Part 15

Neuroimmune Disorders

Multiple Sclerosis

90

Samuel K. Ludwin, Jack Antel, and Douglas L. Arnold

Abbreviations

ADEM	Acute disseminated encephalomyelitis
BBB	Blood-brain barrier
CIS	Clinically isolated syndrome
CNS	Central Nervous System
CSF	Cerebrospinal fluid
DAWM	Dirty-appearing white matter
DIS	Disseminated in space
DIT	Disseminated in time
DSS	Disability status scale
DTI	Diffusion tensor imaging
EAE	Experimental allergic encephalomyelitis
EBV	Epstein-Barr virus
EDSS	Expanded disability status scale
Gd	Gadolinium
GFAP	Glial fibrillary acidic protein
HERV	Human endogenous retrovirus
HHV6	Human herpes virus 6
HLA	Human leukocyte antigen
IFN	Interferon
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein

S.K. Ludwin (✉)
Pathology and Molecular Medicine, Queens University, Kingston, ON, Canada
e-mail: ludwin@cliff.path.queensu.ca

J. Antel • D.L. Arnold
Montreal Neurological Institute and Hospital, McGill University, Montreal, QC, Canada
e-mail: jack.antel@mcgill.ca, doug@mrs.mni.mcgill.ca

D.W. Pfaff (ed.), *Neuroscience in the 21st Century*,
DOI 10.1007/978-1-4614-1997-6_103,

MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
MTR	Magnetization transfer ratio
NAA	*N*-acetylaspartate
NAWM	Normal-appearing white matter
NK	Natural killer
NMO	Neuromyelitis optica
PLP	Proteolipid protein
PPMS	Primary progressive MS
RRMS	Relapsing-remitting MS
SPMS	Secondary progressive MS

Introduction

Almost 200 years since it was first described, multiple sclerosis (MS) remains one of the most intriguing human diseases, with a complexity of clinical, pathological, and etiological features that have so far defied simple explanation. Although there are tempting hypotheses in abundance, it is still unclear which observations are phenomena and which are epiphenomena, what is cause and what is effect. The nature of the disease is a cruel one, striking people in their youth and most promising years, with a slow inexorable course over decades in many, and leading to gradual incapacitation. By its nature, it affects not only the patients themselves, but their families and friends in profound ways. Until modern imaging methods were introduced, the very diagnosis, based on clinical symptoms which were known to mimic many other diseases, was often in doubt.

There has been an exponential explosion of knowledge and understanding of many aspects of the disease encompassing clinical, imaging, and health services and public policy research, and extensive basic experimental investigation. We now know and understand an enormous amount about some of the processes of MS, without understanding altogether how the pieces fit together. Enormous strides have taken place in the therapy of MS, drawing on basic research, which have markedly impacted on the characteristic relapsing phase of disease and ameliorated many of the unpleasant symptoms of patients, but without yet tackling the basic cause or reversing the progressive phase of disease.

Although isolated case reports of cases with symptoms suggestive of MS were described from the fifteenth century onward, it was only in the early to mid-nineteenth century that the disease was described analytically, clinically, and pathologically, by workers in France, Britain, and Germany. Beautiful anatomical depictions of the disease by Cruveilhier, Carswell, Hooper, Dawson, and Charcot show features that are seen to be just as fresh today, and the microscopic descriptions of Dawson, Rindfleisch, Virchow, and Charcot demonstrate the elements of

the plaque which we study currently, namely, demyelination, remyelination, gliosis, inflammation, and axonal damage. It is also of interest to note that discussions concerning the vascular and infectious theories of causation were posited even then. Over the years, the possible environmental, toxic, and immune theories were added, and as this chapter shows, many remain viable today.

More than most other diseases, MS demands a true interdisciplinary approach. Imaging studies are validated by the pathology, but at the same time inform the pathologist as to what needs to be studied. The clinician guides both but at the same time needs imaging and pathology to deliver answers to clinical questions, in order to understand the clinical course and patient management. In the absence of a known etiology, great reliance is placed on analogies from the experimental laboratory. The complex interplay between genetic and environmental factors in both causing and modulating the disease, and the effects on both of these on the immune system that produces the pathologic changes, requires the close collaboration of workers from all fields. This chapter will attempt to describe and elucidate what is currently known about this mysterious and common disease.

Clinical Aspects of MS

MS has been well recognized as a clinical-pathologic entity since the 1870s. The classical descriptions detail a disease course that evolves over many years. The early phases of the disease feature recurrent episodes of neurologic dysfunction (relapses), each followed after days to weeks by variable degrees of recovery and periods of stability (remission). This classical form of the disease is known as relapsing-remitting MS (RRMS). Although the actual frequency of relapses may decline over time, the affected individual can develop a gradual increase in neurologic disability, now referred to as secondary progressive disease (SPMS). The recognition of these features permitted accurate clinical diagnoses of large patient cohorts that could be followed over many years. Standardized clinical criteria were established (Table 90.1) to make the diagnosis of definite MS, permitting standardized selection of patients for entry into clinical trials and for large population-based studies aimed at delineating the complete clinical spectrum of the disease, variations in its natural history, and epidemiologic features of the disease (age of onset, geographic distribution, familial incidence). Such studies were also supported by the relatively wide acceptance of a standardized neurology assessment scale initially termed “the disability status scale” (DSS) later adapted to the expanded disability status scale (EDSS). Other forms of the disease include the clinically isolated syndromes (CISs), benign MS, and primary progressive MS (PPMS, see below).

Progressive forms of MS refer to continued accumulation of neurologic deficits which cannot be ascribed to recurrent inflammatory events that are associated with disease relapses and new MRI-defined lesions. The latter can continue to occur concurrently in patients with progressive disease. Most commonly, progressive

Table 90.1 McDonald diagnostic criteria (2001) with Polman MRI revisions (2005)

Clinical presentation	Additional data needed
• 2 or more attacks (relapses) • 2 or more objective clinical lesions	None; clinical evidence will suffice (additional evidence desirable but must be consistent with MS)
• 2 or more attacks	Dissemination in space, demonstrated by: • MRI
• 1 objective clinical lesion	• or a positive CSF and 2 or more MRI lesions consistent with MS
	• or further clinical attack involving different site
• 1 attack	Dissemination in time, demonstrated by: • MRI
• 2 or more objective clinical lesions	• or second clinical attack
• 1 attack	Dissemination in space demonstrated by: • MRI
• 1 objective clinical lesion (monosymptomatic presentation)	• or positive CSF and 2 or more MRI lesions consistent with MS
	Dissemination in time demonstrated by: • MRI • or second clinical attack
Insidious neurological progression suggestive of MS (primary progressive MS)	One year of disease progression (retrospectively or prospectively determined)
	Two of the following: (a) positive brain MRI (nine T2 lesions or four or more T2 lesions with positive VEP) (b) positive spinal cord MRI (two focal T2 lesions) (c) positive CSF

disease evolves in those with an initial relapsing disease course, usually apparent after several decades after disease onset and then evolving over subsequent years. An estimated 10% of patients have a progressive course from the start.

Additional clinical-pathologic studies helped establish the extremes of the disorder (fulminant or Marburg variant) versus benign MS (asymptomatic lesions discovered at autopsy or no disability after 20 years) and existence of disease variants whose relation to the classic disease form remains under study. These include primary progressive MS, neuromyelitis optica, and recurrent myelitides.

The observation from the late 1940s of increased intrathecal synthesis of immunoglobulin (Ig) in the CNS in cases of MS that could be measured by analysis of the cerebrospinal fluid (CSF) increased amount of Ig that had restricted heterogeneity (oligoclonal bands) provided the first laboratory adjunct to support the diagnosis of MS. The development of electrophysiologic measures to document presence of demyelinated CNS pathways (visual, auditory, and somatosensory evoked responses) provided further laboratory support for the diagnosis leading to their inclusion in revisions made to the original diagnostic criteria that were limited to clinical measures.

The 1980s marked the inclusion of magnetic resonance imaging (MRI) in the evaluation of MS patients leading to further revision of diagnostic

criteria (MacDonald). New lesion formation on MRI examination became accepted as a means to diagnose an individual case as having recurrent disease and qualifying for therapeutic intervention rather than requiring a second clinical event. MRI confirmation of lesions consistent with CNS demyelinating disease has become a sine qua non for acceptance of the diagnosis of MS including for inclusion into therapeutic trials. To date, clinical criteria remain the primary endpoints for clinical trials seeking drug registration, but MRI confirmation is an important adjunct. In the MRI era, the time from first reported symptom to the individual being given the diagnosis of MS has been reduced from a mean of ~8 years to a mean of a few weeks to months with the introduction of the term "clinically isolated syndrome" to bypass the debate as to whether individuals with their first clinical event and an MRI with demyelinating lesions should be called MS or be considered for therapy (see later section regarding early initiation of therapy).

Laboratory criteria to support the diagnosis of the progressive component of MS remain under development. New criteria include MRI measures of brain atrophy and optical coherence tomography that measures retinal nerve fibers, as a surrogate of axonal loss throughout the CNS.

Clinical Features of MS

As multiple sclerosis is defined as a disease whose lesions are disseminated in space within the CNS and over time, the clinical features of the disease need be considered with regard to both the neurologic symptoms and signs that arise from lesions at different sites within the CNS and how these manifest over time. In line with the long-recognized lesions within the white matter tracts of the CNS, individuals affected by MS can show variable combinations of clinical features dependent on the specific tracts affected. Most readily recognized manifestations include disturbance of function of vision (optic nerve), strength (descending motor tracts), sensory dysfunction (ascending sensory tracts), incoordination (cerebellar tracts), and urinary bladder control (usually spinal cord). Most motor deficits reflect involvement of the upper motor neuron pathways and thus manifest with increased tone (spasticity) and exaggerated reflexes. Since MS lesions do not respect anatomical boundaries, combination of dysfunction from involvement of adjacent pathways (e.g., motor and sensory) is common. Severity of the disturbance varies, widely reflecting the extent of insult to the specific pathway. Correlation exists with the extent of demyelination and destruction of the underlying axons. In cases with intact axons but disrupted myelin, aberrant nerve conduction can result in paroxysmal symptoms, e.g., trigeminal neuralgia or exaggerated symptoms such as sensory dysesthesia and Lhermitte's phenomenon. Any myelinated tract or nerve root with a CNS component can be involved including all cranial nerves and spinal motor and sensory nerve roots. A feature of demyelinated nerve is failure to sustain conduction either on a sustained or intermittent basis. Conduction through demyelinated nerve is sensitive to generalized metabolic upsets most readily recognized by effects of increased temperature on enhancing neurologic deficits (Uhtoff's phenomenon).

More complex clinical features of MS are less readily explained by involvement of single pathways or single discrete lesions. The clinical phenotype often reflects a combination of factors including severity of injury at a given site and the effects multiple lesions within the same or different anatomical pathways. For example, impaired balance can reflect involvement of motor, sensory, vestibular, visual, and cerebellar pathways.

Cognitive and psycho-affective dysfunction is well recognized to occur in MS but is variable in severity. Depression is the commonest affective symptom but can reflect a reactive component rather than being a direct result of tissue injury. Attributing deficits in specific cognitive tasks to a single anatomic lesion is unusual in MS given that the majority of affected individuals have multifocal white matter lesions as detected by MR-based imaging. Immunohistochemical analyses of MS tissue sections indicate that there can be extensive gray matter lesions. Current in vivo imaging methods remain insensitive to detect many of the gray matter lesions leaving open the question of how much of the cognitive or affective dysfunction in MS can be attributed to such lesions. The incidence of seizures is marginally above the expected.

Differential Diagnosis of MS

At the time of an initial event with neurologic findings that can be localized to a single anatomic site, one needs to exclude other structural lesions (vascular, tumor). MR imaging will both exclude these diagnoses, and in >50% of cases, there will already be evidence of multifocal lesions. For individuals with multifocal inflammatory lesions, the main differential diagnosis is the presence of systemic inflammatory disorders (e.g., collagen-vascular disorders, sarcoidosis) or multifocal infectious disease of the CNS.

Clinical Spectrum of MS

The combination of clinical and imaging diagnostic criteria has allowed a clearer characterization of the clinical spectrum of MS. The early natural history reports recognized cases in which the initial episodes were followed by an indefinite benign subsequent course with no advance on the disability scale. The fulminant extreme was also recognized. The most characteristic long-term disease course in the pretreatment natural history studies was development of increasing disability without a significant shortening of life span. Negative prognostic factors include a higher initial relapse rate, time to second relapse, development of progression, and multisystem involvement Uncertainty remains about the long-term impact of the immunomodulatory therapies discussed below on long-term disease course, with suggestive evidence that this as well as relapse frequency and relapse-associated disability has been impacted.

The natural history registries also demonstrate that the peak age of initial MS symptoms is in individuals in the third and fourth decade with recognition that disease can have onset at both younger and older extremes. Childhood onset MS,

i.e., prepuberty, is uncommon and more frequently has large inflammatory lesions with encephalopathic (behavioral/cognitive) features. The onset in the older cohort (fifth and sixth decade) more characteristically features a progressive or spinal cord-centered disease although relapsing/remitting disease has now been well documented with the availability of MR imaging. In this group, differential diagnosis of vascular disease becomes a more frequent challenge. A consistent observation is the increasing incidence of MS in females, whereas the rate in males remains constant.

The median time for progression to EDSS 3–4 (restriction of ambulation) for all MS cases as a group varies in different studies from 8 to 12 years, ranging up to 17 years or more in others. Median times to EDSS 6 (needing gait support) ranged from 14 to 28 years in various studies. In SPMS, these figures are slightly shortened, ranging from 8 to over 20 years, whereas for PPMS, these times were shortened to a range of 3–8 years. Finally, estimates of time to EDSS 10 (death) are 33 years for PPMS and up to 40 years in RRMS. Prognosis is affected negatively by early onset of progressive disease, a higher relapse rate, and multiple system involvement. Various geographical and ethnic factors, as well as age of onset and gender, also affect the natural history.

Primary Progressive MS

This entity accounts for ~10–15% of MS cases being more frequent in males and starting at later age than RRMS, being similar to the age when SPMS develops in initially RR patients. Diagnosis of the disease requires supportive imaging/laboratory measures including presence of MRI lesions often more prominent in the spinal cord than the brain, presence of oligoclonal bands in the CSF, and abnormal evoked responses. Clinical trials of PPMS patients indicate that up to 20% of patients meeting these criteria may experience a relapse over a 2-year trial period. This entity would then be termed "progressive-relapsing MS."

Pediatric Multiple Sclerosis

In the last decade, MS clinicians and pediatricians have come to realize that MS occurring in patients below the age of 18, although not common, does not merely behave as it does in adults, but have unique clinical, radiological, and pathological features that present challenges diagnostically and therapeutically. Estimates of incidence range from 1% to 10%, of MS patients, but the higher estimate undoubtedly includes some patients in whom it was realized retrospectively that the first symptoms occurred before the age of 18. Studying these cases offers an opportunity for seeing changes (immunological, pathological, and imaging) which may not only aid in earlier therapy for children but also for providing a window into the first stages of the disease in adults.

Understanding the disease is complicated by the concurrent development of the nervous, immune, and endocrine systems, and by general growth features. For example, the normal female to male ratio is not evident in prepubertal children. In addition, many of the ethnic and racial distinctions seen in the adult disease are

not present in children. Pathologically and on imaging, the lesions appear to be larger, or tumefactive, but biopsies show essentially the same histological and immunohistological features of the more acute lesions seen in adults. Children appear to have an immune makeup which is more activated, not only to CNS-specific antigens such as MOG (myelin oligodendrocyte glycoprotein), but in a more global fashion. EBV virus seropositivity is significantly raised above the normal controls, the difference being more marked than that seen in adults.

The most significant differential diagnosis to be ruled out in making a case for MS in a child is whether the patient has ADEM (acute disseminated encephalomyelitis). This disease, particularly common in the young, is a true immune-mediated process, arising in response to a viral infection or postvaccination and probably operating through molecular mimicry and epitope spreading. It is the human equivalent of EAE in animals and shares much of the characteristics of that disease. The pathology may be very difficult to distinguish from acute MS (see below). Although usually monophasic, it may be recurrent, further complicating the differential. However, it usually has prominent encephalopathic features, which are not a feature of MS. Whether it serves as a substrate for the later development of MS will await the outcome of many long-term studies currently underway. Some of the more detailed descriptions of the clinical features are beyond the scope of this chapter.

Neuromyelitis Optica (NMO)

For many years, this condition, presenting with optic neuritis and extensive myelitis, was considered to be part of the MS spectrum. A recently identified disease-specific autoantibody in the CSF, NMO-IgG, which recognizes the K channel, aquaporin 4, in astrocytes, has served to help definitively distinguish this entity from MS. NMO differs from MS showing a longer segment of spinal cord involvement, but less brain involvement. CSF oligoclonal banding is absent in NMO. Pathologically, the cord lesions show more severe and extensive demyelination and necrosis than do MS cases, with polymorphonuclear and eosinophil infiltrates and complement deposition. The optic nerve lesions may also be more necrotizing than those of MS. The antibody localizes to astrocytic foot processes, especially perivascularly, with subsequent cellular necrosis and edema formation. Many cases of so-called opticospinal MS, commonly found in Japanese patients, have now been reclassified as NMO on antibody testing. In addition, variants of NMO are now described, with relapsing forms and cases showing supratentorial lesions being described. The autoantibody nature of the disease lends itself to therapies such as B-cell depletion and plasma exchange.

Genetic Aspects of MS

One of the most important developments in the study of MS over the last two decades has been the understanding of the primal role of genetic makeup in the

causation and the modulation of the disease. The importance of a differing racial incidence in the disease was an early indication that genetic makeup played a large role in causation (see below). Initial observations began with familial studies in Canada, the United States, and Europe, which established that relatives of patients with MS had a much higher incidence of the disease than the general population. For first-degree relatives, this reaches up to 30 times that of normal population. Significantly, the degree of risk was directly related to the closeness of the relationship, with monozygotic twins having almost double the risk of dizygotic twins. Similarly, the latter carry higher risk than normal siblings and half-siblings in order. Landmark Canadian studies on twins, siblings and half-siblings, and adoptees reared together and apart have shown that in the microenvironment of familial MS, the effect of the environment is small. This statement, however, does not hold for whole populations. Recently, awareness of the role of maternal influence has confirmed that maternal transmission is much higher than that of paternal. Indeed, the risk of maternal half-sibs is the same as that of full siblings.

In spite of the obvious genetic influences, many people of similar genetic makeup will not contract the disease, and the complex interplay between strong environmental and genetic factors is of critical importance in determining who will or will not get the disease.

There has been much discussion as to whether MS is a single gene condition, or one of the polygenic diseases, each of small influence by itself, such as cardiovascular disease. Candidate gene association and linkage analysis showed only one striking association. The HLA gene cluster, particularly the class 11 region, on chromosome 6p21.3 is now recognized to be the dominant single genetic abnormality in MS. HLA-DRB1 and DQB1 are the significant alleles, with DRB1.1501 being of particular importance. These observations were confirmed in large European and Canadian studies using single nucleotide polymorphism technology. Significant in relation to MS, this locus is intimately associated with control of the immune system.

More recently, advances in techniques have resulted in multiple genome-wide association studies (GWAS) using significant banks of patient DNA. As might be expected, a small number of non-HLA candidate genes have been discovered, although none with the power of the HLA class 11 locus. This has reintroduced for some workers, although not all, the notion of a polygenic basis for the disease. Most of these genes are related to the immune system and are responsible for a wide variety of cellular functions such as cell adhesion, T-cell signaling, proliferation, differentiation and activation, B-cell proliferation, apoptosis, and regulation of HLA 1 genes to name a few.

The genes include the receptors for IL-2 and IL-7, CD58, EVI5, RPL5, as well as others. It must also be remembered that genes may interact with one another (epistasis) and even their DNA may interact with other DNA segments to alter function. Future investigations may clarify the relative importance of these genes.

It is very likely that some aspects of the course of the disease may be under genetic control. As noted below, African Americans may have a more aggressive course and Japanese a different form of the disease. However, even within the

Caucasian population, genetic background may influence the age of onset, clinical course, and disability progression. It is possible that some of these differences may depend on variations of the immunological mediators, either within the HLA locus or in some of the more recently identified genes.

Epidemiology

One of the most striking features has been the unique geographical distribution of the disease. The highest incidence of the disease is found in areas corresponding to the so-called temperate zone, with the lowest incidence in regions around the equator. Indeed, this observation has been refined in recent years, with multiple studies in individual countries, such as Australia, (Tasmania to Darwin) and Canada (Northern and Southern Newfoundland), which show an increasing incidence the further the distance from the equator. The prevailing view on this differential for many years has implicated exposure to some infectious agent found in temperate climates. This strong latitude-dependent susceptibility appears to be slightly decreasing, perhaps related to the increasing mobility of populations, or else local factors. For example, in Norway, the incidence appears greatest closest to the sea. However, two features have recently emerged as leading causes. The first is the high prevalence in temperate areas, especially in Europe and North America, of people with a genetic makeup consistent with a Northern European ancestry. The second is the demonstration that high-prevalence areas are subjected to fewer hours of sunlight and consequently lower levels of vitamin D than those closer to the equator. These factors will be discussed in greater detail below.

The influence of race is striking. Prevalence studies show the highest incidence in countries with a higher Caucasian population. However, among Caucasians, the incidence varies with many factors, including latitude. It is less common in blacks and aboriginal peoples. US military records show a significantly higher incidence in Caucasians than in Afro-Americans, Mexicans, Japanese, and Puerto Ricans. While it is rare in Asia and Africa, there are some areas where the incidence is higher, such as Northern China and parts of Japan. In Hawaii, Asians have half the incidence rate of Caucasians. In addition, race appears to play a role in the phenotype of the disease, with African Americans often a more aggressive course and Japanese having a higher incidence of opticospinal disease.

Migration studies have helped to dissect out the relationship between race and environmental studies. These have been carried out in countries where there have been significant shifts in population because of immigration, namely, in South Africa, Hawaii, Israel, and the United Kingdom. Although the details vary, in general, these studies have shown that immigrants retain the incidence of the region of childhood if their immigration occurs after the age of 15–20, whereas if this event occurs prior to that cutoff, they acquire the incidence of their new country. This obvious environmental influence has been ascribed for many years to childhood infections, which may still be valid, but other factors, such as sun exposure, may also play a role.

The influence of gender is also striking. As with many putative autoimmune diseases, there is a high female to male ratio, ranging from two to three. This ratio appears to be increasing, as in Canada, where it is now estimated as over 3.2:1. Hormones, specifically estrogens and progesterone, may play a role in modulating the effects not only on the immune process but also on the potential for remyelination and the age of onset of the disease. It is known that relapses are ameliorated during pregnancy, although in postpartum there is often an upsurge of attacks.

There have been some instances where there have been epidemics of an increase in incidence occurring within a defined period. While some of these have undoubtedly been due to greater awareness of the diagnosis, some may have been genuine. The classic examples of potential epidemics occurred in the Faroe and Channel Islands off the coast of Britain and in Iceland. The spikes in incidence were attributed to the sudden influx of British soldiers during the Second World War and supported the suggestion of a viral etiology. More recent examples have occurred following clusters of MS cases in people who had infectious mononucleosis, or Epstein-Barr virus (EBV) infection, which will be dealt with below.

In recent years, a theory has been posited that MS is caused by venous obstruction to the vessels draining the brain and spinal cord (called chronic cerebrospinal venous insufficiency (CCSVI)), with subsequent leakage of iron causing tissue damage. The venous obstruction has been demonstrated by specialized Doppler studies. This theory has attracted much attention in the popular press. Other studies have failed to show these changes in MS patient more than in controls, especially at early stages in the disease. These observations cast doubt as to whether they bear any relationship to MS or even whether they actually exist. Further well-conducted studies will be necessary to resolve this issue.

Environmental Factors

Since the description of the disease, numerous environmental causes have been postulated. The evidence of the importance of MS patients' genetic makeup has necessitated understanding how these factors may interact with a genetic susceptibility to produce disease. In this context, environmental factors may be operating to cause the disease, or to act as a trigger to the immune system to produce attacks.

Many of the postulated etiologies have shown only weak effects, or have not stood the test of time. Various studies over the years suggesting a role for toxins, such as organic solvents, in etiology have been published, but the evidence for this has not been strong or consistent. Similarly, the role of stress in causing MS is still being questioned. In this regard, it is at times difficult to determine whether reported stress is a cause or a consequence of the disease. Much has been written on the influence of diet in MS development. This factor has potentially some relationship to ethnic group, race, and geography. Dietary factors such as meat and dairy consumption, malnutrition, and levels of linoleic acid have been studied, with variable and inconclusive results as having a wide variety of other, sometimes

common, foodstuffs. Populations with higher fish consumption have been noted to have a lower risk of MS. Pooled data from many studies suggest an association with smoking and MS.

Infectious agents, sunlight, and vitamin D status are the three environmental factors which appear to be more robust as etiological influences capable of interacting with immune and genetic processes. The latter two are probably related and will be considered together.

Infection

Since the identification of MS as an inflammatory disease, infectious agents have always been strong and tempting suspects. The migration and geographic studies have supported this view, suggesting the presence of a past infection in endemic areas. In addition, strong data suggested that viral infection could trigger attacks, and subsequently, a wide variety of agents have been proposed, including measles, parainfluenza, canine distemper, retroviruses, Epstein-Barr virus (EBV), and human herpes virus 6 (HHP6). With the advent of new and sensitive molecular techniques, the search for old and novel pathogens continues. Studies have shown that these agents may cause immune disease, through the mechanisms of molecular mimicry, epitope spreading, and bystander damage. The concentration on viruses should not preclude the possibility that other infectious agents could not play roles, either in the causation or in triggering attacks.

The relationship between infection and immunity is also strengthened by the so-called hygiene theory of susceptibility, which suggests that a vigorous early immune response to infections is necessary to protect against the development of autoimmune disease. In developed countries, improved sanitation and widespread use of antibiotics have interfered with the body's ability to mount a normal response to infection, leading to an increase in autoimmune disease incidence. This credence has gained credibility by the observation that prior infection with parasitic helminths protects individuals from contracting MS.

Currently, most attention is given to the role of EBV in causing MS. Almost 100% of patients are seropositive for EBV, although it should be noted that the rate in the general population is 90%. This difference is even higher in the pediatric population. EBV DNA is higher in patients than in controls, and during relapses, in patients prior to the onset of disease, EBV titers are higher than those in controls. Lymphocytes from MS patients are more likely than those from controls to transform spontaneously under EBV induction, and patients are more likely than controls to have EBV-specific cytotoxic T cells. MS patients are more likely than controls to have had infectious mononucleosis, and clusters of MS cases in Denmark have followed community outbreaks of infectious mononucleosis.

Finally, there is some evidence, not accepted by all, that the meningeal B-cell follicles found in MS cases contain EBV-infected lymphocytes. There is a higher level of intrathecal and CSF EBV than other viruses. As with other viruses, there is

no specificity for EBV, in the CSF oligoclonal bands, a hallmark of MS. However, protein arrays from human dDNA libraries have shown a high reactivity to EBV antigens BRRF2 and EBNA-1.

Although several studies have shown evidence of HHV6 in plaques and in blood and CSF of MS patients, the epidemiological evidence for this virus is not as strong as for EBV. Evidence for the involvement of human endogenous retroviruses (HERVs) is suggested by the finding of HERV proteins in the sera and CSF of MS patients and by the relationship between this virus and both EBV and HHV6, suggesting that infection may depend on the interaction of more than one virus. In this context, it should be pointed out that HHV6 and paramyxoviruses use a common cell receptor, CD46, to enter cells and that numerous other agents, including common bacteria, make use of the same molecule and thus may share common pathways to cellular entry.

Vitamin D and Sunlight

Recently, attention has been focused on the roles of vitamin D and sunlight in the genesis of MS. Studies in Newfoundland, Europe, and Australia have correlated the days of sunshine with latitude. In general, hours of sunshine decrease with increasing latitude away from the equator. The lack of sunshine is, of course, a known cause of vitamin D deficiency. This, together with its known effects on the development of a normal immune system, its ability to act on the HLA DRB1.15, and its disturbance in other immune diseases, led to a systematic examination of vitamin D in MS. One of the most significant studies came from records of the US armed forces, in which samples of blood taken at the time of soldier recruitment were available for the measurement of serum 25-hydroxyvitamin D. Many years later, these levels were measured in soldiers who would later develop MS and compared with those in controls. The levels in future MS patients, many years before onset, were significantly lower. Experimental confirmatory studies in EAE showed an inhibitory effect on the development of the disease, both in vivo, and using activated cells in vitro. The potential for preventing the disease by administering this vitamin perhaps not only to patients but also preventatively to pregnant mothers remains attractive. Some preliminary studies have been inconclusive. However, the known observation that MS patients are more likely to have been born in May than in November suggests that they could have been deprived of vitamin D while in utero during winter periods of low sunlight and gives hope that more definitive studies could be therapeutically preventative.

Immunology

The immune system plays a pivotal role in causing the inflammatory reaction in MS, either as a primary cause or else in reaction to other central nervous system disease. Traditionally, this view has been based on observations on the presence of

pathogenic immunocompetent cells and chemical mediators both within the lesions and in the serum of MS patients, both during and between relapses. Many of these cells have shown a heightened reactivity to myelin and other CNS antigens. The increasing demonstration that many of the environmental, epidemiological, and genetic factors known to be of significance in MS may operate through their impact on the immune system provides strong, if circumstantial, evidence for the immune hypothesis. These will be discussed elsewhere in this chapter. Evidence obtained from immune-mediated experimental animal disease has yielded important information on how immune mechanisms impact the brain and allowed for extrapolation of features common with MS. Of particular importance is experimental allergic encephalomyelitis (EAE), an autoimmune disease induced by the injection of myelin products into susceptible animals, with or without adjuvant. The resulting pathology, although much closer to the human allergic disease of acute disseminated leukoencephalomyelitis, ADEM, (postinfectious or postvaccine encephalomyelitis), a recognized immune-mediated condition, nevertheless shares common features with the acute stages of MS. In support of the autoimmune hypothesis, the therapeutic response to immune-modulating therapy in MS patients has yielded significant information as to the pathogenesis of these processes, in addition to their success in alleviating the disease. In MS, there is a complex interplay of cells, cytokines, and chemokines, as well as other humoral growth factors and toxic molecules, the details of which are not always fully clear. Nevertheless, each of these remains a potential target for therapeutic intervention. The details of these therapies will be described elsewhere in this chapter.

Critics of the autoimmune hypothesis have pointed out the lack of consistent responses of the immune cells to specific myelin or axonal elements and to the fact that many of these immunocompetent cells can be found in normal control patients. Also cited is the failure of immunocompetent cells from MS patients to consistently transfer disease to animals.

Nevertheless, the role of the immune system in producing pathology and disease in MS is accepted by most workers in the field. The major controversies at this time revolve around the origin of the response. Does the immune response develop de novo in the periphery in response to some unknown insult? The immunocompetent cells would then enter the CNS to cause specific targeted disease against CNS antigens using molecular mimicry. Alternatively, does preexisting CNS disease release CNS antigens to the periphery to stimulate a secondary immune response which then enters the CNS? This has also come to be known as the inside-out versus the outside-in theory.

With all its imperfections as an exact model for MS, EAE still provides valuable information as to how immunopathogenic processes act in the brain.

It is important to emphasize that all the processes involving the entry of cells into the brain, their interaction with CNS cells, and the elaboration of both pathogenic and anti-pathogenic humoral factors are potential targets for modulation and therefore therapeutic intervention.

The events leading to the entry of immunocompetent cells into the brain have been well studied in a variety of experimental states, and most of them appear to

hold true for MS as well. It is believed that exposure to myelin or myelin-like antigens is responsible for the activation of CD4 helper T cells either from previously autoactivated or from naïve cells. These cells then recruit a variety of other immune cells, in the periphery (CD8 cytotoxic cells, myeloid cells, and NK cells), which then enter the CNS through the blood vessels. The subsequent attack on myelin, oligodendrocytes, and perhaps axons and other CNS elements may be mediated directly by these cells, or by cytokines and factors generated by some of the endogenous CNS cells, such as macrophages and microglia. Entry into the CNS through blood vessels takes place in the context of classically described processes. These include cellular rolling, endothelial contact, and adhesion using adhesion receptors on the endothelium and corresponding ligands on the infiltrating cells, with subsequent movement through the vessel wall and spread into the parenchyma behind the blood-brain barrier under the influence of cytokines. The activation of CD4 cells in the periphery may be primary, caused by exposure to a foreign antigen, which resembles the autoantigen (molecular mimicry), secondary to leaked antigens from previously damaged CNS tissue, or both. It is important to recognize that our concepts of the brain as an immune deficient or protected organ have changed considerably. Pathways linking the CNS perivascular interstitial space and the CSF and draining to the peripheral lymph nodes through the nose, involving both antigen presenting cells and myelin fragments, have been well described and studied. On the afferent side, the infiltration of immune cells across the blood-brain barrier, as well as through the choroid plexus, allows for both effector mechanisms, as well as immune surveillance of the CNS.

The Cellular Elements

A wide variety of cells are involved in the immune response in MS. T cells include $CD4^+$ (which may differentiate into TH1, TH2, and TH17 cells), CD8 cytotoxic cells, and regulatory T cells (Tregs). B cells are recognized to be playing an increasingly large role in the genesis of the immune process in MS. These cells are part of the adaptive immune system, whereas natural killer (NK) cells, gamma delta T cells ($\gamma\delta$), dendritic cells, and myeloid cells constitute the innate immune system. Resident microglia and macrophages and myeloid cells and possibly astrocytes may act as antigen-producing cells and may elaborate cytokines and other toxic molecules that are important both in the tissue damage found in MS lesions, as well as for potential neuroprotection.

The differentiation of T cells either into pro-inflammatory (TH1 and TH17), or anti-inflammatory (TH2) cells are controlled by specific cytokines and transcription factors, and recently, it has been shown that miRNAs also play a role. In recent years, TH17 cells have been shown to be of increasing importance in the genesis of tissue damage, possibly through a greater affinity for the blood-brain barrier, which they cross more efficiently than do TH1 cells. In addition, these subsets have a distinctive repertoire of secreted products that may produce tissue damage and may also influence the differentiation of other cellular elements. Some specific

differentiation factors include IL-12 and IL-4 for TH1 and TH2, respectively, and IL-1β and TGF-β for TH17. Transcription factors such as T-bet and miRNA-326 also influence T-cell differentiation. Many T-cell activation processes require the participation of co-stimulatory molecules on APCs, such as B7.1 and B7.2, for full differentiation. Pro-inflammatory mediators include γIFN, IL-17, and IL-22, while IL-4, IL-5, IL-10, and TGF-β are anti-inflammatory products of TH2 cells.

T-cell responses may be directed against any of the myelin proteins (MBP, PLP, MOG, and MAG), as well as some nonmyelin epitopes such as αβ-crystallin and some neuronal elements (contactin).

The control of differentiation is also a function of Tregs, which can be natural or induced and which may also serve to dampen or inhibit the immune response through contact or through secretion of IL-10. These cells also contain the nuclear transcription factor FoxP3.

The role of B cells in immunopathogenesis of MS has traditionally been less recognized than that of T cells, but in recent years, more emphasis has been placed on the importance of these cells. In the periphery, they are activated by helper CD4 cells and, together with other activated cellular components, cross the blood-brain barrier into the CNS itself. They elaborate antibodies which may be directed against myelin and nonmyelin components and which may damage tissue elements directly, often through complement mediation, or in conjunction with T cells. They may become resident in the parenchyma, behind the blood-brain barrier, or singly or in follicles in the meninges. In these situations, they may be responsible for ongoing autonomous elaboration of antibodies causing progressive damage.

Markers of Immunological Damage in MS

There are numerous direct indications that the pathogenesis of MS includes a major immune component as described above.

The blood-brain barrier (BBB) plays a critical role in maintaining the integrity of the CNS, but is breached early in the development of the disease. The passage of cells through the abnormal barrier and their subsequent migration through the tissues, mediated by integrins and matrix metalloproteinases and guided by chemo-attraction, are very significant features of MS. Studies on human tissues show many indicators of the abnormalities in the vascular system confirming the existence of these processes in MS. There is an upregulation of endothelial cell adhesion molecules, such as ICAM, VCAM, and ALCAM, which have corresponding ligands on the infiltrating cells. In MS lesions, the chemokines CXCL12, CCR7, CCL19, and others are expressed on various cell types, but each may be specific for different stages of the disease, as well as for specific sites. CCL20, expressed in the choroid plexus, facilitates entry of TH17 cells into the CNS.

Pathological examination of MS lesions shows the presence of most of the elements described above. Lymphocytes, mainly T cells; MHC class 11 macrophages;

B cells; and activated microglia are all found in the parenchyma and around blood vessels. In some lesions, immunoglobulin and complement deposition may be seen in and around the lesions. These changes are also seen away from the lesions in the so-called normal-appearing white matter (NAWM) and probably correlate with progressive illness. Although CD4 cells are seen early in the perivascular infiltrates, CD8 cells are more prominent where they are exposed to resident antigen presenting cells and can subsequently damage tissues directly.

Similarly, the B-cell meningeal follicles may be responsible for ongoing cortical damage in progressive illness. These follicles also contain germinal centers, dendritic cells, and express cytokines such as CXCL13.

T cells autoreactive to myelin and other CNS elements may be found in the blood and CSF of both normal people and in MS patients, but in the latter, they tend to be derived from preexisting memory cells and to be available in a more activated state. Recently, the presence of TH17 cells in the CSF of MS cases, and in the blood of patients undergoing relapses, has bolstered the case for their involvement in the pathogenesis of the disease, given their known ability to damage myelin and neurons in culture.

The presence of oligoclonal banding in the CSF of MS patients has always suggested a prominent role for B cells and plasma cells underlying IgG antibody-mediated damage to the CNS. In addition, they suggest autonomous intrathecal production of antibody. Consistently, the attempts to find single antigens relating to these antibodies have been unsuccessful. However, gene rearrangement and clonal expansion of lesional B cells have pointed toward specific antigens underlying these cellular responses. Myelin-specific antibodies are also found within the lesions. This finding is not universal and is still controversial. Some of the immunoglobulin in the CSF is also IgM and is usually directed at myelin lipid, rather than protein antigens. Some of these antibodies may also be localized to nonmyelin tissue such as the neurons and axons. Finally, $\gamma\delta$ T cells, known to kill oligodendrocytes in vitro and to a lesser degree NK cells, are also found within MS lesions and CSF.

As discussed above, B cells and T cells interact with each other in the generation of pro-inflammatory molecules. However, this interaction is also responsible for the production of anti-inflammatory cytokines such as TNFα, and IL-10, and other active molecules which are also present in MS lesions.

Neuropathology

The understanding of MS depends on a detailed appreciation of the pathology and the extent to which this relates to both clinical and radiological features. Although many of these features were described many years ago, modern techniques now allow for a more precise elucidation of their meaning. In addition, there is a large body of relevant experimental research which allows for the understanding of the etiopathogenesis, the diagnosis, and potential therapeutic measures.

Demyelinating Diseases

A demyelinating disease is one in which the myelin is preferentially destroyed with relative sparing of the axon, in contrast to primary or Wallerian degeneration where myelin loss is secondary to axon destruction. Many so-called demyelinating diseases do in fact have significant axonal damage either as part of the primary disease or secondary to it.

There are four main groups of demyelinating diseases:

Group 1 comprises acquired inflammatory demyelinating diseases with no established infectious etiology, including MS and its variants, ADEM.

Group 2 includes progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, and HIV vacuolar myelopathy and represents examples of inflammatory demyelinating diseases with a proven infectious etiology.

Group 3 contains the acquired noninflammatory demyelinating conditions which include toxic diseases such as vitamin B12 deficiency and central pontine myelinolysis, as well as other environmental agents such as trauma, radiation, drugs, chemotherapy, etc.

Group 4 consists of the hereditary metabolic or dysmyelinating diseases of myelin, including adrenoleukodystrophy, metachromatic leukodystrophy, and Krabbe's disease, for which defective genes have been isolated. They may also at times have a secondary autoimmune response to myelin breakdown.

The Use of Animal Models or of Other Human Conditions in Understanding the Development of Multiple Sclerosis

Dissecting the etiopathogenesis of MS requires referencing both numerous animal models and other human diseases of known etiology. In MS, there is no matching spontaneous or induced models. What animal models have shown extensively are similarities to *elements* of the MS lesion (such as immune-mediated inflammation, demyelination, axonal damage, and remyelination), which has allowed for the inference that the mechanisms in the model may explain events in MS. The most important model is EAE, an immune-mediated neurological disease induced by the injection of myelin, myelin peptides, or myelin-sensitized cells into genetically susceptible animals, with or without adjuvant. It has many features similar to MS. In its original form, EAE is monophasic, resembling acute MS or ADEM. By manipulating the experimental procedures and by using different species and/or strains, other forms of the disease may be produced, including chronic relapsing or progressive patterns, with greater similarities to MS. However, it is still not MS.

Other models demonstrate features of individual *components* of the disease. Exogenous toxins such as cuprizone, lysolecithin, and ethidium bromide are used as demyelinating agents in animals and are also the basis for studies in remyelination. Viruses such as MHV, canine distemper, herpes, and Theiler's virus all produce varying degrees of demyelination and subsequent remyelination, while many may also lead to axonal degeneration. Inflammatory and immune

diseases may also cause abnormalities of the blood-brain barrier (BBB), while all of these conditions may lead to gliosis of varying degrees. The use of gene deletions, transgenic models, or spontaneously dysmyelinating mutants in understanding the genesis of myelin and axon pathology has increased dramatically in recent years. As long as one recognizes the inadequacies of these as pure models of MS, the judicious use of animal models can be of enormous use in trying to understand and dissect mechanisms of causation and progression in MS.

Staging and Timing of Lesion Pathology

MS lesions fall into one of, or a variation of, active/acute, chronic-active and chronic-inactive/classical groups. In discussing, comparing, and defining any lesion, accurate or at least consistent staging becomes very important. The acuity of the lesion as described in different pathological staging schemes is often discordant with the assessment of the acuity of the clinical presentation. Active/acute lesions have traditionally been defined as showing demyelination with inflammatory infiltrates, whereas chronic lesions show demyelination and gliosis with little or no inflammation or myelin breakdown. The subacute or chronic-active lesion is one with a chronic core and active edge, or a plaque with a low level of inflammatory activity. The active edge of plaque may represent continual expansion of a lesion whose core has become inactive, or it could represent new activity around the edge of a preexisting plaque. Cellularity alone may be a misleading sign of acuity, as lymphocytes and macrophages and debris may remain in the tissue for prolonged periods. The contents of macrophages may help to assess the stage of myelin breakdown more accurately. Activated early macrophages may stain positively for MRP14 and 27E10; reactivity for the minor myelin proteins MOG and MAG disappears within a week or two, but MBP and PLP are retained in macrophages for up to 3–4 weeks. To be fully relevant, future staging classifications should include other important elements, including axonal and cortical damage, the gliotic reaction, and remyelination, both in lesions and in the so-called NAWM.

The Morphology of MS Lesions

The immunopathological aspects of the MS lesions have been mainly covered in the immunology section.

The Chronic or Inactive (Classical) Plaque

This is the commonest type of MS lesion seen at autopsy in patients who die after a relatively protracted course. Grossly, the chronic plaque is seen as a firm grayish-brown, well-circumscribed lesion single or multiple. Common sites include the periventricular centrum semiovale and the corpus callosum (Figs. 90.1 and 90.2) and the cerebellum. Plaques are also found at the corticomedullary junction, in the

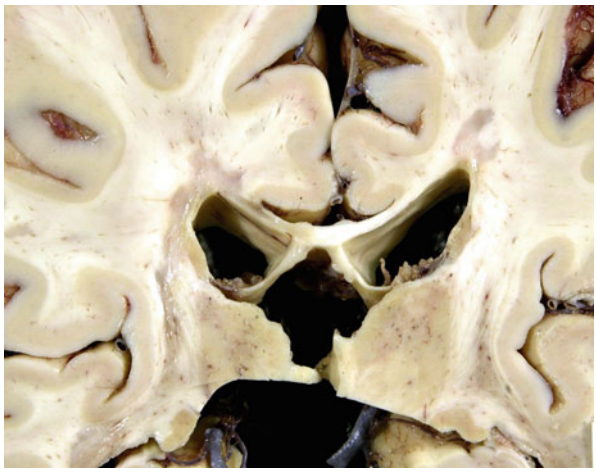

Fig. 90.1 Photograph of a brain with well-demarcated gray/pink MS plaques around the ventricles and extending into the white matter. Adjacent to the plaques, there are areas of less well-defined white matter changes, representing dirty-appearing white matter. Note the atrophy due to myelin and axon loss in the corpus callosum on the left and the ventricular dilatation

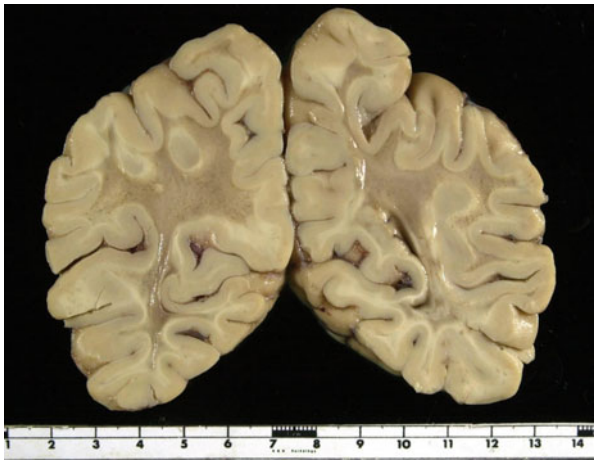

Fig. 90.2 The white matter in the occipital lobes of this brain is almost totally replaced with diffuse gray plaque

cortex and other gray matter structures (see below). The spinal cord is often shrunken and gray white following involvement with primary demyelinated lesions as well as secondary (Wallerian) degeneration (Fig. 90.3). The upper cervical and thoracic cord is usually affected especially in the progressive stages of the disease, either primary or secondary. MRI studies of these areas are difficult but have confirmed the atrophy and have shown good clinical correlation with the progressive forms of the disease. Other sites of predilection include the optic nerves and chiasm and the brainstem (Fig.90.4).

Besides the obvious plaques, there are diffuse alterations in neighboring white matter, including granularity and discoloration, the newly named “dirty-appearing

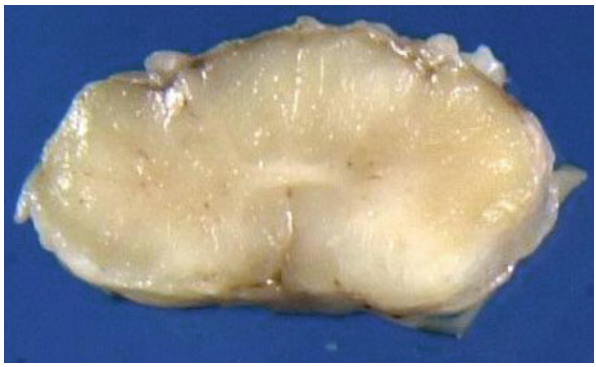

Fig. 90.3 The cervical cord is shrunken and atrophic and largely filled with gray plaque, leaving only the anterior section sector on the right intact

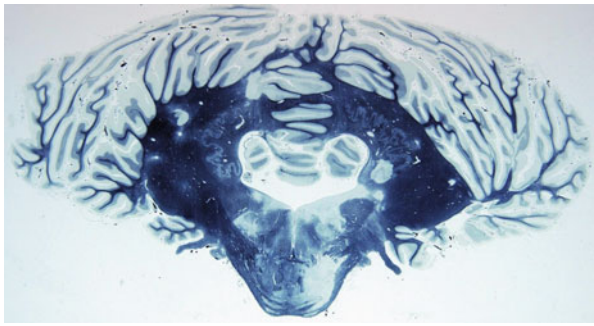

Fig. 90.4 This whole microscopic section of the cerebellum and pons is stained with a *blue* stain for myelin. The base of the pons on the *lower right* shows a large well-defined *white* area, indicative of a demyelinated plaque, and there are other small plaques scattered in the cerebellar white matter

white matter" DAWM (Fig. 90.1). MRI changes at postmortem have guided the pathologist to otherwise grossly normal regions, the normal-appearing white matter (NAWM) which histologically shows significant pathology. It is apparent that many lesions have been underrepresented in the past, emphasizing a need for the use of imaging in the pathological examination of MS cases.

Generalized atrophy is often striking in the brains of MS patients, either focally or diffusely (Figs. 90.1 and 90.2), as manifested by enlargement of the ventricles and shrinkage of structures such as the corpus callosum and the deep white matter, as well as the cortex and, in severe cases, the deep gray matter. This mirrors changes seen on MRI.

Histologically, the hallmark of the disease is loss of myelin (Fig. 90.5) with sharp borders between the demyelinated areas and the normal tissue. Within the plaque, there may be varying degrees of myelin preservation. Demyelination may be detected either with conventional stains such as Luxol fast blue, or with immunochemical stains against myelin components such as MBP (Fig. 90.6) and PLP. Often, axons appear to lose their sheaths as they enter the plaque. There may be almost no myelinated fibers present (Fig. 90.7), or axons with either thin or

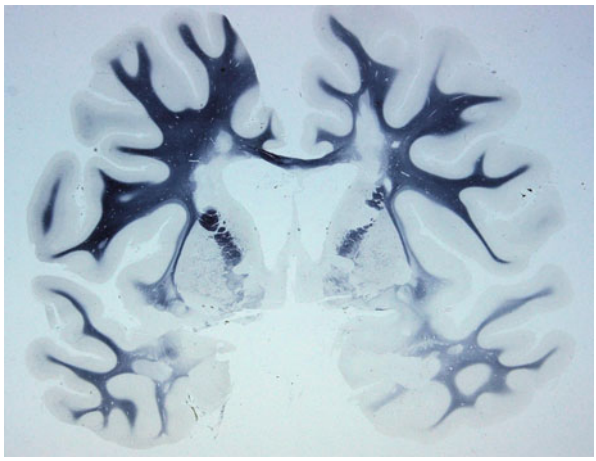

Fig. 90.5 This whole microscopic section of the forebrain, stained as in Fig. 90.5, shows typical plaque distribution around the upper angle of the ventricles. Surrounding the white plaques are regions of less well-stained myelin representing the DAWM

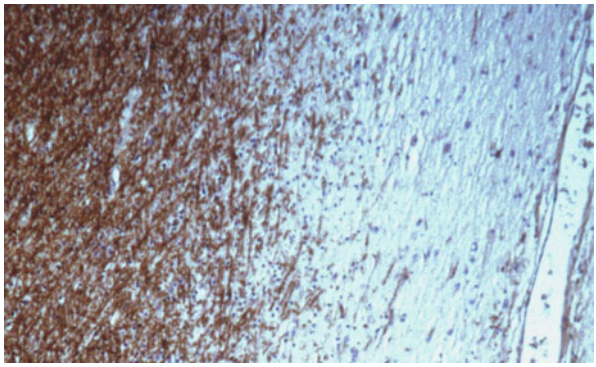

Fig. 90.6 Microphotograph of the edge of a plaque immune stained for myelin basic protein. On the left, the normal myelinated fibers stain *brown*, while on the right, the plaque contains very few fibers. At the rim, some fibers are myelinated

normal myelin sheaths may be present, representing remyelination and myelin preservation, respectively. At times, there is an extension of demyelination following the walls of blood vessels into the surrounding tissue, a feature known as a Dawson's finger. Oligodendrocyte loss, which may be extensive and near total at times, is usually most severe in the center of the lesion and may accompany the loss of myelin. In chronic plaques, there is almost invariably no evidence of ongoing oligodendrocyte necrosis or apoptosis. Around the periphery of the lesion, there is often a rim in which there is a greater density of oligodendrocytes which may be associated with an increase in the number of thin sheaths representing remyelination.

The chronic lesion contains extensive areas of astrocytic gliosis (Fig. 90.8). Ultrastructurally (Fig. 90.7), naked axons are surrounded by astrocytic processes. While inflammation (see below) is not a striking feature of the chronic plaque (indeed this is often used to define the chronic lesion), scanty collections of

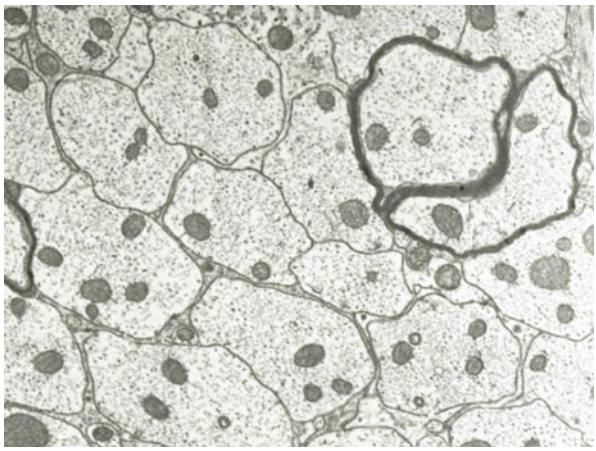

Fig. 90.7 Electron micrograph showing a field of demyelinated axons. On the right, two remyelinated axons, with thin sheaths, are present

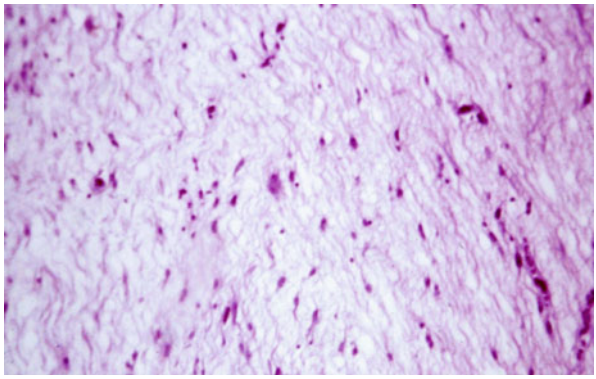

Fig. 90.8 The center of a chronic plaque devoid of myelinated axon shows the fibrillary pattern of astrocytic gliosis

mononuclear cells, including lymphocytes and sometimes plasma cells, mast cells, monocytes, and macrophages, may frequently be seen, usually in a perivascular position. B cells may be more common than T cells. Lipid-laden macrophages may be found scattered throughout the tissue. There is usually prominent loss of axons within the plaque, ranging from mild to severe, even though at first sight this is often less apparent than the more obvious loss of myelin. Blood vessels within the plaque may show sclerosis or perivascular fibrosis (hyalinization) and blood-brain barrier abnormalities. Axonal loss and damage (Fig. 90.9) is common (see below).

New evidence is emerging that the extent of the lesions is far greater than originally believed. Involvement of the deep gray matter is quite common, as well as the NAWM and the DAWM. In addition, evidence suggests that cortical pathology and demyelination is widespread and very common.

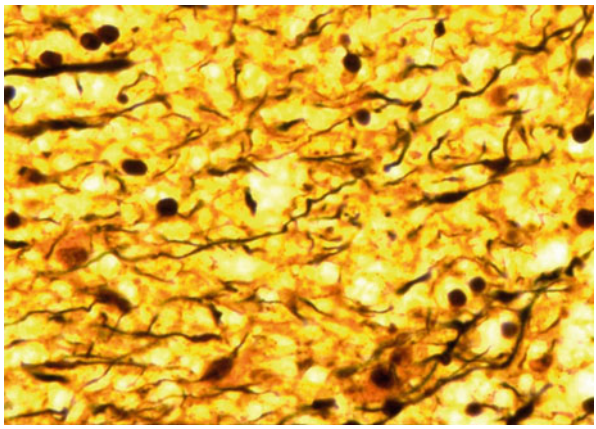

Fig. 90.9 Histology of a plaque silver stained for axons. There is a major dropout of black axons, some of which are thickened and swollen indicating damage

The periplaque, or the rim of tissue around the classical inactive or burnt-out plaque, may show thinner sheaths representing remyelination, or fewer axons, due to Wallerian degeneration from damaged axons in the plaque center. Accompanying this alteration of the myelin is an increase in gliosis, seen best with the astrocytic marker, glial fibrillary acidic protein (GFAP). Macrophage markers will show an increase in these cells in this area.

The Acute or Active Lesion

For some, the term "acute" should be reserved for the clinical situation. However, one can use the term, recognizing that an acute plaque can be found in a chronic clinical case and vice versa. The definition and dating of acute plaques pathologically has been described above. The acute plaque is the battleground of immune and inflammatory damage. The evidence for their presence in MS is covered additionally in the section on Immunology.

Some of these lesions, noted recently with imaging techniques, show an alarming size and confluent nature; these cases of tumefactive MS raise the differential of a rapidly developing neoplasm. It will be of interest to see with prospective imaging studies, whether these lesions will be shown to develop into the more diffuse forms of MS as described below.

Histologically, there is extensive demyelination. The plaques are often less well defined than chronic plaques. In addition, thinly myelinated fibers may be seen within the lesion, suggesting either partially demyelinated or remyelinated fibers. The presence of oligodendrocytes showing the reexpression of developmental myelination proteins suggests that the latter event is occurring in a significant number of these fibers. Striking changes are seen in and around blood vessels corresponding to the breakdown of the BBB seen as gadolinium enhancement on the MRI. These include lymphocytic infiltrates (Fig. 90.10), deposition of serum proteins such as albumin in the vessels and the surrounding tissue, and in very severe cases, mural necrosis. Accompanying the myelin loss is a large infiltrate of

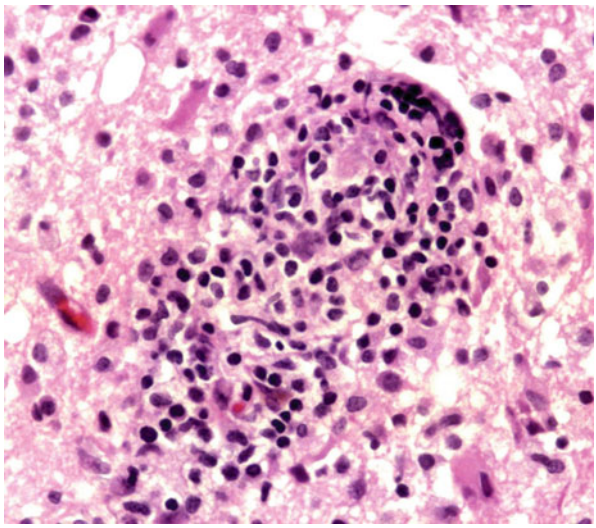

Fig. 90.10 In this acute plaque, the central blood vessel is almost obliterated by a lymphocytic infiltrate, which extended through the wall to lie perivascularly. Inflammatory cells also lie in the tissue away from the vessels

foamy or debris-filled macrophages (Fig. 90.11). They may be associated with denuded axons or axons surrounded by thin sheaths suggestive of remyelination. Depending on the age of the lesion, the macrophages may demonstrate the myelin proteins described above. Many of the macrophages and microglia are MHC-II positive, as are some astrocytes and even endothelial cells. The normal CNS does not express MHC-II, and its presence in diseased tissue suggests an ability to interact with lymphocytes. The inflammatory infiltrate varies but in most acute cases is extensive. Lymphocytes staining with the leukocyte common antigen (CD45) comprise the majority of cells, although plasma cells and even mast cells have been found, together with less well-characterized monocytes, myeloid cells, and some dendritic cells. Both CD4 helper T cells and CD8 suppressor, cytotoxic T cells, may be found in the lesions. Previously, CD4 cells were felt to predominate in early lesions, with CD8 cells taking over at later stages, but this is variable and a fixed pattern has not been defined. γδ T lymphocytes may be seen in these lesions, and their association with acute phase reactant or stress proteins such as heat shock protein on oligodendrocytes has been well recognized.

In some acute lesions, oligodendrocytes are clearly decreased. In general, cell loss is more profound in the center of the lesion than at the periphery, where the numbers may even be increased. The numbers correlate with the extent of remyelination. Apoptotic cell death in oligodendrocytes is somewhat controversial being rare in some studies and common in others. Bcl-2, a marker of cell survival, has been shown to be expressed by oligodendrocytes in MS tissue. Some oligodendrocytes, even in the acute plaque, appear to be reexpressing myelination proteins and markers, suggesting that remyelination is an early phenomenon. The changes in oligodendrocytes extend into the surrounding rim or penumbra.

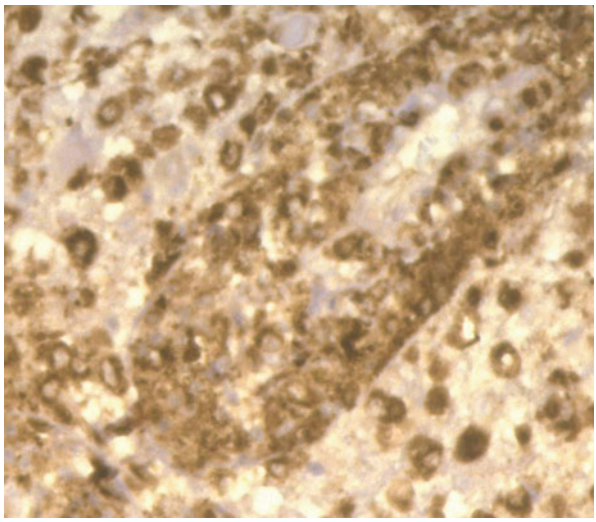

Fig. 90.11 Acute plaque similar to Fig. 90.11, immune-stained for lipoxygenase, a marker of active phagocytosing macrophages. The macrophages are present around the vessel and scattered in the surrounding tissue

Silver stains and neurofilament proteins demonstrate acute axonal injury (Fig. 90.9). Axonal numbers are often decreased, and indicators of axonal damage, such as expression of the precursor protein for beta-amyloid, have demonstrated. Similar changes have been noted in the surrounding periplaque rim.

The astrocytes or gemistocytes show early evidence of hypertrophy with swollen eosinophilic cytoplasm. These cells may be so numerous in biopsies as to raise the possibility of neoplasia on biopsies of the tissue. This astrocytosis can be seen in the periplaque rim and surrounding NAWM, where it usually accompanies the infiltration of macrophages and inflammatory cells.

Although several cDNA microarray studies in MS have attempted to detect gene regulation abnormalities, they have so far demonstrated only upregulation of inflammation-related molecules and have failed to show any definitive etiological agents.

The prototypical acute form of MS, although relatively rare, is Marburg's disease, in which the severe clinical course generally lasts less than 1 year. Some of the more florid cases now being seen on MRI and on biopsy may be examples of this variant. The pathology is generally highly destructive and affects the whole nervous system. A wide range of plaque size is seen, from small to confluent.

Another generally acute variant is Balo's concentric sclerosis which may however become chronic (Fig. 90.12). It is composed of concentric rings of alternating inflammatory demyelination and preserved myelin. This arrangement tends to suggest an anoxic-mediated mechanism, preconditioning some of the tissue to protect from successive wave of demyelination. This pattern may also be seen as part of more typical cases.

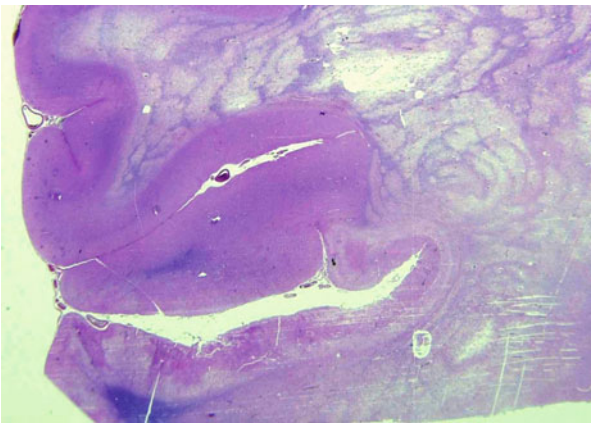

Fig. 90.12 A photomicrograph of a plaque from a case of Balo's concentric sclerosis, a variant of MS. The cortex is relatively spared, but the white matter is replaced by concentric layers of demyelination (*pale* areas), alternating with thin bands of relatively preserved myelin (*blue lines*). The demyelinated areas are partially necrotic, with cavity formation

The Chronically Active or Subacute Plaque

This is a loosely defined entity, and both terms are used almost interchangeably in the literature. These plaques contain an inactive hypocellular gliotic core with the features of a chronic silent plaque described above, surrounded by an active periphery with the features of active or acute plaques. The periphery may display demyelination, edema, increased astrocytes and oligodendrocytes, a mononuclear infiltration, demyelination, and even some remyelination. Frequently, however, the degree of activity is less than that seen in acute plaques. Whether this pattern represents ongoing activity from the original process or a new extension superimposed on old lesion is not clear.

Mechanisms of Demyelination in MS

Demyelination is the dominant feature of the plaque. The perivascular extension of demyelination from the main lesion (Dawson's finger) hints at the role of vascular-mediated inflammation in the pathogenesis. Immunochemical stains for myelin proteins (MBP, PLP, MOG) often reveal more subtle degrees of myelin loss than classical staining methods.

Demyelination may occur by damaging oligodendrocytes or by a direct attack on the myelin sheath. This has been shown experimentally in many models, and in MS, where there is infiltration by T cells and deposition of immunoglobulins, direct myelin breakdown can occur with preserved oligodendrocytes. In other plaques, there are decreased oligodendrocytes, indicating direct damage. Oligodendrocytes and their precursors may be killed by necrosis and by numerous toxic inflammatory mediators. Most authors claim that oligodendrocytes undergoing apoptosis are present in MS. The so-called dying-back gliopathy where the inner cell tongue of the oligodendrocyte is seen to degenerate before the perikaryon of the oligodendrocyte, is also seen in MS tissue. Inflammation-induced mediators of tissue injury that damage oligodendrocytes, myelin, and axons, include direct and indirect attack

by CD8 T cells with the discharge of cytotoxic granules and FAS ligation, excitotoxicity through glutamate, antibody and terminal complement component attack on the membranes, and pro-inflammatory molecules (TNFα, gamma interferon, IL12, lymphotoxin, etc.). Microglia and macrophages are important mediators of this damage, as well as being scavengers. Heat shock proteins and γδ T cells damage oligodendrocytes in MS tissue. Oligodendrocytes in MS tissue can also upregulate TRAIL receptors in response to injury, rendering them more vulnerable when a second injury occurs. Nitrous oxide and other reactive oxygen species, matrix metalloproteinases (MMPs), as well as perforin, granzyme, caspases, and calpain have all been implicated in oligodendrocyte and axonal damage MS. The development of ongoing demyelination in chronic plaques devoid of florid inflammation has been linked to the development of secondary progressive MS.

The periventricular location of many plaques has raised the possibility of circulating CSF agents. In addition, CNS myelin in different areas may vary; different charge isomers of MBP and highly citrullinated forms, generally seen in development, may be found in MS brains and may be more susceptible to attack.

Lucchinetti and colleagues described a large series of acute/active MS, diagnosed mainly on biopsy but also with autopsy material, and suggested new ways of interpreting the pathology and pathogenesis of the demyelination leading to MS lesions. Morphologically, they described four lesion patterns. The patterns varied from patient to patient, but all the lesions in any one given patient were claimed to be of the same type. Cases in pattern 1 (15%) showed inflammatory demyelination marked by T cell and macrophage infiltration. Pattern 2, the most common, consisted of lesions with well-demarcated zones of demyelination and striking T-cell inflammation with simultaneous loss of all myelin proteins. This pattern was marked by the striking deposition of complement around blood vessels and on the myelin. Oligodendrocytes were relatively well preserved, and remyelination often was frequently found. In all, the morphological features resembled those seen in T cell, antibody-mediated, MOG-induced EAE. In pattern 3 lesions, the next most common type, the plaque was less sharply delineated, but demyelination and inflammation still occurred. The prominent feature in these cases is the loss of oligodendrocytes and no remyelination. Another prominent finding is that of a loss of myelin-associated glycoprotein (MAG) greater than that of MBP or PLP. This feature suggested pathology in the inner cell tongue of the oligodendrocyte, perhaps suggestive of a dying-back or distal gliopathy. Significantly, the changes also resembled the damage seen with anoxic and toxic damage to the oligodendrocyte. Pattern 4, a very rare form, showed oligodendronecrosis in the periplaque.

These observations suggest a heterogeneous etiopathogenesis in MS patients. If true, this would also suggest different therapies depending on the etiological background. Few other groups have access to a similar population of acute cases, and the findings have been difficult to confirm.

In contrast, others have suggested on the basis of a small case series that all MS lesions start with oligodendrocyte damage/apoptosis and that the inflammation/immune attack is a secondary phenomenon. Again, this needs confirmation, and the issue needs resolution.

Axonal Damage in MS

Although descriptions of axonal damage in MS are almost as old as descriptions of the disease itself, for many years, this was neglected as a focus of attention. However, axonal damage and loss is seen not only in chronic lesions but also during acute stages of the disease. Most investigators now correlate axonal degeneration both with increasing disability and with the advent of progressive forms both primary and secondary. It is at the basis of brain and especially spinal cord atrophy and is the reason for extensive areas of rarefaction and necrosis. Axonal loss is also one of the substrates for the presence of the "black holes" seen on MRI and the reduction in the neuronal marker, NAA, found on MRS and the reduced MTR.

Axonal loss varies from 20% to 90% as seen with silver stains and antibodies to neurofilaments (Fig. 90.9). Wallerian degeneration emanating from damaged axons within lesions may also lead to axonal degeneration and loss in tracts distal to lesions, especially in the NAWM. Early damage to axons can be demonstrated by an accumulation of beta-amyloid precursor protein, nonphosphorylated neurofilament proteins, and the demonstration of axonal swellings or spheroids.

Inflammatory and immune factors target axons directly, or else damage them as a bystander reaction secondary to an attack on myelin. Although anti-neurofilament antibodies can be demonstrated in MS patients, anti-myelin antibodies are far more prevalent, which suggests that the axon may not be a primary immune target. Axons can be damaged by the agents causing oligodendroglial damage described above. Voltage-gated Ca channels also accumulate at sites of axonal damage and allow for calcium influx leading to damage, suggesting to some a degenerative or an anoxic primary process. The cause of the ongoing axonal degeneration in chronic progressive cases is not certain. It is possible that a slow subclinical inflammatory process continues throughout the disease course, not manifest through the usual indicators of acute inflammation such as clinical relapses or gadolinium enhancement. Progression of disease even when anti-inflammatory therapies control the relapse rate has suggested that other factors are operating in the chronic situation.

Loss of trophic factors from oligodendrocytes and myelin, or loss of a physical barrier protecting the axon from exposure to destructive factors, could also be the cause. In addition, the rearrangement of Na and K channels on demyelinated axons may place undue metabolic stress on axons by increasing the need for ATP. There is therefore an opportunity for therapeutic intervention employing classical neuroprotective strategies, of which remyelination is a potentially important one.

Gray Matter Pathology in MS

Cortical and deep gray matter involvement is widespread in MS cases and may produce major cognitive changes (Fig. 90.13). The cortical lesions which sometimes need to be stained specially may be extensions of white matter plaques, or isolated or about the pia. They are far less inflammatory than white matter lesions, and this has given rise to the suggestion that they are caused by cytolytic substances from cells sequestered behind the BBB, perhaps from the B-cell meningeal follicles. Demyelination, axonal and neuronal damage, and microglial activation are found, and the rate of remyelination is high.

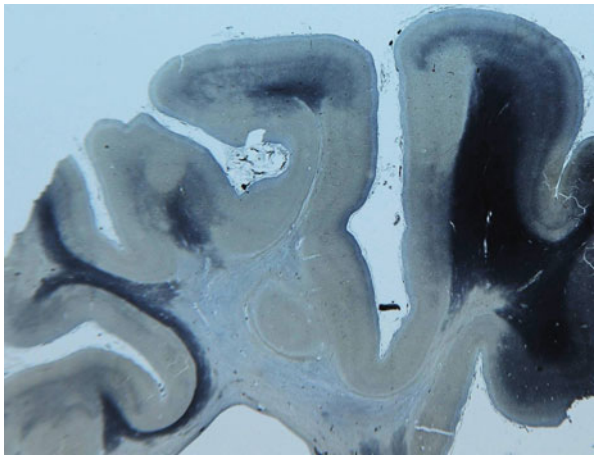

Fig. 90.13 Cortical plaques in a myelin-stained section of gyri with cortex and underlying white matter. In the white matter, there is a large pale plaque (*bottom left*), which extends to multiple gyri. More normal cortex showing some myelin is seen at the tip and right wall of the gyrus in the right. The rest of the cortex shows varying degrees of demyelination, seen best in both walls of the vertical sulcus just to the right of center

Pathology of the Normal-Appearing White Matter

Demyelination, axonal damage, inflammation, macrophage activity, gliosis, as well as metabolic changes have been described for years in the NAWM and may account for many of the widespread clinical effects; altered MBP may also be seen. Modern imaging techniques demonstrate these alterations during life. For some workers, they represent the very first evidence of new lesions, which is borne out by some imaging studies. Other workers suggest that they represent Wallerian degeneration of axons damaged in the plaques.

Remyelination

It is now accepted that remyelination is a prominent feature of the MS brain. Experimental studies proved the presence of this phenomenon, seen morphologically with the presence of thin sheaths, and many of the same processes have been demonstrated in the MS lesions. Indeed, some plaques are so well remyelinated that they are evident on microscopic sections as shadow plaques (Fig. 90.14), composed of thin sheaths (Fig. 90.15). In some studies, up to 28% of plaques and up to 47% of plaque areas have been shown to be remyelinated. Remyelination recapitulates developmental myelination and is carried out by oligodendrocyte precursors showing such markers as NG2 within the lesions. In addition, transcription factors governing repair such as olig-1 are seen on oligodendrocytes, among many other molecules. Netrins, Nogo, neurotrophins, and growth factors such as ILGF, BDNF, and PDGF, all associated with myelination in development, can be seen in MS lesions. It should be pointed out that the inflammatory response in MS does not only cause damage but may also provide growth factors essential for repair. Enhancement of remyelination remains a therapeutic goal. This has been accomplished experimentally both with treatment by growth factors and with the injection of oligodendrocyte precursors.

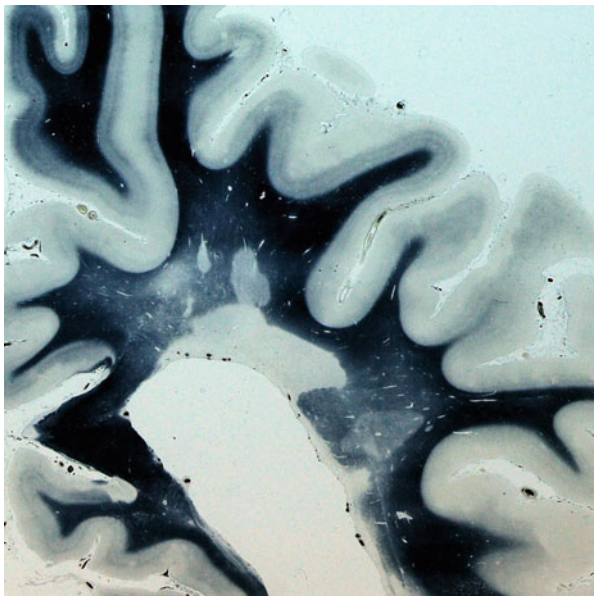

Fig. 90.14 This microscopic section of cortex, white matter, and ventricle is stained with the Loyez method (*dark blue*). In the center, at the bottom of the picture, lies the cavity of the third ventricle. Above it is a well-defined plaque, almost totally devoid of myelin staining. There are also three well-defined plaques around it, which are *pale blue*, representing remyelinated shadow plaques. In addition, around the main plaque, there are diffuse pale-stained areas indicative of dirty white matter (DAWM)

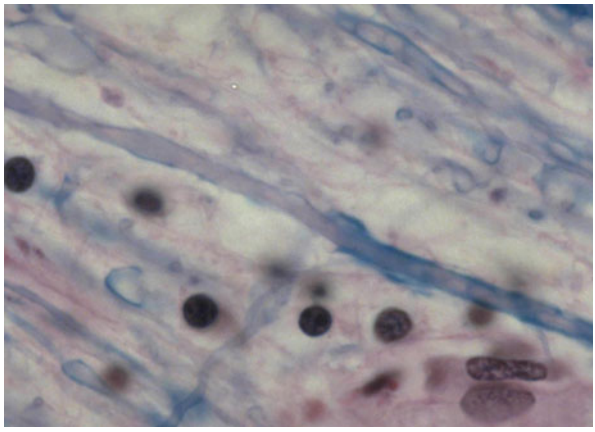

Fig. 90.15 An axon (*pink*) runs obliquely across the figure. On the right, it is surrounded by a relatively *thick blue*-stained normal myelin sheath, whereas on the left, the remyelinated sheath is far thinner

Imaging in MS

MRI permits detection of the focal inflammatory lesions in the white matter of patients with MS with exquisite sensitivity. Thus, it can aid in diagnosis, prognosis, and monitoring of the effectiveness of therapy. The ability to follow the dynamics of white matter lesion formation in vivo has greatly improved our understanding of the pathologic evolution of MS.

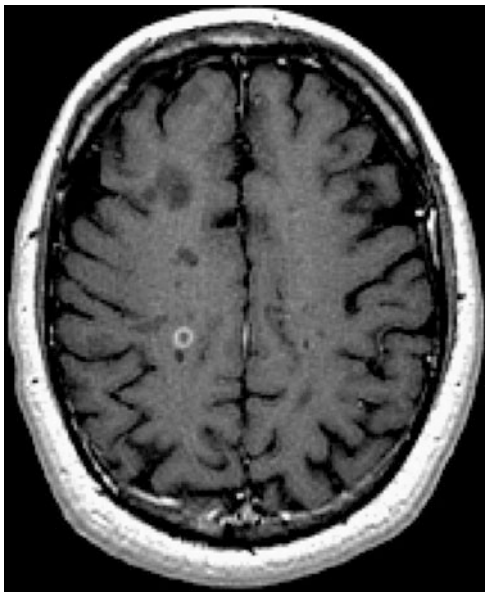

Fig. 90.16 T1-weighted MRI scan showing a *ring-shaped* gadolinium enhancement of one T1-weighted lesion, indicating that this lesion is associated with active inflammation and increased blood-brain barrier permeability. Other hypointense lesions in the slice do not enhance, as they are older and no longer associated with sufficient inflammation and increase in blood-brain barrier permeability

Visualizing the Pathology of MS In Vivo

MRI is very sensitive to the presence of focal lesions, the development of new lesions, and the presence of actively inflamed lesions in the white matter of patients with MS. Actively inflamed lesions can be identified because they are associated with increased permeability of the BBB and enhancement after injection of MRI contrast agents, usually gadolinium-based (Gd) chelates (Fig. 90.16).

Serial MRI scans show that new white matter lesions develop with a frequency approximately ten times greater than clinical relapse (Fig. 90.17). This implies that the majority of lesions are "clinically silent." This dissociation of MRI lesions from clinical symptoms occurs for several reasons: MRI lesions vary in their destructiveness, most are small, and by chance, most are likely to affect regions of the brain that are not "eloquent" and do not produce symptoms that are immediately evident to the patient. In addition, the brain has the capacity to adapt to injury that is why clinically evident disability may accumulate only after substantial injury has occurred and the capacity to adapt is overwhelmed.

Demyelinating lesions in MS can also affect cortical and deep gray matter structures. The cortical demyelinating lesions are much less inflammatory than the white matter lesions and are usually not visible on conventional MRI scans. Specialized acquisition techniques such as double inversion recovery and phase-sensitive inversion recovery increase the visibility of these lesions, but still fail to visualize the majority of them. Very high field strengths (7 T) may be able to provide the contrast and resolution necessary to see these lesions, and a variety of

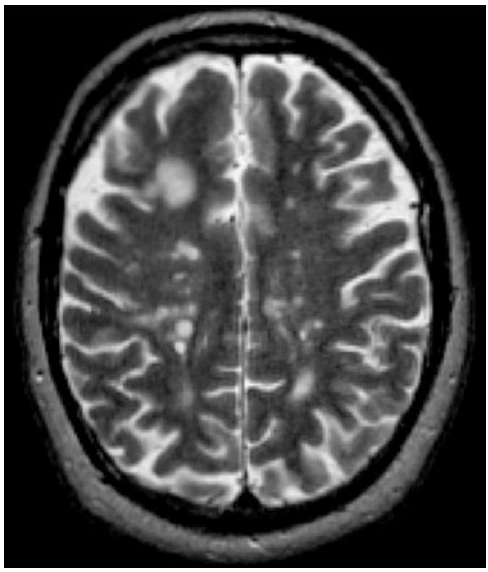

Fig. 90.17 T2-weighted MRI scan showing multiple hyperintense lesions. The T2 hyperintensity reflects increased free water in the lesions associated with either acute or chronic inflammatory injury to myelin and axons and the response to this injury, such as gliosis

nonconventional acquisition methods such as magnetization transfer ratio imaging may be able to visualize them at more conventional field strengths, such as 3 T.

The NAWM is affected by subtle, diffuse pathology that does not show up as "lesion." This may be associated with tissue loss and atrophy that can be detected by quantitative image analysis, even at the earliest stages of MS. Nonconventional, quantitative MRI acquisition methods sensitive to a loss of integrity of myelin or axons or both, such as MRS, MTR, and DTI, can also demonstrate this pathology.

Quantitative analysis of subtle structural changes that affect the volume of whole brain or specific structures, such as the thalamus, has revealed that diffuse tissue loss occurs early in MS and that the thalamus, a relay nucleus, is particularly affected. MTR imaging uses chemical and magnetization exchange at the molecular level to obtain an estimate of changes in macromolecular content, which is dominated by membrane bound macromolecules that are a marker of myelin content due to the large amount of redundant membranes in myelin. MTR images can be used to obtain semiquantitative estimates of changes in myelin content. This is particularly powerful during the demyelination and remyelination phases of acute lesion formation, when the changes are large and rapid. MRS measures signals from metabolites in the brain, rather than water, and can be used to assess neuroaxonal integrity in vivo due to the fact that one of these metabolites, N-acetylaspartate, is almost exclusively present in neurons and axons and is sensitive to change due to neuroaxonal loss or metabolic dysfunction (Fig. 90.18). DTI also seeks to measure axonal loss based on the fact that the water within axons is restricted in its lateral diffusion (diffusion anisotropy). This technique is less pathologically specific, as the pathological correlates of changes in diffusion anisotropy appear to include other sorts of pathology.

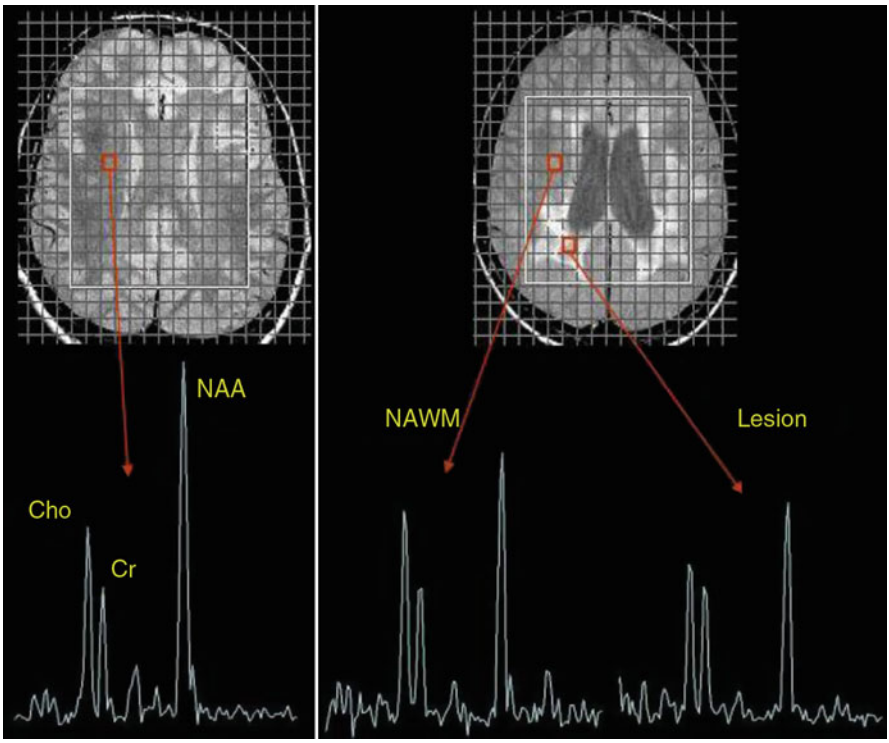

Fig. 90.18 MR spectroscopic image from a normal subject (*left*) and a patient with MS (*right*). Note the low NAA peak intensity in the MS lesion compared to normal white matter and the intermediate intensity in the normal-appearing white matter of the MS patient

Diagnosis of MS Using MRI

The diagnosis of MS is a clinical one, based on a typical clinical picture with the presence of multiple lesions disseminated in space and time for which there is no better explanation (see above). MRI greatly facilitates this diagnosis because of its great sensitivity to (white matter) lesions that are not evident clinically.

Although ultimately a clinical decision, the MRI appearance of MS is relatively specific when age, clinical information, and the full range of MRI abnormalities (including lesion numbers, distribution, size, shape, associated volume changes, and contrast enhancement) are considered. Even considering only the number, location, and size of lesions can provide good sensitivity and specificity for the diagnosis of MS, particularly when other diagnoses have been excluded by appropriate tests.

To make a radiologically based diagnosis of MS, multiple lesions should be present. At least one lesion should be greater than 5 mm in diameter, and at least one should be periventricular, infratentorial, or juxtacortical in location. Contrast enhancement and evidence for lesions in the corpus callosum (particularly with atrophy) add specificity, as these findings are less typical of vascular lesions.

The shape and orientation of corpus callosa lesions is also important. The inflammation in MS tends to run along blood vessels, which run perpendicular to the corpus callosum. This produces elongated ovoid lesions that somewhat resemble the fingers radiating from the palm of a hand, so-called Dawson's fingers.

Various criteria have been proposed and evaluated, in terms of their sensitivity and specificity for the diagnosis of MS. As experience accumulates, it seems that complicated algorithms are not necessary and that, in the right clinical setting, if typical lesions are present, minimal evidence for dissemination in space and time (DIS and DIT) is sufficient.

Specific diagnostic criteria for MS that incorporate MRI findings (the McDonald criteria) were proposed by an international panel in 2001. In this model, one lesion was the optimum number for gadolinium-enhancing and juxtacortical lesions, but three lesions were optimal for periventricular lesions and nine for the total number of T2 lesions. The McDonald criteria also provided an operational definition for dissemination of lesions in time based on the detection of new Gd-enhancement or new T2 lesion formation. In 2005, an international panel revised the McDonald criteria to simplify the criteria for DIT, as well as PPMS, and to clarify to role of spinal cord imaging (Table 90.1). The new criteria for DIT require a second scan showing a Gd-enhancing lesion at least 3 months after onset of the initial clinical event, or a new T2 lesion at any time compared to a reference scan done at least 30 days after onset of the initial clinical event. A minimum of 3 months was chosen as the required interval because Gd-enhancing lesions associated with the initial event are not expected to last longer than 1–2 months. Therefore, any Gd-enhancing lesion present at 3 months after a clinical event is likely to represent a new event.

Concerned by a relative lack of sensitivity of the McDonald criteria, some authors have suggested modification of the original McDonald criteria to use a less stringent definition for DIS (at least one T2 lesion in at least two of four locations) (juxtacortical, periventricular, infratentorial, or spinal) and to allow any new T2 lesion after 3 months as evidence for DIT. There seems little reason not to move to simpler criteria.

Monitoring of Treatment by MRI

The clinical usefulness of routine MRI for monitoring the evolution of patients with MS is less widely accepted. However, routine MRI surveillance of patients with MS in fact provides important information about disease activity that is not evident clinically, and this information can help with prognosis and management decisions.

The value of monitoring patients using conventional MRI depends on the stage of disease and whether the patient is on therapy or not.

Clinically Isolated Syndromes Suggestive of MS

In patients presenting for the first time with symptoms suggestive of MS, if the required evidence of white matter lesions disseminated in space and time is not

present on the initial scan, then performing follow-up scans can provide this evidence and help make the diagnosis of MS sooner than clinical follow-up alone. This is important since early initiation of anti-inflammatory therapy is associated with better clinical outcome.

Untreated Patients with RRMS

Patients or clinicians who may be reluctant to initiate treatment at time of diagnosis may find that evidence of new or active lesions on MRI sways their decision to initiate treatment. In untreated patients with established RRMS, the presence of Gd-enhancing lesions is only weakly correlated with concurrent relapses and disability progression. However, Gd-enhancing lesions leave behind new or enlarging T2w lesions, and the gradual accumulation of T2w lesions is associated with greater disability and greater risk of progression to SPMS over the long term. The rate of lesion growth over 20 years has been estimated by some to be 0.80 cm^3/year in patients who retained an RRMS course and 2.89 cm^3/year in those who developed SPMS.

Treated Patients with RRMS

In treated patients with established RRMS, the presence of Gd-enhancing or T2w lesions is associated with a suboptimal response to therapy and progression of clinical disability.

In a phase III trial of interferon beta-1a, given intramuscularly in order to evaluate criteria for "treatment response," patients were classified as responders or nonresponders using (1) the number of relapses during the 2-year trial, (2) the number of new T2 lesions after 2 years, and (3) the number of gadolinium-enhancing lesions at year 1 and year 2 on study. Outcomes included 2-year change in the EDSS, multiple sclerosis functional composite, and brain atrophy. In the IFN-beta1a arm-treated group, patients with high numbers of new MRI lesions had significantly more disease progression. The authors concluded that new MRI lesion activity during IFN-beta1a treatment correlates with poor response to IFN-beta1a and that MRI classification may facilitate rational therapeutic decisions. A number of other studies have confirmed that Gd-enhancing lesions and new T2 lesions predict relapses and disability progression. The risk of having a suboptimal response to treatment with interferon beta increases eightfold to tenfold in patients who have a single active lesion after 1 year of treatment. Evidence of MRI activity 3–6 months after starting treatment with interferon predicts clinical activity over the next 18 months with a predictive value of 60–70%. Inflammatory activity on the MRI also predicts brain atrophy in treated patients, which in turn predicts disability. In one study, an active scan (a scan with either a Gd-enhancing lesion or a new T2 lesion) in the first 6 months of treatment had a predictive value of 60–70% for clinical activity over the subsequent 18 months. Thus, MRI evidence of ongoing disease activity warrants a reexamination of therapeutic options.

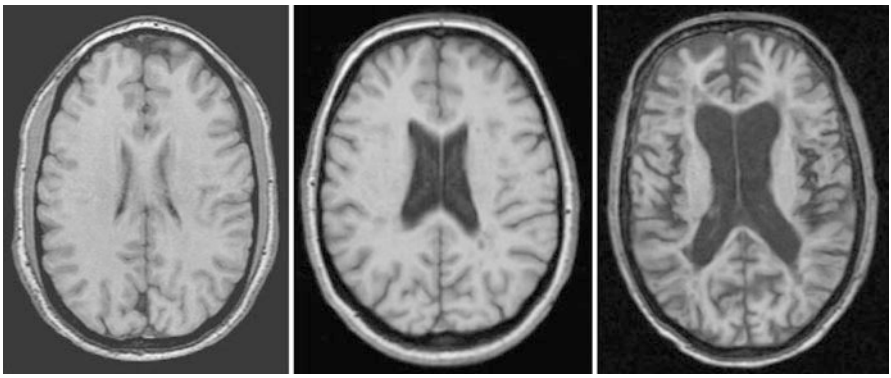

Fig. 90.19 MRI from a normal subject (*left*), a patient with RRMS (*middle*) and a patient with SPMS (*left*). Note the enlargement of the lateral ventricles and sulci, indicative of cerebral atrophy in the MS patients

Progressive MS

Patients with progressive MS may benefit less from MRI monitoring except in specific instances. In patients with SPMS, MRI activity as assessed by white matter lesions can dissociate from disability progression, particularly in patients who no longer have clinical or MRI evidence of active focal white matter inflammation. This appears to be due to the fact that the majority of the progression in these patients has a different pathogenesis that does not involve the concurrent formation white matter lesions. The nature of this alternate pathway underlying disability progression is unknown. Speculation includes compartmentalized inflammation in the CNS, a delayed degeneration of chronically demyelinated axons, and primary degenerative mechanisms. Markers for assessment of these processes are not routinely available in the clinic. They require nonconventional MRI acquisitions techniques, such as MRS (Fig. 90.18), or quantitative measurements of subtle atrophy on conventional MRI (Fig. 90.19).

In patients with SPMS who are transitioning from RRMS and who still have relapses or MRI evidence of focal white matter inflammatory activity, disease-modifying therapy appears to still have a benefit. Thus, the MRI still provides useful information in such patients if therapeutic decisions need to be made.

Data from the rituximab trial in PPMS suggests that focal inflammatory activity in the white matter is also a predictor of treatment response as assessed by slowing of disability progression in patients with PPMS.

Recommendations for MRI Monitoring in the Clinic

In our view, the MRI assessment of MS "activity" based on markers of focal white matter inflammation (Gd-enhancement and T2w lesion formation) is another aspect

of the assessment of the inflammatory activity of the disease, similar to the clinical assessment of relapses. How closely to monitor patients should be individualized in order to answer the specific questions that are relevant to an individual patient at their particular stage of disease and situation. Scans should be done more frequently in early MS than in later disease and more frequently in patients who are not doing well. They should also be done more at baseline and as often as necessary to monitor response to therapy or in whom the presence of ongoing disease activity would influence therapeutic decisions. The timing of scans may have to be adapted to financial and resource limitations. Scans will also be done more frequently in patients on clinical trials.

Comparison of serial MRI data is facilitated if serial scans are obtained on the same scanner using the same acquisition sequences. This is especially important in investigative studies. The use of contrast-enhanced scans is to be encouraged, as this may allow the detection of ongoing focal white matter inflammation that may not be otherwise evident.

Therapy of MS

In his lectures describing the features of MS, Charcot indicated that the time had not yet come to consider therapy for this entity. For the next hundred years, any therapeutic claims were based on treating relatively small group of individuals in a nonrandomized manner (see Therapeutic Claims in MS IFMSS). As potentially cytotoxic immune-suppressant therapies (cyclophosphamide) became available in the 1970s (just preceding the MR imaging era), concern about their toxicity resulted in a strategy in which mainly those with aggressive or advanced disease were treated. A well-controlled clinical trial indicated lack of efficacy of cyclophosphamide in late secondary progressive MS. Based on open-label trials in the 1980s, cyclophosphamide continues to be used as a salvage-type therapy for worsening MS unresponsive to approved therapies. A less toxic agent azathioprine was used for a range of MS patients with only small-scale clinical trial data available. Neither agent was patent protected nor put forward for formal approval for use in MS.

Since the 1960s, there has been use of corticosteroids as a therapy to reduce severity of disease relapses and enhance rate of recovery. Initial controlled trials were conducted using ACTH. Subsequent relatively small controlled trials were conducted comparing different corticosteroid preparations, with or without nontreatment groups being included. A current consensus for therapy and one usually built in as the therapy for relapses occurring in clinical trials involves high-dose intravenous therapy (Solu-Medrol 1 g iv daily for 3–5 days) for relapses associated with disability. Whether such therapy has an impact on the eventual extent of recovery remains debated.

The modern therapeutic era was initiated in the 1980s with fortuitous coming together of technology to produce recombinant molecules or synthesize new molecules, MR imaging, and progress in design of randomized blinded clinical trials. Since that time, a number of agents have received formal regulatory approval, while

a large number of others are moving through the clinical trial process. This chapter describes the agents that have received FDA approval and selective agents from clinical trials that provide insight into the MS disease process.

Regulatory-Approved Agents

Interferon β and Glatiramer Acetate (GA)

Pivotal clinical trial of patients with relapsing-remitting MS led to confirmation of earlier uncontrolled trial results that two classes of agents, interferon β and glatiramer acetate, reduced disease activity as measured by relapse frequency, disability resulting from these relapses, and new MR-defined lesion formation. Each of these agents reduces clinical disease activity by 30–35% in patients with clinically definite MS and pretreatment activity of 1–2 relapses per year. Lack of efficacy with interferon β injections has been linked in some cases with development of neutralizing antibodies. There is extensive data on the mechanisms of actions of interferon β and glatiramer acetate. Both are considered to act as modulators of the immune response within the systemic compartment, with interferon having additional effects on inhibiting migration of lymphocytes across the blood-brain barrier including by inhibiting production of matrix metalloproteinases. Although neither agent is considered to effectively access the CNS, one needs to consider that there may be indirect effects mediated by the lymphocytes whose properties are modulated systemically and then access the CNS. GA is reported to favor anti-inflammatory and neurotrophic molecule production by lymphocytes.

Subsequent clinical trials have indicated that both these agents may be more effective when given early in the disease course including prevention of second events when given to patients with CIS. The agents have insignificant effects in patients with progressive forms of MS although intercurrent relapses continue to be responsive. Long-term follow-up (now 20 years) have established the long-term safety of these agents resulting in their indefinite use in patients without any defined "stopping rules." Side effects relate mainly to need for continued subcutaneous or intramuscular injections and systemic "flu-like symptoms" (IFN) and allergic reactions (GA). Data remain suggestive that long-term disability and even survival may be favorably impacted, but such studies are complex to analyze due to selection bias related to who remains on therapy. A current consensus would favor that these therapies when given early are favorable for long-term outcome.

As these agents became standard therapy in MS, subsequent therapeutic trials with the next generation of potential therapies were conducted on ever more selected populations including those with less active disease. This is reflected in the observation that the control groups in subsequent MS clinical trial have shown a continued decrease in disease activity. One cautions about comparing efficacy across clinical trials.

Natalizumab

This humanized monoclonal antibody delivered intravenously on a monthly basis recognizes the VLA-4 adhesion molecule, interrupting the interaction between

lymphocytes and the barriers (endothelial, meningeal) that inhibit cells entry into the CNS. This agent proved more effective than interferon β in head-head clinical trials for relapsing forms of MS. The subsequent recognition that 1:1,000 treated individual would develop progressive multifocal leukoencephalopathy after 2 years of this therapy has resulted in this agent being a second-line choice for those whose disease is uncontrolled by IFN or GA or who cannot tolerate the latter agents. Data are not yet available on any effect of this agent in progressive MS.

Other adhesion molecule-directed therapies are currently still in clinical trial stages.

Mitoxantrone

This agent received approval for worsening MS. The cumulative dose-producing cardiotoxicity of the agent limits duration of therapy. There is a lifetime risk of leukemia estimated at 1:500.

Fingolimod (FTY720)

Fingolimod is a sphingosine-1-phosphate (S1P) receptor agonist that acts by enhancing chemokine-directed ingress of lymphocytes bearing corresponding chemokine receptors, mainly CCR7 (naïve and central memory T cells) into regional lymph nodes and inhibiting subsequent S1P-directed lymphocyte (T and B cells) egress from the lymph nodes. This results in peripheral lymphopenia including depletion of the putative disease-mediating population. This agent was approved as a therapy for relapsing forms of MS in 2010 based on its positive effects both in placebo-controlled and comparator (interferon β1a) trials. As S1P receptors are expressed on almost all tissues, regulatory guidelines recommend monitoring of effects of fingolimod therapy on heart rate, blood pressure, and pulmonary function. Fingolimod readily enters the CNS leading to ongoing investigations regarding its effects on regulating immune responses within the CNS and tissue protection and repair processes. Clinical trial data indicate that this therapy reduced the rate of tissue loss (MR determine brain volume changes) compared to interferon β1a therapy. To be answered is whether such results reflect direct CNS effects or indirect effects reflecting overall reduction in CNS inflammation as suggested by observations with other agents (monoclonal antibodies – natalizumab, alemtuzumab, rituximab) that do not access the CNS. Clinical trials with fingolimod for progressive MS are ongoing. Published data from extension phases of clinical trials indicated sustained efficacy of fingolimod, but incidence of rare but serious toxicity cannot yet be determined.

Lessons from Clinical Trials

The optimal therapeutic agent for MS would be one which is highly effective, without toxicity, and convenient to use. In absence of such an agent, the choice of therapy in clinical practice will be dependent on reaching a consensus with the patient regarding expectations from therapy with regard to benefit versus risk and

impact of disease versus therapy on quality of life issues including family planning. Experience from treatment protocols involving intense immune suppression (high-dose cytotoxic agents requiring subsequent immune reconstitution (autologous stem cell transplant)) establishes that systemic immune intervention can ablate subsequent inflammatory lesion formation over a prolonged period (>5-year follow-up) if not indefinitely. Such therapy however has not universally prevented continuation or development of progressive disease in absence of new inflammatory lesions. Clinical trials with cytotoxic antibodies that deplete the total lymphocyte pool (anti-CD52 – alemtuzumab) or selective components of the immune system (anti-CD20 (expressed on B cells but not antibody-producing plasma cells) – rituximab) for months followed by spontaneous recovery document their high degree of effectiveness in preventing new inflammatory lesion formation; however, there is disease recurrence as immune reconstitution occurs. Experience with alemtuzumab indicates that manipulation of the immune regulatory balance in MS patients can result in development of other tissue-specific autoimmune phenomena (thyroid, platelet, kidney). The effectiveness of rituximab in relapsing MS occurring prior to any change in serum or CSF immunoglobulin levels has illustrated the role of B cells in regulating the immune network. As mentioned, neither alemtuzumab nor rituximab are effective for progressive MS.

An approach to achieving effective immune therapy without toxicity would be to eliminate or modulate the disease-mediating cells without impacting on the remaining components of the immune system. The approach of targeting disease-relevant antigen-specific lymphocytes is referred to as immunologic tolerance. This approach remains problematic in MS where no dominant disease-relevant antigen has been identified. Even in inbred animals induced to develop an inflammatory/demyelinating disease of the CNS (experimental autoimmune encephalomyelitis (EAE)) by immunization with CNS tissue, there are major strain and species differences with regard to what is the main disease-inducing antigen. Over time, animals with relapsing forms of this disease can develop sensitization to a wider range of antigens (epitope spreading). Attempts to immunize MS patients with specific modified myelin antigens (altered myelin basic protein peptide ligands) or with myelin encoding DNA vaccines designed to induce anti- rather than disease-promoting pro-inflammatory responses have either induced the unwanted immune response or not shown clear efficacy in limited trials. Other strategies to induce tolerance include manipulating how putative disease-relevant antigens are presented to T cells by antigen presenting cells and eliminating specific T cells bearing the receptors that would recognize the antigen.

The clinical trial pipeline for relapsing MS includes a series of agents that address the issue of convenience (oral agents) with agents that will have predicted efficacy in the range of the original immunomodulators with an acceptable safety profile. Updated information on ongoing clinical trials in MS is available via NIH and United States National MS Society websites.

No data currently exist to address whether reducing risk factors associated with development of MS, namely, vitamin D deficiency or smoking will prevent disease

development. Incomplete data are available to assess the effects of vitamin D therapy or elimination of smoking on disease course once established. Good clinical practice favors such interventions while data are being generated.

Neurobiologic-Directed Therapies

As mentioned above, current immunotherapies do not impact on established secondary progressive disease. The frequency and severity of initial relapse-related injury has been linked to subsequent progressive disease development and course. Limited data yet exist on use of agents that could protect tissue from effects of initial injury per se or on subsequent disease course. Examples of such agents would be those directed at inhibiting actions of potential neurotoxic molecules such as glutamate. Demyelinated nerve features redistribution of specific ion channels as a means to maintain electrical conduction. A consequence would be altered intra- and extracellular exchange of ions resulting in excess inflow of calcium with potential deleterious effects. Ion channel blockers are studied in experimental models with only limited translation into the clinic. The potassium channel blocker 4-aminopyridine is currently approved for a therapy to promote sustained activity in MS. Additionally, one seeks means to overcome the heavy energy demand required for demyelinated nerve to maintain conduction. Further awaited for translation are agents that are shown in animal models to enhance the capacity of progenitor cells to mediate remyelination. Candidate agents identified in animal studies include neurotrophic factors and hormones such as prolactin and progesterone and agents that target specific receptors that promote or inhibiting progenitor cell differentiation (retinoic acid, anti-LINGO, and NOGO antibodies).

Emerging data from the rehabilitation field is that there is greater than previously recognized plasticity of the adult human CNS and that sustained rehabilitation and physical and cognitive training can result in enhanced long-term function. Although there are many reports of benefit of short-term intervention weeks in improving function in MS patients, currently, there are no standardized protocols for long-term management.

Symptomatic Therapy in MS

Individuals with MS experience an array of symptoms that reflect the effects of disruption of normal function of the CNS. Many of these are also encountered in those with other neurologic disease that disrupts CNS pathways. Common problems are spasticity, reduced endurance/fatigue, pain, depression, and bladder/bowel/sexual dysfunction. Discussion of the complex problem of managing MS patients and optimizing quality of life for the individual and her (his) family is beyond the scope of this chapter.

Future Directions in MS

In spite of the tremendous accrual of knowledge about MS over the last 50 years, there still remains much to investigate before we can understand the causes, diagnosis, and treatment of this mysterious disease. Future directions will need to answer the questions as to the definition of the disease itself. Is it a single entity, or a spectrum of conditions leading to a similar syndrome or presentation? What are the underlying causes of the disease, and how is the immune component influenced by the complex interaction of genes, environment, and other known epidemiological factors? How can we better diagnose, not only the disease itself, but also its components so that the in vivo pathology can be examined and patients can be treated according to the stage, or pathological process they are undergoing?

Understanding MS requires the detailed correlation between clinical presentation, imaging analysis, pathology, genetics, and epidemiology, all informed by relevant experimental studies. Emerging fields in science, such as pathogen mining to look for infective organisms, and ever-expanding sophisticated gene analysis and epidemiological techniques will be required to answer some of these questions. Therapies designed to better protect the axon, and to repair myelin, will be added to the ever-improving modalities to treat and halt the inflammatory process. Molecular imaging techniques that correlate with pathological changes will help the clinician decide on a time-sensitive treatment course.

Further Reading

Bar-Or A, Darlington PJ. The immunology of multiple sclerosis. In: Cohen J, Ruddick R (eds) (2011) Multiple sclerosis therapeutics, 4th edn. Cambridge University Press, New York.

Burrell AM, Handel AE, Ramagopalan SVF et al (2011) Epigenetic mechanisms in multiple sclerosis and the major histocompatibility complex (MHC). Discov Med 11:187

Compston AS, Confavreaux C, Lassmann H et al (eds) (2006) McAlpine's multiple sclerosis, 4th edn. Churchill Livingstone Elsevier, Philadelphia

Degenhardt A, Ramagopalan SV, Scalfari A et al (2009) Clinical prognostic factors in multiple sclerosis: a natural history review. Nat Rev Neurol 12:672–682

Koch-Henricksen N, Sorensen PS (2010) The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol 9:520–532

Maneiro C, Bemmer T, Radding A et al (2009) In vivo imaging of cortical pathology in multiple sclerosis using ultra-high field MRI. Neurology 73:941–948

Miller DH (1994) Magnetic resonance in monitoring the treatment of multiple sclerosis. Ann Neurol 36(Suppl):S91–S94

Montalban X, Tintore M, Swanton J et al (2010) MRI criteria for MS in patients with clinically isolated syndromes. Neurology 74:427–434

Polman CH, Rheingold SC, Edan G et al (2005) Diagnostic criteria for multiple sclerosis. Ann Neurol 58:840–846

Raine CS, McFarland H, Hohfeld R (eds) (2008) Multiple sclerosis: a comprehensive text. Saunders Elsevier, New York/Philadelphia