoding. Indirectly, pain can produce changes in mood, including depression, and medications used to treat pain frequently have adverse effects on cognitive functioning. In MS, spasticity and muscle tension are often treated with benzodiazepines, which have a direct negative effect on cognition. Antiepileptic medicines frequently used to manage neuropathy also can produce changes in cognitive functioning, particularly difficulties with attention and processing speed.

## *Fatigue*

However, the most common clinical symptom of MS is fatigue, and people with MS can experience worsening of cognitive symptoms both as a direct result of physical fatigue and as a result of cognitive fatigue. Cognitive fatigue is defined as a decline in cognitive performance during a brief time period, such as a neuropsychological testing session or part of the patient's workday. This decline occurs even though the patient is not participating in any physically fatiguing activities. Krupp and colleagues found that patients with MS demonstrated clear declines in performance on measures of verbal memory and executive functioning for a 4-hour session, whereas medically healthy controls demonstrated improvement in memory and executive functioning during the same time period, likely because of practice effects (32). As such, it is particularly important for patients with MS to adequately pace themselves to keep fatigue to a minimum.

## **ASSESSMENT OF COGNITIVE DYSFUNCTION IN MULTIPLE SCLEROSIS**

### *Clinical Neuropsychological Assessment*

Complete and accurate assessment of cognitive dysfunction in patients with MS is important, particularly in those patients who do not have dementia but who, nonetheless, may be experiencing social and occupational difficulties as a result of MS-related cognitive dysfunction. There are several issues related to the assessment of patients with MS. The "gold standard" for assessment is a full neuropsychological battery administered and interpreted by a clinical neuropsychologist. There are several strengths associated with this approach, including an in-depth understanding of the neurological and neuropathological causes of the difficulties the patient is experiencing and the ability to compare the patient against normative standards for the population as a whole, other similar patients with MS, and against the patient's premorbid functioning. Neuropsychologists are able to work with the patient and his or her family or caretakers to develop an individualized plan to understand, manage, and, when possible, remedy the difficulties the patient is experiencing. Neuropsychologists can determine what types of compensatory and rehabilitative approaches will be most helpful for the patient. In addition, neuropsychologists can continue to follow the patient while assessing progression of difficulties and effectiveness of strategies to manage and remediate them.

However, there are numerous potential problems associated with neuropsychological assessment. A full battery usually takes between 6 and 8 hours to administer. This can lead to several difficulties, including problems related to the patient's fatigue, issues with cost and reimbursement, and issues of accessibility, given the limited number of neuropsychologists in many geographic areas. Therefore, numerous other options must be considered.

Recently, a group of prominent MS neuropsychologists have recommended a minimal neuropsychological battery for use with patients with MS. This battery, well-described elsewhere, specifies the need to assess a number of areas of cogni-

tive functioning, including attention, processing speed, mood, verbal and visual learning and memory, language, and verbal and visual-spatial reasoning and judgment (47). Although these measures are still administered by a neuropsychologist or trained psychometrician, most patients should take between 90 and 120 minutes to complete them. This is an excellent option for screening patients, or tracking known progression of deficits in patients who have undergone a more thorough assessment in the past. The downside of this approach is that it still requires a neuropsychologist or trained psychometrician, and issues related to access may still pose barriers for the patient.

One solution under consideration for this access problem is the use of a brief screening questionnaire in the neurologist's office. The MS Neuropsychological Questionnaire, a 15-item measure designed by Benedict and colleagues, can be administered to both patients and significant others in any clinic setting (48). Interestingly, the patient report form of this questionnaire correlates significantly with depression, rather than cognitive functioning. However, the significant-other version has good sensitivity and specificity to cognitive dysfunction in the patient.

### *Assessment in Research and Clinical Trial Settings*

Several outcome measures, are used in randomized controlled clinical trials to assess cognitive functioning as a secondary outcome. Of these, the most commonly used is the Multiple Sclerosis Functional Composite (MSFC). This measure consists of an assessment of lower-limb function/ambulation, the 25-foot walk, a measure of upper-limb function, the nine-hole peg test, and a measure of attention/working memory, the Rao version of the PASAT (49). The MSFC is generally used in conjunction with Expanded Disability Status Scale (EDSS) scores as an outcome measure for clinical trials. It has numerous strengths but also some significant weakness. The PASAT is highly sensitive to MS-related cognitive dysfunction, but it is not specific. Poor performance on the PASAT can be attributed to several factors other than MS cognitive dysfunction, including anxiety, poor math skills, or difficulties in attention and working memory secondary to fatigue, pain, or medication.

## **TREATMENT**

### *Effect of the Disease Modifying Medications on Cognitive Functioning*

Some of the current disease-modifying medications slow the progression of cognitive dysfunction in MS. Intramuscular IFN- $\beta$ -1a (Avonex) and IFN- $\beta$ -1b (Betaseron) help preserve cognitive functioning, whereas no effect on cognition is seen for glatiramer acetate (Copaxone) (50–52). IFN- $\beta$ -1b has a positive effect on visual memory in a small group of patients with RRMS (51). Intramuscular IFN- $\beta$ -1a has several preservative effects on a much larger group of patients with RRMS (50). Specifically, intramuscular IFN- $\beta$ -1a helps preserve cognitive functioning during a 2-year period in many key areas, including processing speed, visual-spa-

tial learning and memory, and executive functioning (50). No data on the effects of subcutaneous IFN- $\beta$ -1a (Rebif) on cognitive functioning are available at this time. The effect of chemotherapy agents commonly used in MS on cognitive functioning has not yet been studied.

### ***Other Approaches to Managing Cognitive Dysfunction in Multiple Sclerosis***

In addition to treatments designed to slow the progression of MS itself, several approaches to managing or remediating cognitive symptoms themselves have been suggested.

Symptomatic treatments have been examined in the hope that they would lead to a beneficial effect on cognitive function. No effect on cognition has been seen for pemoline and amantadine or 4-aminopyridine. There have been small, non-randomized, open-label trials of donepezil hydrochloride in patients with MS with cognitive dysfunction and dementia, finding some benefit, but no randomized placebo-controlled trials as yet in this class of medications (53). Clinically, the treatment of depression and pain may also have a significant effect on the patient's experience of cognitive dysfunction.

The research into the application of cognitive rehabilitation techniques has been limited. One study finds no benefit on cognition in a small trial of a cognitive rehabilitation program (54), although this study finds benefit of their program on quality of life for patients. Plohmann and colleagues demonstrated some benefit for a computerized attention training program in patients with MS with mild attentional deficits (55). Canellopoulou and colleagues demonstrated some benefit for memory training in a clinical setting, although these strategies did not independently generalize to the patients' daily lives (33). Further research into the application of cognitive rehabilitation techniques, particularly newer, computer-assisted techniques, is clearly needed.

Currently, because the benefits of medication and rehabilitation are fairly limited, the most common strategies for managing cognitive difficulties associated with MS require the patient to compensate for these difficulties and accommodate them when unable to compensate. There are numerous strategies that patients may find useful for managing specific types of cognitive problems. For patients with memory and organizational difficulties, the use of personal organizers, particularly those with alarms, can be helpful. Compensating for these kinds of difficulties frequently involves a fair amount of assistance from others in the patient's life, including both family members and coworkers. The use of written reminders and directions, telephone logs, and help maintaining a clean and organized home environment can be important. In addition, pacing to manage physical and cognitive fatigue can be helpful. For patients who wish to keep working outside the home, appropriate pacing can be crucial. Several accommodations designed to allow the person with MS adequate opportunities to rest, such as telecommuting and job-sharing, can help keep patients productive in the workforce.

## SUMMARY

Cognitive dysfunction is a common symptom of MS, present in as many as three-fourths of patients. It frequently appears early in the disease course. Cognitive dysfunction arises from many of the neuropathological changes seen in the brains of patients with MS, including atrophy, discrete lesions, and changes to NAWM. Cognitive dysfunction is a particularly disabling symptom because of its effects on a patient's social functioning and ability to continue paid employment, as well as the support services that it requires. Cognitive symptoms can vary a great deal from patient to patient and are affected by several other MS symptoms. Depression, fatigue, anxiety, and pain can worsen or mimic cognitive symptoms. Immunomodulating therapies have some positive effect on cognition, but currently, most patients will receive most benefit from developing individualized plans to manage and compensate for these problems.

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## Multiple Sclerosis, Genetics, and Autoimmunity

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### INTRODUCTION

Multiple sclerosis (MS) afflicts approximately 250,000 to 350,000 individuals in the United States and is the most common autoimmune disease involving the nervous system. Multifocal immune-mediated destruction of the myelin sheath and secondary axonal damage in the central nervous system (CNS) results in variable neurological dysfunction, most commonly altered vision, incoordination, gait ataxia, paralysis, and sensory disturbances (1). Relapsing-remitting MS (RRMS), where patients have attacks of neurological dysfunction lasting days to weeks followed by complete to near complete recovery, accounts for approximately 85% of patients with MS. After approximately 10 years, the majority of patients with RRMS have entered secondary-progressive MS (SPMS), where there is gradual accumulation of neurological dysfunction without recovery. This may represent a neurodegenerative phase of the disease that results from axon transection and neuronal loss (2,3). Primary-progressive MS (PPMS), accounting for approximately 15% of patients with MS, is similar to SPMS, except that these patients do not have a preceding relapsing-remitting phase.

Although the cause of MS is unknown, both environmental and genetic factors are believed to interact to induce the development of disease (4,5). The strongest evidence for a genetic role in susceptibility to MS comes from a study of Canadian twins (6,7). This work demonstrated that the risk of MS within a family increases directly proportional to the amount of shared genetic information, reaching a concordance rate of approximately 30% in monozygotic twins, compared to approximately 3 to 5% for first-degree relatives and 0.1% for the general population. Whole genome screens in patients and similar studies in mice have identified several loci associated with MS and its animal model experimental autoimmune encephalomyelitis (EAE) (8–12). However, except for the class II major histocompatibility complex (MHC) alleles HLA-DR and HLA-DQ, specific candidate genes having a

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strong association with MS have yet to be identified. A heterozygous point mutation in PTPRC, the gene encoding the tyrosine phosphatase CD45, was associated with MS, but this was not confirmed in subsequent studies (13–15) and is of uncertain significance.

The dominant theory for the pathogenesis of MS centers on the formation of abnormal T-helper cells that are autoreactive against myelin antigens. These T-cells cross the blood–brain barrier (BBB) via adhesion molecules, such as very late antigen-4 (VLA-4), and are stimulated by myelin antigens presented by local antigen presenting cells and differentiate predominantly into T-helper type 1 (T<sub>H</sub>1) cells that secrete proinflammatory cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). This, coupled with activation of B-cells, cytotoxic T-cells, and macrophages, results in inflammatory demyelination and associated neurological symptoms (1,2,4,5). Although each step in this model is important, the formation of abnormal myelin-specific T-cells is the first key event, without which disease cannot develop. This is best demonstrated by two key experiments in mice. First, myelin-specific T-cell clones isolated from mice with EAE, unlike B-cells, can adoptively transfer disease to naïve mice (16,17). Second, transgenic mice, where the only lymphocytes present in the mouse are transgenic T-cell clones specific for myelin basic protein (MBP) (i.e., Rag1<sup>-/-</sup> mice with a MBP-specific T-cell antigen receptor [TCR] transgene) (18), uniformly develop spontaneous EAE. Combined, these studies indicate that abnormal myelin-specific T-cells are necessary and sufficient to induce and sustain EAE in the absence of any other lymphocyte, including B-cells. Although B-cells may worsen disease severity, they are not required to initiate or sustain disease.

T-cells directed against myelin antigens can be detected in the blood of normal individuals at a frequency similar to that of patients with MS (19,20). This is not surprising because the major myelin proteins are expressed in the thymus during T-cell maturation (21), and T-cells only exit the thymus into the periphery if bind with low affinity to self-peptide/MHC complexes (22). Once in the periphery, T-cells continue to require low-affinity stimulation by self-peptide/MHC for survival. Despite this, self-tolerance is maintained in normal individuals and autoimmune disease does not develop. Therefore, the simple presence of myelin-specific T-cells is not abnormal; rather, a qualitative change in these T-cells is likely required to induce disease. As such, the discovery of genes and associated molecular mechanisms that regulate T-cell function and self-tolerance should provide prime candidates for MS susceptibility genes and new targets for disease therapy.

## THE ADAPTIVE AND INNATE IMMUNE SYSTEMS

The immune system can be divided into two broad categories: the innate immune system and the adaptive immune system. The former consists predominantly of phagocytic cells (e.g., macrophages and polymorphonuclear cells), whereas the latter are lymphocytes (e.g., T-cells and B-cells). The defining distinction between these two arms of the immune system is the unique ability of lymphocytes to gener-

ate a huge repertoire of high-affinity antigen receptors and the maintenance of the encounter with antigen as immunological memory. Although innate immune system cells also have cell surface antigen receptors, termed toll-like receptors (TLRs), these receptors are invariant and only recognize conserved pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide, peptidoglycan, CpG DNA motifs, dsRNA, and bacterial flagellen (23). In contrast, adaptive immune system cells have marked variation in their antigen receptors, with each cell's antigen receptor recognizing a degenerate number of antigenic determinants with varying affinity. Recognition of antigen by these receptors leads to clonal expansion of that cell, providing both immunological specificity and memory.

The adaptive system is divided into humoral and cellular immune responses, which are mediated by B-cells and T-cells, respectively. Antigen recognition by B-cell antigen receptors (i.e., membrane-bound immunoglobulin [Ig]M) induces B-cell proliferation and differentiation into antibody-secreting plasma cells, which, in turn, secrete high-affinity antibodies. These antibodies help clear extracellular infectious agents by promoting complement fixation and targeting pathogens for phagocytosis via interaction with Fc receptors on phagocytic cells. In contrast to B-cells, T-cells cannot recognize soluble antigen, but rather require a peptide antigen to be bound to and presented by an MHC. T-cells are divided into two main subtypes: CD4<sup>+</sup> T-helper cells, which require MHC class II, and CD8<sup>+</sup> cytotoxic T-cells, which require MHC class I. T-helper-cells activate the innate immune system and B-cells via cytokine release, while cytotoxic T-cells kill virally infected cells. Although cytotoxic T-cells, B-cells, and innate immune system cells contribute to inflammatory demyelination, it is the T-helper cell that is key to the initiation and pathogenesis of autoimmune demyelination and is the focus of this chapter.

## T-CELLS, SELF-TOLERANCE, AND AUTOIMMUNITY

For many years it was believed that the adaptive immune system evolved to recognize the difference between self and nonself, but we now know that this model is incorrect. In fact, the adaptive immune system not only normally recognizes self-antigens but also requires them for normal homeostasis. Rather, the defining characteristic of interactions with self vs nonself is that lymphocytes are tolerant of self-antigen and nontolerant to foreign antigen. Although lymphocyte antigen receptors bind and recognize self-antigen, this does not normally induce lymphocyte activation and proliferation. Autoimmune disease, including MS, develops when this tolerance to self-antigen is lost. Multiple mechanisms have been proposed for the maintenance of T-cell tolerance to self-antigen (24), several of which are discussed.

T-cells develop in the thymus from stem cells that originated in the bone marrow. Once in the thymus, T-cell receptor (TCR) genes in individual cells undergo gene rearrangement, leading to a large repertoire of TCR specificities. Once this TCR gene rearrangement has occurred, immature T-cells or thymocytes undergo one of three fates: positive selection (5%), negative selection (5%), or death by

neglect (90%). Negative selection occurs when a newly rearranged TCR binds to self-antigen/MHC with high affinity, inducing cell death by apoptosis (i.e., programmed cell death). The elimination of T-cells with high affinity to self-antigen in this manner represents an important mechanism for the maintenance of self-tolerance. In contrast, if TCR does not bind self-antigen/MHC at all, then the cells also die by apoptosis. This is termed death by neglect and occurs in approximately 90% of thymocytes. Low-affinity interactions of TCR with self-peptide/MHC provide a survival signal to the developing thymocytes, and only these cells mature, leave the thymus, and enter the periphery. Thus, all mature T-cells are positively selected to bind self-antigen/MHC with low affinity, which explains why normal individuals have myelin-reactive T-cells.

Once in the periphery, naïve T-cells continue to engage self-antigen/MHC. Although these interactions promote T-cell survival (25–27), they normally do not induce significant T-cell proliferation (28). An exception to this occurs in the presence of lymphopenia, when self-peptide-MHC interactions induce a strong proliferative response (29), albeit less vigorous than stimulation with foreign antigen. This has been termed homeostatic proliferation because it is believed to be a mechanism to quickly repopulate an individual to near-normal numbers of T-cells.

Why do these physiological self-peptide-MHC interactions not induce T-cell activation, breakdown of self-tolerance, and the development of autoimmunity? Activation of naïve T-cells normally requires not only the recognition of peptide/MHC by the TCR but also other cosignaling events. The combination of these two signals initiates a signaling cascade that results in actin cytoskeleton reorganization and the formation of a specialized contact area between the T-cell and the APC termed the immune synapse (30–33). The formation and integrity of the immune synapse is essential for the activation and proliferation of T-cells (34,35). Although several “cosignals” have been described, the most important is the interaction of the T-cell surface receptor CD28 with its ligands B7-1 and B7-2, which are present on the surface of activated APCs (36,37). Costimulation by CD28 recruits protein kinase-enriched lipid rafts to the immune synapse (32,33), thereby enhancing TCR-mediated signaling and lowering T-cell activation thresholds. TCR signaling in the absence of CD28-B71/2 costimulation not only results in reduced T-cell proliferation but also induces a state of anergy or nonresponsiveness to subsequent stimulation via TCR and CD28 (38). The absence of CD28 in mice leads to T-cell hypoproliferation in response to antigen receptor stimulation (39) and resistance to the induction of EAE (40) and other autoimmune diseases, indicating that anergy plays an important role in the maintenance of self-tolerance.

Resting APCs, such as dendritic cells and macrophages, express minimal levels of the CD28 receptor B7-1 and no B7-2, thus providing only limited costimulatory signals to T-cells. Recognition of nonself microbial products by dendritic cell and macrophage pattern recognition receptors, such as TLRs, leads to a marked upregulation of the CD28 costimulatory receptors B7-1/B7-2, as well as the secretion of inflammatory cytokines, such as TNF- $\alpha$  and interleukin (IL)-1. This signifi-

cantly enhances their ability to activate naïve T-cells (23). Because self-antigens do not bind TLRs and do not activate APCs, the innate immune system, via TLR signaling and associated upregulation of costimulatory molecules, plays a key role in communicating to the adaptive immune system whether the antigen it presents is self or nonself (41). The inability of self-antigen to upregulate costimulatory signals in APCs prevents the normal physiological interactions of TCR with self-peptide/MHC from inducing T-cell activation and represents another important mechanism for the maintenance of self-tolerance.

Although T-cell anergy is initiated by TCR signaling in the absence of CD28 costimulation, it also requires the subsequent expression of the CD28 homolog cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) to maintain it. CTLA-4 is upregulated after T-cell activation (42) and engagement of CTLA-4 with B7-1 and B7-2 transduces a negative signal that inhibits T-cell proliferation. Mice that are deficient in CTLA-4 develop a lethal inflammatory/autoimmune disease characterized by severe inflammatory infiltrates in multiple organs (43). Antibody blockade of CTLA-4 interaction with B7-1 and B7-2 in vivo exacerbates EAE severity (44). In humans, a functional role for CTLA-4 in the prevention of autoimmunity has recently been established by the association of Grave's disease, autoimmune hypothyroidism, and type 1 diabetes with polymorphisms in CTLA-4 that significantly reduce its expression (45). A study of the same polymorphisms in patients with MS has yet to be reported. Other polymorphisms in CTLA-4 have been assessed in patients with MS by multiple groups, for the most part with contradictory results (46–52).

PD-1 and ICOS are two additional members of the CD28/CTLA-4 costimulatory family that, like CTLA-4, are upregulated after T-cell activation and negatively regulate autoimmune disease susceptibility (53). However, they have distinct receptors and do not interact with B7-1 or B7-2. PD-1 is similar to CTLA-4 in that it inhibits T-cell activation, and mice that are deficient in this gene spontaneously develop autoimmune disease (53). In contrast, ICOS regulates T-cell differentiation without significantly altering the initial activation thresholds of naïve T-cells. Deficiency or blockade of ICOS in mice promotes EAE, possibly by inhibiting differentiation of naïve T-cells into autoimmune suppressing T<sub>H</sub>2 cells (53). T<sub>H</sub>2 cells secrete immunosuppressive cytokines, such as IL-4, IL-5, IL-10, and IL-13, which inhibit proinflammatory T<sub>H</sub>1 cells and suppress autoimmunity.

Although CD28 costimulation markedly lowers T-cell activation thresholds, it is not absolutely required to induce full T-cell stimulation. It has been estimated that clustering of approximately 8000 TCRs is sufficient to activate T-cells, a number that is reduced by approximately fivefold by CD28 coreceptor stimulation (54). Thus, one potential mechanism for the abnormal activation of T-cells by self-peptide would be a gene mutation that directly enhances TCR clustering, thereby allowing T-cell activation in the absence of CD28 cosignals. Indeed, two mouse genes, *Mgat5* and *cbl-b*, display this precise phenotype. Deficiency in either gene results in enhanced ligand-induced TCR clustering, CD28 independent T-cell acti-

vation, increased susceptibility to EAE, and the development of spontaneous autoimmune disease. Although mutations in these two genes produce the same phenotype, the molecular mechanisms involved are distinct. Mgat5 is an enzyme in the Asn (N)-linked protein glycosylation pathway that modifies cell surface receptors (e.g., TCR) with a high-affinity ligand for a family of carbohydrate-binding proteins termed galectins. Multivalent interactions of galectins with Mgat5-modified glycoproteins sequester the TCR complex within a cell surface galectin-glycoprotein lattice that restricts ligand-induced TCR recruitment to the immune synapse. The absence of Mgat5 glycans disrupts this lattice, thereby enhancing agonist-induced TCR clustering, downstream signaling, T-cell activation, and, in mice, spontaneous autoimmunity. In contrast, *cbl-b* is an E3 ubiquitin ligase that negatively regulates the guanine nucleotide exchange factor Vav by targeting phosphatidylinositol 3 (PI3)-kinase for ubiquitination. This inhibits actin cytoskeleton reorganization and associated TCR receptor clustering at the immune synapse (55–57). The key point is that in the absence of either Mgat5 or *cbl-b*, TCR clustering is enhanced, leading to antigen-induced T-cell hyperproliferation and spontaneous autoimmunity in mice. Although mutations in Mgat5 and *cbl-b* have not been reported in humans, they represent prime candidate susceptibility genes for autoimmune diseases, such as MS.

Taken together these data strongly indicate that multiple genes involved in the T-cell costimulatory pathway can independently regulate the same endpoint: T-cell hyperproliferation and autoimmune disease. Therefore, it is likely that dysregulation of T-cell costimulatory pathways play a key role in the loss of immune tolerance in MS and represent prime targets for therapeutic intervention.

## **MOLECULAR MIMICRY, IMMUNE TOLERANCE, AND MULTIPLE SCLEROSIS**

The previous discussion centered on molecular mechanisms in the regulation of immune tolerance. Because they are not specific for a particular antigen, they can be applied to the pathogenesis of multiple autoimmune diseases. However, they do not address why one individual develops MS and another develops type 1 diabetes. Although the answer to this question is unknown, it is likely differences in environmental risk factors, or more precisely exposure to different pathogens. It is well established that numerous human pathogens contain peptide sequences that are molecular mimics of endogenous self-antigens (58,59). For example, the myelin antigen MBP shares homology with multiple microbes, including measles, hepatitis B, influenza, adenovirus, herpes simplex virus (HSV), Epstein-Barr virus (EBV), PPV, and pseudomonas (60). In addition, TCR binds peptide/MHC with very low-affinity ( $K_D$  approximately  $10^{-5}M$ ), resulting in a highly degenerate specificity for peptide/MHC. Thus, in a genetically susceptible individual who has the appropriate MHC haplotype and dysfunction in one or more of the pathways described, infection with one of these pathogens may lead to cross-activation of myelin-reactive T-cells and the development of MS. This model is supported by the fact that

relapses in MS are frequently triggered by viral infections, that pathogen peptides with homologies at specific MHC and TCR contact sites are able to stimulate MBP-specific T-cell clones from patients with MS (60), and that clinical and pathological EAE can be induced by immunization with peptide from a microbe that has homology to a myelin antigen (61–64). However, the strongest evidence for this model is work with a transgenic mouse that expresses a MBP-specific TCR (65). When these mice were housed under pathogen-free conditions, they displayed no phenotype. However, when they were housed in a nonsterile animal colony with mouse pathogens, such as mouse hepatitis virus, pinworm, and trichomonas-type flagellates, approximately 14–44% of the mice spontaneously developed clinical and pathological EAE. This indicates that exposure to pathogens and associated activation of the MBP-specific T-cells were required to trigger spontaneous EAE and provides a model to define how genetic susceptibility and environmental factors can interact to induce autoimmune demyelination. Because humans do not live in sterile environments, a similar experiment in humans is not possible. Instead, we must rely on these types of animal experiments to provide the best evidence for molecular mimicry in the pathogenesis of MS.

## SUMMARY

The best available evidence, both from human studies and animal models, such as EAE, strongly indicate that MS is initiated and sustained by T-cells. Other leukocytes, such as B-cells, may regulate severity of disease but are insufficient to cause disease. Complex interactions between an individual's genetic background and his or her environment are required for disease to develop. Although we do not yet know the precise genetic and environmental factors required, mutations in genes that regulate T-cell activation thresholds and/or costimulatory pathways in combination with exposure to pathogens with cross-reactivity to myelin antigens currently represents the best model for the pathogenesis of MS. Confirmation of this model and the determination of these factors should eventually lead to better preventative and treatment strategies for this disabling disease.

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## B-Cell Immunity in Multiple Sclerosis

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### INTRODUCTION

Multiple sclerosis (MS) is the most common chronic neurological disorder in young Caucasian adults (1–3), but after more than a century of active research, its etiology remains unknown. It is a peculiar disease, because it is restricted to the central nervous system (CNS), is not associated with any other neurological or systemic disorder, and has no animal counterpart. In the typical form, MS evolves through relapses and remissions, although, in the majority of patients, a secondary progressive phase ensues (4–7). Its classic pathological hallmark is the plaque, which, in the acute form of the disease, shows perivascular infiltration and contains macrophages filled with myelin debris (8–16). This classic view is challenged by magnetic resonance imaging (MRI) and pathological studies, which reveal substantial early axonal damage.

MS was formerly described as a strictly demyelinating disease but is now known to affect both axons and myelin (17–19). Several MRI studies have shown signs of preplaque anomalies, the pathological nature of which is controversial. MS is considered to have an inflammatory and a degenerative component, although whether the degenerative component is secondary to inflammation or is caused by primary axonal damage, remains unclear.

The prevailing etiological hypothesis for MS is that it is a multifactorial disorder, affecting individuals predisposed by a combination of several susceptibility genes and environmental factors (which are mainly infectious in nature) (20–30). Plaque formation is attributed to immune mechanisms, triggered by an autoimmune attack directed against antigens in the myelin membrane. Experimental autoimmune encephalomyelitis (EAE) is an animal model induced in mammals by injection with either whole-brain tissue or specific myelin proteins, such as myelin basic protein (MBP) (31–33), myelin oligodendrocyte glycoprotein (MOG) (34–38), myelin-associated glycoprotein (MAG) (39,40), or proteolipid protein (PLP) (41,42). The disease can be transmitted from an affected to a naïve animal by

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T-cell transfer, which has led to the identification, in humans, of T-cell clones specific for these myelin proteins (43–46). These T-cell clones, which can be isolated from normal individuals and patients with MS, are believed to be central to the initiation of the immune process that culminates in plaque formation. Consequently, MS has been seen mainly as a T-cell disease, and therapeutic strategies are based on this concept (47,48).

Recent publications have challenged this view and focused on the possible involvement of B-cells in the immune pathogenesis of MS. This chapter reviews the biology of B-cells in relation to their implication in MS. Findings from various groups, including the author's own, are summarized and their implications discussed.

## IMMUNOGLOBULIN IN CEREBROSPINAL FLUID

MS is considered to be a cell-mediated autoimmune disease, and involvement of the humoral immune system is indicated by the presence of oligoclonal immunoglobulin (Ig) proteins (oligoclonal bands) (OCBs) in the CSF of the majority of patients. These proteins are produced by B-cells in the CNS (49–53).

Intrathecal Ig synthesis, reflected by an increased IgG ratio and presence of OCBs, is a general feature of chronic brain inflammation and is of diagnostic use in MS. Naturally produced intrathecal OCBs are restricted to the IgG1 and IgG3 subclasses in most patients (54,55). Much lower amounts of IgM and IgA are sometimes present (56), and local production of IgM is often detected in the CSF of patients with early MS (57,58). A significant relationship between IgA and a progressive disease course (59) and the presence of OCBs has been reported, the latter in approximately 90 to 95% of patients with clinically definite MS (CDMS). Presence of OCBs is important in the diagnosis of early MS, particularly in those patients with normal brain magnetic resonance imaging (MRI) scans (60,61); it has been suggested that they appear early in the course of MS. The frequency of OCBs in patients with optic neuritis (ON), one of the earliest manifestations of MS, is between 34 and 72%, depending on the detection technique used (62–64).

Several approaches have been used to identify the antigens recognized by these IgG antibodies (Abs). Intrathecal Ig bands recognize several myelin proteins (MBP, PLP, MOG, and MAG) (65–70) and viruses (measles, rubella, varicella zoster, and herpes simplex) (71,72). Heterogeneity of antigen specificity of intrathecal IgG has been observed, but only a small proportion of total Ig may be MS-related. Attempts to absorb non-MS-specific Ig with viral or endogenous antigens have proved unsuccessful (73). These early findings raised the question of whether OCBs resulted after an MS-related antigen-driven B-cell response or an MS-unrelated B-cell response. Different groups have shown more recently that an antigen-driven adaptive immune response occurs in the CNS. By examining B-cell Ig genes, it was found that MS lesions and CSF contain B-cells with characteristics of an antigen-driven adaptive immune response, including T–B-cell interaction, somatic hypermutation, immune memory development, and B-cell clonal selection and expansion (74–80).

## INNATE AND ADAPTIVE IMMUNITY

Immune responses have both an innate and an adaptive component. The defense mechanisms involved in innate immunity consist of phagocytes (neutrophils and macrophages), natural killer (NK) cells, natural IgM-secreting natural autoreactive B-cells, and plasma proteins (including complement system proteins). NK cells produce interferon (IFN)- $\gamma$ , a macrophage-activating cytokine. Natural autoreactive B-cells, which react with one or more self-antigens independent of T-cell help, account for most of the B-cell repertoire in the fetus and healthy adult individuals. These B-cells have no memory and are usually encoded by unmutated germline variable region genes. Macrophages secrete cytokines that stimulate inflammation and immune responses, whereas the complement system lyses and opsonizes microbes for phagocytosis. The adaptive immune component involves specificity for distinct macromolecules, and an ability to remember and respond more vigorously to repeated exposures to the same molecule. Adaptive immune responses are initiated by the interaction of a foreign antigen with mature T- and B-lymphocytes that express diverse, clonally expressed antigen receptors. T-cells mature in the thymus, where they express antigen receptors and coreceptors (CD4 or CD8), the latter recognizing peptides displayed by the body's own major histocompatibility complex (MHC) molecules. Immature B-cells mature in bone marrow where they express antigen receptors (IgM and IgD) and become functionally competent. Immature T- and B-cells expressing high-affinity receptors for self-antigens, which are present in the generative lymphoid organs, are eliminated; this process is referred to as negative deletion, or central tolerance to self-antigens.

## LYMPHOID TISSUES

All adaptive immune responses are initiated in the peripheral lymphoid tissues, namely the lymph nodes, spleen, and mucosa. Mature naïve T- and B-cells leave the generative lymphoid organs (thymus and bone marrow) and circulate through the peripheral lymphoid tissues, where foreign antigens are filtered out and concentrated. The net result of adaptive immune responses is generation of effector and memory cells. Effector CD4 and CD8 T-cells secrete cytokines that activate B-cells, macrophages, granulocytes, and vascular endothelial cells. Plasma and memory B-cells secrete antibodies into the circulation, and both the cells and the antibody bind to antigen in the tissues.

## HUMORAL IMMUNITY

The majority of humoral immunity results from an antigen-driven T-cell-dependent B-cell reaction in lymphoid tissue. Adaptive humoral immune responses against lymph-borne proteins are initiated in the lymph nodes. Circulating mature naïve T- and B-cells migrate through venule walls and take up temporary residence in lymphoid tissue. T- and B-cells are sequestered in particular areas: the follicles are B-cell-rich areas (B-cell-zones); primary follicles contain mainly mature, naïve B-cells; and T-cells are located between the follicles (in T-cell zones). Antigen-

activated follicular B-cells migrate toward the interface between the follicles and T-cell zones, where they present antigen to helper T-cells specific for that antigen (81,82). B-cells are activated by T-cell generated lymphokines to enter the cell cycle and undergo mitosis to generate a clone of cells with identical B-cell receptors. These form germinal centers (GCs) in the follicles, called secondary follicles. GCs are the sites of B-cell proliferation, B-cell Ig V gene mutation, B-cell clone selection, and memory B-cell generation. Selection of antigen receptors is based on antigen affinity (83–85). B-cells with the highest affinity receptor are selected to survive and differentiate into antibody-secreting plasma cells (86–88). GCs play a critical role in the generation of high-affinity humoral immune responses via Ig gene somatic hypermutation.

### IG GENE SOMATIC HYPERMUTATION

Unlike T-cells, B-cells are subject to antigen-dependent mutations and selection processes in the secondary lymphoid tissues, designed to increase the affinity and functional efficiency of the memory Ig repertoire (83,89). Like T-lineage repertoire selection in the thymus, B-lineage repertoire selection is closely regulated and functionally linked with a specialized microenvironment—the GC (85,90–94).

Both Ig gene somatic hypermutation and B-cell clonal expansion play critical roles in the generation of high-affinity humoral immune responses. B-cell surface Ig is composed of heavy and light chains encoded by the variable (V), diversity (D), and joining (J) genes in the case of the heavy chains ( $V_H$ ,  $D_H$ , and  $J_H$ ) and by the  $V_L$  and  $J_L$  genes in the case of the light chains (95–98). Each  $V_H$  or  $V_L$  segment contains three regions of especially high variability (hypervariable regions) corresponding to the protein loops that contact the antigen and are known as complementarity determining regions (CDRs) (99). The genes  $V_H$  and  $V_L$  code for CDR1 and CDR2, respectively, whereas CDR3 is encoded by a combination of the  $V_H$ ,  $D_H$ ,  $J_H$ ,  $V_L$ , and  $J_L$  genes. The regions between CDRs are the framework regions, and they are responsible for maintaining the structure. Each B-cell expresses a particular pair of heavy and light V regions, representing the molecular basis of the clonal selection theory, which postulates that each B-cell and its progeny are predestined to have a single antibody specificity (100).

In T-cell-dependent antibody responses, antigen-specific B-cells undergo rapid and extensive clonal expansion in GCs. During this process, somatic point mutations are introduced into the Ig gene of the proliferating B-cells. Mutations are concentrated in the hypervariable region that forms the antigen binding site (85,86,101). Some of these may generate a binding site with increased affinity for its antigen, leading to antibodies of improved binding affinity after further antigen selection. Therefore, repeated antigenic challenge leads to “affinity maturation” of the response, resulting in production of antibodies with high affinity for the antigen. B-cells with the highest affinity are selected to survive and differentiate into memory and antibody-secreting B-cells. Rechallenge with a persistent antigen results in massive proliferation of clonal memory B-cells into high-affinity anti-



body-secreting plasma cells. These cells dominate the humoral immune response after vaccination and in autoimmunity and B-cell malignancy.

Somatically mutated B-cell Ig V genes are considered to be the hallmark of antigen-driven humoral immunity.

## IMMUNE MEMORY AND B-CELL CLONAL EXPANSION

Studies have shown that clonal expansion is based on immune memory and results in an enhanced response on restimulation with the same antigen. Memory is generated by an increased affinity of B-cell clones for antigen, resulting from somatic mutation of the Ig V gene. The B-cells undergo a Darwinian positive selection process based on their affinity for antigen held on follicular dendritic cells in GCs. High-affinity mutants survive, whereas low-affinity mutants cannot bind antigen and die by apoptosis (102,103). Positively selected B-cells differentiate to short-lived plasma cells, which secrete antibody, and to long-lived memory plasma cells that persist in the lymph nodes, spleen, and bone marrow (90,104–106). Long-lived memory cells are clones that maintain the established phase of the T-cell-dependent antibody response. Under persistent antigen stimulation, memory B-cell clones proliferate and dominate a specific B-cell mediated humoral immune response throughout the organism's lifetime. Such antigen stimulation might be necessary to maintain antigen-specific serum antibody in inflammatory tissue at high levels for long periods. Therefore, B-cell clones require antigen for their maintenance (106–108) and in the absence of antigen are lost from the adoptive host (107). The role of antigen in memory maintenance and clonal expansion is important, and antigen persistence might be a key factor in (auto)immune diseases.

## THE ROLE OF B-CELLS IN MULTIPLE SCLEROSIS

For the last two decades, T-cell-mediated immunity has dominated studies of MS pathogenesis. This emphasis on T-cells mainly resulted from detection of activated T-cells in MS lesions and analogies with the animal model EAE. T-cells can transfer EAE between animals with particular MHC class II alleles and are believed to break down the blood–brain barrier (BBB) in early MS and predominate within acute lesions. The type 1 helper T-cell (Th1), cytokines, interleukin IL-2, IFN- $\gamma$ , and tumor necrosis factor- $\alpha$ , are involved in the inflammatory reaction in active MS lesions (109,110). The  $\alpha$ -chemokines, IFN-inducible protein 10, and monokine induced by IFN- $\gamma$  (Mig), which attract Th1 cells, are mainly secreted by macrophages and reactive astrocytes within actively demyelinating MS lesions (111). Overexpression of type 5 Chemokine C-motif Receptor and type 3 Chemokine XC-motif Receptor, the receptors for these chemokines, is seen in lesion-derived T-cells and peripheral T-cells in patients with MS (112–114). Recently, there has been considerable progress in this field (74–77) using investigations based mainly on molecular studies of the Ig V<sub>H</sub> genes expressed by B-cells in CSF or lesions in patients with MS. These studies have shown that B-cell immunity, including antigen-driven B-cell somatic hypermutation, T–B-cell

interaction, memory B-cell and plasma cell development in GCs, tissue-specific migration, and B-cell clonal expansion in the CNS, are actively involved in MS development. They occur during the early stages of MS and continue through later disease stages. Increased intrathecal oligoclonal Ig synthesis, the most common abnormality detected in patients with CDMS, suggests ongoing B-cell proliferation in the CNS. The specific binding of antibodies to their antigen in brain tissue could cause tissue damage by activation of innate immunity, including complement and phagocytes.

### **B-CELL CLONAL EXPANSION: COMMON AND CONSISTENT FEATURE OF MULTIPLE SCLEROSIS**

The advent of the polymerase chain reaction (PCR) and sequencing techniques and knowledge of characteristics of the GC differentiation pathway have enabled adaptive humoral immune responses to be studied during MS pathogenesis. Real-time PCR and sequence analysis of Ig V<sub>H</sub> genes demonstrated a dominant B-cell clonal expansion in CSF of 10 out of 12 patients with MS and 3 out of 15 patients with other neurological diseases (74). The nucleotide sequences of V<sub>H</sub> genes in clonally expanded CSF B-cells from patients with MS shows preferential usage of the V<sub>H</sub> IV family. Numerous somatic mutations were seen, mainly in the CDRs with a high replacement-to-silent ratio, distributed in a way suggestive of migration into the CNS after positive selection of B-cells in the GCs through their antigen receptor. Another report also showed preferential use and somatic hypermutation of the V<sub>H</sub> IV family genes in lesion biopsies from two patients with acute MS (75). These results were confirmed by observations of B-cell clonal expansion in frozen plaque sections from 10 patients with MS and increased use of VH1-69, VH4-34, and VH4-39 in these lesions (76). Accumulation of oligoclonal B-cells was seen in 10 out of 10 patients with MS, but only 3 out of 10 patients with other neurological disorders. The V(D)J genes used by B-cells in the CSF were much less represented in peripheral blood lymphocytes, indicating tissue-specific clonal B-cell localization (77). Recently, the authors have shown B-cell clonal expansion in 11 out of 12 patients with chronic progressive MS (unpublished data). These findings indicate that most patients with MS with early or chronic disease have predominant B-cell clonal expansion and confirm the significance of intrathecal B-cell clonal expansion in the clinical and pathobiological aspects of MS. Analysis of CSF B-cell clonality provides an objective assessment of an autoimmune process in MS evolution.

### **B-CELL CLONAL EXPANSION IN EARLY MULTIPLE SCLEROSIS**

Several studies demonstrate that permanent tissue damage occurs in early MS (11,115–117). The ability to recognize an autoimmune reaction in the CNS during the early disease stages is important in establishing a diagnosis, and early therapy may prevent or delay early tissue damage. Analysis of Ig V<sub>H</sub> genes in CSF B-cells means that a wider range of patients with different clinical courses can be studied.

It is particularly suitable for patients in the early stages of disease, who have a first attack suggestive of MS (clinically isolated syndrome [CIS]). Progress in imaging techniques and laboratory tests have improved MS diagnosis, but MS-like lesions or OCBs are seen in only 50–70% of patients with CIS (118–122). The presence of both lesions and OCBs is highly predictive of CDMS (60,120,123–125), and the predictive value of OCBs for CDMS development is important in patients with CIS with normal brain MRI scans (61,126,127). However, a special diagnostic problem is presented by patients with CIS with recurrent myelitis in one locus but no other signs of demyelination on MRI imaging (128) and by patients with normal MRI results and no OCBs (129,130).

The authors' ongoing studies examine the clinical diagnostic value of B-cell clonal expansion in predicting the development of MS after CIS. Of 20 patients with CIS suggestive of MS examined, 8 had 3 or more high-signal lesions (2 of whom had no CSF OCBs [group 1]), 4 had 1 high-signal lesion in the spinal cord (2 of these had no OCBs [group 2]), and 8 had normal MRI and no OCBs (group 3). Nucleotide sequencing of the Ig V<sub>H</sub> genes showed that intrathecal B-cell clonal expansion had occurred in all 8 patients in group 1 (including the 2 patients negative for OCBs), in 3 out of 4 patients in group 2 (including 1 with no OCBs), and in 2 out of 8 patients in group 3. CDMS developed within the 5-year follow-up in 10 of the 13 patients who showed intrathecal B-cell clonal expansion: 6 from group 1 (2 with no OCBs), 2 from group 2 (1 with no OCBs), and 2 from group 3 (normal MRI and no OCBs). None of the 7 patients who did not show intrathecal B-cell clonal expansion demonstrated any further neurological manifestations within the 5-year follow-up. These results indicate that clonal B-cell infiltration precedes the appearance of MRI lesions and OCBs.

Early histoimmunostaining studies of MS lesions showed an inflammatory, primarily lymphocytic, infiltration of the vessels and capillaries of the brain parenchyma that did not lead to changes in the myelin sheaths and could not be demonstrated by gadolinium (Gd)-enhanced MRI. This "vasculitis" may allow B-cells to penetrate the CNS, where they produce Igs that are detected as OCBs. These changes have been well demonstrated in normal white matter (8,131–133). Such findings support the hypothesis that clinical symptoms and intrathecal B-cell clonal expansion can occur before CNS dissemination, indicating the importance of B-cell-mediated humoral immunity during the early stages of lesion development. The authors' results confirm and extend those of previous reports and indicate the value of detection of intrathecal B-cell clonal expansion at presentation as a predictive and prognostic indicator in patients presenting with CIS suggestive of MS.

## ANTIBODY-MEDIATED INNATE AND ADAPTIVE IMMUNE RESPONSES IN MULTIPLE SCLEROSIS

### *Antibodies in Demyelinated Lesions of Multiple Sclerosis*

Infiltrated B-cell clones are clearly required for development of humoral immunity-induced demyelination. These clones produce monoclonal (auto)antibodies

that damage myelin by several mechanisms. They can specifically bind antigen in myelin, then activate the classic complement cascade as a result of binding complement component C1q to the Fc region of the antigen-bound antibody (134). This induces production of the membrane attack complex (MAC), which inserts itself into cell membranes causing lysis and cell death (135,136). The Fc region of the antibody can also bind to Fc receptors on macrophages, neutrophils, and NK cells, causing these innate immune cells to specifically attack myelin by antibody-dependent cell-mediated cytotoxicity.

The role of autoantibodies in demyelination was first noted in the EAE model. Classical EAE, induced in susceptible animal strains (i.e., Lewis rats) by immunization with myelin antigens and Freund's adjuvant, is usually monophasic with acute inflammation in the CNS and no demyelination. However, when anti-MOG antibody is given as a single injection after immunization with MBP, demyelination is induced. During the last 30 years, myelin protein-specific B-cells and antibodies have been extensively studied in MS. High-affinity antibodies to MBP and MOG have been purified from MS lesions (137), and B-cells and antibodies to PLP (138), MAG, and other CNS antigens (139,140) are found in the CSF of patients with MS (the antibodies also being found in serum) (139). Genain et al. used immunogold-labeled MOG and MBP to locate antigen-specific plasma cells and antibodies in MS lesions and demonstrated that MOG- and MBP-specific antibodies are present on the surface of plasma cells in the parenchyma, in areas undergoing demyelination, on the myelin networks around scattered axons, and on droplets of myelin debris in the parenchyma (70). These findings strongly support the idea that infiltration of MOG- and MBP-specific plasma cells and the local secretion of autoantibodies play an important role in demyelination and lesion formation. Deposition of antibodies and complement and infiltration of T- and B-cells and macrophages into the perivascular region are coupled with the destruction of the myelin sheath and axons (141)—the most common pattern of pathology in the majority of MS lesions.

### ***Complement in Demyelinated Lesions of Multiple Sclerosis***

On binding antigen, an (auto)antibody can activate proinflammatory complement components, such as C1, C3, and C9, and cause tissue damage. Activation of the first part and terminal components of the classic complement cascade can be assessed by measuring activation of the C3 and C9 terminal complement complex, respectively. Deposits of antibody and complement, activated macrophages and microglial cells, and T- and B-cell infiltration are seen in the CSF in MS cases and are considered to be involved in lesion formation (142–146). Full activation of the complement cascade, especially of C9, during MS attacks may be restricted to patients with more advanced disease and is significantly correlated with degree of neurological disability (147). In the brain, oligodendroglial cells may be more sensitive to complement damage (148) than microglia and astrocytes (149).

In MS, complement in the peripheral blood may be transported into the CNS as a result of leakage of the BBB. Alternatively, there may be local synthesis of

complement by astrocytes, microglia, and infiltrated macrophages, especially in an environment containing high levels of proinflammatory cytokines (150). The most convincing data for a role of complement activation in MS come from histopathologic studies showing presence of IgG and complement protein C1q within the plaque (151), C3 around the demyelinating borders of MS plaques (152), and C3d on macrophages in the most active lesions (132). There is evidence that initial demyelination is caused by binding of Ig to myelin, which results in activation of complement, phagocytes, and resident microglia. However, the antigens involved are unknown; further analysis of the oligoclonal B-cell response may help to identify the primary provoking antigens (131). In MS, the activated lytic complement complex generated by C9 activation is exclusively deposited in areas of active myelin and oligodendrocyte destruction (153).

### ***Antibody-Dependent Cell-Mediated Cytotoxicity***

The Fc region of the molecule mediates many effects of antibodies. This region of IgG1 and IgG3 binds Fc receptors on phagocytes (macrophages and neutrophils) and promotes phagocytosis of antibody-bound antigen in different tissues. The consequences of phagocytosis are activation of the enzyme phagocyte oxidase and secretion of hydrolytic enzymes, which induce tissue inflammation and tissue damage. The hyperactive early plaque is usually infiltrated around its central vein with monocytes, lymphocytes, and plasma cells (154). The phagocytic element contributes to myelin breakdown (155,156); phagocytosis was seen in the periplaque white matter of autopsy plaques from two patients with secondary-progressive MS (SPMS). Immunohistochemical staining showed microglia engaging short segments of disrupted myelin bearing C3d deposits (157,158). Phagocytosis of myelin by macrophages, triggering the production of reactive oxygen species (ROS), has been observed in EAE (157). These studies show that antibody can cause demyelination by three main mechanisms:

1. Opsonization and phagocytosis of cells.
2. Complement- and Fc-receptor-mediated inflammation and tissue injury.
3. Interference with normal cellular functions by antibody binding to physiologically important molecules or cellular receptors.

## **NEW APPROACH TO MULTIPLE SCLEROSIS-RELATED ANTIBODY**

The finding that clonal expansion of hypermutated B-cells occurs in lesions and in the CSF during different disease stages could mean that persistent antigen-driven adaptive immunity is responsible for disease progression. MS-related antigens have been investigated extensively using patient CSF and serum antibodies. Antibodies against infectious agents and autoantigens have been identified in the CSF and in the lesions, but the data are not convincing, because most of them might not be MS-related. The clonal expansion of B-cells in CSF and lesions could indicate that MS is driven by persistent restricted antigen(s) and provides the opportunity to study MS-related antigens using in vitro recombinant antibody synthesis techniques

developed in the last decade. This approach, which makes it possible to synthesize antibodies using the Ig V<sub>H</sub> and V<sub>L</sub> genes expressed in a B-cell clone, uses PCR and sequential cloning of gene sequences coding for the Ig V<sub>H</sub> and V<sub>L</sub> chains in a phage display vector to produce single-chain Fv antibody. Brain cDNA banks and phage display peptide libraries have provided peptide ligands that can be used to identify the antigen recognized by the antibody, using an *in vitro* selection process called panning. This technique allows the study of antigens recognized by clonally expanded CSF-B-cells. The feasibility of this approach was recently demonstrated in studies using an antibody phage display library, generated from brains of humans with subacute sclerosing panencephalitis (SSPE) or MS, which showed that recombinant antibody prepared from SSPE brain reacted with the nucleocapsid protein of measles virus (159–161). Williamson et al. showed that recombinant antibody, engineered from the Ig genes of B-cells obtained from either active plaques and periplaque regions in MS brain or from the CSF of a patient with MS, bound tightly and specifically to double-stranded DNA (162). These early findings demonstrate that such techniques may have a significant effect on the study of MS-related antigens in the CSF and lesions of MS.

## CONCLUSIONS

The outstanding fact of this review is that the presence of B-cell clonal expansion, in CSF and plaques of patients with MS, is indicative of an ongoing antigen-driven response in the CNS. These somatically hypermutated B-cells are found early in the disease, sometimes in the absence of OCBs and MRI lesions, as well as in latter stages. These findings have several possible implications:

- Increased diagnostic sensitivity.
- Prognosis.
- Role in immunopathogenesis.
- Role in primary and secondary progression.
- Effect on therapy.

Evidence that MS is an autoimmune disease remains inconclusive, but one can at least presume that humoral immunity plays a role in lesion formation and perpetuation; alternatively, it could be involved in tissue-repair mechanisms.

The antigen inducing this humoral response remains unknown, and it is important to identify it as either a self- or foreign-protein—a goal that several laboratories are actively pursuing. Given the complexity of MS and what we currently know about CSF IgGs, many antigens could be involved.

The authors believe that the paradigm of MS as a T-cell disease must be revisited, because B-cells are involved during the initial and late diseases. There is mounting evidence that a degenerative process, in addition to and possibly even preceding inflammation, is also implicated, but currently the biological basis of this degeneration, as distinct from inflammation, is obscure. Finally, it is suspected that inflammation as a whole may play a part in tissue reconstitution and B-cell immune responses might be involved in all or part of these processes.

These studies will be pursued and will hopefully unravel further aspects of the perceived complexity of MS.

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## Multiple Sclerosis

### *A Case for Early Treatment*

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Alex C. Tselis, PhD, MD and Omar A. Khan, MD

#### INTRODUCTION

Multiple sclerosis (MS) has emerged as a treatable disorder with several disease-modifying therapies (DMTs) now licensed in the United States for MS treatment. These are interferon (IFN)- $\beta$ -1a (two formulations, Avonex and Rebif), IFN- $\beta$ -1b (Betaseron), glatiramer acetate (GA) (Copaxone), and mitoxantrone (Novantrone). Although all five DMTs were tested in phase III randomized controlled trials (RCTs) and shown to be superior to placebo, no therapy has ever been shown to halt disease progression (1–5). Because MS is a chronic, unpredictable disease and clinically variable from patient to patient, treatment choices for patients with MS can pose a dilemma, despite the plethora of information. Nonetheless, the use of DMTs in definite relapsing-remitting MS (RRMS) is well established.

More recently, clinicians and scientists have focused on the early treatment of MS, which presents a new challenge in MS therapeutics. Several lines of evidence suggest that early treatment should be considered. Despite positive outcomes in treatment trials of clinically isolated syndromes (CIS) in patients considered at high risk of developing definite MS, many debate whether early treatment of patients with CIS affects long-term disability. The lack of data demonstrating an effect of DMT on long-term disability in patients with CIS, combined with the economic consequences of these medications, argue in favor of a conservative approach in this population. This argument is further strengthened by the well-established efficacy of IFN- $\beta$  and GA in RRMS; it is also well recognized that existing DMTs are only partially effective.

On the other hand, a growing body of evidence suggests that treatment with a DMT in patients presenting with CIS is beneficial and may delay the development of clinically definite MS (CDMS). The authors examine data supporting early treatment of MS and also consider the revised diagnostic criteria for MS (6), which enables clinicians to make the diagnosis and initiate treatment early.

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A picture of MS pathogenesis is emerging, based in part on the data discussed in this chapter, in which the initial stages of the disease are dominated by inflammatory demyelination, leading to concomitant axonal damage. As the disease progresses, the inflammatory component declines significantly, but a degenerative process is triggered with axonal transection and degeneration proceeding independently (and probably irreversibly) of inflammation. The disease thus evolves into a neurodegenerative process and is less responsive to treatment by immunomodulatory medications, which are primarily directed against the inflammatory component of the disease (7).

### **APPLICATION OF THE NEW MULTIPLE SCLEROSIS DIAGNOSTIC CRITERIA: SUPPORTING EARLY DIAGNOSIS AND TREATMENT**

The diagnosis of MS depends on the demonstration of the presence of at least two separately located lesions (spatial dissemination) in the white matter of the central nervous system (CNS) occurring at two separate times (temporal dissemination), in the absence of any better explanation for these lesions. Formal criteria for the diagnosis were first proposed in 1965 and reflected the understanding of the disease and diagnostic technology available at that time (8). As new diagnostic methods evolved (e.g., measurements of oligoclonal banding and immunoglobulin [Ig] G index in cerebrospinal fluid [CSF], evoked potential tests), new diagnostic criteria were published to incorporate these methods in the diagnosis of MS (9). With the advent of magnetic resonance imaging (MRI) scanning in patients with MS, the diagnostic criteria for MS had to be modified to consider this new method (6).

The importance of MRI for the diagnosis of MS became apparent when patients with CIS who had brain MRIs were followed long-term. In one study of 89 patients with CIS, 57 (64%) had abnormal and 32 (36%) had normal MRIs. After a 5-year follow-up, 72% of patients with an initially abnormal scan had developed CDMS, using the Poser criteria, whereas only 2 of 32 patients (6%) with normal MRIs had CDMS (10). The presence of oligoclonal bands (OCBs) in the CSF was also a risk factor for the development of CDMS, but it was not as strong as that of an abnormal MRI. Further follow-up of 81 of these patients, over 10 years, confirmed and strengthened these results. The risk of progressing to CDMS was 83% (45 out of 54 patients) in those with an abnormal initial MRI and 11% (3 out of 27 patients) in those without (11).

In an important follow-up study by the same group (12), 71 patients with CIS were followed out to an average of 14 years. Those with initially abnormal brain MRIs were at high risk (88%) of developing CDMS, with a median time of 2 years. Of those who developed MS, the degree of disability at 14 years, as measured by the Expanded Disability Status Scale (EDSS) (13), correlated with the increase in lesion volume from onset to 5 years but not the increase in lesion volume from 5 to 14 years. This suggests that disease activity early in the course of MS, within the first 5 years, may have significant prognostic implication. A higher level of early

disease activity, as measured by the degree of accumulation of new lesions, may be more damaging to the brain than a similar accumulation of lesions later in the course of the disease.

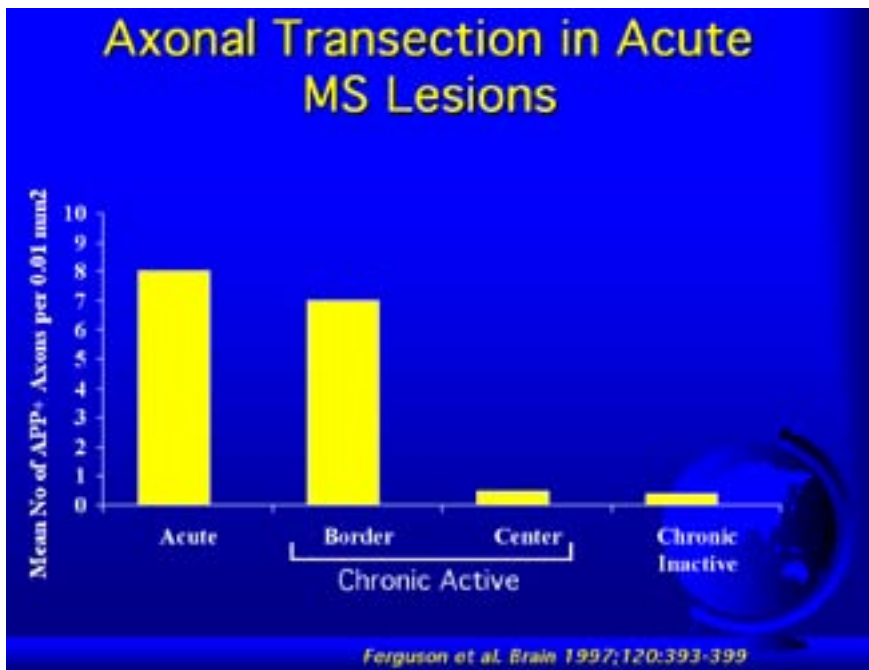
An international panel on MS diagnosis has published new diagnostic criteria for MS, popularly referred to as the McDonald criteria (6). These new diagnostic criteria have largely replaced the previous MS diagnostic criteria published by Poser and colleagues 20 years ago (9), although the new recommendations have retained the requirement of disease dissemination in time and space. However, the new criteria incorporate paraclinical tools, primarily MRI, to facilitate the diagnosis of MS for patients presenting with CIS suggestive of MS. In addition to a second clinical attack in a different location in the CNS, the McDonald criteria also allow the presence of new lesions on T2 or gadolinium (Gd)-enhanced T1 images 6 or 3 months after onset, respectively, to confirm dissemination in time and space.

Tintore and colleagues (14) examined the usefulness of the McDonald criteria in 139 patients with CIS. All patients underwent a brain MRI within 3 months of their attack and again 12 months later. After 12 months, 11% had developed CDMS according to the Poser criteria, compared to 37% with the McDonald criteria. Of the 37%, 80% experienced a second attack within a mean follow-up of 49 months. The new criteria showed a sensitivity of 74% and a specificity of 86% in predicting conversion to CDMS. Dalton and associates (15) reported similar findings in 80 patients presenting with CIS. Another study (12) demonstrated that 80% of patients presenting with CIS and MRI lesions go on to develop CDMS. Filippi and colleagues (16) also found that the presence of multiple T2-weighted lesions on brain MRI scans in patients are highly predictive of progression to clinically definite MS.

The McDonald criteria allow for diagnosis of MS to be made before the patient experiences a second clinical attack, with significant practical implications. Most importantly, we can now identify patients after their first clinical attack by use of MRI methods and predict the likelihood that they will develop CDMS with a reasonably high degree of sensitivity and specificity. This raises the issue of initiating treatment early in these so-called “high-risk” patients, as discussed in this chapter. Further advances in the early diagnosis of MS will sharpen the urgency of making these therapeutic decisions. For example, in a recent study, 103 patients with CIS were evaluated for the presence of anti-myelin basic protein (MBP) and anti-myelin oligodendrocyte glycoprotein (MOG) antibodies (17). Of the 39 patients with no such antibodies, only 9 (23%) had a relapse, in a mean time of 45 months, whereas 21 of 22 (95%) with antibodies to both MBP and MOG relapsed in an average of 7.5 months. The rest of the patients had only anti-MOG antibodies, and 35 of 42 (83%) of them had relapses in 14.6 months. This interesting pilot study implies that serologic tests may be developed to detect MS at early stages of the disease (18).

## EVIDENCE BASED ON HISTOPATHOLOGICAL DATA

Irreversible axonal damage in MS has been recognized for more than a century (19). However, only recently has it been eloquently shown with modern histopatho-



**Fig. 1.** Axonal transection in acute MS lesions as measured by the area density of amyloid precursor protein (APP)-positive axons.

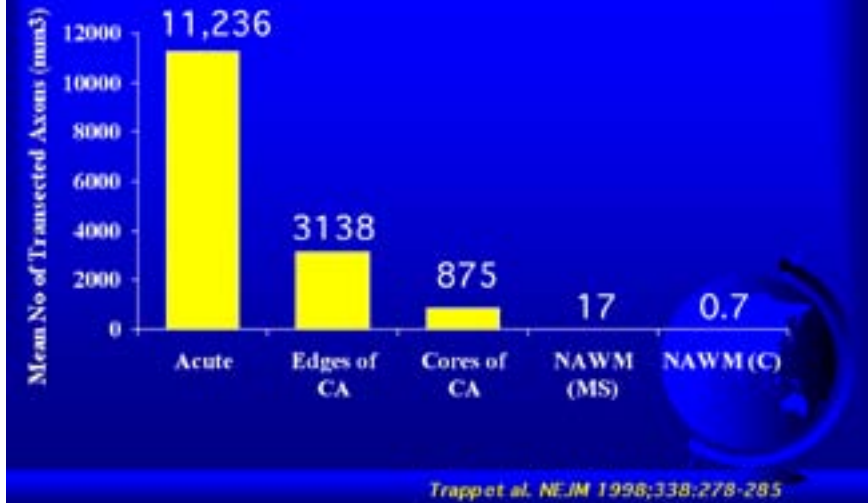
logical techniques. Two studies revived the concept of axonal damage as a consequence of inflammation occurring during episodes of acute inflammation that may occur even in the early stages of the disease course in MS (20,21). Ferguson and colleagues (20) studied 18 MS brains and examined 19 lesions of varying ages. Lesions were evaluated for the presence of amyloid precursor protein (APP), a sensitive marker of axonal damage. They found a high number of APP-positive axons in acute lesions and the edges of chronic active lesions (Fig. 1). Trapp and colleagues (21) studied 47 lesions from 11 patients with MS and a disease duration ranging from 2 weeks to 26 years. Using immunohistochemistry and confocal microscopy, they found transected axons to be a consistent feature throughout active lesions, with an average density of 11,236 per mm<sup>3</sup> (Fig. 2).

The presence of axonal transection in patients with a short disease duration implies that axonal transection occurs in MS lesions at an early stage of the disease. These two studies in particular led MS experts to reconsider their approach to treating MS, especially because of the availability of effective and relatively safe DMT. With compelling histopathological data demonstrating irreversible axonal damage early on in MS, the early initiation of antiinflammatory DMT renders itself to be a logical therapeutic approach.

## EVIDENCE BASED ON MAGNETIC RESONANCE IMAGING DATA

There has been considerable work examining the severity and extent of brain tissue damage in MS using MRI. Most studies have focused well-established cohorts of patients with MS with considerable disease activity or disability.

## Axonal Transection in Acute MS Lesions

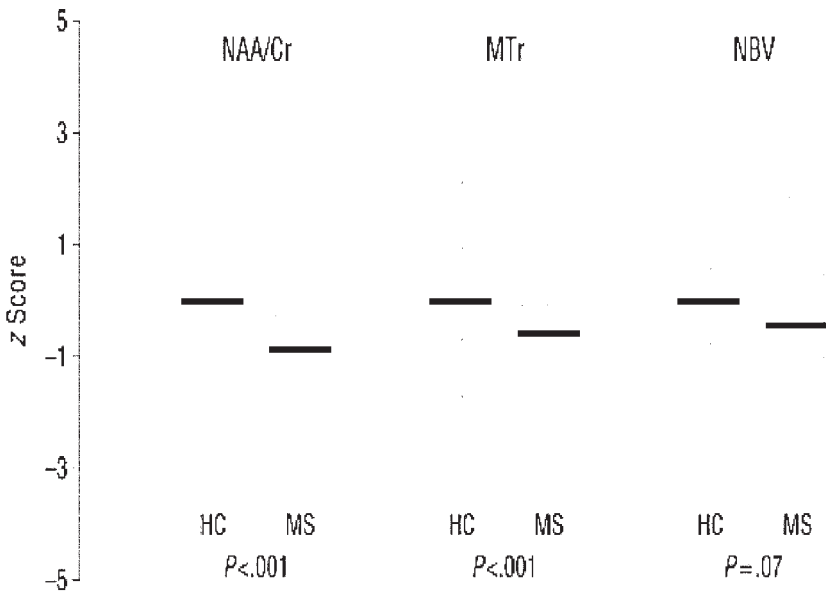


**Fig. 2.** Axonal transection as measured by confocal microscopy of axonal retraction balls, detected by immunohistochemical staining.

However, more recently much interest has been placed on disease pathology in early MS. MRI studies in MS have shown demyelination and axonal injury in patients with relatively mild disease of short duration. Newer techniques for measuring MS pathology with MRI, including MR spectroscopy and magnetization transfer ratio, have also identified destructive tissue pathology in the early stages of RRMS (22–25).

Rudick and colleagues (22) examined brain parenchymal fraction (BPF) in patients with RRMS who had minimal disability and short disease duration and compared them with healthy controls (22). The BPF is the fraction of intracranial volume occupied by brain parenchyma only (i.e., brain parenchyma volume divided by whole-brain volume). They found that, on average, the patients with MS had a BPF that was 2 to 3 standard deviations below the level seen in healthy controls. Chard et al. (26) studied brain atrophy in patients in the stage of MS having minimal disability, with a mean EDSS of 1.0 and mean disease duration of 1.8 years. Three-dimensional fast-spoiled gradient recall images were obtained and segmented into gray and white matter, in addition to measuring whole-brain volumes. Patients with MS were compared to 27 normal matched controls. Significant brain atrophy affecting both gray and white matter was seen in the MS patients, suggesting that destructive disease pathology may occur even at an early stage of the disease.

Magnetic resonance spectroscopy (MRS) enables the study of *in vivo* tissue metabolites that represent axonal integrity. *N*-acetylaspartate (NAA) has been widely evaluated as a marker of neuronal and axonal viability in MR spectroscopic studies. These studies have consistently shown decreased NAA levels and NAA to creatine (Cr) ratios (NAA/Cr) in MS lesions, as well as in the normal-appearing white matter (NAWM) of patients with MS (27–30). In a study by De Stefano and



**Fig. 3.** Comparison of *N*-acetyl-aspartate to creatinine ratio (NAA/Cr), magnetization transfer ratio (MTr), and normalized brain volume (NBV) between healthy controls (HC) and patients with MS. NAA/Cr and MTr values are significantly reduced in patients with MS.

colleagues (25), 60 patients with RRMS, a median disease duration of 2.7 years, and an EDSS score of  $<2$  underwent combined MRIs/MRSs, including magnetization transfer ratios (MTR) and brain volume measurements. They compared these values with those of healthy controls. The patients with MS demonstrated significantly lower NAA/Cr ratios and reduced MTR levels. Brain volume was also decreased (Fig. 3). Subanalysis of patients with a disease duration of  $<3$  years ( $n = 36$ ) and a T2 lesion load  $<2 \text{ cm}^3$  ( $n = 26$ ) showed lower NAA/Cr, MTR values, and brain volumes than those of healthy controls. This study provided strong evidence of a destructive tissue process as shown by MRS, MTR, and measurements of brain volume in early and patients with minimally affected RRMS. The demographics of the patients in the De Stefano study have been compared to those in the pivotal trials demonstrating efficacy of DMT (Table 1). It is evident that patients with established RRMS participating in phase III trials had greater disability (mean EDSS) and disease duration than the cohort studied by De Stefano. Thus, it would be logical to assume that treatment of MS at an early stage should confer greater advantage to the patient than waiting for the disease to exhibit clinical evidence of disease activity (i.e., occurrence of relapses).

Whole-brain atrophy as measured by BPF, as well as atrophy of the corpus callosum, has previously been observed in relatively milder stages of RRMS (22). Ranjeva and colleagues (31) studied the corpus callosum volume in patients with

**Table 1**  
**Baseline Demographics:**  
**Phase III Pivotal Trials vs DeStefano Study**

Phase III trials	Age	Disease duration	EDSS
Betaseron®	35.2	4.7	3.0
Avonex®	36.7	6.6	2.4
Copaxone®	34.6	7.3	2.7
Rebif®*	35.6	6.4	2.5
Stefano study	35.0	2.7	0–2

CIS suggestive of MS. Forty-six patients with CIS and 24 matched healthy controls underwent various techniques employing MRI, including MRS, MTR, and mean diffusivity (MD) to measure corpus callosum volume and tissue pathology. Decreased MTR and increased MD were consistently seen in patients with CIS, whereas no atrophy was observed, with no statistically significant difference in normalized brain volume between patients with MS and healthy controls. This pattern was seen even in the absence of lesions visible on conventional MRI. These findings suggested that diffuse structural and metabolic changes indicative of myelin pathology occur in the corpus callosum at the earliest stage of MS, even before any atrophy can be detected.

**EVIDENCE BASED ON CLINICAL TRIALS IN CLINICALLY ISOLATED SYNDROMES AND MULTIPLE SCLEROSIS**

Studies of treatment at the earliest clinical manifestations of MS are of special interest in understanding the stagewise response of the disease to DMTs (Table 2). In particular, the earliest clinical manifestations of MS present with the CIS of optic neuritis (ON), transverse myelopathy, and brainstem demyelinating lesions (typically, internuclear ophthalmoplegia). As discussed, patients with CIS with multiple (though silent) lesions on the MRI are at high risk of developing CSMS within the next few years. The MRI data have been so compelling as to force the incorporation of MRI criteria in the diagnostic criteria for MS, as discussed (6).

Two double-blind, placebo-controlled trials of patients presenting with the first CNS demyelinating event and abnormal MRI scans have shown a benefit of initiating DMT (32,33). In the multi-center Controlled High-Risk Subjects Avonex Multiple Sclerosis Prevention Study (CHAMPS), 383 patients were randomized to receive 30 µg intramuscular (IM) IFN-β-1a weekly vs placebo for 3 years. The primary endpoint was conversion to CDMS, as defined by the occurrence of a second attack. The study was halted at the time of an interim analysis, before its scheduled 3-year duration, when it was shown that treatment with IFN-β-1a significantly delayed time to second attack compared with placebo. The probability of developing CDMS was 50% in the placebo group vs 35% in the IFN-β group ( $p = 0.002$ ). The Food and Drug Administration (FDA) has now approved the use of IFN-β-1a



**Table 2**  
**Summary of DMT Pivotal Trials in CIS, RRMS, and SPMS**

Agent	Study (ref.)	Primary outcome	Primary outcome achieved		
			CIS	RRMS	SPMS
Interferon (IFN)-β-1a (Avonex)	Controlled High-Risk Subjects Avonex MS Prevention Study (CHAMPS) (32)	Second demyelinating event, defining clinically define multiple sclerosis (CDMS)	Yes		
IFN-β-1a (Rebif)	Early Treatment of MS (ETOMS) (33)	Second demyelinating event, defining CDMS	Yes		
Glatiramer acetate (Copaxone)	(4)	Proportion of patients relapse-free		Yes	
IFN-β-1b (Betaseron)	(1)	Rate of MS relapses		Yes	
IFN-β-1a (Avonex)	(2)	Accumulation of deficit, defined by Expanded Disability Status Scale (EDSS)		Yes	
Glatiramer acetate (Copaxone)	(4)	Rate of MS relapses		Yes	
IFN-β-1a (Rebif)	Pregnancy in Multiple Sclerosis Study (PRIMS) (3)	Rate of MS relapses		Yes	
Glatiramer acetate (Copaxone)		Time to confirmed progression			No
IFN-β-1a (Rebif)	SPECTRIMS (40)	Progression of deficit defined by EDSS			No
IFN-β-1b (Betaseron)	European Study Group secondary-progressive MS (SPMS) Study (42)	Progression of deficit defined by EDSS			Yes
IFN-β-1b (Betaseron)	North American Study Group SPMS Study (39)	Progression of deficit defined by EDSS			No
IFN-β-1a (Avonex)	IMPACT (41)	Progression of deficit defined by Multiple Sclerosis Functional Composite (MSFC), a composite index			Yes (No effect on EDSS)

given at a dose of 30 µg IM weekly after the first demyelinating episode in patients with CIS suggestive of MS. The Early Treatment of MS (ETOMS) study used an identical primary endpoint in study patients with a first demyelinating event. Investigators randomized 309 patients to receive 22 µg SC IFN-β-1a weekly or placebo for 2 years. Compared with placebo, treatment with IFN-β-1a resulted in fewer patients converting to CDMS (34% vs 45%) ( $p = 0.047$ ).

In one of the earlier trials of GA, 50 patients with RRMS were randomized to either GA 20 mg SC daily for 2 years or placebo, with a primary endpoint of proportion of patients who were exacerbation-free during the course of the trial (34). The results were that 56% of patients in the active treatment arm were exacerbation-free, whereas only 26% of the placebo group had no exacerbations ( $p = 0.045$ ). Interestingly, in a posthoc analysis comparing patients with mild disease (EDSS = 0–2) to those with more severe disease (EDSS = 3–6), there was a much greater reduction in relapse count in the milder patients than the more disabled ones. These results suggest that pharmacologic intervention early in the course of the disease may be more efficacious in the prevention of accumulation of deficit than such intervention later in the disease course, when there is already fixed deficit or after the onset of secondary progression.

Can the initiation of DMT be delayed with no loss of benefit to a patient with CDMS? A reasonable way to test this would be to continue a trial in which patients in a placebo arm are rerandomized to an active treatment arm and followed carefully. Two such studies have been done.

In the first study, a continuation of the PRISMS trial, in which patients with RRMS were randomized to 1 of 3 arms, placebo, 22 µg and 44 µg of IFN-β-1a SC 3 times a week (tiw), patients in the placebo arm were rerandomized, at 2 years, to one of the active IFN-β-1a arms, and followed for another 2 years (35). At the 4-year time point, the high-dose 44 µg tiw group statistically had significantly less progression of disability (defined as an increase of EDSS by at least one step, sustained for at least 3 months) than either of the crossover groups, along with the lower dose 22 µg tiw group. There was also less accumulation of MRI burden of disease during the 4 years of the study in the high-dose 44 µg tiw group than the lower dose group or either of the crossover groups (35).

In the second study, with the longest follow up among all MS treatment trials conducted to date, 251 patients with RRMS were randomized to GA or placebo. At the end of the randomized phase, 209 (83%) of the patients continued onto open-label GA and were followed for up to 10 years after the initial randomization. A total of 128 patients completed 10 total years on the study. Of these, 64 were originally randomized to GA and 69 to placebo, before the open-label phase. Most of the patients originally on GA had stable or improved EDSS scores, although the average EDSS increased by 0.9 points to 3.67. However, the relevant finding was that fewer patients in the GA-at-randomization group ( $n = 50$ ) had progression during the entire study period than those in the placebo-at-randomization group ( $n = 72$ ) ( $p = 0.015$ ) (36).

These studies highlight two significant findings. First, DMT early in the course of disease, at the first clinical attack, significantly delays the occurrence of a second attack, which would satisfy the criteria for CDMS. Second, early treatment results in significantly less progression in the disease than late treatment, and late treatment does not restore lost function (37).

## DMT IN SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS

Although the efficacy of DMT in RRMS is firmly established (1,3,4,32), results of studies examining the efficacy of DMT for patients with secondary-progressive MS (SPMS) have either been negative or shown only modest benefit at best (38–42) (Table 2).

In one of the first studies of GA, 106 patients with “chronic progressive MS,” a mixture of patients with SPMS and primary-progressive MS (PPMS), were recruited at two centers (Albert Einstein College of Medicine, Bronx, NY, and Baylor College of Medicine, Houston, TX) and randomized to GA 20 mg SC every day for 2 years or placebo (38). The primary outcome was time to progression (defined by a 1.0-step increase in EDSS for baseline EDSS > 5 and a 1.5-step increase in EDSS for baseline EDSS <5) sustained for 3 months. There was no difference in outcome between the 2 groups for the 2 years.

In the SPECTRIMS study, 618 patients with SPMS were randomized to 1 of 3 arms: placebo, IFN- $\beta$ -1a 22 or 44  $\mu$ g subcutaneously tiw for 3 years. The primary outcome was time to confirmed progression of disability (defined by an increase in EDSS by one point if baseline EDSS <5.5 and by 0.5 point if baseline EDSS >5.5, sustained for 3 months). The primary outcome was not different between the patients in the three arms, although there was a hint of efficacy in a posthoc subgroup analysis favoring patients with prestudy relapses compared to patients without (40).

In the North American Study Group on Interferon  $\beta$ -1b in Secondary Progressive MS study, 939 patients with SPMS were randomized to placebo or IFN- $\beta$ -1b 5 MIU or 8 MIU/m<sup>2</sup> of body surface area, subcutaneously every other day, for 3 years, with a primary outcome of disease progression defined by a 1.0 point increase in EDSS (or 0.5-point increase if baseline EDSS >6.0). There was no difference on the primary outcome variable between the arms of the study (39).

However, another study of IFN- $\beta$ -1b showed a positive effect on SPMS, again using the conventional measure of disability, the EDSS, as a primary endpoint. In the European Study Group SPMS study, 718 patients with SPMS with baseline EDSS between 3.0 and 6.5 were randomized to placebo or IFN- $\beta$ -1b 8 MIU SC every other day for 2 years, with a primary outcome of a 1.0-point increase in EDSS sustained for 3 months (or a 0.5-point increase for baseline EDSS of 6.0 or 6.5). The study found that the drug delayed progression by 9 to 12 months ( $p = 0.0008$ ) during a study period of 2 to 3 years (42). Although the study design and patient populations were similar to the North American SPMS study, there were differences. Subjects in the European SPMS study had more recent relapses than subjects in the North American SPMS study (43). It is interesting that a posthoc analysis of

the subjects in the SPECTRIMS trial also showed a hint of efficacy in those SPMS patients with prestudy relapses (*see above*).

CAMPATH-1H, a strong lymphocyte-depleting monoclonal antibody directed against CD52, was used to reduce MRI activity of disease in patients with SPMS. In this study, 25 patients with SPMS, baseline EDSS 4.0–6.0, were infused with CAMPATH-1H and followed clinically and with serial MRIs. Despite a profound decrease in the number and volume of Gd-enhancing lesions on MRI, disability progression continued in 50% of the patients. These patients also had increasing brain atrophy. The atrophy correlated with a decrease in the NAA signal on MRS, indicating that the atrophy most likely resulted from axonal degeneration (44,45).

The effect of CAMPATH-1H may be quite different when used in an earlier stage of disease. In an open-label preliminary study of 22 patients with RRMS, there was a substantial decrease in relapse rate and virtual cessation of accumulation of disability (46). Although these results were preliminary and in a small, selected group of patients, this may be an example of the dichotomy of the response of MS to therapy, depending on the stage of the disease, with earlier disease (RRMS) showing better response than later disease (SPMS).

Some studies found benefit in SPMS only by using sensitive outcomes. For example, the IMPACT study recruited 436 patients with SPMS and randomized them to either placebo or IFN- $\beta$ -1a 60  $\mu$ g IM once a week for 2 years. In this study, the primary outcome was change in the MS functional composite (MSFC), which consisted of a combination of measures of ambulation (timed gait), cognition (paced auditory serial addition test), and upper extremity function (nine-hole peg test), from baseline to 24 months. There was a modest but statistically significant effect on the composite score, largely driven by the “nine-hole peg test,” but there was no effect on ambulatory ability (timed gait component of the MSFC) or EDSS (41).

## CONCLUSIONS

Recognizing the partial efficacy of available DMT in RRMS and modest benefit in SPMS, initiating therapy in patients considered at high risk of developing MS appears reasonable. Given the evidence discussed, one may argue that delaying DMT in this group of patients offers no advantage to the patient and may even be detrimental. However, longer follow-up in patients presenting with CIS and abnormal brain MRI scans is needed to unequivocally settle the issue of early treatment in MS.

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## Pregnancy and Multiple Sclerosis

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### INTRODUCTION

Numerous autoimmune diseases affect women during their reproductive years, including multiple sclerosis (MS), rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis, and Sjögren's syndrome (1–3). Most autoimmune diseases become clinically silent or are pushed into so-called “remission” during pregnancy (4). Pregnancy poses a challenge for the immune system because the fetus is essentially an allograft harboring antigens from the father. In response, pregnancy induces immune deviation in the mother, protective to the fetus, and, at the same time, alters autoimmune responses to the benefit of the pregnant woman. Studies examining disease course support the protective effect of pregnancy in the context of autoimmune diseases.

### IMMUNOLOGICAL PROFILE OF MULTIPLE SCLEROSIS DURING PREGNANCY

MS is widely believed to be a T-cell-mediated autoimmune disease directed against myelin antigens in the central nervous system (CNS) (5). Although the precise cause and antigens have not been identified, several are candidates in MS. It is also believed that other factors, including genetic susceptibility, exposure to infectious agents, and environmental factors, may interact and render a person susceptible to developing the disease.

Given the autoimmune nature of MS and its predominant occurrence in young women, investigators have started to examine the effect of pregnancy on the immunological profile. T-helper (Th) lymphocytes are classified into Th1 (proinflammatory) and Th2 (antiinflammatory) cell types based on the type of cytokines they secrete (2). Th1 cytokines include interleukin (IL)-1, -2, and -12; tumor necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$ , whereas Th2 cytokines include IL-4, -5, -6, and -10 (6). This highly simplified view of Th1 and Th2 responses is the focus of several therapeutic strategies in autoimmune diseases, including MS. Currently

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available therapies in MS and numerous therapies that are under investigation are targeted at inducing Th2 responses, diminishing Th1 responses, or both (7).

Studies have shown that the immunological profile in MS undergoes numerous distinct changes during pregnancy. Specifically, pregnancy creates a state of natural immunomodulation paralleling the therapeutic effect of currently available disease-modifying therapies (DMT). A protective effect of pregnancy in experimental autoimmune encephalomyelitis (EAE), a commonly studied animal model of MS, has been repeatedly demonstrated. Induction of EAE is diminished in pregnant guinea pigs, rabbits, and Lewis rats (8–10). More specifically, late pregnancy suppresses disease activity in EAE. In one study, serum obtained from mice during late pregnancy decreased proliferative responses and diminished inflammatory Th1 cytokine production (11). Others have shown an altered state of cytokine imbalance favoring Th2 responses during pregnancy (12,13). In pregnancy, humoral immune responses are increased, whereas cellular-immune responses are decreased. Minimizing a strong cellular response against fetal antigens during pregnancy is critical in maintaining pregnancy. Administration of Th1 cytokines causes abortion in normal pregnant mice (14). Moreover, a recent study showed that production of IL-12 and TNF- $\alpha$  was significantly reduced during late pregnancy (15).

Several lines of evidence suggest that estrogens are important immunoregulators and that their relatively high levels during pregnancy promote the development of an antiinflammatory Th2 response (16). After the first 4 weeks of pregnancy, virtually all estrogen production is driven from fetal trophoblast (17). In late pregnancy, there is a disproportionate amount of estriol produced. This has been the focus of intense research as one of the factors conferring a protective effect of pregnancy in autoimmune diseases. In one study, estriol was compared to progesterone and placebo pellets in mice induced with EAE (18). Estriol-treated mice showed reduced severity of EAE compared to no effect with progesterone. This led to a study in human nonpregnant patients with MS treated with 8 mg of orally administered estriol daily for 6 months (19). Pretreatment observation for each patient was used as the control period. During estriol treatment, patients showed reduced relapse frequency, as well as brain magnetic resonance imaging (MRI) lesions. Blood obtained from patients receiving 8 mg of daily estriol showed a distinct deviation toward a Th2 response. Endometrial biopsies showed no evidence of hyperplasia or abnormal menstrual bleeding, suggesting the safety of this therapeutic approach in MS. These preliminary observations warrant a large multicenter, placebo-controlled study to further explore the therapeutic effect of oral estriol in relapsing-remitting MS (RRMS).

Progesterone and prolactin have also been studied as potential immunoregulators during pregnancy. In the presence of progesterone, lymphocytes from pregnant women produce a protein factor capable of inhibiting Th1 cytokine production and stimulating a Th2 shift (20). In contrast, prolactin induces humoral- and cell-mediated immune responses, and its increased secretion during lactation (postpartum) may potentially render women with MS to a higher relapse rate (21).

## DISEASE COURSE DURING PREGNANCY

That young women of child-bearing potential are at highest risk for the development of MS has drawn much interest and debate. These patients with MS are often faced with difficult decisions regarding pregnancy and parenthood. Numerous studies have examined the clinical course of MS during pregnancy (Table 1). However, there is a lack of large well-designed studies examining the effect of pregnancy on MS. Furthermore, pregnancy studies in MS are likely to be biased because women with high disease activity and frequent relapses are expected to avoid pregnancy. Until 1990, virtually all studies reported were retrospective. Nevertheless, the majority of studies reported the relapse rate to decrease during pregnancy and increase during the postpartum period. In 1990, the first prospective study by Birk et al. (22) was reported, which confirmed previously published retrospective observations. The authors studied eight women and followed them for up to 6 months after delivery. The annualized relapse rate during pregnancy decreased to 0.17 and increased to 1.74 during the 6-month postpartum period with an 18-fold increase in the relapse rate in the first 3 months after delivery. Autoreactive T-lymphocytes were significantly decreased in pregnant women with MS compared to nonpregnant women with MS. Although the study was small, the authors noted no complications as a result of epidural anesthesia or breast-feeding. Sadovnick and colleagues (23) observed the relapse rate in 53 women with MS during pregnancy and up to 6 months after delivery. The relapse rate during pregnancy was compared to the prepregnancy annual relapse rate (self-control), as well as the annual relapse rate in 64 matched controls. Results of this study showed that the number of observed relapses during pregnancy was not significantly different when compared to the prepregnancy rate (self-controls). When compared to matched controls, the relapse rate was significantly lower ( $p < 0.02$ ) only during the third trimester. After delivery there was an increase in the relapse rate; however, this was not significant when compared to either the self- or matched controls. The authors concluded that neither pregnancy (entire gestation period) nor the 6-month period after delivery increased the risk of relapses. The results were conflicting with other published reports, suggesting that pregnancy is associated with a decrease and puerperium with an increase in the relapse rate. Study methodology raised questions if the study was truly prospective and data missing from case controls may have affected the outcomes reported in this study (24). In a longitudinal prospective study conducted by Rouillet et al. (25), 125 women with RRMS were followed for a mean duration of 10.3 years. During this time, 33 women became pregnant and 32 full-term pregnancies were observed. Women had been followed for at least 1 year at the clinic before pregnancy. Patients were used as their own controls and also compared to nonpregnant controls. Compared to prepregnancy baseline, there was no increase in the relapse rate during the entire pregnancy or any individual trimester. In contrast, when compared to control patients with MS, the relapse rate was lower during pregnancy (0.86 vs 0.54,  $p = 0.07$ ). During the first 3 months after delivery, the relapse rate increased compared to baseline (1.62 vs 0.51,  $p = 0.05$ ). During the next 3

**Table 1**  
**Summary of Studies Examining the Relapse Rate of Patients With Multiple Sclerosis During Pregnancy**

Published study	Type of study	Number of live births (N)	Control group	Relapse rate prepregnancy	Relapse rate during pregnancy	Relapse rate 6 months postpartum
Confavreux et al., 1998	P	227	Nonpregnant self	0.70	0.43	1.05
Worthington et al., 1994	P	15	Nonpregnant self	0.57	0.48	1.1
Sadovnick et al., 1994	P	58	Nonpregnant self	0.63	0.57	0.93
			Matched controls	0.82	0.57	0.95
Roulet et al, 1993	P	32	Nonpregnant self	0.51	0.79	1.06
Bernardi et al, 1991	R-questionnaire	66	Nonpregnant self	0.65	0.1	0.79
Birk et al., 1990	P	8	Anticipated relapse			
			rate	0.66	0.17	1.74
Frith & McLeod, 1988	R-interview	85	Nonpregnant self	0.53	0.3	0.99

*Abbr:* P, prospective; R, retrospective.

months, the relapse rate returned to the baseline level (0.46 vs 0.51). The authors also noted that relapses were generally mild or moderate during pregnancy in contrast to the postpartum period when relapses were more severe. Neurologic disability did not increase during follow-up after pregnancy. Another prospective observation reported the effect of pregnancy on the relapse rate in 52 patients with RRMS who had 66 pregnancies during an 8-year period (26). Compared to baseline, the relapse rate during pregnancy decreased significantly (0.65 vs 0.10,  $p < 0.01$ ). Interestingly, a rebound effect was observed in this study. There were no relapses observed during the last trimester of pregnancy in contrast to the puerperium where a relapse rate of 0.79 was observed.

Worthington et al. (27) also examined the relationship between pregnancy and MS in a prospective fashion. They compared the relapse rate in 15 women who became pregnant to 22 matched controls for up to 3 years after pregnancy. The relapse rate showed a decrease from 0.57 before pregnancy to 0.48 during pregnancy. During the postpartum period, there was a significant increase in the number of relapses in the first 6 months ( $p < 0.02$ ), with 75% of the relapses occurring in the first 3 months after delivery. However, at the end of the 3-year follow-up period after pregnancy, the annual relapse rate was 0.50 and similar to prepregnancy relapse rate (0.50 vs 0.57,  $p = \text{NS}$ ). Relapses occurring during the 6 months postpartum were more severe than those during or before pregnancy. However, compared to matched controls, there was no significant difference between the relapse rates (0.50 vs 0.48), the severity of relapses, or in neurologic disability at 3 years after pregnancy.

The Pregnancy in Multiple Sclerosis Study (PRIMS) was the most comprehensive study to date examining the effect of pregnancy on MS disease activity (28). The PRIMS study was a multicenter prospective study in which 254 women from 12 centers in Europe participated. A total of 269 pregnancies were observed, and participants were followed for up to 12 months after delivery. Full data on pregnancy and delivery were available for 256 pregnancies in 241 women. The relapse rate during each trimester of pregnancy and 1 year after delivery were compared to the baseline relapse rate during the year before pregnancy (self-control). Compared to the baseline relapse rate of 0.7, there was a slight decrease in the relapse rate during the first and second trimesters (0.5 and 0.6,  $p = 0.03$  and  $p = 0.17$ , respectively). However, in the third trimester, the relapse rate was reduced to 0.2 (0.2 vs 0.7,  $p < 0.001$ ). The relapse rate during the first 3 months after delivery increased significantly to 1.2 (1.2 vs 0.7,  $p < 0.001$ ) and returned to prepregnancy baseline level 12 months after delivery (0.6 vs 0.7,  $p = 0.59$ ). Forty-two women received epidural analgesia during labor, which was not associated with an increased risk of a relapse during puerperium. Women who breast-fed ( $n = 122$ ) had a significantly lower relapse rate compared to women who did not ( $p = 0.02$ ). Neurologic disability assessed at 36 weeks of pregnancy and at 3, 6, and 12 months after delivery showed mild worsening. The use of epidural analgesia or breast-feeding did not correlate with increased disability. This study confirmed previously published

observations on the effect of pregnancy on MS and provided clinicians and patients with reassuring information. Women with MS who are contemplating pregnancy may be informed that conception, gestation, epidural analgesia, and breast-feeding are not likely to alter the natural history of the disease.

A unique study in which serial brain MRI scans were performed in pregnant women with MS showed that MRI activity, including new and enlarging lesions, decreased during pregnancy (29). Two patients participating in a monthly brain MRI protocol became pregnant and were imaged during and after pregnancy. Both patients demonstrated a significant decrease in the number of lesions during pregnancy, with no active lesions seen during the third trimester. During postpartum, both patients exhibited several new lesions. Although the study had only two patients, serial brain MRI scans supported the clinical evidence from other prospective studies showing the protective effect of pregnancy on MS, particularly during the third trimester.

In conclusion, most observations, both prospective and retrospective, indicate a reduction in the relapse rate during pregnancy, particularly during the third trimester. This is followed by an increased risk of a relapse during the postpartum period, especially in the first 3 months after delivery. Overall, pregnancy does not seem to affect the long-term course of MS.

## DISEASE MANAGEMENT DURING PREGNANCY

### *Management of Pregnancy*

#### *Prepregnancy Counseling*

Three important aspects of prepregnancy counseling for patients with MS need consideration. First, couples contemplating pregnancy may need counseling regarding the effect of pregnancy on MS and vice versa. Second, potential parents often face the anxiety of “transmitting the disease” to the newborn and are eager to learn about the chances of the baby developing MS. It is important to note that there are no prenatal tests or amniotic fluid tests for MS. There is a well-defined genetic predisposition, and the incidence of familial MS is estimated to be 10% to 15% (30). In general, if one parent has MS, the lifetime risk of a child developing MS is between 3 and 5%, compared with the general population of 0.5% (31). However, there are no well-defined modes of transmission associated with MS (i.e., autosomal dominant or recessive). The third aspect of preconception counseling involves fertility and the ability to conceive. MS has no known physiologic effect on fertility, and retrospective studies have shown that MS does not increase the risk of ectopic pregnancies (32), spontaneous abortions (33), or stillbirths when compared to the general population. However, sexual dysfunction may occur in 50 to 90% of patients (34). Psychological stress associated with sexual dysfunction may be challenging to treat if it impairs the ability to conceive. Sexual dysfunction can occur in males or females with MS and includes difficulties such as erectile dysfunction, vaginal dryness, bladder and bowel incontinence, or positional difficulties. In one

study, more than 70% of the patients reported improved relationship with their partner after discussing sexuality related issues with the healthcare professional (35). If the issues surrounding sexual dysfunction have been addressed and the couple continues to have difficulties conceiving, fertility may have to be assessed by fertility disorder specialists. Although no large well-designed studies have been conducted to examine the effect of fertility-enhancing medications on disease activity in MS, one study did report an increased relapse rate in three women who used clomiphene citrate, a commonly used fertility-enhancing drug (36). Finally, patients who were previously treated with immunosuppressive agents for MS may have prolonged periods of amenorrhea and infertility (37). Although patients who receive intense immunosuppressive and chemotherapy agents have clinically aggressive disease and are more likely to avoid pregnancy, potential infertility as a result of these interventions should be carefully discussed.

### *Obstetrical Care*

Prenatal care of patients with MS should not differ from that of women who do not have MS (38). Women with MS may experience aggravation of their bowel and bladder symptoms during pregnancy. This may warrant close monitoring of urinary tract infections. Mueller et al. studied pregnancy-related complications in 198 women with MS and found that they were no more likely to have pregnancy or delivery complications than more than 1500 women without MS observed for 2 years after delivery (39). However, they did note a possible increased risk of maternal anemia. With respect to infants, there was no difference in the incidence of low birth weight, congenital malformations, or higher infant mortality rates in the two groups. Patients with relatively greater neurologic disability at the time of pregnancy or unexpected disease activity during pregnancy may require special care, including a multidisciplinary approach involving the obstetrician and the neurologist. More specifically, preexisting motor deficits and increased fatigue may cause difficulty during labor and require assisted delivery (38). Overall, there is no evidence to suggest that pregnant women with MS should be considered “high-risk pregnancy” simply because of the diagnosis of MS. High risk should be based primarily on obstetrical status or in the event of neurologic worsening.

### *Delivery and Anesthesia*

The method of delivery should be determined by the obstetrician as deemed appropriate. Caesarean section should not be considered as the preferred route of delivery in patients with MS unless clinically indicated (40). Similarly, there is no conclusive evidence contraindicating the use of anesthesia for delivery in patients with MS. The use of epidural anesthesia in patients with MS has been the focus of much debate. Two studies have suggested that spinal anesthesia may increase the risk of relapses (41,42). However, others have not found epidural or other forms of anesthesia to increase the risk of relapses (28,43,44). A modest association between an increase in the relapse rate and the use of bupivacaine regional blockade given at concentrations higher than 0.25% for a prolonged period was reported in one study

(45). However, there was no increased risk observed with epidural anesthesia. These findings were confirmed in the PRIMIS study (28). Currently, there is little evidence to support an association between either epidural anesthesia or regional blockade and increased risk of relapses. Larger well-designed studies are needed to provide conclusive information regarding the use of analgesia and anesthesia in MS during labor.

### **Management of Multiple Sclerosis During Pregnancy (Fig. 1)**

The Food and Drug Administration (FDA) designates safety categories to drugs based on risk to fetus and overall safety during pregnancy (Table 2). There are five categories currently assigned by the FDA. These are discussed in descending order of safety.

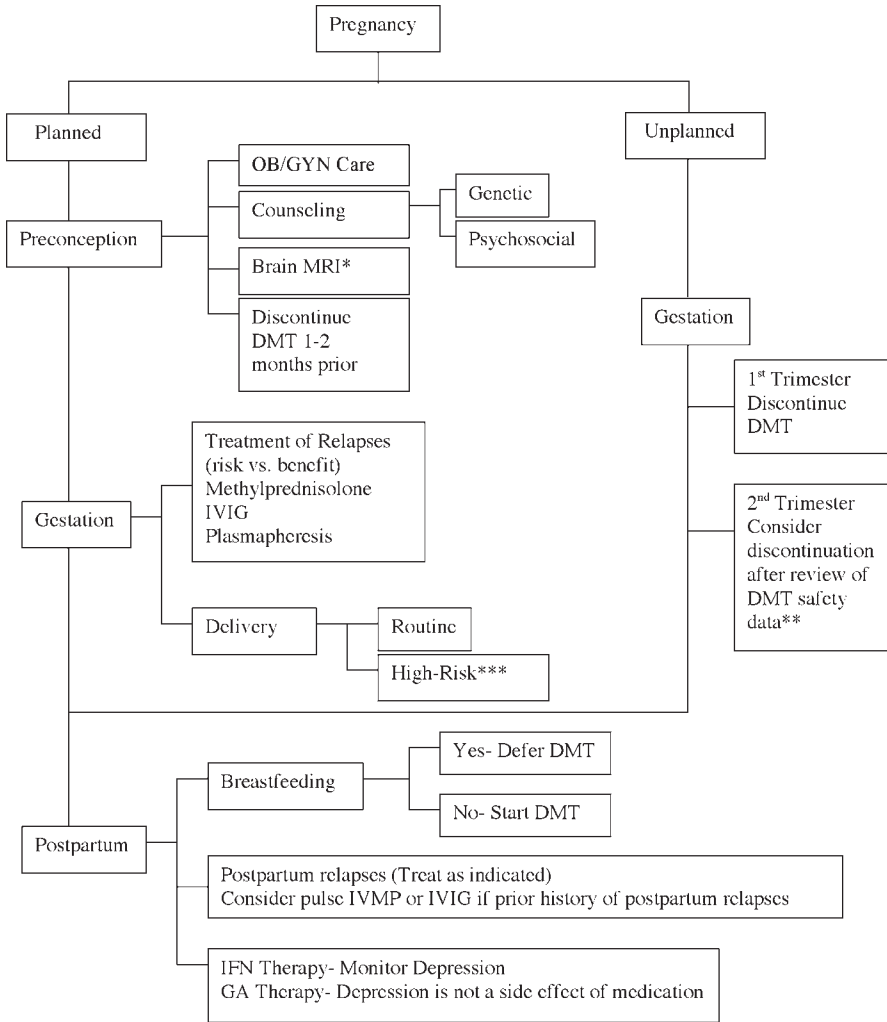
- Category A: Controlled human studies have shown no fetal risks. This is the safest category.
- Category B: No controlled human studies are available. Animal studies show either no risk or minimal risk to the fetus.
- Category C: No human studies are available. Adverse fetal effects have been shown in animals.
- Category D: Evidence of human risk has been shown, but benefits may outweigh risks.
- Category X: Well-described and proven fetal risks have been shown. Use in pregnancy is contraindicated.

### *Treatment During Pregnancy*

*Relapses:* Treatment of relapses should depend on a risk–benefit assessment. Relapses characterized by severe motor, cerebellar, or sensory deficits leading to gait ataxia should be considered for treatment because these deficits are more likely to cause injury to the mother and potentially harm the fetus. Intravenous (IV) methylprednisolone (IVMP) (category C) given at a dose of 1 g for 3 to 5 days is an appropriate first-choice treatment (46). Cleft lip and palate have been associated with the use of IVMP during pregnancy, although the overall incidence is low (47). Alternate treatment strategies include plasmapheresis; this is considered to be a safe alternative to IVMP for the treatment of relapses with virtually no risk to the fetus and can be used safely during breast-feeding (48). It poses minimal risk to the mother, mostly related to shifts in fluid volume during pheresis (49). Several investigators have also studied the effect of IV immunoglobulin (IVIg) (category C) as a preventive therapy for relapses in the postpartum period and found IVIg to be effective in preventing relapses during the immediate postpartum period (50,51).

*Disease-modifying therapies:* Currently, two categories of DMT,  $\beta$ -(IFN) (category C) and glatiramer acetate (GA) (category B) are effective and are available for long-term use in the treatment of RRMS (52). At this time, there is insufficient data to support elective or continuous use of DMT during pregnancy. Some reports have suggested that DMT, particularly glatiramer acetate, may be safe during pregnancy (53,54).  $\beta$ -IFNs (category C) are abortifacient (55) and are best avoided during pregnancy. For couples contemplating pregnancy, it is generally





**Fig. 1.** Flow chart showing the management of multiple sclerosis in pregnancy. \*Obtain brain MRI scan prior to conception in patients with clinically active disease. \*\*GA is category B in pregnancy and may be used in some patients. IFNs can be abortifacient and should be discontinued. \*\*\*High risk delivery should be determined by obstetrical status. Worsening of neurologic disability may also place a patient at high risk.

recommended that DMT be discontinued at least one to two menstrual cycles before planned conception. It may be helpful to obtain a brain MRI scan before the decision to conceive, especially in patients with a history of clinically active disease. Brain MRI scans with multiple active lesions may predict relapses (56). There-

**Table 2**  
**US Food and Drug Administration Categorization**  
**of Medications Used in Multiple Sclerosis**

Category B	Category C	Category D
Bupropion	Amitriptyline	Azathioprine
Glatiramer acetate	Avonex	Benzodiazepines
Loperamide	Baclofe	Carbamazepine
Sildenafil	Betaseron	Cyclophosphamide
Oxybutynin	Donepezil	Mitoxantrone
Pemoline	Fluoxetine	Phenytoin
	Gabapentin	
	IVIg	
	Methylprednisolone	
	Nortriptyline	
	Paroxetine	
	Rebif	
	Sertraline	
	Symmetrel	
	Tizanidine	
	Venlafaxine	
	4-aminopyridine	

*Notes:* Category A: controlled human studies have shown no fetal risks; category B: no controlled human studies are available. Animal studies show either no risk or minimal risk to the fetus; category C: no human studies are available. Adverse fetal effects have been shown in animals; category D: evidence of human risk has been shown but benefits may outweigh risks; category X: well described and proved fetal risks have been shown. Use in pregnancy is contraindicated.

fore, in the presence of disease activity on a brain MRI scan or significant worsening of brain MRI lesions compared to a previous scan, it may be advisable to postpone pregnancy and repeat the brain MRI after a 6-month interval. Even more challenging for the clinician is the issue of unplanned pregnancy or if pregnancy is confirmed after the critical first trimester. Information on the risks and benefits of continuing therapy should be discussed with the patient so that an informed decision can be made. Although discontinuation of DMT in these circumstances is commonly practiced, one may consider elective use of DMT, particularly with Copaxone (category B), after the first trimester, given the safety profile of this agent. However, multicenter MS pregnancy registries may be the only practical method to examine the safety of DMT during pregnancy.

### ***Postpartum Management***

Management of MS in the postpartum period can be a challenge, given the responsibility of a newborn, along with the uncertainty about the disease. A multidisciplinary approach involving the neurologist, pediatrician, psychosocial

counseling, and, most importantly, family support (57) may be needed in some instances. In one study, despite deciding to become pregnant, patients with MS perceived this decision as risky behavior (58).

### *Depression*

Depression is frequently encountered during postpartum (59) and is also well-recognized in MS (60). Patients with a history of depression or “pregnancy blues” should be carefully monitored. Despite the joy of having a newborn, providing care to the infant can place enormous strain on the mother, the couple, and their resources. Patients with MS may be more susceptible to such stress and thus require closer monitoring. Up to 30% of patients with MS may be unable to provide adequate care for the infant because of disability (61). This, in turn, may precipitate depression in addition to the higher risk of relapses making the postpartum period challenging and highly stressful necessitating the importance of a good family support system (62). Finally, feeling depressed and stressed may need to be carefully distinguished from a true relapse to avoid unnecessary use of steroids. Antidepressants and counseling should be considered. IFN- $\beta$  may precipitate or cause depression (63). Its use during the postpartum period is not contraindicated but should be closely monitored.

### *Breast-Feeding*

Breast-feeding is not contraindicated in patients with MS. Pregnant women may be concerned about transmitting the disease to the infant through breast-feeding (64), which is not the case. Furthermore, there is no conclusive evidence to suggest that breast-feeding has a harmful effect on the disease course or precipitates exacerbations. Nelson et al. (65) conducted a study where 435 women were interviewed regarding breast-feeding and postpartum relapses. Information was collected on 191 pregnancies, and no significant difference in exacerbation rate was found between women who breast-fed and those who did not. In the PRIMIS study, breast-feeding was associated with a lower risk of a relapse during the postpartum period (28). A rare condition affecting vision, known as “lactation optic neuritis” (ON), has been reported in women during the postpartum period (66). This should not deter patients to breast-feed but merely alert them to report symptoms for careful evaluation. Overall, patients who are contemplating breast-feeding may need to be advised regarding the use of medications during breast-feeding. DMT for MS may be secreted in the breast milk and therefore should not be prescribed during breast-feeding. However, history of clinically active disease or occurrence of relapses during postpartum may warrant immediate resumption of DMT. Breast-feeding should be avoided in these cases.

### *Treatment of Relapse*

As discussed, the bulk of data indicate that there is an increase in the relapse rate during the postpartum period. Relapses should be treated with steroids if warranted. Higher doses of IVMP are generally the most efficient and fastest method to treat a

relapse, although there is no consensus on the ideal dosing regimen of steroids. The use of an oral prednisone taper after high doses of IVMP makes no difference on neurologic outcome (67). This is useful to know because prolonged use of steroids can lead to weight gain, mood irritability, and worsening of depression. Thus, eliminating unnecessary use of steroids during postpartum may be beneficial to the patient. In cases of caesarean section deliveries, treatment with high doses of IVMP may impair wound healing. Plasmapheresis remains as the only alternative to steroids for the treatment of a relapse, especially in steroid-resistant cases (68). Currently, there is insufficient data to support the routine use of IVIg during postpartum to prevent relapses. Only patients at high risk of relapses during puerperium should be considered as a candidate for IVIg treatment. High risk may be arbitrarily defined as history of relapses after previous pregnancies or relapses during pregnancy. These decisions require careful consideration and should be made on a case-by-case basis until studies are performed that provide more conclusive data.

### *Resumption of Disease-Modifying Therapies*

In general, resumption of treatment with DMT should occur soon after delivery if breast-feeding is not planned. Ideally, patients should resume the same DMT as before pregnancy. Treatment-naïve patients or newly diagnosed patients should be given information on all available DMTs to make an informed choice. Consideration should be given to two aspects regarding the use of DMT after delivery. First, all currently available DMT are injectable medications requiring self-administration by the patient. This may be somewhat trying for many patients during the postpartum period when they are already undergoing significant stress and strain. Thus, compliance with the injections and management of side effects should be given special attention. Second, patients need to be informed that DMT may not show immediate clinical benefits and their effect on reducing the relapse rate is generally observed after several months (69–72). Therefore, one may still experience a relapse during therapy with DMT, especially in the first few months after initiating therapy. The purpose of DMT is to alter the long-term natural history of the disease and minimize ensuing disability. Consequently, the most important message delivered to the patient should emphasize that some form of DMT is better than none, which is a view strongly endorsed by the National Multiple Sclerosis Society (1998).

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## Treatment of Progressive Multiple Sclerosis

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### INTRODUCTION

Treatment directed at the progressive phase of multiple sclerosis (MS) is the most difficult (Table 1). Immunosuppressive therapies, such as total lymphoid irradiation (TLI), cyclosporine, methotrexate, 2-chlorodeoxyadenosine (2-CdA), cyclophosphamide, mitoxantrone, azathioprine, interferon (IFN), steroids, intravenous immunoglobulin (IVIg), and plasma exchange have shown some positive clinical effects in progressive disease. However, all of these nonspecific immunosuppressants suffer from the same basic defect; they may temporarily halt a rapidly progressive downhill course, but it is difficult, or dangerous, to employ them for more than a few months to 1 or 2 years. Thus, because MS is an illness of decades, not months, immunosuppressive therapy is only at best a temporary solution.

### TOTAL LYMPHOID IRRADIATION

TLI has potent immunosuppressive effects and is beneficial in patients with progressive MS (1). The absolute lymphocyte count is an indicator of therapeutic efficacy, with greater efficacy in patients with lower counts. Many patients begin reprogressing after initial therapy, and a major limitation is that use of TLI may preclude the use of other treatments that affect the immune system at a later time if the patient reenters the progressive phase.

### CYCLOSPORINE

A large multicenter trial of cyclosporine in the United States (2) and a trial in London (3) found that cyclosporine has a beneficial, although modest, effect in ameliorating clinical disease progression. However, its clinical use is limited because of a narrow benefit-to-risk ratio.

**Table 1**  
**Treatment Strategies for Multiple Sclerosis**

Disease course/stage	Treatment
Monosymptomatic (e.g., optic neuritis)	Intravenous (IV) methylprednisolone, 100 mg/day for 5 days
Relapsing-remitting, no disease activity for several years and no magnetic resonance imaging (MRI) activity	IV corticosteroids if attacks occur
Relapsing-remitting, current disease	IV steroids plus Avonex (30 µg IM weekly) activity, and/or MRI activity or Betaseron (1 mL SC QD) or Copaxone (20 mg SC QD)
Relapsing-remitting, disease activity while on interferon (IFN) or Copaxone	Add monthly bolus of IV methylprednisolone, consider increasing IFN dose
Relapsing-remitting, accumulating disability (IFN/Copaxone/steroid nonresponders)	IV monthly cyclophosphamide/steroid pulse therapy, consider IV mitoxantrone
Rapidly progressing disability	IV cyclophosphamide and corticosteroid eight day induction, followed by pulse maintenance
Very rapidly progressing disability	Plasma exchange
Secondary progressive	IV steroid monthly pulses or IV cyclophosphamide/steroid monthly pulses or methotrexate (PO or SC, 7.5 to 20 mg/week) with or without steroid pulses or consider addition of IFN-β if not already taking or consider IVIg monthly
Primary progressive	IV steroid pulses or methotrexate (PO or SC, 7.5 to 20 mg/week) with or without steroid pulses or Cladribine IV or SC or IVIg monthly or consider mitoxantrone or consider azathioprine

IM, intramuscularly; SC, subcutaneously; QD, every day; PO, administered orally.

## METHOTREXATE

Methotrexate is a well-known immunomodulator used extensively in other conditions, such as rheumatoid arthritis. Weekly low-dose oral methotrexate (7.5 mg) was studied in a randomized, double-blind, placebo-controlled trial of 60 patients with chronic progressive MS (4). Methotrexate positively affected measures of upper extremity function, such as the nine-hole peg test and a block-in box test; these tests are a sensitive measure of repeated use of digits. However, lower

extremity function as measured by ambulation and disability scales were not affected. There was no clinically significant toxicity.

The safety of methotrexate also has been established in patients receiving 20 mg SC weekly (5). Whether higher doses given intravenously or intrathecally would be more effective in MS is unclear.

## **CLADRIBINE**

Cladribine (2-CdA, Leustatin), a potent immunosuppressive agent useful in the treatment of hairy cell leukemia, was reported to be of benefit in a study of patients with chronic progressive MS (6). In this 1-year, double-blind trial, 48 matched pairs received 4 monthly courses of 2-CdA through a central venous catheter for a 7-day period. Seven out of 23 evaluable patients receiving placebo experienced a one point or more worsening in their Expanded Disability Status Scale (EDSS) score at 1 year, whereas only one out of 24 patients taking cladribine had worsened. Treated patients had improvement on disability scores, no increase in brain MRI lesions, and decreased cerebrospinal fluid (CSF) oligoclonal bands (OCBs), whereas the placebo group experienced the opposite in all of these categories. Side effects included two cases of herpes zoster, a fatal case of hepatitis (not clearly related to the treatment), and persistently lowered CD4 counts.

In a subsequent multicenter trial, 159 patients with progressive MS (30% of whom had primary-progressive MS [PPMS] and the remainder secondary-progressive MS [SPMS]) were randomly assigned to one of two doses of SC cladribine (0.07 mg/kg per day for 5 consecutive days every 4 weeks for either two or six cycles (total dose 0.7 mg/kg and 2.1 mg/kg, respectively) or to placebo (7). There was no significant difference between the groups in terms of EDSS score during the course of the study. However, the results of magnetic resonance imaging (MRI) studies were significantly different between the treatment and placebo groups. At baseline, approximately 35% of patients in each group had enhanced T1 lesions on MRI; this remained unchanged in the placebo group, while decreasing to 10% and 6% in the low- and high-dose treatment groups, respectively. The cladribine groups also had a 90% reduction in the mean number of enhanced T1 lesions at month 6 that persisted for 24 months of follow-up, whereas the placebo group had a 33% and 50% reduction at the 6-month and final evaluation, respectively. Cladribine was generally well tolerated. The high-dose group reported more cases of upper respiratory infection, pharyngitis, back pain, arthralgia, and skin disorder, compared with the low-dose and placebo groups; no serious infections occurred. Dose-related decreases in the mean lymphocyte count occurred with cladribine therapy. A disadvantage of the SC method of administration is that the total lymphocyte and CD4 counts, as measures of effectiveness, do not fall for a number of months and may remain low for months after discontinuing therapy.

One explanation for the difference in outcome between these two studies in terms of the EDSS score is that the patients in the second study had relatively high EDSS scores at baseline (median score-6.0); the size and duration of the study were not

powered to detect results in patients with this severity of disease (7). In addition, a significant proportion had PPMS, and this subgroup did not respond as favorably on MRI as the SPMS.

A third study of 159 patients with progressive MS (70% SPMS, 30% PPMS) found that treatment with cladribine did not prevent brain atrophy compared with placebo (8). Furthermore, the change in brain volume did not correlate with other MRI measures of disease (e.g., number and volume of enhancing lesions).

## CYCLOPHOSPHAMIDE

Cyclophosphamide (Cytoxan) has been used for the treatment of MS since the early 1980s despite conflicting data. An early study compared an induction course of cyclophosphamide with steroid treatment and found impressive differences; however, in retrospect, the patients were all in a rapidly progressive phase of the illness (9). By comparison, a Canadian study several years later found no effect from an induction course of cyclophosphamide; these patients and controls were more representative of the chronic progressive patients treated in other trials (10).

Cyclophosphamide is most effective in patients younger than age 40 years, especially in those who have been in the progressive phase for <1 year. The drug is ineffective for PPMS.

Cyclophosphamide is administered in monthly bolus injections and maintained for a year or more, often concurrently with IV steroids. The duration of treatment is limited by the risk of bladder cancer, which appears to rise with time and may depend on the total accumulated drug dose.

## MITOXANTRONE

Mitoxantrone is an anthracycline analog that is an effective chemotherapeutic agent for many cancers. The efficacy of mitoxantrone in MS was evaluated in a trial of 42 patients with active disease who were treated monthly with either IV methylprednisolone (1 g) plus IV mitoxantrone (20 mg) or IV methylprednisolone alone for 6 months (11). Although the number of patients was small, there was a statistically significant reduction in the number of relapses and an increase in the number of patients free of attack associated with the combined therapy. In addition, 90% of the methylprednisolone/mitoxantrone group showed no new enhancing lesions on MRI vs only 31% in the methylprednisolone group.

These results prompted a multicenter, placebo-controlled, randomized, observer-blind trial of 194 patients with progressive MS that was completed in Europe (12). Patients received IV treatment (5 mg/m<sup>2</sup> or 12 mg/m<sup>2</sup>) every 3 months for 2 years. Treatment with mitoxantrone was associated with significant clinical benefits compared with placebo on multivariate analysis, reducing progression of disability and clinical exacerbations.

The risk of cardiotoxicity with mitoxantrone prevents prolonged usage, although 24 months of use in the randomized trial did not result in clinically significant cardiac dysfunction (12). Mitoxantrone is now Food and Drug Administration

(FDA) approved for use in MS, but further studies are required to determine the optimal treatment protocols and frequency of long-term side effects.

## AZATHIOPRINE

Azathioprine has been studied in both relapsing-remitting MS (RRMS) and chronic progressing MS (CPMS) since 1971. A meta-analysis of the results of five double-blind and two single-blind, randomized, control trials of azathioprine use in MS showed only a small difference after 2 years in favor of azathioprine (13). The study concluded that the side-effect profile outweighs any small clinical gain.

## INTERFERON

The efficacy of IFN has been well documented in patients with RRMS (*see above*). A small randomized trial of IFN- $\beta$ -1a in PPMS found no benefit (14).

On the other hand, IFN- $\beta$ -1b is effective in patients with secondary progressive MS. In one study of such patients in 32 centers in Europe, for example, 358 patients received placebo and 360 received IFN- $\beta$ -1a every other day subcutaneously for up to 3 years (15). There was a 22% relative reduction in the proportion of patients with progression in the IFN- $\beta$ -1b group compared with placebo. The time to becoming wheelchair-bound was also significantly delayed in the treatment group, equivalent to 12 months ( $p < 0.01$ ). The placebo group had a mean 8% increase in MRI lesion volume, compared with a 5% decrease in the IFN- $\beta$ -1b group.

The authors assert that the positive effect of treatment could not be ascribed merely to a reduction in disability as the result of relapses; there was also a direct effect on progression. Whether this results from suppression of some ongoing low-level inflammatory process could not be answered directly.

Side effects were manageable; 45 patients taking IFN- $\beta$ -1b stopped because of adverse effects, compared with 15 taking placebo, but twice as many stopped because of inefficacy of treatment in the placebo group (*see Side Effects of Interferons*). Neutralizing antibodies were seen in 28% of patients (*see Neutralizing Antibody Formation*).

A North American study of IFN- $\beta$ -1a b in secondary progressive MS found no effect on time to confirmed progression to disability, although significant benefit was shown on all other outcomes (16). Two subsequent randomized, placebo-controlled studies of IFN- $\beta$ -1a found similar results (17,18). Subgroup analysis of one suggested that the maximal benefit occurred in women and in patients who were still experiencing relapses when treatment started (17).

The reason for the disparity in the studies in terms of progression to disability is not clear. Factors that may be important include a shorter observation period (2 vs 3 years) and shorter duration of the prerandomization secondary progressive phase (2.2 vs 4 years) in the European study. In addition, there was a higher proportion of patients with prestudy relapses in the European study, a finding associated with improved outcomes in the most recent report (17).

Additional hope for immunomodulatory therapy in patients with secondary progressive MS was provided by an MRI study that found that compared with placebo, treatment with IFN- $\beta$ -1a for 3 years resulted in significant improvements in all MRI measures, particularly in patients with relapses in the 2 years prior to the study (19).

These data have major implications for the treatment of MS. Secondary progressive disease is the single largest category of MS. The immunomodulatory drugs are now approved only for the treatment of relapsing MS; the FDA has approved IFN- $\beta$ -1b (Betaseron) for use in patients with secondary progressive disease who continue to have relapses (20) and is considering approval of IFN- $\beta$ -1b and IFN- $\beta$ -1a for other patients with secondary progressive disease. These drugs may become standard therapy for secondary progressive MS, particularly for women and patients still experiencing relapses. Additional data may further clarify the appropriate use of these agents.

## CORTICOSTEROIDS

Monthly bolus IV corticosteroids, typically 1000 mg of methylprednisolone, are used at many institutions for treatment of primary or secondary progressive MS alone or in combination with other immunomodulatory or immunosuppressive medications. A trial of methylprednisolone in secondary progressive MS was conducted, in which a total of 108 patients received either 500 mg or 10 mg of IV methylprednisolone every 8 weeks for 2 years (21). Although there was no difference in the proportions of patients in each treatment group who experienced sustained progression of disability, the time to onset of sustained treatment failure was delayed in the high-dose group. Further studies are planned.

## INTRAVENOUS IMMUNOGLOBULIN

Immunoglobulin may help patients with several autoimmune diseases, including chronic relapsing polyneuropathy and dermatomyositis, and also has been studied in MS. Its mechanism of action is unclear but may relate to antiidiotypic effects and suppression of tumor necrosis factor (TNF)- $\alpha$ . A randomized, placebo-controlled trial of monthly IVIg in RRMS involved 150 patients over 2 years (22). IVIg was associated with fewer relapses (62 vs 116 with placebo) and a greater likelihood of being relapse-free (53 vs 36%). Only three patients receiving IVIg (4%) experienced side effects, including cutaneous reactions and depression; these manifestations were not unequivocally related to the IVIg.

Observations in animal models of MS revealed that IVIg could promote new myelin synthesis (23). However, a double-blind, placebo-controlled study of 67 patients with an apparently irreversible motor deficit found that IVIg (0.4 g/kg for 5 days, then single infusions every 2 weeks for 3 months) did *not* reverse established weakness in MS (24).

## **PLASMA EXCHANGE**

Plasma exchange has been investigated in patients with acute central nervous system (CNS) inflammatory demyelinating disease who did not respond to steroid therapy (25). Moderate or greater improvement in neurological disability occurred during eight of 19 (42%) courses of active treatment, compared with one of 17 (6%) courses of sham treatment. Improvement occurred early in the course of treatment and was sustained on follow-up. However, four of the patients who responded to the active treatment experienced new attacks of demyelinating disease during 6 months of follow-up.

## **BONE MARROW TRANSPLANT**

Recent strategies have focused on more aggressive approaches to immunosuppression, such as autologous bone marrow transplantation (BMT). This approach has shown promise in experimental models of MS (26,27). There has been one case report of a patient with MS who developed chronic myelogenous leukemia and was treated with allogeneic BMT (28). Another patient with MS had a BMT after she failed to respond to corticosteroids, IFN- $\beta$ , azathioprine, methotrexate, and/or cyclophosphamide (29). The patient showed mild improvement in strength after autologous BMT but died 7 months after transplant from acute bronchopneumonia associated with cardiocirculatory insufficiency and elevated liver enzymes.

In one controlled trial, two patients with rapidly progressive disease were given cyclophosphamide followed by total body irradiation and methylprednisolone (30). The patients then had an autologous stem cell transplantation. At the 2-month follow-up period, both patients improved clinically and were off of all immunosuppressive medications. Larger trials are awaited in order to evaluate the risk–benefit ratio of this approach.

## **ANTIINTEGRIN ANTIBODIES (NATALIZUMAB)**

The formation of inflammatory lesions in patients with MS may involve lymphocytes and monocytes that gain access to the brain parenchyma from the circulation by first adhering to vascular endothelial cells (31). The glycoprotein  $\alpha$ -4 integrin is expressed on the surface of inflammatory lymphocytes and monocytes and may play a critical role in their adhesion to the vascular endothelium (32,33).

These observations have led to investigations of  $\alpha$ -4 integrin antagonists. Treatment with these agents in rodent models of MS resulted in reduced signs of disease activity and inflammation (34–36). A small preliminary, placebo-controlled study in humans using the monoclonal antibody natalizumab, an  $\alpha$ -4 integrin antagonist, also found that the drug reduced the frequency of new gadolinium (Gd)-enhancing lesions (37).

These findings have now been extended in a larger and longer double-blind trial in which 213 patients with RRMS or relapsing secondary progressive MS were randomly assigned to receive 3 mg/kg or 6 mg/kg of IV natalizumab, or placebo,



every 28 days for 6 months (38). Both natalizumab groups had significant reductions in the mean number of new brain lesions on monthly Gd-enhanced MRI (9.6 for placebo vs 0.7 and 1.1 in the 3 mg/kg and 6 mg/kg natalizumab groups, respectively). This effect was evident 1 month after the first infusion and was sustained throughout the treatment period. In addition, there were significantly fewer relapses in the natalizumab groups (27, 13, and 14, respectively).

Although no direct comparative studies have been performed, these results suggest that natalizumab may be more effective than the IFNs or glatiramer acetate. Therapy with natalizumab was well tolerated, with a safety profile similar to that of placebo.

Longer term data and comparison studies, as well as the effect of natalizumab on the progression of disability, are necessary before firm recommendations can be made regarding the optimal use of this agent. Nevertheless, these data are promising.

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# Cyclophosphamide Therapy for Multiple Sclerosis

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## INTRODUCTION

Cyclophosphamide has long been a widely used alkylating agent in the treatment of malignancies, notably hematologic malignancies and, despite its long history, continues to find new uses in immune-mediated nonmalignant processes. Although cyclophosphamide has been studied as a treatment for multiple sclerosis (MS) for the past 30 years, it is only after successful trials of mitoxantrone with improved designs employing magnetic resonance imaging (MRI) as a surrogate marker that clinical researchers have become highly confident of the use of this class of drugs. Trial results suggest that it is efficacious in cases of worsening MS that have an inflammatory component as evidenced by relapses and/or gadolinium (Gd)-enhancing lesions on MRI or in patients in earlier stages of progression. There is no evidence of efficacy in primary-progressive (PPMS) or later secondary-progressive MS (SPMS). Although they are usually considered a general immunosuppressive agent, immunologic studies indicate that proliferating myelin-specific T-cells may be preferentially eliminated in MS. Side effects include nausea, alopecia, infertility, bladder toxicity, and risk of malignancy. Cyclophosphamide is usually given as every 4- to 8-week outpatient intravenous (IV) pulse therapy with or without corticosteroids and is usually well tolerated. Cyclophosphamide is recommended for patients with MS whose disease is not controlled by first-line agents and those with rapid worsening.

## BACKGROUND

Cyclophosphamide is transformed in the liver to active alkylating metabolites, which then react with replicating DNA, destroying susceptible rapidly proliferating malignant and nonmalignant cells. It is widely used concurrently with other antineoplastic drugs for the treatment of leukemias and lymphomas, adenocarcinomas,

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and other malignancies. It is also used in marrow ablative regimes for both malignant and nonmalignant diseases. Cyclophosphamide has been used in numerous regimens for the treatment of immune-mediated diseases (1,2). Initially, out of concern for potential toxicities, cyclophosphamide use was restricted to the most aggressive immune-mediated diseases, such as Wegener's granulomatosis, polyarteritis nodosa, refractory polymyositis or inflammatory neuropathies, and primary central nervous system (CNS) vasculitis (3,4). A landmark study by Gorley et al. demonstrated that pulse cyclophosphamide therapy was much more effective than standard therapy in the treatment of lupus nephritis (5). This study demonstrated that cyclophosphamide could be well tolerated over time and highly effective in chronic immune-mediated diseases. It is the recommended form of therapy for the treatment of idiopathic nephrotic syndrome and for lupus nephritis, where it appears to have additive efficacy when give concomitantly with pulse methylprednisolone (6). Pulse cyclophosphamide is now regularly used in the treatment of severe systemic-onset juvenile rheumatoid arthritis (7), interstitial lung disease associated with collagen vascular diseases (8), and idiopathic thrombocytic purpura. Cyclophosphamide has been adopted in the treatment of numerous immune-mediated disorders of the peripheral nervous system (9).

## CYCLOPHOSPHAMIDE TREATMENT OF MULTIPLE SCLEROSIS

In the past, the rationale that led investigators to study cyclophosphamide as an MS treatment rested on the hypothesis that MS is an inflammatory, cell-mediated autoimmune disease affecting the CNS (10–12). The immunosuppressive effects of cyclophosphamide have proved to be effective for the treatment of other putative autoimmune diseases (1,2). More recently, it has become clear that a primary autoimmune hypothesis is not essential to the rationale for the use of immunosuppression, because inflammation is often the predominant cause of injury in viral infection. In this regard, Rodriguez et al. have investigated the effect of immunosuppression on CNS remyelination in a chronic virus-induced demyelinating disease (13). They have reported that treatment of animals with cyclophosphamide or anti-T-cell monoclonal antibodies enhanced new myelin synthesis, suggesting that factors associated with immune T-cells somehow impair remyelination and interference with the function of immune T-cells enhances CNS remyelination by oligodendrocytes.

Cyclophosphamide was first tested in MS in 1966 (14) and has since been used in selected patients with MS. Its use has gradually increased recently among clinicians who are confronted with persistent active inflammation in patients with MS treated with US Food and Drug Administration (FDA)-approved disease-modifying drugs, interferon (IFN)- $\beta$  and glatiramer acetate. With FDA approval in 2000 of a related drug, mitoxantrone, for patients with worsening MS, the decision to treat patients with MS with drugs of this class has become standard practice (15,16). According to a recent survey regarding the use of immunosuppressive drugs,

cyclophosphamide is being used by many neurologists for the treatment of MS (17). There have been more than 40 published reports on the clinical and immunological effects of cyclophosphamide in MS (Tables 1 and 5), including many that indicate cyclophosphamide is efficacious in MS. Not all studies have shown positive effects. This has created differing opinions regarding its use, especially because two placebo-controlled trials conducted in the 1980s did not show positive effects (18,19). Many questions remain unanswered regarding how cyclophosphamide should be used in the treatment of MS.

## **STAGES OF MULTIPLE SCLEROSIS**

Later in the course of MS it has been proposed that a separate process of neurodegeneration may occur independent of active cell-mediated inflammation and thus may not be amenable to antiinflammatory therapies. With time, MS becomes less inflammatory, as measured by Gd-enhancing lesions on MRI (20,21). It has been suggested that PPMS may represent an entirely different pathological process and thus respond differently to therapy (22). Immunomodulatory drugs with proven efficacy in MS may not show efficacy in all clinical trials, depending on the patient populations being studied. This is best illustrated in studies of IFN- $\beta$ -1b. Positive clinical effects were observed in the European trial of IFN- $\beta$ -1b (23), whereas there were no positive clinical effects in the North American trial IFN- $\beta$ -1b trial (24). Analyses of patient demographics demonstrate that those in the European trial were at an earlier stage of their disease. Cyclophosphamide results should be evaluated according to the stage of MS and the degree to which inflammation plays a role in the pathology of patients being treated. One reason for conflicting reports regarding the efficacy of cyclophosphamide in MS is that the patient populations treated were at different stages of their disease.

## **CLINICAL TRIALS OF CYCLOPHOSPHAMIDE IN MULTIPLE SCLEROSIS (TABLE 1)**

In 1966, Aimard et al. described the successful arrest of a progressive case of MS using cyclophosphamide (14). This led to an open uncontrolled clinical trial in which 30 patients with MS were treated with cyclophosphamide 200 mg/day IV for 4–6 weeks so that a total of 4–9 g of medication were administered. At the end of 2 years of follow-up, 50% of the patients were improved or stable (25).

In 1969, Milac and Miller described their experience in treating 16 patients with oral cyclophosphamide (26). Initially, patients were given sufficient cyclophosphamide to reduce their white blood cell count to 2000/mm<sup>3</sup>, but the rate of complications was considered too great at this level, so the dose was reduced to maintain the leukocyte count at 3000/mm<sup>3</sup>. The daily dose varied from 75 to 150 mg. Seven patients dropped out of the study because of adverse effects. In 1971, Wieczorek et al. reported that cyclophosphamide combined with azathioprine was efficacious in an open uncontrolled trial (27). Gopel et al. reported a similar experience (28). However, Cendrowski reported that the cyclophosphamide was no better than corti-

**Table 1**  
**Reports on the Clinical and Immunological Effects of Cyclophosphamide**

Date	Author	No. of patients	Type of multiple sclerosis	Regimen	Comments
1966	Aimard	1	Progressive		Arrest of disease in patient with progressive multiple sclerosis (MS). First reported use.
1967	Girard	30	Progressive	200 mg/day IV for 4–6 weeks; 4–9 g total	50% improved or stable at 2 years. Open label.
1969	Millac	16	Progressive	Oral, 75–100 mg/day	Toxicity associated with low white blood cell (WBC) counts.
1973	Cendrowski	23	Relapsing and progressive	100–300 mg IV for 16–33 days plus 50 mg hydrocortisone	No difference in comparison to patients treated with adrenocorticotrophic hormone (ACTH) or cortisol.
	Drachman	6	Acute attacks	4–5 mg/kg IV for 10 successive days	No effect observed on recovery from relapse.
1975	Hommes	32	Progressive	100 mg daily +50 mg prednisone bid; 8 g total over 20 days	Stabilization in 69% of patients. Open label, uncontrolled.
1977	Gonsette	110	Relapsing-remitting	IV over 2 weeks to achieve leukopenia of 2000 and lymphopenia of 1000. Dose = 1–12 g. No corticosteroids	Stabilization in 62% of patients over 2–4 years. Decrease in relapse rate. Open label, uncontrolled.
1980	Gonsette	134	Relapsing-remitting	Identical IV regimen as in 1977 report	Stabilization in relapse rate in 76% of patients. Open label, uncontrolled.
	1980	Hommes	39 Chronic progressive	400 mg cyclophosphamide (CTX) plus 100 mg	8 g total. Stabilization in 69% of patients. Open label, uncontrolled. Factors associated with response: patients with disease onset before 28 years of age, short duration of disease before



					treatment, rapid progression of disease, low initial disability and HLA-DRw2 positivity.
1981	Theys	21	Progressive	6–8 g given over 3–4 weeks	No effect in patients with moderately advanced MS over 2 years
1983	Hauser	20	Progressive	400–500 mg/day IV for 10–14 days plus ACTH	16/20 stabilized at 1 year vs 4/20 prescribed with ACTH and 9 of 18 prescribed with plasma exchange regimen. Randomized, control. No blinding or placebo control.
ACTH					
1987	Goodkin	27	Chronic progressive	Inpatient induction for 10–14 days with IV CTX/ACTH or outpatient induction with 700 mg/m <sup>2</sup> weekly for 6 weeks plus prednisone. Maintenance therapy of 700 mg/m <sup>2</sup> daily 2 months for 24 months	Stabilization in 59% of patients induced at 12 months vs. 17% in nonrandomized controls. Trend favoring maintenance therapy vs randomized controls. Nausea and vomiting a limiting side effect of maintenance therapy.
1987	Myers	14	Chronic progressive	Monthly therapy with 400–800 mg/m <sup>2</sup> oral or IV escalating to 1200–2000 mg/m <sup>2</sup> monthly; 5–13 doses given over 5–14 months to reduce B-cell and CD4 <sup>+</sup> cells. With and without steroids.	Three improved, 9 unchanged, 2 worsened. Open label, uncontrolled. Regimen found too toxic for long-term use.
1987	Siracusa	14	Chronic progressive	Short course of intensive CTX until WBC count reached 3000	Five patients discontinued because of side effects. Patients stable, although not improved. Regimen believed to be too toxic without marked clinical benefit.

(continued)

**Table 1 (Continued)**  
**Reports on the Clinical and Immunological Effects of Cyclophosphamide**

Date	Author	No. of patients	Type of multiple sclerosis	Regimen	Comments
1988	Carter	164	Progressive	2-week IV CTX/ACTH regimen	Eighty-one percent improved or stable at 1 year. Repression in 69% of patients at mean of 17.6 months. Improvement seen in younger patients with shorter disease duration. Open label, uncontrolled.
1988	Killian	14	Relapsing-remitting	Monthly 750 mg/m <sup>2</sup> IV pulses for 1 year	A trend showing decreased relapses in 6 treated patients vs 8 placebo patients ( $p = 0.6$ ). Positive response in placebo patients treated in open label continuation study. Pilot-randomized, double-blind, placebo-controlled trial.
1989	Mauch	21	Chronic progressive	8 mg/kg IV at 4-day intervals until lymphocyte count was half the initial value. 1.9 g average total dose	20 out of 21 patients stable at 1 year vs 7 out of 21 patients receiving ACTH. Open label, nonrandomized controls.
1989	Canadian	55	Progressive	1 g IV on alternate days up to 9 g plus oral prednisone	No difference vs placebo ( $n = 56$ ) or plasma exchange regime. ( $n = 57$ ). Randomized, double-blind, placebo controlled
1989	Trouillas	10	Progressive	IV (450 mg/day) for 20 days 3 weeks plus methyl-prednisolone (MP)	6 out of 10 stabilized at 3 years vs 9 out of 10 in plasma exchange regime vs. 0/10 in untreated or azathioprine controls. Open label, nonrandomized controls.

1990	Millefiorini	15	Remitting-progressive	IV CTX followed by booster daily 2 months for 2 years	50% clinically stable at 2 years. No major side effects.
1990	D'Andrea	7	Relapsing-remitting	IV induction (11 doses 300 mg/m <sup>2</sup> ) then daily 6 months for 3 years	Decrease relapse rate in all patients at 1 year; in subsequent 2 years 2 patients worse, others clinically stable.
1991	Likosky	22	Chronic progressive	IV (400–500 mg) 5 days/week until leukocyte count fell below 4000/mm <sup>3</sup>	No difference vs placebo ( <i>n</i> = 21) at 12, 18, or 24 months. Randomized, single-blind, placebo-controlled
1993	Weiner	256	Progressive	Published IV CTX/ACTH induction vs. modified IV CTX/ACTH induction (600 mg/m <sup>2</sup> on days 1, 2, 4, 6, and 8) followed by 700 mg/m <sup>2</sup> IV pulses daily 2 months for 2 years or no prescription	No difference between published or modified induction (56% stable at 12 months); Benefit of booster vs no boosters at 24 and 30 months. Better response in patients Randomized, single-blinded, 40 years or younger. Non-treatment control for boosters.
1997	Weinstock-Guttman	17	Fulminant	IV 500 mg/m <sup>2</sup> plus IV MP for 5 days followed by maintenance therapy with CTX/methotrexate, MP or interferon (IFN)-β-1b	13 of 17 (75%) patients improved or stable at 12 months; 9 of 13 (69%) at 24 months Open label, uncontrolled, consecutive patients
1998	LaMantia	30	Chronic progressive	Every 2 month IV pulses (600 mg/m <sup>2</sup> ) × 12 months with or without induction (300 mg/m <sup>2</sup> IV × 9 days)	At 12 months 75% stable if induction given; 35% stable if no induction. Increased response to treatment in remitting-progressive patients
1999	Gobbini	5	Refractory relapsing-remitting disease	Monthly pulses of CTX given for 12 months	MRI outcome: decrease in gadolinium (Gd)-enhancing lesions following pulse cyclophosphamide in all patients treated

(continued)

**Table 1 (Continued)**  
**Reports on the Clinical and Immunological Effects of Cyclophosphamide**

Date	Author	No. of patients	Type of multiple sclerosis	Regimen	Comments
1999	Hohol	95	Progressive	Induction with 1 g IV MP for 5 days followed by IV pulse CTX/MP every 1 month for 1 year, every 6 weeks × 1 year and every 2 months for 1 year	Response to therapy linked to duration of progressive disease; a trend favoring responses in secondary vs primary progressive disease
2001	Zephir	111	Progressive	IV CTX/Mp 700 mg/m <sup>2</sup> monthly for 1 year	Response in patients with clinical attack in 2 years prior to therapy
2001	Perini	26	Secondary progressive	IV CTX/mp 800–1250 mg/m <sup>2</sup> monthly for 1 year then every 2 months × 1 year	Clinical improvement at 2 year/ reduction in Gd+lesions and T2 lesion volume
2001	Khan	14	Rapidly deteriorating refractory patients	Pulse CTX 1000 mg/m <sup>2</sup> given monthly plus 20 mg IV dexamethasone	Clinical improvement or stability in 14/14 patients at 6 months sustained at 18 months following treatment
2001	Patti	10	Rapidly progressive	Monthly pulses CTX 500 mg/m <sup>2</sup> –1500 mg/m <sup>2</sup> for 18 months	Reduction in relapses, disability plus T2 MRI burden

**Table 2**  
**Factors Associated With a Response to Therapy**

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1	Rapidly progressive course
2	Gadolinium-positive lesions on magnetic resonance imaging
3	Relapses in the year before therapy
4	Less than 2 years in progressive phase
5	Younger age
6	Absence of primary progressive course

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costeroids or adrenocorticotrophic hormone (ACTH) alone (29). In addition, Drachman found no effect of 4–5 mg/kg cyclophosphamide given for 10 successive days for the treatment of acute attacks (30).

After these initial studies, Gonsette and Hommes reported positive effects in both patients with relapsing-remitting MS (RRMS) and PPMS treated with cyclophosphamide. Hommes et al. published three reports on his experience with cyclophosphamide in pilot studies of chronic progressive MS (31–33). He treated 86 patients with a short course of cyclophosphamide (400 mg/day) plus prednisone (100 mg/day) given to induce a leukopenia below 2000/mm<sup>3</sup>. Patients received a total dose of 8 g of cyclophosphamide and were treated in an uncontrolled open-label fashion. He reported on groups of 32 and 39 patients with varying times of follow-up and analyzed those factors associated with a response to therapy. He reported stabilization of disease for a period of 1 to 5 years in 69% of the patients. The factors that predicted a good response to therapy included disease onset before 28 years of age, short duration of disease before treatment, rapid progression of disease, low initial disability, and HLA-DRw2 positivity (Table 2). These factors were consistent with a report by Theys, who found no benefit of 6–8 g of cyclophosphamide given for 3 to 4 weeks in patients with moderately advanced MS (34).

Gonsette et al. treated 201 patients with RRMS with cyclophosphamide and reported on groups of 110 patients with follow-up for 2 to 6 years (35) and 134 patients with follow-up for 2 to 10 years (36). Patients were treated with IV cyclophosphamide without corticosteroids for a 1- to 2-week period and received between 1 and 2 g to maintain a leukopenia of 2000 and a lymphopenia of 1000 for 2–3 weeks. In summarizing their experience, Gonsette et al. reported a 75% decrease in the annual relapse rate in 70% of patients treated, compared to the relapse rate 1 to 2 years before treatment with the most pronounced effects in those with shortest disease and no effect in patients who were already severely handicapped. Of the patients, 30% failed to respond to cyclophosphamide. Stabilization as measured by time to next relapse was approximately 2.5 years. In addition, 60% of patients experienced improvement in neurologic signs and disability. The study was open label and uncontrolled. The authors discussed the known decrease in relapse rate that occurs in patients without treatment and stated that the decrease they observed was more than expected. They also reported that the effect of a short 2- to 3-week treatment was of limited duration (lasting 2 to 3 years) and discuss the need for a strategy to prolong the remission period.

A randomized, controlled study of the use of cyclophosphamide was reported in 1983 by Hauser et al. (37). Patients with progressive MS were treated with a 2- to 3-week course of cyclophosphamide given intravenously 400–500 mg/day 1 to achieve leukopenia of  $2000/\text{mm}^3$  plus ACTH and compared to a group that received ACTH alone or a group that received plasma exchange, ACTH, and oral cyclophosphamide. All patients were alopecic after the treatment. Although the study was a randomized and controlled trial, it was neither blinded nor placebo controlled. The results showed that 80% (16 out of 20) of the patients treated with cyclophosphamide were improved or stable at 1 year, compared to only 20% (4 out of 20) in the ACTH-treated group. The plasma exchange group showed an intermediate (50%) response. Positive clinical results were observed in terms of Expanded Disability Status Scale (EDSS), functional status scales, and numerous treatment failures. Analysis of the patient profiles demonstrates the patients were relatively young (35 years) and disease duration before treatment was between 2 and 3 years. It was reported that 11 of the 16 patients who were stable or improved at the 1-year period in the *New England Journal of Medicine* study experienced reprogression of their disease in the second or third year after treatment. Subsequent follow-up by Carter et al. of 164 patients treated with cyclophosphamide plus ACTH induction demonstrated that almost all patients began to reprogress, with the average time to reprogression being 18 months, and some patients reprogressed as soon as 6 months after cyclophosphamide therapy (38).

The Northeast Cooperative Treatment Group (39) was formed to answer two questions regarding the use of cyclophosphamide in MS. First was a modified induction regimen with a fixed dose of cyclophosphamide equivalent to a published regimen in which dose was adjusted according to white count. Second, would an every-2-month booster ( $700 \text{ mg}/\text{m}^2$ ) therapy with pulses of cyclophosphamide prevent or delay reprogression? Patients were randomized into one of four groups and evaluated in a single blinded fashion. Patient groups received either the published or modified induction, followed by outpatient pulse therapy or no pulse therapy. There was no placebo group, and differences between the booster and nonbooster groups were blunted because both groups received an initial induction. The results demonstrated no difference between the published- vs the modified-induction regimens in either initial stabilization (56%) or subsequent progression in those not receiving boosters. A modest effect of boosters in delaying reprogression was observed at 24 and 30 months in patients receiving boosters ( $p = 0.04$ ). Post hoc subgroup analyses showed an age effect with boosters delaying time to treatment failure in patients ages 18–40 years ( $p = 0.003$ ) but not in patients ages 41–55 years ( $p = 0.97$ ). In addition, patients with PPMS had a poorer prognosis than patients with SPMS ( $p = 0.04$ ). Patients with recent-onset progression responded better to boosters ( $p = 0.02$ ) than patients with progressive disease for more than 7 years ( $p = 0.58$ ). In addition, Goodkin conducted a similar study in 27 patients with PPMS who were treated with every-2-month boosters of  $700 \text{ mg}/\text{m}^2$  after induction and compared to nonrandomized controls in which a trend favoring boosters was observed (40).

Two studies have been reported in which a placebo control group was used and no positive clinical effects observed with a 2-week induction regimen (18,19). In the Canadian study, 55 patients with PPMS received 1 g of cyclophosphamide on alternate days until the white blood cell count fell below 4.5 or until 9 g were administered plus 40 mg prednisone orally for 10 days. The study was placebo controlled and single masked and included another treatment group that received plasma exchange. In the Kaiser study, 22 patients with PPMS received 400–500 mg cyclophosphamide IV 5 days per week until the leukocyte count fell below 4000/mm<sup>3</sup> and were compared to a group receiving folic acid in a randomized, single-blind study. The results of these two studies and the 1983 *New England Journal of Medicine* study and the Northeast Cooperative Treatment Group have been extensively debated in the literature (41–43). Placebo-controlled trials in MS have been subject to type 2 errors (false-negative). For example, a type 2 error occurred in a pilot study of mitoxantrone in progressive MS, which reported minimal efficacy and concluded that mitoxantrone was not worthy of further study in MS (44). Analysis of the Canadian and Kaiser results suggests that patient groups that are not responsive to cyclophosphamide were different from patient groups reported to be responsive. The placebo-treated groups were stable. In the Kaiser study at 1 year, 70% of the placebo group was stable, and 53% were stable at 2 years. The Canadian study reported 67% of its placebo group stable at 2 years. In the Canadian study, 60% of the cyclophosphamide-treated patients were classified as chronic-progressive, whereas 40% were relapsing-progressive. The Canadian and Kaiser studies do not address the use of pulse cyclophosphamide therapy in MS, which is the most common regimen currently used in patients with MS.

### ***Pulse Therapy and Magnetic Resonance Imaging Trials***

Although there have been other reports of short 2- to 3-week courses of cyclophosphamide for the treatment of MS with both positive (45,46) and negative results (47), most physicians currently use intermittent pulse therapy for the treatment of MS. Meyers treated 14 patients with escalating monthly doses to as high as 2000 mg/m<sup>2</sup>, a regimen he found too toxic for long-term use (48). Killian treated 14 patients with RMS with monthly pulses of 750 mg/m<sup>2</sup> for 1 year in a pilot-randomized, double-blind placebo-controlled trial that showed a positive trend ( $p = 0.06$ ) in six treated vs eight placebo patients (49). D'Andrea treated 7 patients with RRMS with every-6-month pulses after induction (50), and Millefiorini treated 15 remitting-progressive patients with every-2-month boosters for 2 years after IV induction (51), and both reported positive effects in open-label trials. Lamantia reported in 30 progressive patients a better response in relapsing-progressive patients and in those receiving induction plus boosters (600 mg/m<sup>2</sup>) every 2 months for 12 months (52).

With the widespread use of IFN- $\beta$  and glatiramer acetate, physicians have been confronted with refractory patients and have reported positive results after treatment with cyclophosphamide in open-label studies (52–56). These patients have been called “fulminant,” “refractory relapsing-remitting,” and “rapidly deteriorat-

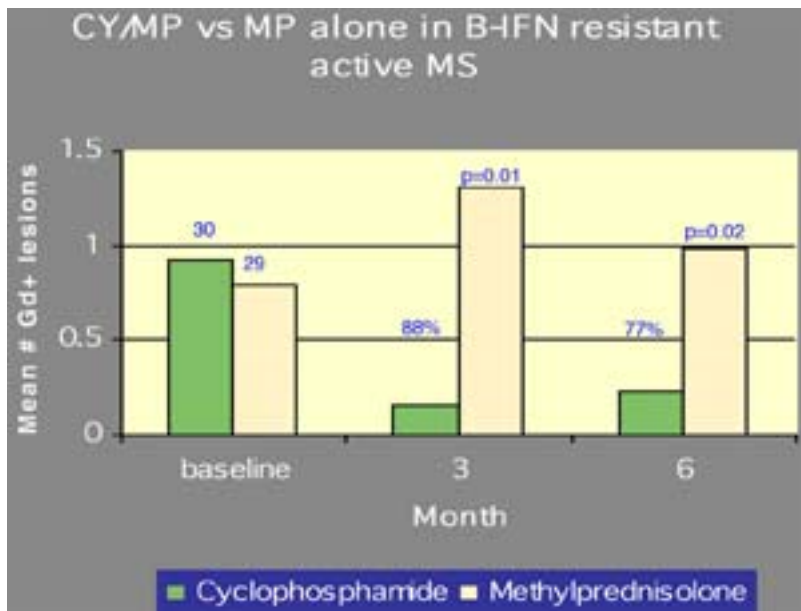


ing refractory” patients. Weinstock-Guttman et al. reported that 75% of patients improved or were stable at 12 months after IV cyclophosphamide for 5 days followed by maintenance therapy. Khan et al. reported clinical improvement or stability in 14 consecutive patients given monthly cyclophosphamide pulses (1000 mg/m<sup>2</sup>).

In the first MRI-based study of cyclophosphamide, McFarland’s group at the National Institutes of Health (NIH) treated patients with RRMS who were not responsive to other immunomodulatory drugs with monthly pulses of cyclophosphamide (1000 mg/m<sup>2</sup>). Patients were followed with monthly MRI and clinical evaluation for a mean of 28 months. All patients showed a rapid reduction in contrast-enhancing lesion frequency, and three patients showed a decrease in T2 lesion load within the first 5 months of starting cyclophosphamide treatment. The treatment was safe and well tolerated (54). MRI studies in patients receiving cyclophosphamide before bone marrow transplantation demonstrate a marked decrease in Gd-enhancing lesions (57). Perini et al. reported significant reduction of T2 lesions and Gd enhancement on MRI in 26 patients with SPMS given monthly IV cyclophosphamide pulses at 800–1250 mg/m<sup>2</sup> for 1 year and then every 8 weeks the second year (58). Significant clinical improvement was also observed, and the treatment was safe and well tolerated. Zephir et al. reported on 111 consecutive patients with progressive disease (21 primary progressive and 90 secondary progressive) treated with pulse cyclophosphamide for 12 months (59). They found that the response to cyclophosphamide was linked to whether patients with SPMS had superimposed relapses during the year before treatment. Patti et al. have recently reported on the effectiveness of a combination of cyclophosphamide and IFN- $\beta$  in patients with rapidly progressive or “transitional” MS, characterized by frequent and severe attacks plus worsening on the disability status scale (56). They treated 10 such patients with monthly pulses of IV cyclophosphamide (500 mg/m<sup>2</sup> to 1500 mg/m<sup>2</sup>) to obtain a lymphopenia of between 600/mm<sup>3</sup> and 900/mm<sup>3</sup> for 12 consecutive months and then at 2-month intervals for a further 6 months and found a significant reduction the number of relapses, disability, and T2 MRI burden of disease. They found the treatment safe and well tolerated.

### ***Combination Therapy Trial***

A trial completed in 2003 evaluated the efficacy and safety of concomitant therapy with IFN- $\beta$ -1a, cyclophosphamide, and methylprednisolone in patients with MS with active disease during IFN- $\beta$  monotherapy (60). This was a randomized, single-blind, parallel-group, multicenter trial in patients with MS with a history of active disease during IFN- $\beta$  treatment. Patients were randomized to either cyclophosphamide 800 mg/m<sup>2</sup> plus methylprednisolone 1 g IV (CY/MP) or methylprednisolone once monthly for 6 months and then received IFN- $\beta$ -1a monotherapy for an additional 18 months for a total of 24 months. The primary endpoint was change from baseline in the mean number of Gd-enhancing lesions. Secondary endpoints included the percentage of patients with Gd-enhancing lesions, change in T2 lesion



**Fig. 1.** Mean number of gadolinium (Gd)-enhancing lesions in patients with interferon (IFN)- $\beta$ -resistant active multiple sclerosis (MS) randomized to either 6 months of monthly cyclophosphamide plus methylprednisolone infusions or 6 months of methylprednisolone only infusions. Cohort sizes are given over the baseline data, which is 1 month after an initial 3-day methylprednisolone course. Magnetic resonance imaging (MRI) was obtained 1 month after infusion treatments. Patients treated with cyclophosphamide showed significantly fewer Gd-enhancing lesions vs. methylprednisolone alone at both 3 and 6 months. Percent reduction and  $p$  values are given.

burden, change in brain parenchymal fraction (BPF), time to treatment failure, and cumulative probability of relapse. Safety was assessed by the incidence of adverse events and the results of blood and urine testing.

A total of 59 patients were randomized to treatment: 30 to the CY/MP group and 29 to the MP group. Change from baseline scores in the number of Gd-enhancing lesions was significantly different between treatment groups at 3 ( $p = 0.01$ ), 6 ( $p = 0.04$ ), and 12 months ( $p = 0.02$ ) (Fig. 1). The cumulative rate of treatment failure was significantly lower in the CY/MP group compared with the MP group (rate ratio, 0.30; 95% CI, 0.12 to 0.75;  $p = 0.011$ ). CY/MP treatment was well tolerated. These results demonstrate that concomitant therapy with IFN- $\beta$ -1a, cyclophosphamide and methylprednisolone decreased the number of Gd-enhancing lesions and slowed clinical activity in patients with active disease on IFN- $\beta$  and is an option as rescue therapy for patients who are poorly responsive to IFN- $\beta$  alone.

## TREATMENT REGIMENS

As described, numerous treatment regimes have been employed, including oral administration, induction regimens given for a 2- to 3-week period, and pulse therapy given at various doses and intervals. These are summarized in Table 3 and

**Table 3**  
**Treatment Regimens**

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1. IV induction therapy: 600 mg/m<sup>2</sup> cyclophosphamide (CTX) given on days 1, 2, 4, 6, 8 plus methylprednisolone (MP) given daily for 8 days
  2. IV pulse therapy with CTX/MP after MP induction (1 g daily for 5 days): CTX pulses begun at 800 mg/m<sup>2</sup> with dose escalation designed to produce leukopenia of 2000/mm<sup>3</sup>; every 4 weeks for 12 weeks, every 6 weeks for 12, every 2 months for 12 months.  
1 g MP given with CTX. Maximum dose 1600 mg/m<sup>2</sup>
  3. IV pulse therapy with CTX at a fixed dose: CTX pulses given at 800–1000 mg/m<sup>2</sup> every 4–8 weeks for 12–24 months; given with or without MP
  4. Combination therapy: IV pulse CTX therapy given concomitantly with INF- $\beta$  or glatiramer acetate in nonresponders for various time periods
- 

contained in Table 1. In the 1990s, an outpatient protocol was adopted by many centers beginning with 5 consecutive days of IV methylprednisolone (1 g/day) and a single dose (800 mg/m<sup>2</sup>) of cyclophosphamide, followed by monthly pulses of cyclophosphamide/methylprednisolone given at increasing doses until a midmonth nadir of 1500–2000 total white blood cell/mm<sup>3</sup> was reached, from 800 mg/m<sup>2</sup> up to a maximum dose of 1600 mg/m<sup>2</sup>. Pulses were given monthly for the first year, every 6 weeks during the second year, and every 3 months during the third year. This protocol was established so that treatment would not necessarily induce alopecia, and the doses of cyclophosphamide pulses were adjusted to uniformly affect white blood cells. A retrospective study of 95 consecutive patients treated by this protocol attempted to identify factors associated with clinical response and found length of time in the progressive stage linked to a positive response, with the suggestion that patients with PPMS were a nonresponsive subgroup (61). In some instances of fulminant MS, induction therapy may be administered (62). In some studies, including the NIH MRI study of Gobbini and McFarland, pulse cyclophosphamide was given without steroids with beneficial effect, whereas most groups have administered them together. Studies from the lupus literature suggest that the addition of steroids may be superior to cyclophosphamide alone (5) and may lessen the side effects. Long-term oral use is limited by greater risk of bladder toxicity.

## TOXICITY

The adverse effects of cyclophosphamide are well known because the drug has been used for more than 30 years in the medical community (Table 4). Apart for alopecia, infertility, and nausea associated with the administration of the drug, the most frequent complication is hemorrhagic cystitis. This has been also seen in lupus nephritis protocols and has been the major reason why long-term oral cyclophosphamide has been avoided in MS. In addition, cases of bladder cancer have been observed in patients who have been treated long-term with cyclophosphamide. One should routinely obtain urine cytology at yearly intervals in patients treated with

**Table 4**  
**Side Effects**

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1	Nausea
2	Alopecia
3	Infertility
4	Infection
5	Bladder toxicity
6	Cancer risk

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cyclophosphamide and recommend yearly cystoscopy after 2 years of therapy. In addition, patients are hydrated with 3 L of fluid on the day of treatment and the day after treatment. Gonadal failure occurs in both men and women receiving alkylating agents such as cyclophosphamide. Most of the available data concern the rate of ovarian failure in cancer survivors in whom alkylating agents were used as part of a multidrug regimen and at different doses than for immunologic disease. In a controlled trial in lupus nephritis (5), a setting more relevant to MS, 23 of 46 women (50%) developed amenorrhea after receiving cyclophosphamide for 6 monthly courses then every 3 months for at least 2 more years beginning with a dose of 0.75 mg/m<sup>2</sup> then adjusting the dose based on the nadir. Risk factors for persistent amenorrhea and premature menopause include age >30 years and cumulative dose >300 mg/kg (63,64). Numerous approaches have been considered to attempt to preserve ovarian function (64). The rate of amenorrhea in women with MS treated with cyclophosphamide (approximately 40–80% in large series) appears similar to that reported for rheumatic diseases (19,39). There are few data concerning the frequency of infertility in men with immune-mediated diseases treated with cyclophosphamide. An increased incidence of subsequent malignancies has been reported in patients both with cancer treated with cyclophosphamide and with rheumatic diseases (65–67), and this may occur after therapy cessation. The magnitude of increased risk appears to increase as a function of total dose. Care must be taken when the cumulative lifetime dose exceeds 80–100 g (65–67). Based on this, at a dose of 1000 mg/m<sup>2</sup>, patients may receive approximately 50 doses during their lifetime. Some investigators have reported that cyclophosphamide is too toxic to administer because of side effects and patient discomfort (24,68). This appears related to dosing schedules, concomitant use of steroids, and use of appropriate antiemetics, because most groups have reported cyclophosphamide generally well tolerated and easy to administer (54–56,58,61).

## IMMUNOLOGICAL EFFECTS

MS may be a Th1 type cell-mediated autoimmune disease (10–12). Cyclophosphamide probably has selective effects on the immune system (Table 5). Cyclophosphamide enters the nervous system because it can be recovered from the cerebrospinal fluid (CSF) (69,70) and it reduces CSF myelin basic protein (MBP)

**Table 5**  
**Immunology**

Date	Author	Comments
1980	Bahr	Cyclophosphamide appears in the spinal fluid after 3 weeks treatment with 400 mg/day.
1982	Ten Berge	Lymphopenia involving T- and B-cells; serum levels of immunoglobulin and primary and secondary antibody responses depressed.
1983	Brinkman	Alteration in lymphocyte populations in peripheral blood and CSF.
1983	Hommes	Cyclophosphamide present at same levels in serum and CSF.
1986	Wender	Decreased synthesis of CNSIg, though not as pronounced as with high doses of prednisone.
1987	Moody	47% reduction in CD4 cells, 22% reduction in CD8 cells; magnitude of reduction of CD4 cells correlated with total dose received. B-cells reduced 50% and PHA responses reduced. No reduction in natural killer cells. Recovery of immune function occurred after 4 months.
1988	Lamers	Increased levels of CSF myelin basic protein (MBP) and intrathecally produced immunoglobulin (Ig) G reduced after treatment with cyclophosphamide and prednisone.
1989	Uitdehaag	Decreased CD4 <sup>+</sup> cells after short-term therapy (8 g in 20 days) present as long as 13.5 years after treatment.
1997	Smith	Immune deviation after pulse cyclophosphamide/methylprednisolone treatment of multiple sclerosis: increased interleukin (IL)-4 production and associated eosinophilia.
1998	Comabella	Elevated IL-12 in progressive multiple sclerosis correlates with disease activity and is normalized by pulse cyclophosphamide therapy.
1998	Takashima	Pulse cyclophosphamide plus methylprednisolone induces myelin-antigen-specific IL-4-secreting T-cells.

and intrathecally produced immunoglobulin (Ig) G (71,72). Early studies demonstrated lymphopenia involving T- and B-cells (73,74), with a more pronounced effect on CD4 cells (75,76). These changes reversed after 4 months, although others reported changes as long as 13.5 years (77). Patients treated with cyclophosphamide demonstrate increased IL-4 production and eosinophilia (78). Cyclophosphamide has a pronounced effect on interleukin (IL)-12, which may be linked to response to therapy (79). Takashima et al. found that cyclophosphamide preferentially deviates myelin-reactive cells to those secreting IL-4 (80). They reported an increased frequency of both MBP and proteolipid protein (PLP) cells secreting IL-4 in patients treated with cyclophosphamide. No such increase was observed in tetanus toxoid-secreting cells, and this change was not observed in patients treated with methylprednisolone. Cyclophosphamide therapy induced myelin antigen-specific Th2 responses.

## CONCLUSIONS

Based on the body of literature, cyclophosphamide probably reduces inflammation in patients with MS with an active inflammatory component to their illness. This inflammatory component may not be prominent in later stages of progressive disease or in patients with PPMS. It is ineffective in later stages of the disease when there is less inflammation, as is true of other immune modulators. Because early inflammatory events correlate with later disability, strong antiinflammatory drugs, such as cyclophosphamide or mitoxantrone, may have an effect on later degenerative changes if given early in the disease to halt inflammation. However, drugs such as cyclophosphamide and mitoxantrone remain limited by their toxicity for widespread use in early stages of MS.

With the approval of mitoxantrone for worsening forms of MS, the question remains of the place of cyclophosphamide in this patient group. There have been no formal comparisons between mitoxantrone and cyclophosphamide. Because there are no reported studies of mitoxantrone in later stages of MS, one might expect that like cyclophosphamide, mitoxantrone may be less effective in later progressive MS. Mitoxantrone is easier to administer than cyclophosphamide but because of cardiac toxicity, can only be given for a 2-year period and cannot be given again if patients begin to reprogress. Sequential use of these agents has been done by some investigators, but toxicity profiles are unknown at this time. Cyclophosphamide has been tested as a rescue therapy in patients not responding to the IFNs (60). Bone marrow transplantation, which is being tested in severe MS, involves treatment with cyclophosphamide. Cyclophosphamide can be administered for longer periods of time, although it too may be limited by cumulative dose considerations.

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# Repair and Neuroprotective Strategies in Multiple Sclerosis

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## INTRODUCTION

Multiple sclerosis (MS) is a demyelinating and inflammatory disease of the central nervous system (CNS). The destruction of the oligodendrocyte-derived myelin lamellae leads to loss of nerve insulation and results in impairment of fast saltatory conduction. Axons and neurons are also damaged and lost in the disease process (1), and cell repopulation is hampered by the inability of mature neurons to undergo cell division. An attractive approach for reestablishing electrochemical circuits in MS would be to restore the function or number of myelin-generating oligodendrocytes in early lesions, to act as bridges that guide and promote axonal regeneration.

MS lesions contain inflammatory cells, astrocytic scarring, myelin debris, myelin-engulfing macrophages, degraded extracellular matrix, naked axons, oligodendrocyte progenitor or stem cells, and a tumefactive nature resulting from blood-brain barrier (BBB) breakdown. Cytotoxic factors released by immune cells and the attack by antibodies and complement all act in concert, leading to the devastating white-matter lesions (2). The dynamic nature and heterogeneity of this complex milieu must be understood in more detail to better guide neuroprotective and repair attempts (3), and new technologies are being applied to elucidate the responsible pathways (4,5). In this chapter, the current knowledge of potential repair approaches, pitfalls, and challenges for research that lie ahead for achieving protection or regeneration in MS are discussed.

## MYELIN REPAIR

Because of environmental influences and the presence of established regulatory mechanisms within cells in charge of generating myelin sheaths, remyelination at MS lesion sites is not expected to be governed by exactly the same processes that operate in developmental myelination. The endogenous remyelination that occurs

in MS is incomplete. The poorly remyelinated axon has myelin sheaths of reduced length and thickness (6). This incomplete remyelination in MS could be the result of decreased progenitor cell numbers, impaired migration, or poor axonal receptiveness (7). Proliferative signals from the axon or glia to the oligodendrocytes may also fail and lead to poor remyelination. For instance, the molecule Jagged1 inhibits process outgrowth and differentiation by oligodendrocytes that express the Notch1 receptor (8). Inability of neural or glial cells to maintain downregulation of Jagged1 would, in theory, be deleterious in MS lesions.

Remyelination occurs spontaneously in animal models of demyelination and is associated with improved neurological function (9–11). The Theiler's murine encephalomyelitis virus (TMEV) SJL/J model exhibits a chronic progressive demyelinating disease and minimal spontaneous remyelination. The TMEV virus is harbored by oligodendrocytes, which are eventually destroyed during the ensuing inflammatory process. If immunosuppressed with cyclophosphamide or via genetic manipulation, however, TMEV-infected animals have enhanced myelin repair and partial functional recovery. Axonal preservation is key in ensuring that myelin repair has a positive functional outcome in this model. Other experimental models of demyelination used to study remyelination in MS include toxin (cuprizone or lyssolecithine)-induced and myelin antigen-induced demyelination. Interestingly, although antibodies against myelin proteins, such as myelin oligodendrocyte glycoprotein (MOG), play an important role in demyelination in experimental autoimmune encephalomyelitis (EAE) (12) and are found within MS lesions (13,14), other antibodies have been found to have promyelinating properties in EAE and in TMEV-induced demyelination (15–17).

Mature oligodendrocytes provide trophic support to axons and protect neurons from apoptosis (18,19). It is believed that during an autoimmune attack which targets myelin, progenitor cells can mature and migrate to areas of tissue damage to replace damaged oligodendrocytes. Identification of markers of oligodendrocytes as they mature *in vivo* is an essential piece of the puzzle that must be understood before a rational strategy for myelin repair is implemented. Oligodendrocyte progenitor cells (OPCs) express the O-2A marker, are found along the subventricular and ventricular zones, and are capable of differentiating into mature oligodendrocytes (7). Although O-2A cells are also able to differentiate into type 2 astrocytes *in vitro*, their ability to differentiate into astrocytes *in vivo* is in question. The QKI RNA-binding proteins have been recently described as markers of actively myelinating oligodendrocytes in rat brain (20). The platelet-derived growth factor- $\alpha$  (PDGF- $\alpha$ ) receptor has also been used to identify OPCs in adult human brain and in MS lesions (21). During this differentiation and migration process, these cells acquire an elaborate morphology and express various cell surface markers. Although the study of these markers is beginning and has been fraught with difficulties in correlating *in vitro* with *in situ* data, it is expected that understanding the details of this differentiation and its maintenance at the genetic regulation level will yield important therapeutic options for MS. Mature oligodendrocytes express

myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein (MAG), and MOG, whereas less mature cells express CNP and Gal-C. The presence of adult progenitor cells within the MS lesions has been demonstrated (21), but inflammation itself may promote the migration of OPCs (22). However, questions arise regarding whether the primary cells leading to partial remyelination of the MS plaque are recruited OPCs or actually represent spared oligodendrocytes or OPCs already residing within or near the lesion zone. Studies of human spinal cords with MS reveal that the numbers of mature oligodendrocytes is diminished in chronic lesions and that OPCs are present in a quiescent state (23), indicating a loss of dynamic recruitment of these cells to areas of damage (24,25).

The CNS exhibits a natural capacity for remyelination as part of a general defense and repair mechanism after injury. Oligodendrocyte precursors repopulate areas of lyssolecithin-induced demyelination in experimental rats (26). Clearly, studies in EAE have shown that spontaneous remyelination correlates with recovery (9). After MS-related optic neuritis (ON), which results in prolongation of the visual evoked potentials (VEPs), a gradual shortening of the VEPs is seen over several months, presumably resulting from remyelination events (27). This remyelination is seen shortly after acute demyelination occurs, and OPCs are actively involved in this process. This remyelination is incomplete, despite the evidence that repair at the molecular level is being promoted. For instance, genomic studies have shown that growth factor and repair genes are recruited after CNS injury (28).

The use of antibodies that promote remyelination (29) has also been proposed as a treatment for MS (30). Antibodies against glatiramer acetate (Copaxone) enhance remyelination of axons in a rat model (31). Copaxone also reduces the degree of axonal loss in the mouse model of EAE (32). Antibody-dependent complement damage to myelin could be inhibited *in vivo* by currently used therapies such as intravenous (IV) immunoglobulin (IVIg), by a protective effect already demonstrated *in vitro* (33).

## NEURONAL AND GLIAL PROTECTION

Growth factors such as fibroblast growth factor 2 (FGF2) and platelet-derived growth factor (PDGF) have been implicated in priming of cells in charge of remyelinating areas of damage (34). Others have shown that glial growth factor 2 (GGF2) (35), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), cardiotrophin 1, and oncostatin M have promyelinating function (36). Insulin-like growth factor 1 (IGF-1) has been shown to promote remyelination by some investigators (37) but not others (38). IGF-1 may, in addition, have antiapoptotic properties for oligodendrocytes (39).

Antioxidant therapies, because of their ability to interfere with pathways that culminate in apoptosis, will likely be included in the neuroprotection armamentarium for EAE (40). For instance, metallothionein-II has antioxidant, antiinflammatory, neuroprotective, and oligodendrocyte-generating action in the Lewis rat model of EAE (41). The author has demonstrated that the histone deacetylase

(HDAC) inhibitor small molecule drug Trichostatin A (TSA) has antiinflammatory properties and ameliorates MOG-induced EAE (42). In addition, TSA has neuroprotective action, which, in part, results from inhibition of caspase activation (i.e., an antiapoptotic role). In vitro studies with TSA have demonstrated that indeed this drug is neuroprotective, inhibits caspase activation, and prevents neuronal death induced by prooxidants (43). Interestingly, the histone deacetylase inhibition properties of the anticonvulsant drug valproic acid may underlie its in vitro neuroprotective function (44). Furthermore, HDAC inhibitors enhance production of the immunosuppressive and regulatory factor TGF- $\beta$ 1 (45), and regulatory T-cells have been proposed to exert neuroprotection in EAE (46).

Neurotransmitters have also been implicated in oligodendroglioneogenesis. The role of the muscarinic, dopamine, and  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptors is being studied (47), and antiglutamatergic drugs ameliorate EAE (48). Others have shown that the antibiotic minocycline blocks caspase activation and also has neuroprotective function in EAE (49–51). A combination of drugs with purported neuroprotective effects will likely lead to rational approaches to preventing disability in MS.

Axonal loss correlates with disability in the EAE model (52). It has become clear that even in acute MS lesions there is axonal and neuronal loss (1,53) and that, at least in part, this loss results from programmed cell death. Therefore, attempts at protecting neurons and the existing pools of progenitor cells should be aimed at counteracting the predominant proapoptotic pathways involved in the disease. The fas and TNF- $\alpha$  proapoptotic pathways are primary effectors for neuronal death in EAE. Downstream cascades culminate in activation of caspases 8, 9, and 3, effectors in the signaling of cell death. Recent studies in EAE have shown that overexpression of the prosurvival gene Bcl2 protects mice primed to develop EAE (54). Methods of enhancing prosurvival pathways (i.e., neurotrophin-triggered transduction signals) are also being investigated (55).

Neuronal growth factor (NGF) enhances neural growth and also has the ability to switch the T-cell phenotype from Th1 (proinflammatory) to Th2 (prohumoral, modulatory) (56). This strategy may be beneficial in neuroprotection, because the Th2 cytokine interleukin (IL)-10 reduces CNS tissue loss after contusion in experimental models (57). However, even factors that play proinflammatory roles, such as IL-1 $\beta$ , may trigger remyelination (37). Most likely, the combined and rational use of neurotrophins and cytokines will be beneficial to any attempts to preserve integrity of neurons and oligodendrocytes.

Besides resulting in damage to oligodendrocytes and neurons, tissue destruction by infiltrating immune cells eventually leads to extracellular matrix (ECM) disintegration and gliosis. Matrix metalloproteinases (MMPs) from perivascular macrophages have been implicated in the proteolytic damage to MS BBB and brain tissue, leading to a milieu that counteracts myelin or axonal repair (58), and, therefore, inhibitors of these molecules have been proposed for the treatment of MS (59). Similarly, ECM proteoglycans participate differentially in plaque tissue, delimiting



the border zones and presumably affecting repair ability at the center of the lesion (60). The role of axon-glia interactions is crucial to homeostasis in the white matter, and understanding these interactions may lead to better approaches at preventing chronic demyelination (i.e., creating the ideal environment for tissue repair to occur). Furthermore, understanding the key molecules that participate in axonal guidance after injury will be important for any repair strategies.

## CELL TRANSPLANTATION

The use of cell transplants is increasingly being proposed to repopulate areas of brain damage in MS, stroke, and neurodegenerative disorders (61). Unfortunately, the use of progenitor cells is limited by serious ethical and technical considerations related to their potential use in humans. Their general use in animal models to highlight their potential is discussed.

### *Embryonic or Adult Neural Stem Cells and Hematopoietic Stem Cells*

These cells are pluripotent and can be expanded and differentiated in vitro. Unfortunately, multiple limitations exist for their use, including the inability to direct their migration, the multifocality of lesions, and the presence of ongoing inflammation in MS. Furthermore, it is unknown if transplanted stem cells may, in some individuals, trigger a more aggressive autoimmune reaction, resulting in disease worsening, or if concomitant treatment with immune modulators or immunosuppressors may alter the ability of transplanted cells to repopulate lesion areas. Another caveat is the current inability to monitor the progress of repopulation by stem cells or their ability to repair myelin in vivo. Studies in rats have shown that neural stem cells injected intrathecally become engrafted into the lesioned spinal cord and differentiate into oligodendrocytes and astrocytes, with some behaving as Schwann cells near the adjacent nerve roots (62). Neural stem cells isolated from adult brain biopsies could potentially be used for repopulating neurons in areas of tissue damage. Importantly, recent data suggest that stem cell transplantation is protective in EAE (63) and suppresses inflammation in MS (64), but more studies regarding its long-term effects are needed.

### *Oligodendrocyte Progenitor Cells*

OPCs from both newborn and adult mouse brains have been used for transplantation experiments and are ideal for myelin repair because of their preserved migratory and mitotic capabilities. Oligodendrocyte precursors can have beneficial effects in a canine myelin mutant model, whether transplanted in neonate or adult animals (65). Recent reports have shown that in rats, it is also possible to deliver genetically transduced oligodendrocyte precursors into the ventricles and document their spreading throughout different brain regions (66). This technique shows the versatility of the cells and their ability to penetrate transependymally to potential areas of damage.

### *Schwann Cells*

Because these cells can be isolated from the sural nerve of a patient with MS and can be grown in vitro (67,68), it is feasible to transplant them into the brain to accomplish remyelination (69). A potential advantage, which remains speculative, would be that an autoimmune response may not be triggered by the autologous graft. Schwann cells have the ability to remyelinate areas of lysolecithin-induced demyelination in mouse spinal cord (70). Recently, a Yale study led by Dr. Timothy Vollmer transplanted Schwann cells into the brain of three patients with MS (summary available at <http://www.myelin.org>). This study showed that Schwann cell transplantation into the CNS is safe, but no evidence of long-term engraftment could be proved with the available techniques.

### *Olfactory Ensheathing Cells*

Recently, the use of transplants of olfactory ensheathing cells (OECs), glial cells that wrap around the axons of the first cranial nerve, has been proposed as an alternative for delivering myelinating cells into MS lesions (71,72). However, these techniques are fraught with multiple barriers, including the realization that the use of too many cells could be paradoxically deleterious (73), bringing into question how it would be possible to learn to titrate the numbers of cells for the treatment of individual lesions. OECs exhibit characteristics of Schwann cells and astrocytes and could be obtained from the nasal mucosa of a patient with MS for autologous transplantation. OECs remyelinate the spinal cord of adult rats (74) and restore functions, such as breathing and climbing, in these animals (75).

## **LESSONS LEARNED FROM NEUROPROTECTION STUDIES IN ISCHEMIA, INFECTION, TOXICITY, AND INJURY: POTENTIAL APPLICATION TO THE EAE MODEL**

Both MS and EAE are influenced by inflammatory molecules, hormonal changes, excitotoxicity, and oxidant stress mechanisms. Manipulation of these pathways, some of which culminate in neuronal apoptosis, will likely lead to neuroprotection across CNS diseases that course with degeneration or inflammation. The following sections describe neuroprotective mechanisms in animal models of ischemia, infection, excitotoxicity, and injury, because some may have potential relevance to the EAE model.

### *Immune System Modulation*

The promotion of regulatory immune cells that can penetrate the CNS and induce neuroprotection is achievable in animal models. For instance, activated myelin-specific regulatory T-cells can have neuroprotective properties in an animal model of CNS axotomy (76) via their ability to secrete neurotrophins (77). Therapies that reduce TNF- $\alpha$  also have neuroprotective effects. For instance, cytokines, such as IL-10, reduce the production of TNF- $\alpha$  and exert neuroprotection via this pathway,

as demonstrated in models of spinal cord injury (78). SB239063, a p38 mitogen-activated protein kinase (p38MAPK) inhibitor, reduces the production of IL-1 and TNF- $\alpha$  and has neuroprotective effects in a focal ischemic stroke rat model (79). Immunosuppressants, such as FK506, which binds the intracellular proteins FKBP, also have neuroprotective properties in rat models of ischemia (80). Furthermore, cyclosporin A inhibits calcium-induced mitochondrial damage and prevents axonal loss in traumatic injury models (81). Finally, NR58-3.14.3, a broad-spectrum chemokine inhibitor, exerts neuroprotection in a rat model of brain ischemia-reperfusion injury (82).

### ***Blockade of Excitotoxicity or Calcium Entry***

Intracortical injections of *N*-methyl-D-aspartate (NMDA) results in tissue damage that can be ameliorated by oral triflusal, and the underlying mechanism may involve attenuation of expression of inducible nitric oxide synthase (iNOS) expression and proinflammatory molecules, such as TNF- $\alpha$ , IL-1 $\beta$ , and COX-2 (83). Ifenprodil and eliprodil are noncompetitive NMDA receptor antagonists that inhibit calcium channel currents and protect brain tissue from experimentally induced hippocampal ischemia in gerbils (84). It has also been demonstrated that the combined use of antagonists for the excitotoxicity receptors AMPA (GYKI-52466) and NMDA (MK-801) achieves better neuroprotection than the use of each agent alone (85). MK-801 ameliorates hippocampal degeneration induced in hamsters by the measles virus (86). Drugs that inhibit glutamate release presynaptically, such as 2-chloroadenosine, also exert neuroprotection in kainic acid-injured rat striatum (87). Unfortunately, drugs that block excitotoxic pathway receptors, such as AMPA, have not been successful in human trials, resulting, in part, from poor water solubility and renal toxicity (88).

Strategies to counteract downstream pathways that mediate the effects of glutamate are also neuroprotective. These include blockade of the glutamate-induced calpain activity with the calpain inhibitor MDL-28170 and blockade of the NOS enzyme with its antagonist NG-nitro-L-arginine (89). Apolipoprotein E (apo E) also protects neurons against NMDA excitotoxicity in vitro (90). In a rat model of kainic acid-induced neurodegeneration, estradiol or estradiol plus progesterone prevents hilar neuronal loss when injected simultaneously with kainic acid in ovariectomized animals (91). Estrogen-induced neuroprotection is related to its antioxidant properties (92,93) by acting as a free radical scavenger and preventing the intracellular accumulation of peroxides. Estrogen treatment is also effective in ameliorating EAE (94,95).

### ***Blockade of Calcium or Sodium Channels***

Calcium channel blockers reduce the extent of brain or spinal cord ischemia in animal models (96,97). Flunarizine is a calcium channel blocker that at low doses can prevent ischemia-induced brain injury in fetal sheep (98). Similarly, the selective N-type calcium channel blocker ziconotide reduces ischemia-induced spinal cord injury (99).

The rise in intracellular sodium is intimately linked to calcium entry and increased cellular energy demands. Thus, sodium channel blockers may also be used as neuroprotective agents. Indeed, drugs that redistribute regional cerebral blood flow and block sodium channels such as vinpocetine (ethyl apovincamate) have been shown in experimental models to exert neuroprotection (100). Antiepileptic drugs that modulate sodium channels, such as lamotrigine, riluzole, verapamil, and diphenylhydantoin, are neuroprotective in slices of hippocampus injured by the sodium channel activator veratridine (101). In addition, the sodium-potassium adenosine triphosphate (ATP)ase pump, which extrudes intracellular sodium, and drugs that reduce extracellular sodium can be manipulated to exert neuroprotection (102). Blockade of the sodium-hydrogen exchanger may result in reduction in intracellular acidosis and thus lead to neuroprotection (102).

### ***Drugs With Mitochondrial Effects***

Creatine monohydrate has neuroprotective effects, when injected subcutaneously, in a transient cerebral hypoxia-ischemia model. Creatine may work by maintaining energy metabolism (prevent energy failure), preventing calcium overload, counteracting mitochondrial permeability transition pore opening, enhancing cytoplasmic high-energy phosphates (103), and exerting antioxidant effects (104).

### ***Antioxidants and Metal-Ion Chelators***

Studies in immature brains (105) have shown that administration of  $\alpha$ -phenyl-*N*-*tert*-butylnitron (PBN), a known superoxide scavenger, attenuates both  $H_2O_2$ - and NMDA-mediated toxicity. However, desferrioxamine (DFX), an iron chelator, is only effective when given before the administration of  $H_2O_2$ . TPEN, a metal chelator with higher affinities for a broad spectrum of transition metal ions, protects against  $H_2O_2$  toxicity but not against NMDA-induced damage.

### ***Neurotransmitter-Mediated Protection***

Neurotransmitters from specific regions of the brain can also exert neuroprotective effects on neurons. For instance, dopamine serves as a trophic factor to striatal neurons, protecting them against glutamate receptor-mediated excitotoxicity (106). Potentiators of  $\gamma$ -aminobutyric acid (GABA) receptor-mediated ion fluxes, such as isoflurane, also have neuroprotective properties (107).

### ***Vitamins and Neuroprotection***

Vitamin D hormone (1,25-dihydroxyvitamin D[3]) modulates L-type voltage-sensitive  $Ca^{2+}$  channels (L-VSCCs) and confers neuroprotection in stroke animal models and in hippocampal slice cultures (108). Antioxidants such as vitamin E exert neuroprotection via their ability to modulate activity of the transcription factor NF- $\kappa$ B (NF- $\kappa$ B), which is implicated in survival pathways (109). Ascorbic acid may reduce NMDA toxicity via blocking of calcium influx, resulting in reduced nitrous oxide induction (110).

### *The Role of Trophic Factors in Neuroprotection*

Cell populations may have different responses to excitotoxic or oxidant insult by upregulating the expression of neurotrophic factors that may be disease- or brain region-specific. Glial-derived neurotrophic factor (GDNF) and neurotrophins 3 and 4 become upregulated following intrastratial injection of glutamate receptor agonists (111). FGF2 mRNA is upregulated in astrocytes after brain injury and, when tested in animal models of stroke or excitotoxic damage, has neuroprotective action. This action results, in part, from its ability to modulate ROS detoxifying enzymes and calcium homeostasis and promote neurogenesis (112). Finally, GM1 gangliosides and their semisynthetic component LIGA20 activate the Trk neurotrophin receptors in cerebellar granule cells and thus prevent glutamate induced injury (113).

### **CONCLUSIONS**

MS has puzzled investigators for its complexity and heterogeneity. Genomic techniques such as DNA microarrays, proteomics, and the study of single nucleotide polymorphisms (SNPs), are now increasingly being used to decipher potential molecular clues of this multifactorial disease (4,5). The unveiling of these disease-associated biomarkers and pathways will likely translate into identifying potential targets of treatment. Recognizing patients with MS who are at risk for oligodendrocyte apoptosis, for instance, will help tailor individual myelin repair techniques exclusively for this group of patients. Identification of molecular pathways involved in neural protection could be harnessed for the early rescue of neurons to prevent disability in MS. Therefore, the identification of genetic polymorphisms associated with clinical disability and neuronal damage is key to identifying targets of treatment. An example is the apoE  $\epsilon$ 4 allele, which has been correlated with enhanced MS severity (114,115) and enhanced susceptibility to developing MRI T1 holes (116).

Animal research brings important insight into mechanisms playing a role in disease processes, but, unfortunately, extrapolating beneficial effects of animal treatments into human disease, especially in the field of MS, has been disappointing. A more thorough understanding of how truly versatile stem cells are for their use in human neurodegenerative diseases is needed. This includes a better understanding of how stem cells are influenced by the tissue milieu into which they are transplanted, the optimal routes of delivery, and the potential occurrence of untoward effects. Knowledge of these pitfalls will facilitate strategies for making the environment permissive for remyelination attempts. Methods for monitoring degree of remyelination in humans are also needed; magnetization transfer ratios (MTRs) in experimental models of demyelination have shown that indeed magnetic resonance techniques may be used for this purpose (117), but more accurate techniques that detect remyelination at the cellular level are required. The application of known neuroprotective drugs to the EAE model will yield new information regarding novel strategies to prevent neuronal and axonal loss. Finally, the exploration of the use of

embryonic stem cells as repair and protective agents in humans is complicated by serious ethical and technical considerations.

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## Future Therapies for Multiple Sclerosis

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### INTRODUCTION

Future immunotherapy for immune-mediated neurologic diseases is in a state of rapid development. The current therapeutic arsenal consists of a mixture of older and new agents whose uses are actively being redefined. Our current understanding of mechanisms of action of established medications and developing approaches will be discussed in relation to disease pathogenesis models. Also, we will present a framework for incorporating current and future therapies into rational treatment strategies and will be addressed together with a review of relevant clinical studies. Mechanisms of immunotherapy and clinical strategies will be discussed in the context of multiple sclerosis (MS).

### MECHANISTIC STRATEGIES FOR IMMUNE INTERVENTION

Most researchers would now agree that a T-cell mediated cellular immune response is central to the pathogenesis of MS. Although MS has not formally been proven to be a cell-mediated autoimmune disease directed against central nervous system (CNS) myelin, it is this hypothesis on which most investigators base their therapeutic strategies. Animal models for these more common neurologic immune mediated diseases have been developed. The approaches of immunotherapy or immunomodulation that are being applied to MS derive from treatment of experimental autoimmune encephalomyelitis (EAE), which has become the primary animal model for MS and from the application to MS of immunosuppressant and/or immunomodulatory drugs used for cancer, transplantation and other organ-specific inflammatory conditions. Whether EAE is a true model for MS remains an open question, but the application of immunotherapeutic strategies to MS has often stemmed from their success in EAE (1). Furthermore, independent of its relationship to MS, EAE has become one of the prime immunologic paradigms for studying cellular mechanisms of experimental autoimmune disease used by immunologists. Animal models for the other neurologic immune mediated diseases will likely also serve a useful purpose in understanding the role of new therapeutic approaches in these diseases. There are a number of ways in which immune intervention can affect

T-cell biology. Our current understanding of T-cell activation is that T-cells recognize antigens in the context of the major histocompatibility complex (MHC). Activation requires appropriate costimulatory signals by an antigen presenting cell. In MS, activated T-cells then migrate to the nervous system where they recognize the myelin antigen to which they were sensitized, release inflammatory cytokines, and initiate the destruction of the myelin sheath. Figure 1 depicts mechanisms by which immunotherapy may affect the autoimmune process in multiple sclerosis.

### *Anergy and Apoptosis*

If disease is initiated by an antigen reactive T-cell then one strategy is to block the activation of such a cell by inducing anergy. As shown in Fig. 1, the triggering of a myelin reactive cell in MS may involve antigen presentation of a cross reactive antigen such as a virus or stimulation of the autoreactive T-cell by other mechanisms such as a superantigen (2). The induction of anergy implies that the myelin reactive cell is paralyzed in a way that it is not functional and cannot therefore be activated to cause damage in the nervous system. Anergy can be induced if the myelin reactive cells encounter free antigen or an antigen is presented by a cell that does not have costimulatory properties such as B7. Of note is that orally administered antigens can induce anergy if free antigen enters the blood stream (3). The B7/CD28 interaction is required for a disease-inducing T-cell (TH<sub>1</sub> type) to be activated. Alternately, some conditions (e.g., repeated exposure of activated cells to high doses of antigen) to agonists of some tumor necrosis factor (TNF) superfamily receptors (4), or to superantigen can result in programmed cell death or apoptosis (5). This strategy could be used to eliminate autoreactive cells and may also play a role in oral tolerance (6).

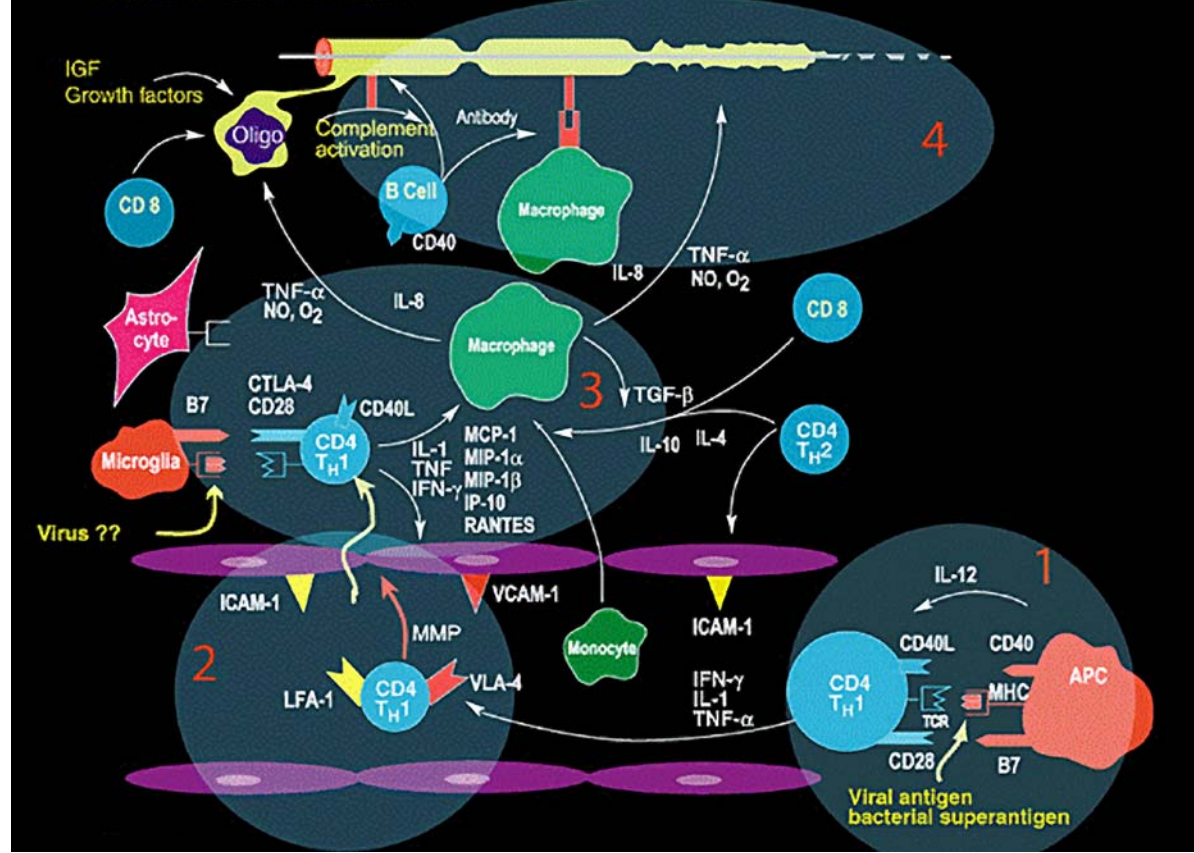
### *Receptor Blockade*

Three classes of receptor can be targeted for blockade: (1) adhesion molecules, (2) the T-cell receptor/major histocompatibility complex (TCR/MHC) complex, and (3) costimulatory molecules. In MS, myelin reactive T-cells include those with reactivity against proteolipid protein (PLP), myelin basic protein (MBP) or other

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**Fig. 1.** (*opposite page*) Immune mechanisms and strategies of immunotherapy in MS. The major steps of the pathogenetic cascade are shown: (1) activation of autoreactive T-cells in the periphery/anergy induction, (2) transmigration of proinflammatory T-cells and monocytes through the blood-brain barrier/receptor blockade leading to autoreactive T-cells, (3) generation of regulatory cells to inactivate myelin-reactive cells or secrete cytokines that suppress inflammation at the target organ, and (4) effector stage of the disease, with damage of oligodendrocytes, myelin sheath and axons. A possible further stage, the resolution of lesions by regulatory mechanisms and remyelination, is not highlighted separately. *Abbr:* APC, antigen presenting cell; TNF, tumor necrosis factor; TCR, T-cell receptor; ICAM, intracellular adhesion molecule; MHC, major histocompatibility complex; MMP-matrix metalloProtease; IL, interleukin ;VCAM, vascular cell adhesion molecule; IFN, interferon; LFA, leukocyte function antigen; TGF, transforming growth factor.

### Elements of the MS lesion





myelin antigens such as myelinoligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG), or other undefined antigens (7). These cells can be blocked with compounds that affect their ability to migrate into the nervous systems by interfering with their adhesion to endothelial cell surfaces. In addition, receptor blockade by antagonists for components of the MHC/TCR complex or costimulatory molecules could prevent myelin specific cells from being activated.

### ***Regulatory T-Cells and Immune Deviation***

Although the biology of regulatory or suppressor T-cells is not completely understood, there are two types of regulatory T-cells that have been characterized and found effective in the EAE model and can be applied for the treatment of neurologic immune mediated diseases. One is an anti-clonotypic or an anti-idiotypic T-cell that reacts with antigen specific autoreactive T-cells (8). These cells may be generated during T-cell vaccination. The second is an antigen specific regulatory T-cell. These regulatory T-cells can migrate to the CNS or cervical lymphatics where they encounter antigen and secrete antiinflammatory cytokines such as IL-4, IL-10 and transforming growth factor (TGF)- $\alpha$  that suppress inflammation (9,10). These cells can be generated by oral toleration. As more is learned about immune mechanisms of inflammation and regulation, it appears there is a balance between TH<sub>1</sub> and TH<sub>2</sub> type responses. Disease inducing cells, or TH<sub>1</sub> type cells, can secrete inflammatory cytokines such as IL-2, TNF, and interferon (IFN)- $\gamma$  which recruit other T-cells and macrophages that induce inflammation. Regulatory TH<sub>2</sub> type cells secrete cytokines that suppress inflammation. Strategies to block TH<sub>1</sub> type cytokines or administer or enhance TH<sub>2</sub> type cytokines are being developed. Similarly, anti-clonotypic T-cells may also function by secreting antiinflammatory cytokines.

Although it is possible to interrupt an immune response in the EAE model with the aforementioned strategies of immune manipulation, not all of these strategies are easily translated into humans. For example, not all compounds cross the blood-brain barrier and some compounds need to be administered by intravenous or subcutaneous injection over prolonged periods of time. In animal models, the time of immunization and disease induction is known so that therapeutic agents can be given at critical points in the immune response. In MS, the intermittent chronic activation of the immune system makes such time dependent targeted therapy difficult. Furthermore, one of the difficulties with immune-specific therapies is that, although the specific therapy may suppress animal models of disease in which inbred strains are used, patients represent an outbred population. Thus, individual patients may recognize different portions of an autoantigen and use a relatively nonrestricted set of T-cell receptors.

## **ANTIGEN-SPECIFIC IMMUNOTHERAPY**

Antigen-specific modulation of the immune response is an attractive approach for treating an autoimmune disease (Table 1). The difficulty with any antigen-specific therapy, however, is the presumption that one knows the autoantigen that patho-

**Table 1**  
**Antigen Specific Immunotherapy**

1. Glatiramer acetate (Copaxone)
2. T-cell receptor
3. Antigen-MHC complexes
4. Oral tolerance

genic T-cells attack and that there is only one autoantigen being attacked. Furthermore, once a tissue is inflamed, multiple reactivities may develop and inflammatory cells that are not specific for myelin antigens could play an important role in the ongoing disease process. The spreading of autoimmune responses has been shown in EAE and diabetes models. In rheumatoid arthritis, even though it is initiated by T-cells, joint inflammation and destruction may become T-cell independent processes. In addition,  $\gamma$ - $\delta$  T-cells reactive to heat shock proteins may be secondarily generated and participate in CNS inflammation and damage.

**Glatiramer Acetate (Copaxone)**

Glatiramer acetate (GA) (Copaxone) is a random polymer designed initially based on the amino acid composition of MBP. The mechanism by which Copaxone may work in humans is unknown. It could function by generating regulatory cells as this has been shown in animals treated with altered myelin peptides (11,12). Altered myelin peptides will likely also reach clinical trials. In addition, proliferation of MBP specific T-cell clones has also been reported to be suppressed by Copaxone and it theoretically could interfere with binding of MBP or other peptides to the MHC cleft (13,14).

Experimental studies in EAE have shown that orally administered GA can suppress disease and that the mechanism of suppression is identical to that when administered subcutaneously (15). A multinational trial of oral GA (the CORAL study) has recently been completed. Patients ( $n = 1650$ ) with clinically definite relapsing-remitting (RR) MS and one or more attacks in the year prior to entry were randomized to 5 or 50 mg of either GA or placebo tablets, given daily for 56 weeks, and were evaluated every 2 months. Magnetic resonance imaging (MRI) scans were done at baseline and at completion of the study. A subset of those patients had MRIs every 2 months. The primary outcome measure was the total number of relapses per group. This study has been completed recently, and although the results have not been published, a statistically significant effect was not observed, although the group receiving the higher dose demonstrated a tendency toward a therapeutic benefit. New formulations of oral GA are currently being developed for future testing. A study of GA in primary-progressive MS (PPMS), carrying the acronym PROMISE, was launched approximately 2.5 years ago. The results of this study are eagerly awaited. The oral GA trial in RRMS was found to be negative (no benefit) although the results have not been published to date.

### *T-Cell Vaccination and T-Cell Receptor Therapy*

In animal models, restricted T-cell receptor usage by MBP reactive cells has been demonstrated and it is possible to suppress EAE by vaccinating or immunizing with MBP reactive clones or T-cell receptor peptides (8,16,17). This approach induces regulatory cells that interact with the injected clones or peptides. It has been shown that the mechanism for this protection involves both an "anti-activated T-cell" response which is short lived, and anti-clonotypic T-cell responses, which are longer lived. Moreover, animal experimentation has suggested the safety of T-cell vaccination using either fixed or irradiated T-cell clones. Two phase 1 studies of T-cell vaccination have been performed in MS patients. In the first, four subjects with progressive MS were given a total of seven inoculations of attenuated, autologous T-cell clones isolated from the cerebrospinal fluid (CSF) (18). These cells were not antigen-specific. There were no untoward side effects and immunologic studies suggested that the inoculation of autologous T-cell activated clones was associated with partial, short-term immunosuppression as evidenced by downregulation of subsequent stimulation via the CD2 pathway of activation. In addition, the autologous mixed lymphocyte response, which is reduced in about half of the patients with MS, was enhanced for a short period of time after the T-cell vaccination. In a second study, six patients were inoculated with MBP specific T-cells (19). It was found that T-cell responses to the inoculants developed and there was a depletion of MBP reactive cells in the recipients. Furthermore, CD8<sup>+</sup> anti-clonotypic T-cell lines were isolated from treated patients that recognized the CD4<sup>+</sup> MBP-specific T-cells. In addition to using T-cell clones to vaccinate, in a phase 1 trial investigators have inoculated MS patients with immunogenic regions of the T-cell receptor that recognizes immunodominant regions of MBP; changes in MBP reactive cells have been reported. The conceptual problem with T-cell vaccination and T-cell receptor therapy is that, in MS, T-cell receptor usage of MBP reactive cells is not likely to be as restricted as in animals. Furthermore, there are reactivities to other myelin antigens such as PLP in MS (20). Thus, T-cell vaccination or T-cell receptor therapy may be too specific, downregulating only a small proportion of autoreactive cells. Although a subcategory of patients could theoretically benefit from such a therapy treatment, it would have to be administered very early in the disease course. There is a possibility that treatment may not be effective over time in a chronic disease such as MS.

### *Anergy Induction*

An alternative approach to inactivating autoreactive T-cells is to use free antigen, antigen coupled to autologous cells, or antigen-MHC complexes. This approach induces anergy and has been used successfully in the EAE model. It is well known that intravenous administration of soluble antigen can lead to tolerogenic signals to the immune system. Investigators attempted phase I trials to induce tolerance to MBP by injecting MBP into MS patients but were unsuccessful and, in some instances, sensitized patients. Although less specific than T-cell receptor

therapy, because all cells reacting to a particular antigen are suppressed, anergy induction does not address the issue that there is reactivity to more than one myelin antigen in MS. However, it is theoretically possible to anergize against a number of different myelin antigens. An important issue for clinical use is how often the treatment would need to be given, by what route, and whether sensitization could occur with repeated treatment. Similar issues are raised by approaches which attempt to induce apoptosis of autoreactive cells and which are as yet less developed.

### **Oral Tolerance**

Oral tolerance refers to the long recognized observation that proteins that pass through the gastrointestinal tract generate systemic hyporesponsiveness. Thus, if animals are fed a protein such as ovalbumin or MBP and are subsequently immunized, the immune response against the fed antigen is reduced. Oral tolerance evolved to allow the mucosal immune system (gut-associated lymphoid tissue [GALT]) to absorb proteins without becoming sensitized to them. In recent years, a great deal has been learned about the mechanism of oral tolerance and the use of oral tolerance has been successfully applied to several animal models of autoimmune diseases including animal models of MS, arthritis, uveitis, diabetes, thyroiditis, myasthenia, and in transplantation (21). Oral tolerance is also being tested clinically in the human disease states of MS, rheumatoid arthritis, uveitis, and juvenile diabetes. Depending on the amount of antigen fed, orally administered antigens result in the generation of active suppression or anergy. Low doses favor active suppression whereas higher doses favor anergy or apoptosis (3,6). The doses and strategy being used in clinical trials are aiming to generate regulatory T-cells that suppress inflammation at the target organ. Antigens that pass through the gut preferentially generate TH<sub>2</sub> type cells that secrete IL-4, IL-10, and TGF- $\beta$ . Such cells leave the gut and migrate to the organ that contains the fed antigen. The regulatory cells are then stimulated to release antiinflammatory cytokines. Oral tolerance can be conceptualized as a natural drug delivery system in which physiologic antiinflammatory cytokines are delivered to the target organ by one's own cells, depending on the fed protein. Thus, it is not necessary to know what the autoantigen is in an autoimmune disease in order for oral toleration to be effective. In this regard, it has been shown that MBP or PLP induced EAE in the mouse can be suppressed by MBP feeding (22). Recombinant human proteins and the use of compounds that enhance the generation of TH<sub>2</sub> type regulatory cells may enhance the biologic effects of oral tolerance. Of note is that in animals, IFN- $\beta$  enhances the protection by orally administered myelin antigens (24).

### **ANTIGEN-NONSPECIFIC IMMUNOMODULATION**

A number of approaches of antigen-nonspecific immunotherapy have been shown to be successful in animal models and are being considered for clinical trials (Table 2). They illustrate approaches that are designed to affect the immune system in a number of different ways. They are listed as having minimal to moderate systemic toxicity.

**Table 2**  
**Antigen-Nonspecific Immunomodulation**

- 
1. Interferons
    - a.  $\alpha$  (IFN-  $\beta$ a-n3 [Alferon] and Interferon-  $\beta$ a-2a [Roferon])
    - b.  $\beta$  (Avonex, Betaseron, Rebif)
    - c.  $\tau$
  2. Drugs to alter lymphocyte traffic
    - a. Selective Anti-adhesion Molecule MAbs (anti-VLA-4 MAb [Antegren])
    - b. Matrix metalloproteinase inhibitors
    - c. Antivirals
    - d. Nitric oxide synthase inhibitors
  3. Monoclonal antibodies
    - a. Campath
    - b. Anti-CD-40/Anti-CD2/Anti-CD4/Anti-T12/and others
    - c. CNTO 1275
    - d. Zenapax
    - e. Antegren
    - f. Rituxan
    - g. WA-J695
  4. IL-2 toxin
  5. TGF- $\beta$ /IL-4/IL-10
  6. TNF antagonists (Pentoxifylline/anti-TNF MAbs)
  7. Immunoglobulin
  8. Plasmaphoresis
  9. Unclassified
    - a. Hormone therapy (estrogen, testosterone)
    - b. Anticholesterol medications (Statin drugs)—(Lipitor, Zocor)
- 

*Abbr:* IFN, interferon; MAbs, monoclonal antibodies; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

### *Interferons*

IFN- $\alpha$ , - $\beta$ , and - $\tau$  are type I interferons that appear to act through the same receptor. It is not yet clear whether there are immunologically significant differences in the way they activate this receptor. The intracellular events following receptor binding are being rapidly elucidated. Whereas IFN- $\beta$  has been shown to affect the course of relapsing-remitting MS, the important question of its mechanism of action at the level of cellular interactions is currently unknown (24). The possibilities are as follows: (1) IFN- $\gamma$  augments immune responses and has been shown to increase MS attacks (25). IFN- $\beta$  decrease IFN- $\gamma$  production by activated lymphocytes (26). (2) IFN- $\beta$  may help reverse a suppressor cell defect that has been described in MS (27), although the defect is more common in progressive MS patients (28). (3) Viral infections have been associated with increased MS attacks (29) and IFN- $\beta$  could be working through its antiviral effects, although there is no evidence from the trial that IFN- $\beta$  worked by this mechanism (24). (4) As described in Fig. 1, a balance between TH<sub>1</sub> and Th<sub>2</sub> type immune responses may be important

in MS and IFN- $\beta$  may favor the generation of TH<sub>2</sub> type responses (30). (5) IFN- $\beta$  has been shown to down regulate IL-2 receptor on lymphocytes of treated MS patients, and thus may act to block the expansion phase of an immune response (31). (6) IFN- $\beta$  can stimulate IL-10 production by macrophages which can suppress immune responses (32). Oral IFN- $\tau$  was shown to be effective in EAE (33). An open-label phase I clinical trial in humans revealed safety up to a dose of 1.8 mg administered daily for 29 days (34). There was also a dose dependent rise in IL-10. No neutralizing antibodies were detected. A large multi-center phase II trial will begin enrollment in July 2004.

### *Drugs to Alter Lymphocyte Traffic*

It is known that there is rapid trafficking of cells from the peripheral immune system into the CNS in MS (35). In this regard, total lymphoid irradiation had an ameliorating effect on progressive MS and only involved manipulation of the peripheral immune system. EAE can be suppressed by monoclonal antibodies (MAbs) and other drugs that alter traffic of the cells into the nervous system (36,37) and such compounds are being considered for treatment of MS. Such treatment has the advantage of not being dependent on antigen specificity of the migrating cells. However, one potential difficulty with this approach is that it is given at a specific point in time when the disease is active in animals and such time-dependent therapy is difficult in MS. The degree to which it could be applied to the chronic progressive type of MS remains to be seen.

#### *Anti-Integrin Antibodies (Natalizumab/Antegren)*

The formation of inflammatory lesions in patients with MS may involve lymphocytes and monocytes that gain access to the brain parenchyma from the circulation by first adhering to vascular endothelial cells (38). The glycoprotein  $\alpha$ 4 integrin is expressed on the surface of inflammatory lymphocytes and monocytes and may play a critical role in their adhesion to the vascular endothelium (39,40). These observations have led to investigations of  $\alpha$ 4 integrin antagonists. Treatment with these agents in rodent models of multiple sclerosis resulted in reduced signs of disease activity and inflammation (41–43). A small preliminary, placebo-controlled study in humans using the monoclonal antibody natalizumab, an  $\alpha$ 4 integrin antagonist, also found that the drug reduced the frequency of new gadolinium-enhancing lesions (44). These findings have now been extended in a larger and longer double-blind trial in which 213 patients with relapsing-remitting or relapsing-secondary-progressive MS were randomly assigned to receive 3 mg/kg or 6 mg/kg of intravenous (IV) natalizumab, or placebo, every 28 days for 6 months (45). Both natalizumab groups had significant reductions in the mean number of new brain lesions on monthly gadolinium-enhanced MRI (9.6 for placebo vs 0.7 and 1.1 in the 3 mg/kg and 6 mg/kg natalizumab groups, respectively). This effect was evident 1 month after the first infusion and was sustained throughout the treatment period. In addition, there were significantly fewer relapses in the natalizumab groups (44,45). Whereas no direct comparative studies have been performed, these

results suggest that natalizumab may be more effective than the interferons or glatiramer acetate. Therapy with natalizumab was well tolerated, with a safety profile similar to that of placebo. On the basis of the above study results, Biogen-Idec is sponsoring two large multi-center double-blind placebo-controlled trials with Antegren. One trial uses IV Antegren as monotherapy monthly for 2 years. The other study combines Antegren with Avonex. One-year data has been submitted to the Food and Drug Administration (FDA) and early approval will be determined in late 2004.

### ***Monoclonal Antibodies***

Phase I studies of anti-T-cell MAb infusions have been undertaken in MS studying the immunologic effects of three anti-T-cell MAbs: anti-CD2, anti-CD4, and anti-T12 (46,47). Investigators reported that anti-T-cell monoclonal antibody infusions suppressed *in vitro* measures of the human immune response. Specifically, an anti-CD2 monoclonal decreased T-cell activation by phytohemagglutinin, and anti-CD4 MAb infusions abolished pokeweed mitogen-induced immunoglobulin synthesis without lysis of the CD4<sup>+</sup> T-cell populations. With repeated infusions, human anti-mouse antibodies were found in the circulation. Although most of the human anti-mouse antibodies were not immunoglobulin isotype-specific, significant anti-idiotypic activity was observed after repeated infusions. Other investigators reported that anti-CD3 infusions were associated with significant toxic effects in MS patients (48). Although the clinical usefulness of currently available anti-T-cell murine MAbs in chronic disease such as MS is hampered by human anti-mouse antibodies, there are a number of potential approaches to solve the problem. First is the use of humanized MAbs with a human Fc and mouse Fab region. A second approach is to attach a toxin to the murine MAb, resulting in both a greater elimination of targeted T-cell population and potentially preventing human anti-mouse antibodies. More extensive studies with anti-CD4 have been carried out by Steinman and colleagues (unpublished data) at Stanford using a humanized anti-CD4 MAb, with a larger trial undertaken at the Institutes of Neurology at Queen's Square, London. At Stanford, patients with chronic progressive MS were treated with a chimeric anti-CD4 antibody with a humanized Fc region (1). Results of the large trial were essentially negative despite very marked reduction in CD4<sup>+</sup> cells (49,50). Possible explanations are that MS becomes a T-cell independent disease late in the course or that regulatory T-cells were also removed by this therapy. The effects of anti-CD52 (Campath 1H), a lymphocyte depleting humanized MAb, on brain and spinal cord atrophy were investigated in a crossover trial of 25 patients with SPMS and an EDSS score between 4.0 and 6.0. Patients received intravenous Campath 1H 20 mg for 3 to 4 hours for 5 consecutive days and were followed for 18 months. Campath 1H markedly reduced the mean number and volume of Gd<sup>+</sup> lesions from baseline over the course of the study. Despite these findings, 13 of 25 patients had substantial decreases in brain volume. Of the 14 patients with



data available, the mean spinal cord area was reduced from 94 mm<sup>2</sup> at baseline to 87.6 mm<sup>2</sup> at 18 months ( $p < 0.001$ ) (51).

In a multi-arm, multiple dose, 6-month phase 2 trial (47 treated, 12 placebo) (52), the recombinant humanized immunoglobulin G<sub>1</sub> monoclonal antibody ATM-027 was shown to deplete V $\beta$  5.2/5.3+ T-cells, reduce the expression of T-cell receptors, and reduce T-cell reactivity to some myelin antigens. This treatment was without apparent toxicity. Serial studies showed a minor degree of reduced MRI activity (perhaps 10-30%), but not to a level of statistical significance. Prolonged (18 months) V $\beta$  5.2/5.3+ T-cell depletion was observed in a second, small study of 14 patients (53).

### ***IL-2 Toxin***

IL-2 toxin targets activated cells and has been shown to be effective in some animal models. It is being tried in diseases such as rheumatoid arthritis and in multiple sclerosis.

### ***TGF- $\beta$***

This naturally suppressive cytokine that has been shown to suppress animal models of autoimmune diseases including EAE and arthritis (54). As discussed previously, it mediates the effect of regulatory cells generated by oral tolerance. Trials of injectable TGF- $\beta$  have been initiated in MS. There has been some interest in trying IL-4 or IL-10 similarly.

### ***TNF Antagonists***

Because TNF may be a mediator of tissue damage in MS, investigators are considering treatment with TNF antagonists in MS. This includes the use of pentoxifylline, a drug which favors Th2 type responses and downregulates TNF production (55) and the use of anti-TNF monoclonal antibodies. Such antibodies have shown some usefulness in rheumatoid arthritis (56).

### ***Immunoglobulin***

Intravenous immunoglobulins (IVIg) mechanism of action is unclear but may relate to anti-idiotypic effects (57). One hypothesis based on animal work, which serves as basis for an IVIg trial in MS, is that it can promote remyelination (58). A detailed discussion is presented in the next section.

### ***Plasmaphoresis***

This process removes plasma proteins and replaces them with colloid and will be discussed in the next section.

### ***Unclassified***

The results of an open pilot study of estradiol in MS (59) and a report describing the immunomodulating effects of cholesterol-lowering statins (60) have fueled support for definitive large-scale MS trials (61).

**Table 3**  
**Antigen-Nonspecific Immunosuppression**

1. Azathioprine (Imuran)
2. Cyclophosphamide (Cytosan)
3. Mycophenolate Mofetil (Cellcept)
4. Cyclosporine
5. Methotrexate
6. Mitoxantrone (Novantrone)
7. Total lymphoid irradiation (TLI)
8. Cladribine
9. Bone marrow transplant

## ANTIGEN-NONSPECIFIC IMMUNOSUPPRESSION

Table 3 lists immunosuppressive agents which have been used in neurologic immune mediated diseases. The mechanisms of immunosuppression of these agents are likely multiple. One shared component is probably a direct cytotoxic effect on pathogenic leukocytes. However, this cannot explain the entire effect as severely leukopenic treated patients with very active autoimmune disease have been seen. Therefore, other mechanisms should be involved. Data suggests that some pulse cyclophosphamide treated chronic progressive MS patients have a shift to from Th1 to Th2 type cytokine production (30). On the other hand, in diseases characterized by hypergammaglobulinemia and exaggerated B-cell responses these functions seem to be selectively reduced by cyclophosphamide therapy (62). An explanation for these findings is that proliferating cells are selectively vulnerable to the cytotoxic effects of cyclophosphamide as is the case in *in vitro* studies. In addition, monocyte functions and cytokine production are inhibited and this may impact both antigen presentation and effector mechanisms. Azathioprine has similar effects to cyclophosphamide except that cytokine production seems to be spared. The main effects of cyclosporine seem to be the result of a down regulation of IL-2 and IL-2 receptor and thus lymphocyte activation. Mycophenolic acid (Cellcept) is also being studied in MS patients and results are pending. Mitoxantrone (Novantrone) is a potent immunosuppressant agent given intravenously every 3 months that was recently approved for use in worsening relapsing-remitting and secondary-progressive MS. A detailed discussion can be found in Chapter 9. Although mitoxantrone is now FDA approved for use in MS, a 2003 report of the Therapeutics and Technology Subcommittee of the American Academy of Neurology recommended that, because of its toxicity and the somewhat limited evidence of benefit, mitoxantrone should be reserved for patients with rapidly advancing disease who have failed other therapies (63).

## CORTICOSTEROIDS

Corticosteroids have been used in autoimmune disease since the 1940s but their mechanism of action remains poorly understood. Similarly to type I interferons, the

intracellular events following glucocorticoid receptor binding are being elucidated. The principle immunomodulatory effects seem to involve binding of the activated glucocorticoid receptor to immunoregulatory transcription factors, thus preventing them from activating genes, as well as increasing expression of inhibitors of these transcription factors (64). At the level of cellular interaction there are effects on leukocyte circulatory kinetics, functional capabilities, and cytokine production, all of which probably play a role in their therapeutic benefit. The benefits of intravenous corticosteroids in acute inflammation in MS likely result from decreasing the entry of cells into the brain and by preventing proliferation and perhaps inducing apoptosis of activated cells. These agents deplete CD4<sup>+</sup> cells, decrease cytokine release including TNF and IFN- $\gamma$ , and decrease class II expression (65). In animal models, glucocorticoids have been shown to cause a shift from Th1 to Th2 cytokine production (66). In humans, DTH-type responses are suppressed after only 2 weeks of therapy. In MS, steroids have also been shown to decrease IgG synthesis in the CNS and reduce CSF antibodies to MBP and oligoclonal bands. The factors underlying the difference in response to oral vs intravenous glucocorticoids brought out in the optic neuritis trial are unknown (67,68). Intravenous methylprednisolone (IVMP) is often used for the treatment of MS relapses. The effects of IVMP on BPV were studied in a randomized, single-blinded trial of 88 patients with RRMS and EDSS scores of less than or equal to 5.5. Patients received regular pulses of IVMP (1 g per day for 5 days, with an oral prednisone taper) and the same treatment for relapses as required ( $n = 43$ ) or IVMP (same regimen) only for relapses ( $n = 45$ ). Over the 5-year study, pulsed doses of IVMP were administered every 4 months for the initial 3 years and then every 6 months thereafter. At baseline, mean BPV was 1254.8 ( $\pm 71.8$ ) mL and 1263.4 ( $\pm 66.4$ ) mL in the two treatment groups, respectively ( $p = \text{NS}$ ). At 5 years, the absolute change from baseline in BPV was +2.6 mL in the pulsed IVMP group compared with -74.5 mL in the relapse-only group ( $p = 0.003$ ). Thus, pulsed IVMP prevented progressive brain atrophy in this study, although randomized, double-blind, placebo-controlled trials are needed before definitive conclusions can be made (69).

## PRINCIPLES FOR CLINICAL APPLICATION OF THERAPIES

The following principles are important for immunotherapy and can serve as a framework from within which to view the various treatment modalities that are currently being used and tested (Table 4). In essence, a treatment hierarchy is given with the least toxic therapy given first. Some of the new therapies now available to MS patients are beginning to make an impact on immunotherapy of other neurological immune-mediated diseases. Advances in MRI and a clearer understanding of how the immune system functions have made an impact on developing therapy for MS.

### *First-Line Therapy*

IFN- $\beta$ s (Avonex, Betaseron, Rebif) and Glatiramer Acetate (Copaxone) represent a class of first-line drugs for the treatment of MS. Some forms of antigen-specific therapy such as T-cell or T-cell receptor vaccination could also represent

**Table 4**  
**Approach to Immunotherapy for Neurological Immune Mediated Diseases**

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1. First-line therapy
  2. Corticosteroid usage
    - a. Acute exacerbations
    - b. Chronic therapy
  3. Second-line immunosuppression to halt refractory or progressive disease
    - a. Immunosuppressants
    - b. Plasma exchange/IVIg
    - c. Bone marrow transplant
  4. Reinstitution of first-line therapy
  5. Combination and pulse therapy
  6. Neuroprotection
  7. Disease monitoring
- 

first-line therapy. Ultimately, drugs such as Copaxone must be compared to IFN- $\beta$  in terms of efficacy, ease of administration, and side-effect profile. Based on the results of the recent CHAMPS trial, Avonex has been approved for patients with a clinically isolated syndrome (CIS) and an abnormal MRI. In addition, the cost of treatment will be an important factor in therapy if there are no major differences in efficacy or toxicity profile between first-line drugs. These immune modifying medications are discussed in detail in Chapter 7.

### ***Appropriate Use of Steroids***

#### *Steroid Use in Exacerbations*

Based on what is currently known about IFN- $\beta$  and other relatively nontoxic drugs that could be given early in the course of MS, it is expected that patients will continue to have relapses. Thus, short courses of IV methylprednisolone are given when a significant relapse occurs to speed recovery. Given what was reported in the optic neuritis study, IV methylprednisolone may have a prolonged salutary effect on the course of MS. Acute attacks are either not treated or are treated with a short course of corticosteroids. Indications for treatment of a MS relapse include functionally disabling symptoms with objective evidence of neurological impairment. Thus, mild sensory attacks are typically not treated. In the past adrenocorticotropic hormone and oral prednisone were primarily used. More recently, physicians are treating with short courses of IV methylprednisolone (500–1000 mg daily for 3–7 days) with or without a short prednisone taper (65). Optic neuritis may occur during the course of MS or be one of the initial symptoms. A recent trial of optic neuritis demonstrated that patients treated with oral prednisone alone were more likely to suffer recurrent episodes of optic neuritis as compared to those treated with methylprednisolone followed by oral prednisone (67,68). These results now make intravenous methylprednisolone the primary treatment used

for optic neuritis and has further supported its use for major attacks. Furthermore, as part of the optic neuritis study, it was found that treatment with a 3-day course of high-dose methylprednisolone reduced the rate of development of MS over a 2-year period (70). The protective effect was most apparent in patients at highest risk for MS, those with multiple focal brain MRI abnormalities. Although these results need to be confirmed in a larger series of patients, they support the use of high-dose IV methylprednisolone for acute MS attacks. High-dose IV methylprednisolone appears to be accompanied by relatively few side effects in most patients although a number of side effects have been reported including mental changes, unmasking of infections, gastric disturbances, and an increased incidence of fractures. Anaphylactoid reactions and arrhythmia may also occur.

### *Chronic Steroid Usage*

The goal is to achieve the lowest possible dose that will maintain quiescence of disease to avoid the long term side effects of steroids. Monthly pulses of intravenous methylprednisolone for the treatment of relapsing-remitting progressive and progressive MS has been investigated and shown to have some benefit. Long-term side effects of corticosteroids include cataracts, osteoporosis, aseptic bone necrosis, Cushing's syndrome, hypertension, and exacerbation of diabetes. Corticosteroids are discussed in detail in Chapter 9.

### ***Second-Line Immunotherapy to Halt Refractory or Progressive Disease***

Although some patients may respond to first-line treatments, it is expected that others will be refractory to therapy or enter a progressive stage. In these instances, immunotherapy or immunosuppression with stronger medication may be warranted depending on the individual case. Drugs in this category would be classified as second- or third-line treatment depending on the risk–benefit ratio of the therapy. Second- or third-line therapy could find utility even if such treatment could only be given effectively on a short-term basis if such treatment allowed reinstatement of first-line therapy. The neurologic immune mediated diseases being discussed all have progressive forms and experienced clinicians vary in their preferences for which agents to use for these. Treatment directed at the progressive phase is the most difficult because the immune mediated diseases may be harder to affect once a progressive stage has been initiated. For this reason, there is a trend toward using these agents either prior to or earlier during the progressive stage in some autoimmune diseases such as rheumatoid arthritis.

### *Immunosuppressive Agents*

Cyclophosphamide (CTX) total lymphoid radiation (TLI), Cellcept, Novantrone, cyclosporin, azathioprine, methotrexate, and cladribine have shown some positive clinical effects in neurologic immune mediated diseases. Cytoxan is discussed in detail in Chapter 10. TLI has potent immunosuppressive effects and a double-blind study of lymphoid irradiation reported benefit in patients with progressive MS

(reviewed in ref. 71). The absolute lymphocyte count appeared to be a crude indication of therapeutic efficacy, with greater efficacy in patients with lower counts. Many patients started progressing again after initial therapy and a major limitation of the use of TLI is whether it, or other treatments that similarly affect the immune system, can be given to those who reenter the progressive phase despite therapy. Mycophenolate-mofetil (MMF/Cellcept) is an inhibitor of inosine monophosphate dehydrogenase, which is necessary for *de novo* purine biosynthesis in lymphocytes. The drug is being successfully and increasingly used for prevention of organ transplant rejection. Beneficial effects in the treatment of autoimmune diseases have been shown in Crohn's disease. The inhibition of lymphocytes by the drug may therefore result in improvement in patients with refractory MS. In this open surveillance trial, we treated seven patients with chronic progressive or relapsing MS. The patients were informed about the drug and its attendant risks, and all gave informed consent before initiation of treatment. Entry criteria in this trial included the diagnosis of MS, disease-progression in terms of EDSS despite established treatment, and no other diseases such as infections or liver or kidney disorders. All patients received the usual dose employed in the treatment of transplanted patients (2 g/day). The administration of MMF led to improvement or stopped progression in five cases. Three of the five patients emphasized improved movement, although EDSS did not change. One of the five patients had to reduce the dose from 2 to 1.5 g per day because of frequent infections, one discontinued the treatment owing to uncontrolled nausea. Two patients without beneficial effects stopped the drug through nausea and/or nonresponse. MRI findings before and at least 6 months after treatment start were available in two patients and showed fewer lesions and no change, respectively. The four patients, who still receive MMF, are feeling well without progression and would like to continue this therapy. In all seven patients treated there was no deterioration concerning EDSS. Although the beneficial effects in five patients demonstrate that MMF may be of value in the treatment of MS, this open-label study does not allow a final conclusion. The question of whether more responsive patients would be found in an unselected group requires further study (72). A large multicenter trial of cyclosporin in the United States (73) and a trial in London (74) suggest that cyclosporin has a beneficial, albeit modest effect in ameliorating progressive MS, but it has not found clinical use because of the narrow benefit-to-risk ratio. Azathioprine has been the subject of a large number of studies in MS (reviewed in ref. 75) and meta-analysis of the results of all published blind, randomized controlled trials showed a statistically significant benefit in reducing frequency of relapses over a 3-year period, but minimal effect on disability (76). Methotrexate has found widespread use in the treatment of autoimmune diseases, such as rheumatoid arthritis, which has led to its use in neurologic immune-mediated disease. A double-blind trial of 7.5 mg/week of oral methotrexate in 60 chronic-progressive MS patients showed a significant beneficial effect on measures of upper extremity disability (77). Further experience may help clinicians to avoid a drawback to the use of methotrexate, which is its ability to induce an irreversible hepatotoxicity. Cladribine, an immunosuppressive

agent approved for the treatment of hairy cell leukemia, showed positive effects in a recent trial in which 7 of 23 placebo treated patients vs 1 of 24 cladribine treated patients had a worsening of one point or more on the EDSS scale at 1-year follow-up (78). The effects of cladribine, a lymphocytotoxic agent, on whole-brain volume were studied in a randomized, double-blind, placebo-controlled trial of 159 patients with progressive MS and an EDSS score from 3.0 to 6.5. Patients received one of three regimens: (1) eight courses of placebo; (2) two courses of cladribine (0.07 mg/kg daily subcutaneously for 5 consecutive days, total dose = 0.7 mg/kg) followed by six courses of placebo; or (3) six courses of cladribine (0.07 mg/kg daily subcutaneously for 5 consecutive days, total dose = 2.1 mg/kg) followed by two courses of placebo. At baseline, brain volume was 1042 ( $\pm 18.8$ ) mL, 1053 ( $\pm 18.1$ ) mL, and 1082 ( $\pm 19.9$ ) mL, respectively, in the three treatment groups. Cladribine did not significantly affect brain volume over time. At 12 months, the mean reduction from baseline in brain volume was 0.4% with placebo, 0.4% with the lower dose of cladribine, and 1.5% with the higher dose of cladribine (79,80).

### *Plasmaphoresis and Intravenous Immune Globulin*

These approaches have been used in fulminant neurological-immune mediated diseases with variable success which has led to some extension of their use into more chronic diseases. IVIg is easier and generally safer to use than plasmaphoresis, but both remain expensive. IVIg showed promise in early MS trials (81). Immune globulin may help patients with a number of autoimmune diseases, including chronic relapsing polyneuropathy and dermatomyositis, and has also been studied in relation to MS. Its mechanism of action is unclear but may relate to anti-idiotypic effects and suppression of TNF- $\alpha$ . A randomized, placebo-controlled trial of monthly IVIg in RRMS involved 150 patients over 2 years (81). IVIg was associated with fewer relapses (62 vs 116 with placebo) and a greater likelihood of being relapse-free (53 vs 36%). Only 3 patients receiving IVIg (4%) experienced side effects including cutaneous reactions and depression; these manifestations were not unequivocally related to the IVIg. Observations in animal models of MS revealed that IVIg could promote new myelin synthesis (58). However, a double-blind, placebo-controlled study of 67 patients with an apparently irreversible motor deficit found that IVIg (0.4 g/kg for 5 days, then single infusions every 2 weeks for 3 months) did not reverse established weakness in MS (83). Plasma exchange has been investigated in patients with acute central nervous system inflammatory demyelinating disease who did not respond to steroid therapy (84). Moderate or greater improvement in neurological disability occurred during 8 of 19 (42%) courses of active treatment compared with 1 of 17 (6%) courses of sham treatment. Improvement occurred early in the course of treatment and was sustained on follow-up. However, 4 of the patients who responded to the active treatment experienced new attacks of demyelinating disease during 6 months of follow-up.

### *Bone Marrow Transplantation*

Recent strategies have focused on more aggressive approaches to immunosuppression such as autologous bone marrow transplantation (BMT) or stem cell trans-



plantation (SCT). This approach has shown promise in experimental models of MS (85,86). In a study assessing safety, 26 patients with severe MS were treated with autologous SCT; one patient had a flare of MS during treatment with granulocyte-colony stimulating factor for stem cell mobilization, one patient had a syndrome of fevers and progressive neurologic deterioration and died 2 years after high-dose immunosuppressive therapy, and there was a relatively high rate of bladder complications and engraftment syndrome (a noninfectious syndrome of fever, rash, and fatigue) (87). A second uncontrolled study of SCT in 21 patients with rapidly progressive MS also found a high rate of engraftment syndrome (88). Patients with high pretransplantation disability scores did not appear to benefit from SCT and the study was unable to assess whether there was benefit in the subgroup of patients with lower baseline disability scores. A recent article on stem cell research in MS involved 14 patients with severe MS (EDSS 4–6.5) receiving autologous hematopoietic SCT. The median follow-up was 3 years. The probability of progression-free survival was 85.7% and the probability of activity-free disease was 46.4%. The number of relapses 1-year prior was 48 and the number of relapses at the end of the 3 years was 2. On MRI there was a 20.2% reduction in T2 lesion burden and a 13.75% reduction in corpus callosal volume. No T1 enhanced lesions were seen after transplantation. After 36 months the EDSS was stable in eight, improved in four, and worsened in two patients. No deaths or grade III/IV complications occurred (89). Larger controlled trials are awaited in order for the risk–benefit ratio of this approach to be calculated.

### ***Reinstitution of First-Line Therapy***

The principle of second- or third-line therapy may be used to induce remission of disease activity with an immunosuppressive agent, followed by reinstitution of first-line therapy, nontoxic therapy to maintain quiescence. The degree to which subsequent first-line therapy will be effective is unknown. Furthermore, a major problem that confronts clinicians is being able to determine when a remission has indeed occurred.

### ***Combination and Pulse Therapy***

Until the pathogeneses of neurological immune-mediated diseases are precisely understood, it is unlikely that a single treatment will be effective in all patients. The principle of combination therapy has found utility in cancer treatment and is becoming an important principle for the therapy of MS and other neurological-immune mediated diseases. Rationales for combining different mechanistic approaches exist. Combining systemic immune deviation with immune deviation of antigen-specific cells or molecular blockades are interesting possibilities. Pulse steroids or relatively nontoxic immunomodulation may find utility in conjunction with other antigen-specific forms of therapy. As it becomes clear which mechanistic strategies work best, more rational combinations can be designed. Because some forms of therapy cannot safely be given chronically, intermittent pulse therapy with

**Table 5**  
**Combination Trials in Multiple Sclerosis**

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1. Avonex and Mitoxantrone	16. Betaseron and Methotrexate
2. Avonex and Fludarabine	17. Betaseron and Copaxone
3. Avonex and Methotrexate	18. Betaseron and Mitoxantrone
4. Avonex and Cytosan	19. Betaseron and Azathioprine
5. Avonex and Provigil	20. Rebif and Cytosan
6. Avonex and CellCept	21. Rebif and Corticosteroids
7. Avonex and Prednisone and Azathioprine	22. Rebif and Azathioprine
8. Avonex and Azathioprine	23. Rebif and Copaxone
9. Avonex and Antegren (SENTINEL)	24. High-dose cyclophosphamide and total body irradiation with stem cell or bone marrow rescue
10. Avonex and corticosteroids or methotrexate	25. Copaxone and Albuterol
11. Avonex and IVIg	26. Copaxone and Antegren
12. Avonex and Copaxone (CombiRx)	27. Copaxone and Mitoxantrone
13. Avonex and Selegiline	28. Mitoxantrone plus either interferon or Copaxone
14. Avonex and Minocycline	
15. Betaseron and Cytosan	

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drugs that affect the immune system may become an important principle for therapy. Current combination trials in MS are listed in Table 5.

An open-label study was performed to evaluate the safety and efficacy of combination therapy with weekly oral methotrexate (20 mg) and IFN-β-1a in 15 patients with MS who had experienced exacerbations while receiving IFN-β monotherapy. Nausea was the only major side effect. A 44% reduction in the number of gadolinium-enhanced lesions seen on MRI scan was observed during combination therapy ( $p = 0.02$ ). There was a trend toward fewer exacerbations. This combination therapy appears to be safe and well tolerated, and should be studied in a controlled trial (90). A recent pilot trial of combining Avonex and Copaxone enrolled 33 subjects with relapsing-remitting MS, an MRI consistent with MS, and an EDSS between 0 and 5.5 who had been taking Avonex for 6 months or more. Previous results proved safety of the combination. This report showed additional safety as well as no evidence for increased risk in terms of new lesion load. The number and volume of gadolinium enhanced lesions showed a decline from baseline to 6 months, as well as from 6–12 months, suggestive of increased efficacy. Larger trials are in progress (91).

**Neuroprotection**

A potential benefit of the neuroprotective agent riluzole on cervical cord atrophy was suggested in a pilot study of 16 patients with PPMS and EDSS scores from 3.0 to 7.5. After receiving no treatment for the first year, patients were treated with riluzole 100 mg daily during the second year. MRI scans were analyzed in a blinded fashion. At baseline, mean spinal cord area was 66.7 mm<sup>2</sup>, which was reduced to

65.4 mm<sup>2</sup> (-2.0% median change) at 1 year. After treatment with riluzole during the second year, little further change was seen in mean spinal cord area (65.2 mm<sup>2</sup>, -0.2% median change vs first year) (92).

### *Disease Monitoring*

The major problems confronting therapy of neurological immune-mediated diseases are unpredictable courses and the length of time over which disability accumulates. Even for treatments that have proven efficacy, it may be difficult to determine in an individual patient whether the treatment is having a therapeutic effect unless the disease becomes truly quiescent clinically or the patient improves on therapy. In some instances it is clear that a patient is either a responder or nonresponder to a particular treatment. We have occasionally seen such positive clinical effects in younger patients with rapidly progressive, steroid unresponsive MS treated with pulse cyclophosphamide. The hope is that surrogate markers, which are linked to the underlying disease process, will allow a more rational approach to assessing therapy apart from clinical assessment. MRI has provided such a surrogate marker in MS and was one of the major factors in the approval of IFN- $\beta$  for the treatment of relapsing remitting MS (93). Even though MRI is not a perfect correlate of disease activity, it may ultimately serve as the best objective measure of ongoing disease in the nervous system given that it is now known that MS is chronically active even when clinical activity is not present (94,95). However, apart from formal clinical trials, MRIs are costly and impractical to perform on a frequent basis in a general neurology practice. There is a large body of evidence showing abnormalities of immune function in MS including activated T-cells, loss of suppressor influences, the presence of activated myelin-reactive T-cells in the peripheral blood and abnormal cytokine patterns. One intriguing recent observation is that the cytokine TNF- $\alpha$  may correlate with disease activity and disease disability (96,97). TNF- $\alpha$  can be measured in whole peripheral blood following stimulation with PHA and TNF- $\alpha$  message can be measured in frozen blood samples. TNF- $\alpha$  may play a role in the disease process as it is an important inflammatory cytokine that can result in damage of CNS myelin. Ultimately, however, immune measures such as TNF must be correlated with response to therapy as measured by clinical and MRI criteria. As with other recombinant protein drugs used for the treatment of a number of diseases, antibodies commonly develop to IFN- $\beta$  products during the treatment of patients with MS. Neutralizing antibodies (NAbs) are a subset of antibodies that reduce or diminish the biologic activity of IFN- $\beta$ . Individual Phase III clinical trials and direct comparison studies with IFN- $\beta$ -1b (Betaseron), intramuscular (IM) IFN- $\beta$ -1a (Avonex), and subcutaneous IFN- $\beta$ -1a (Rebif) have shown that NAbs develop more frequently during treatment with IFN- $\beta$ -1b than IFN- $\beta$ -1a and that between the two IFN- $\beta$ -1a products, NAbs develop more frequently during treatment with subcutaneous IFN- $\beta$ -1a than IM IFN- $\beta$ -1a. Data from clinical trials of IFN- $\beta$  products indicate that clinical efficacy of IFN- $\beta$  is reduced in NAb-positive patients. In light of these data, the immunogenicity of IFN- $\beta$  products should be considered prior to initiating treatment with IFN- $\beta$ . Also, ongoing laboratory

**Table 6**  
**Current Clinical Trials in Multiple Sclerosis**

1. 3,4 DiAminoPyridine/4-AminoPyridine (Fampridine)	34. Low-fat diet with fatty acids
2. Acupuncture	35. Lymphocytapheresis
3. Albuterol (Proventil)	36. Marijuana derivatives
4. $\alpha$ -Lipoic acid	37. Methotrexate
5. Altered Peptide Ligand (NBI-5788)	38. Methylprednisolone
6. Antibiotic therapy	39. Micellar paclitaxel (PAXCEED)
7. Anesthetic cream (EMLA)	40. Mitoxantrone (Novantrone)
8. Aspirin	41. Modafinil (Provigil)
9. Azathioprine (Imuran)	42. Monoclonal antibodies (Campath, anti-CD-40, CNTO 1275, Zenapax, Antegren, Rituxan, WA-J695)
10. Baclofen	43. Mycophenolate mofetil (CellCept)
11. Bone marrow/peripheral stem cell transplant	44. Myelin basic protein peptide
12. CTLA 4-Ig	45. Pain medication (Advil, Aleve, Tylenol)
13. Cyclophosphamide (Cytosan)	46. Phosphodiesterase inhibitor (Ibudilast)
14. Dexrazoxane (Zinecard)	47. Phosphodiesterase-4 inhibitor (Rolipram)
15. Dextromethorphan/quinidine	48. Pirfenidone (Deskar)
16. Donepezil HCL (Aricept)	49. Pixantrone
17. Estriol	50. Plasma exchange
18. Fluoxetine (Prozac)	51. Prednisone
19. Gabapentin (Neurontin)	52. Rehabilitation
20. Ginkgo biloba	53. Riluzole (Rilutek)
21. Glatiramer acetate (Copaxone)	54. Rosiglitazone maleate (Avandia)
22. Immunoglobulin (IVIg)	55. Schwann cell transplantation
23. Inosine	56. Sildenafil citrate (Viagra)
24. Insulin-like growth factor-1 (IGF-1)	57. Cholesterol-lowering medications (Statin drugs-Zocor/Lipitor)
25. IFN- $\alpha$ -n3 (Alferon)	58. T-cell receptor peptide
26. IFN- $\alpha$ -2a (Roferon)	59. T-cell vaccination
27. IFN- $\beta$ -1a (Avonex, Rebif)	60. Teriflunomide
28. IFN- $\beta$ -1b (Betaseron)	61. Testosterone
29. IFN- $\tau$ (oral)	62. Thalamic stimulation
30. IL-1 receptor blocker (anakinra; Kineret)	63. Valacyclovir (Valtrex)
31. IL12 monoclonal antibodies	64. Yoga
32. Laquinimod	
33. Levitracetam (Keppra)	

monitoring of patients treated with higher-dose IFN- $\beta$  is recommended for early detection of NAbs (98). Apart from formal studies, the treating neurologist must decide on therapy based on clinical assessment and accumulation of disability. Furthermore, a decision must be made as to whether a person has responded to therapy or whether additional treatment should be given. These decisions are generally made based on the frequency with which the patient is having attacks and level of progression. MS therapy remains a dynamic everchanging field. The explosion of new trials have stimulated and accelerated immunologic research in this field and has

lead to the development of new trials compounds. Current clinical trials are listed in Table 6.

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