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Multiple sclerosis

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). The lesions of MS were first depicted in 1835 by the Scotsman, Robert Carswell (Compston, 1988). The cause of MS became a matter of great interest and speculation. In 1940, Ferraro & Jervis noted the close pathological similarities between experimental autoimmune encephalomyelitis (EAE) and certain cases of acute MS. These similarities gave rise to the theory that MS is an autoimmune disease, a theory further supported by the remarkable similarities between chronic relapsing EAE and MS (Lassmann & Wisniewski, 1979). Advances in the understanding of the immunology of EAE have been rapidly applied to research on MS. Indeed, our current knowledge of the immunology of MS is largely based on studies inspired by insights obtained from research on EAE.

Clinical features

General clinical features

MS generally first presents itself clinically between the ages of 15 and 50 years, but may commence as early as three years (Hanefeld *et al.*, 1991) or as late as the seventh decade. It is about twice as common in females as in males. MS typically results in neurological symptoms and signs indicative of involvement of the white matter of the CNS. The most common clinical features are: monocular visual loss, due to optic neuritis; weakness of the lower limbs, with or without upper limb weakness; sensory loss or paraesthesiae of the limbs or trunk; sensory or cerebellar ataxia; cranial nerve symptoms and signs, such as diplopia, facial sensory disturbance, oscillopsia and nystagmus, due to brainstem involvement; bladder and bowel disturbance; and memory and cognitive impairment. The typical course is one of relapses and remissions, with clinical evidence of involvement of the same or different regions of the CNS in different attacks. This relapsing–remitting

pattern often later changes to a gradually progressive pattern of neurological deficit (secondary progression). About one-third of patients follow a progressive course from the onset without experiencing any obvious discrete attacks or remissions (primary progression). Rarely, MS takes an acute fulminant monophasic course, leading to death within three weeks to six months after the onset of the first clinical signs (Marburg's disease) (Lassmann, Budka & Schnaberth, 1981; Lassmann, 1983; Johnson, Lavin & Whetsell, 1990).

Diagnosis

The clinical diagnosis of MS requires the demonstration of involvement of different regions of the CNS at different times (dissemination in time and place) in the absence of any better explanation for the clinical findings (Poser *et al.*, 1983). The history of the illness and the clinical neurological examination have key roles in the diagnostic process, and laboratory investigations are often also necessary to establish a diagnosis. Examination of the cerebrospinal fluid (CSF) by isoelectric focusing typically shows oligoclonal immunoglobulin G (IgG) bands, which are not present in the serum, although such a pattern is not specific for MS and may be present in any inflammatory CNS disease (McLean, Luxton & Thompson, 1990). A mild mononuclear pleocytosis may also be present in the CSF. Electrophysiological studies of signal transmission through visual, somatosensory, auditory and motor pathways (evoked potential studies) are useful in demonstrating subclinical involvement, but do not show changes specific for MS. Magnetic resonance imaging (MRI) of the brain and spinal cord is highly sensitive for detecting MS lesions, although non-specific, and may also be valuable in excluding other pathology (Ormerod *et al.*, 1987). The CSF and MRI findings in MS and the information they provide about MS pathogenesis are discussed in detail later in this chapter.

Association with other autoimmune diseases

MS has been reported to occur concurrently with other autoimmune diseases, including ankylosing spondylitis (Khan & Kushner, 1979; Seyfert *et al.*, 1990), rheumatoid arthritis (Baker *et al.*, 1972; De Keyser, 1988; Seyfert *et al.*, 1990), scleroderma (Trostle, Helfrich & Medsger, 1986), inflammatory bowel disease (Rang, Brooke & Hermon-Taylor, 1982; Sadovnick, Paty & Yannakoulias, 1989; Seyfert *et al.*, 1990), autoimmune thyroid disease, especially Graves' disease (Baker *et al.*, 1972; De Keyser, 1988; Seyfert *et al.*, 1990; McCombe, Chalk & Pender, 1990), type I diabetes mellitus (Wertman, Zilber & Abramsky, 1992), Addison's disease (Baker *et al.*, 1972), autoimmune gastritis (Baker *et al.*, 1972), myasthenia gravis (Somer, Muller & Kinnunen, 1989), pemphigus vulgaris (Baker *et*

al., 1972), psoriasis (Cendrowski, 1989), alopecia areata (Seyfert *et al.*, 1990) and primary biliary cirrhosis (Pontecorvo, Levinson & Roth, 1992). To determine whether the association of MS with other autoimmune diseases is higher than that expected to occur by chance, Seyfert *et al.* (1990) conducted a prospective case–control study of MS patients and healthy volunteers and found 13 of 101 MS patients and two of 97 controls with such diseases ($P = 0.009$). They also found that MS patients have a significantly increased overall frequency of a variety of serum autoantibodies, particularly anti-thyroid-microsomal antibodies, anti-TSH-receptor antibodies, anti-pituitary antibodies, anti-parietal-cell antibodies, anti-smooth-muscle antibodies, anti-nuclear antibodies, anti-double-stranded-DNA antibodies and rheumatoid factor (Seyfert *et al.*, 1990). Other studies have also found a significantly higher frequency of serum organ-specific (especially anti-thyroid) antibodies (Kiessling & Pflughaupt, 1980; De Keyser, 1988; Ioppoli *et al.*, 1990; Tomasevic *et al.*, 1990) and non-organ-specific antibodies (De Keyser, 1988; Tomasevic *et al.*, 1990) in MS patients than in patients with other neurological disorders. Wertman *et al.* (1992) found that the prevalence of type I diabetes mellitus was significantly higher in MS patients under the age of 30 years than in the general population of the same age group. An anti-DNA antibody idiotype termed 16/6, which occurs with high frequency in the sera of patients with systemic lupus erythematosus, is also present at an increased frequency in the sera of patients with MS and of patients with other autoimmune diseases (Shoenfeld *et al.*, 1988). Collectively, the increased occurrence of other autoimmune disease and of serum autoantibodies in MS indicate that MS is also an autoimmune disease.

Uveitis

Anterior and posterior uveitis occur in patients with MS more frequently than would be expected by chance (Archambeau, Hollenhorst & Rucker, 1965; Breger & Leopold, 1966; Porter, 1972; Bamford *et al.*, 1978; Lightman *et al.*, 1987; Meisler *et al.*, 1989; Graham *et al.*, 1989). The concurrence of uveitis and MS may simply be another example of two autoimmune diseases occurring in patients with a susceptibility to autoimmunity, as discussed above. However, the frequency of this association is considerably higher than the association of MS with other individual autoimmune diseases, suggesting that the concurrence of uveitis and MS may also be due to cross-reactivity between uveal and CNS antigens. This hypothesis is supported by the finding that uveitis occurs in pigs and rabbits with EAE induced by inoculation with CNS tissue (Fog & Bardram, 1953; Bullington & Waksman, 1958). Recently, circulating antibodies to the uveitogenic retinal protein, arrestin (S-antigen), and to the homologous brain protein, β-

arrestin 1, have been found in eight out of 14 patients with MS but not in normal controls or patients with other neurological diseases (Ohguro *et al.*, 1993). Furthermore, in two patients with MS, serum antibody titres were higher during relapse than in remission. Cross-reactivity between uveal and CNS antigens may explain the close temporal relationship between the onset of uveitis and the onset or exacerbation of MS in some patients (Archambeau *et al.*, 1965).

Involvement of the peripheral nervous system

MS has classically been considered a disease restricted to the CNS; however, there have been several studies demonstrating subtle electrophysiological or neuropathological evidence of peripheral nervous system (PNS) involvement in patients with typical MS (Waxman, 1993), as well as reports of the concurrence of MS with clinically apparent chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (Thomas *et al.*, 1987; Rubin, Karpati & Carpenter, 1987; Mendell *et al.*, 1987). Furthermore, PNS involvement is frequent in acute MS (Marburg's disease) (Lassmann, 1983). As discussed in Chapter 3, involvement of the PNS, especially the proximal PNS, is usual in EAE induced by inoculation with whole CNS tissue or myelin basic protein (MBP), but not with proteolipid protein (PLP). Based on the findings in EAE, it can be hypothesized that the degree of PNS involvement in MS depends on whether the autoimmune attack is directed only against antigens confined to the CNS (for example PLP and myelin/oligodendrocyte glycoprotein [MOG]) or against antigens present in both the CNS and the PNS (for example MBP, galactocerebroside and myelin-associated glycoprotein [MAG]). As with the concurrence of MS and uveitis, some cases of concurrent MS and CIDP may simply be due to the tendency for different autoimmune diseases to occur in the same susceptible individual.

Genetics

A major genetic component in the susceptibility to MS has been clearly demonstrated by a population-based study of MS in twins. The concordance rate for MS in monozygotic twins (25.9%) was found to be much higher than that in dizygotic twins (2.3%) and non-twin siblings (1.9%) (Ebers *et al.*, 1986). Multiple genes appear to be involved in this genetic susceptibility, including class II HLA genes and possibly T cell receptor (TCR) genes.

Class II HLA genes

In 1973 Jersild *et al.* reported that MS is associated with the cellular specificity HLA-Dw2. However, the subsequent widespread use of serologi-

cal typing techniques, which fail to distinguish Dw2 from the other DR2 haplotypes, resulted in the impression that this association was confined to Caucasian populations originating from Northern Europe (Hillert & Olerup, 1993). With the introduction of genomic typing techniques, it has now become clear that the DRw15,DQw6,Dw2 (DRB1*1501-DQA1*0102-DQB1*0602) haplotype is associated with MS, irrespective of ethnic origin (Olerup *et al.*, 1989; Hao *et al.*, 1992; Serjeantson *et al.*, 1992; Hillert & Olerup, 1993). The Dw2 haplotype segregates closely with MS in multiplex MS families, indicating that it plays an important role in determining susceptibility to MS (Hillert *et al.*, 1994). The relative contributions of the DR and DQ loci remain unclear; however, studies in Hong Kong Chinese (Serjeantson *et al.*, 1992) and French Canadians (Haegert & Francis, 1992) have implicated DQB1*0602 as a susceptibility allele. It has been suggested that DQ β chain polymorphisms at a single residue (26) contribute to the development of MS in the latter population (Haegert & Francis, 1992).

In Swedish and Norwegian patients there is evidence of immunogenetic heterogeneity between the relapsing–remitting and the primary progressive forms of MS. Whereas both clinical forms are associated with the DRw15,DQw6,Dw2 haplotype, the relapsing–remitting form is also associated with the DQB1 allelic pattern observed in the DRw17,DQw2 haplotype (Olerup *et al.*, 1989; Hillert *et al.*, 1992*a*).

TCR genes

A linkage between MS and the TCR β chain complex was found in one study of American MS multiplex families (Seboun *et al.*, 1989) but not in another family study (Lynch *et al.*, 1991). Population studies of North American Caucasian MS patients have indicated the existence of an MS susceptibility gene(s) within the region of the TCR β chain gene complex (Beall *et al.*, 1989) and more specifically within the TCR Vβ region (Beall *et al.*, 1993). In the latter study the TCR Vβ subhaplotype frequencies differed significantly from the control population only in the $DR2^+$ MS patients and not in the $DR2^-$ MS patients, providing the first evidence for gene complementation between an HLA class II gene and TCR Vβ gene(s) in conferring susceptibility to MS (Beall *et al.*, 1993). There is also evidence for an association with TCR Vβ and Cβ genes in French (Briant *et al.*, 1993) and Spanish (Martinez Naves *et al.*, 1993) MS patients. On the other hand, population studies of Scandinavian MS patients have not found an association between susceptibility to MS and TCR β chain haplotypes (Fugger *et al.*, 1990; Hillert, Leng & Olerup, 1991). An association between MS and a restriction fragment length polymorphism of the TCR Vα and Cα gene segments has also been reported (Oksenberg *et al.*, 1989; Sherritt *et al.*, 1992), but this was not confirmed by

another study which found evidence that the seemingly polymorphic fragments may have resulted from incomplete cleavage of DNA by the restriction enzyme (Hillert, Leng & Olerup, 1992*b*).

Familial occurrence of MS with other autoimmune diseases: evidence for a primary autoimmune gene

In the families of patients with MS there appears to be an increased occurrence of other autoimmune diseases, including systemic lupus erythematosus, scleroderma, thyroid disease and inflammatory bowel disease (Trostle *et al.*, 1986; Minuk & Lewkonia, 1986; Bias *et al.*, 1986; Sloan *et al.*, 1987; Sadovnick *et al.*, 1989; McCombe *et al.*, 1990; Doolittle *et al.*, 1990). On the basis of a genetic analysis of 18 autoimmune kindreds (three containing a member with MS), Bias *et al.* (1986) have proposed that autoimmunity is inherited as an autosomal dominant trait with secondary genes, including HLA genes, determining the specific type of autoimmune disease.

Other genes

Evidence has been presented that an MBP gene or some other MBP-linked locus influences susceptibility to MS (Boylan *et al.*, 1990; Tienari *et al.*, 1992); however, another study did not demonstrate linkage between MS and the MBP gene (Rose *et al.*, 1993). In contrast to earlier studies, Walter *et al.* (1991) and Hillert (1993) found no evidence that Ig constant region genes confer susceptibility to MS. However, Walter *et al.* (1991) found an association between MS and an Ig heavy chain variable region gene segment. There is also a report of a significant association between MS and the M3 allele of α-1 antitrypsin, the major circulating protease inhibitor (McCombe *et al.*, 1985). Harding *et al.* (1992) have reported the occurrence of an MS-like illness in women with a mitochondrial DNA mutation found in Leber's hereditary optic neuropathy and have suggested that mitochondrial genes may contribute to susceptibility to MS.

In conclusion, the only confirmed genetic factor predisposing to MS is the HLA-DR-DQ haplotype DRw15,DQw6,Dw2. There is suggestive evidence of roles for the TCR β chain genes and a primary autoimmune gene in determining disease susceptibility, but further studies are needed to confirm their roles.

Neuropathology

Primary demyelination is the key morphological feature of the MS lesion (Périer & Grégoire, 1965; Prineas, 1985). Primary demyelination is a

process resulting in loss of the myelin sheath with preservation of the underlying axon, in contrast to secondary demyelination, where myelin loss is a consequence of axonal loss. Other important characteristics of MS lesions are a mononuclear inflammatory infiltrate (see below), the presence of myelin breakdown products within macrophages, and astrocytic gliosis. The lesions of MS can occur virtually anywhere within the CNS, but the most common sites of involvement are the optic nerves, spinal cord and periventricular regions of the cerebral hemispheres. An essential feature is the occurrence of lesions of different ages, as indicated by varying degrees of inflammation, ongoing demyelination, remyelination and gliosis.

An important question concerning the pathogenesis of MS is whether the primary demyelination results from direct damage to the myelin sheath itself or whether it results from destruction of the oligodendrocyte, the cell that produces and maintains myelin. It is generally agreed that the oligodendrocyte is lost in the longstanding MS lesion, but there has been controversy concerning its fate in the early lesion. However, Prineas *et al.* have recently presented evidence that there is oligodendrocyte loss in the early lesion (Prineas *et al.*, 1989, 1993*a*).

Contrary to previous opinion, significant remyelination by oligodendrocytes does occur in MS (Lassmann, 1983; Prineas *et al.*, 1984, 1993*a*). Remyelination has been observed ten weeks after clinical onset (Prineas *et al.*, 1993*a*). It may well commence much earlier, as in rats with acute EAE it commences as early as six days after clinical onset (Pender, 1989; Pender, Nguyen & Willenborg, 1989). Remyelination of a demyelinating CNS lesion (possibly due to MS) has been observed in a brain biopsy from a 15-year-old boy about two weeks after the onset of neurological symptoms (Ghatak *et al.*, 1989). Prineas *et al.* (1993*a*) have suggested that new MS lesions normally remyelinate unless interrupted by recurrent disease activity. It is likely that shadow plaques (groups of thinly myelinated fibres) represent remyelination after a single previous episode of focal demyelination (Lassmann, 1983; Prineas *et al.*, 1993*a*). The finding that new demyelinating lesions may be superimposed on old shadow plaques supports the MRI evidence (see below) that local recurrence may be at least as important as progressive edge activity in determining plaque growth (Prineas *et al.*, 1993*b*). It also indicates that recurrent demyelination of the same area may be a factor underlying failed remyelination in MS.

Although primary demyelination is the hallmark of MS, axonal loss also occurs and may be severe in longstanding lesions (Barnes *et al.*, 1991). Occasionally, frank necrosis occurs. As mentioned earlier, PNS demyelination sometimes develops in patients with MS. All the above morphological features of MS are observed in chronic relapsing EAE (Lassmann & Wisniewski, 1979; Lassmann, 1983; see Chapter 3).

Pathophysiology

Evoked potential studies of signal transmission through visual, auditory, somatosensory and motor pathways reveal functional abnormalities in patients with MS. Although these studies are useful for clinical diagnosis, their contribution to understanding the pathophysiology of MS is limited by difficulties in interpretation. The typical evoked potential findings in MS are a prolongation of latency and a reduction in amplitude. In peripheral nerve conduction studies, a prolongation of latency indicates conduction slowing, whereas a reduction in amplitude (without temporal dispersion) indicates focal conduction block or complete conduction failure. However, evoked potential studies of CNS function are dependent on signal transmission through pathways containing one or more synapses where signals are normally delayed, integrated and amplified. Hence, prolongation of the latency of an evoked potential may be caused by increased synaptic delays due to presynaptic axonal conduction block as well as by conduction slowing. Furthermore, a reduction in the amplitude of the evoked postsynaptic field potential is an unreliable indicator of presynaptic axonal conduction block (Stanley, McCombe & Pender, 1992). Therefore, at present our understanding of the pathophysiology of MS has to rely mainly on experimental studies of demyelination in animals.

It is highly likely that the main mechanism producing neurological symptoms and signs in the early stages of MS is nerve conduction block due to primary demyelination. It is well established that primary demyelination *per se* in the CNS causes focal conduction block or conduction slowing at the site of demyelination (McDonald & Sears, 1970). Neurological symptoms and signs will result if conduction block occurs simultaneously in a significant proportion of fibres within a given pathway. In clinical attacks of EAE there is CNS conduction block due to demyelination (see Chapter 3).

Conduction slowing due to demyelination may have no significant clinical consequences, although it is possible that slowing of conduction in presynaptic axons may alter spatiotemporal integration in postsynaptic neurones and thus produce clinically apparent disturbances of function. However, because conduction is insecure in slowly conducting fibres, intermittent conduction block may occur and lead to neurological symptoms. For example, demyelinated fibres may be able to transmit signals at low frequencies but not at higher frequencies (McDonald & Sears, 1970), owing to an increase in threshold through the hyperpolarizing effect of the electrogenic Na^+/K^+ pump (Bostock & Grafe, 1985). An inability to sustain high-frequency transmission may contribute to the fading out of vision after looking at an object continuously for several seconds, and to the fatiguability of muscle strength experienced by some patients with MS. Conduction in demyeli-

nated fibres is also susceptible to small changes in body temperature. A temperature increase of 0.5 °C can reversibly induce conduction block in demyelinated fibres by shortening the duration of the action potential and thus reducing the current available to excite the demyelinated region (Rasminsky, 1973). Cooling has the opposite effect. Reversible conduction block accounts for the temporary clinical deterioration that occurs in patients with MS with an increase in body temperature, for example due to fever. Demyelinated fibres may also generate ectopic impulses, either spontaneously or after mechanical stimulation (Smith & McDonald, 1982). Ephaptic transmission (lateral spread of excitation from one axon into an adjacent one) occurs in the congenitally dysmyelinated spinal root fibres of the dystrophic mouse (Rasminsky, 1980) and may possibly occur in demyelinated CNS fibres. Ectopic impulse generation and ephaptic transmission are likely to contribute to the paroxysmal phenomena that occur in MS, namely Lhermitte's sign, trigeminal neuralgia, painful tonic seizures and paroxysmal dysarthria.

Conduction can be restored in demyelinated CNS fibres by remyelination, although conduction is slow and insecure until the remyelination is well established (Smith, Blakemore & McDonald, 1981). However, remyelination is not essential to restore nerve conduction: nerve conduction can be restored in fibres that are still demyelinated, possibly by alterations in the distribution of Na^+ channels within the demyelinated axolemma, by reduction in the diameter of demyelinated axons or by glial ensheathment (Bostock & Sears, 1978; Smith, Bostock & Hall, 1982; Waxman *et al.*, 1989; Shrager & Rubinstein, 1990). During clinical recovery from EAE there is restoration of CNS conduction due to glial ensheathment and remyelination (see Chapter 3). The extent to which remyelination contributes to clinical recovery after attacks of MS remains to be determined.

It is possible that cytokines or other inflammatory mediators may also contribute to acute neural dysfunction in MS, but there is little evidence to support this suggestion. Oedema is unlikely to contribute to the neurological deficit, except when it occurs within a confined space, for example the optic canal, where it may result in secondary ischaemia. Axonal loss is likely to be an important cause of persistent neurological dysfunction in MS (Barnes *et al.*, 1991), as it is in chronic relapsing EAE (Stanley & Pender, 1991).

Magnetic resonance imaging and spectroscopy

Magnetic resonance imaging is a sensitive technique for the detection of CNS lesions in MS. The typical findings are regions of increased signal on T_2-weighted images, which correspond with histologically defined plaques (Ormerod *et al.*, 1987). It is likely that this increased signal is due to oedema

in acute lesions and to gliosis in chronic lesions; demyelination per se is unlikely to make an important contribution (Ormerod *et al.*, 1987). Enhancement of T_1-weighted images after the intravenous administration of gadolinium diethylenetriaminepentaacetic acid (gadolinium) reflects breakdown of the blood–brain barrier and is a useful indicator of disease activity (Miller *et al.*, 1988). Serial studies have shown that gadolinium enhancement of T_1-weighted images precedes other MRI abnormalities in the evolving new lesion (Kermode *et al.*, 1990) and that enhancement can also occur in old lesions that have been non-enhancing on previous scans (Miller *et al.*, 1988). Although disease activity as indicated by gadolinium enhancement is usually asymptomatic, clinical deterioration in patients with relapsing–remitting MS is significantly associated with increased frequency and area of gadolinium-enhancing lesions (Smith *et al.*, 1993). Similar changes in gadolinium enhancement on MRI also occur in chronic relapsing EAE (see Chapter 3).

Serial MRI studies of MS have indicated a difference in the dynamics of disease activity between secondary progressive MS and primary progressive MS, particularly in relation to the inflammatory component of the lesions (Thompson *et al.*, 1991). Patients in the secondary progressive group had 18.2 new lesions per patient per year and 87% of these enhanced. Enhancement also occurred within and at the edge of pre-existing lesions. In contrast, patients in the primary progressive group had only 3.3 new lesions per patient per year and only 5% of these enhanced (Thompson *et al.*, 1991). MRI studies have demonstrated considerable expansion of the extracellular space in longstanding lesions, which probably reflects axonal loss (Barnes *et al.*, 1991).

Although MRI has provided important information about the temporal profile of inflammation in MS, it has not yielded information about the time course of demyelination, because it does not reveal normal myelin or myelin breakdown products. Proton magnetic resonance spectroscopy (MRS) can detect increased lipid resonances at 0.9 and 1.3 parts per million which probably indicates myelin breakdown products (Davie *et al.*, 1993, 1994; Koopmans *et al.*, 1993). Serial proton MRS of acute MS lesions has demonstrated such increased resonances in lesions which had been enhancing with gadolinium for less than one month, indicating that myelin breakdown occurs during the initial inflammatory stage of lesion development (Davie *et al.*, 1994). Increased choline signals also occur in MS lesions (Arnold *et al.*, 1992; Davie *et al.*, 1994) and were initially attributed to recent demyelination; however, a study on EAE has indicated that such an increase can be produced by the increased membrane turnover associated with inflammation in the absence of demyelination (Brenner *et al.*, 1993). Proton MRS of MS lesions has also demonstrated decreased *N*-acetylaspartate signals, which have been attributed to neuronal or axonal damage (Arnold *et*

al., 1992), although this change is partially reversible over 4–8 months and therefore cannot be explained solely by axonal loss (Davie *et al.*, 1994).

Immunopathology of the CNS lesions

Characteristics of the inflammatory infiltrate in the CNS

Immunocytochemical studies of CNS tissue sections from patients with MS have shown that the perivascular inflammatory cell cuffs and the parenchymal inflammatory cell infiltrate consist predominantly of T lymphocytes and macrophages (Traugott, Reinherz & Raine, 1983*a*,*b*; Booss *et al.*, 1983; Hauser *et al.*, 1986; Woodroofe *et al.*, 1986; Esiri & Reading, 1987; McCallum *et al.*, 1987; Sobel *et al.*, 1988; Boyle & McGeer, 1990). Generally $CD8^+$ T cells have been found to be more frequent than $CD4^+$ T cells (Booss *et al.*, 1983; Hauser *et al.*, 1986; Woodroofe *et al.*, 1986; McCallum *et al.*, 1987; Hayashi *et al.*, 1988), although one study found that $CD4^+$ T cells outnumbered $CD8^+$ T cells in the normal-appearing white matter adjacent to active chronic lesions (Traugott *et al.*, 1983*a*) and another found that there were slightly more $CD4^+$ T cells than $CD8^+$ T cells in plaques as well as in the adjacent white matter (Sobel *et al.*, 1988). The variations in cellular composition of MS lesions are likely to be due to variations in the pathological stage of the lesions studied (Sobel *et al.*, 1988). The preponderance of $CD8^+$ T cells over $CD4^+$ T cells in MS lesions is in contrast to the findings in EAE lesions, where $CD4^+$ T cells predominate (see Chapter 3). The numbers of both $CD4^+$ T cells and $CD8^+$ T cells are maximal at the borders of MS plaques, with the numbers falling off inside the plaque and in the adjacent normal-appearing white matter (McCallum *et al.*, 1987). Some of the infiltrating cells express the interleukin-2 receptor (IL-2R), indicating that they are activated T cells (Bellamy *et al.*, 1985; Hofman *et al.*, 1986; Sobel *et al.*, 1988). Compared with the lesions of viral encephalitis, the lesions of MS have a selective reduction in the number of cells expressing CD45RA, which is found on naive T cells (Sobel *et al.*, 1988).

$\gamma\delta$ T cells are also present in chronic MS lesions, where they co-localize with immature oligodendrocytes expressing the 65-kDa heat shock protein (hsp65) (Selmaj, Brosnan & Raine, 1991), and in acute lesions where hsp60 is present in foamy macrophages and hsp90 in reactive astrocytes (Wucherpfennig *et al.*, 1992*b*). Human $\gamma\delta$ T cells have been shown to lyse human oligodendrocytes *in vitro*, possibly by targeting hsp which are differentially expressed by oligodendrocytes compared to astrocytes and which can be recognized by $\gamma\delta$ T cells (Freedman *et al.*, 1991, 1992). It has been proposed that, after initiation of the inflammatory process in the CNS by $\alpha\beta$ T cells reactive with a myelin antigen(s), hsp may be overexpressed at the inflam-

matory site with resultant recruitment of $\gamma\delta$ T cells that induce demyelination (Wucherpfennig *et al.*, 1992*b*).

In some cases of MS there is a prominent accumulation of plasma cells in the perivascular spaces of the CNS, and plasma cells are also present in the parenchyma (Prineas & Wright, 1978). Esiri (1980) found that immunoglobulin-containing cells (the great majority of which were considered likely to be immunoglobulin-producing) are numerous in MS plaques. In recent plaques these cells were commonly found within the parenchyma as well as in perivascular cuffs, while in chronic plaques and normally myelinated tissue they were almost entirely confined to the perivascular spaces (Esiri, 1980). Using an MBP–enzyme conjugate technique, Gerritse *et al.* (1994) found B cells forming anti-MBP antibody in the brains of five out of 12 MS patients. Prineas & Graham (1981) found capping of surface IgG on macrophages contacting myelin sheaths and interpreted this as evidence that anti-myelin antibody opsonizes myelin for phagocytosis by macrophages. Granular deposits of the C9 component of complement and of the terminal complement complex have been demonstrated immunocytochemically in association with capillary endothelial cells, predominantly within plaques and adjacent white matter from MS patients but not from controls (Compston *et al.*, 1989). With the exception of the apparent predominance of $CD8^+$ T cells over $CD4^+$ T cells, the findings in MS are similar to those in EAE (see Chapter 3).

Major histocompatibility complex (MHC) class II antigen expression in the CNS

It is well established that MHC class II antigen is expressed on macrophages and microglia in MS lesions (Traugott & Raine, 1985; Woodroofe *et al.*, 1986; Hayes, Woodroofe & Cuzner, 1987; Cuzner *et al.*, 1988; McGeer, Itagaki & McGeer, 1988; Boyle & McGeer, 1990; Lee *et al.*, 1990; Bö *et al.*, 1994). Using double-labelling techniques and confocal microscopy, Bö *et al.* (1994) found that class II antigen is expressed not only by parenchymal macrophages within the CNS lesions but also by macrophages within the perivascular spaces (perivascular macrophages) of blood vessels both inside and outside the lesions. MHC class II antigen expression by microglia is found in many non-inflammatory neurological diseases (McGeer *et al.*, 1988), indicating that it represents a non-specific reactive phenomenon. Astrocytes in MS lesions have been reported to express MHC class II antigen (Traugott & Raine, 1985; Traugott, Scheinberg & Raine, 1985; Hofman *et al.*, 1986; Traugott & Lebon, 1988; Lee *et al.*, 1990); however, Boyle & McGeer (1990) and Bö *et al.* (1994) could not confirm this. Oligodendrocytes do not express MHC class II antigen in MS lesions (Lee & Raine, 1989; Lee *et al.*, 1990). Vascular endothelial cells have been reported

to express MHC class II antigen (Traugott & Raine, 1985; Traugott *et al.*, 1985) but this was not confirmed by Bö *et al.* (1994).

In conclusion, it would appear that in MS lesions MHC class II antigen is expressed by microglia and macrophages but not by astrocytes, oligodendrocytes or endothelial cells. A similar cellular distribution of MHC class II antigen expression is found in EAE (see Chapter 3). As perivascular macrophages are the only MHC class II-positive cells in MS lesions that contain abundant cytoplasmic MHC class II immunoreactivity, it is likely that they act as antigen-presenting cells in MS (Bö *et al.*, 1994), as they do in EAE (see Chapter 3). At present it is unknown whether microglia upregulate or downregulate the immune response in MS.

Adhesion molecule and cytokine expression in the CNS

In MS lesions there is increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 and E-selectin on CNS vascular endothelium (Sobel, Mitchell & Fondren, 1990; Washington *et al.*, 1994), indicating that adhesion molecules may play a role in T cell entry to the CNS, as in the case of EAE (see Chapter 3). ICAM-1 is also expressed on some glial cells, raising the possibility that inflammatory cells expressing the ICAM-1 ligand, lymphocyte function-associated molecule-1 (LFA-1), may also interact with glial cells through LFA-1/ICAM-1 binding (Sobel *et al.*, 1990).

Cells expressing tumour necrosis factor (TNF) are present in the brain lesions of MS but have not been detected in the normal brain (Hofman *et al.*, 1989). Studies using the polymerase chain reaction detected IL-1 mRNA in the majority of acute and subacute MS plaques, and IL-2 and IL-4 mRNA in some acute lesions (Wucherpfennig *et al.*, 1992*a*).

TCR gene usage in the CNS

Following the demonstration of restricted TCR Vβ gene usage by MBP-specific T cells in mice and rats (see Chapter 3) and in some patients with MS (see below), TCR gene usage by infiltrating T cells has been studied in MS brain tissue by the polymerase chain reaction to determine whether there is restricted usage, which might indicate a specific autoreactive response. Oksenberg *et al.* (1990) reported restricted TCR Vα gene usage in MS brain tissue, but a subsequent more detailed study demonstrated heterogeneous TCR Vα and Vβ gene usage in active MS lesions (Wucherpfennig *et al.*, 1992*a*). Some of the infiltrating T cells use Vβ5.2 (Oksenberg *et al.*, 1993), which has been reported by one group, but not others, to be selectively used by MBP-specific human T cells (see below). Interestingly, 40% of the TCR Vβ5.2 N(D)N rearrangements in the lesions of MS patients with the HLA-DRB1*1501-DQA1*0102-DQB1*0602-DPB1*0401 haplotype have been

found to comprise VDJ sequences used by a cytotoxic T cell clone specific for MBP peptide 89–106 from an MS patient with this HLA haplotype or by encephalitogenic rat T cells specific for MBP peptide 87–99, suggesting that pathogenic MBP-specific T cells may be present in MS brain tissue (Oksenberg *et al.*, 1993). Further studies will be needed to determine whether this is a common and specific finding in MS lesions. It also remains possible that the infiltrating T cells using these Vβ-Dβ-Jβ sequences do not recognize MBP but other antigens.

Wucherpfennig *et al.* (1992*b*) found an accumulation of $\gamma\delta$ T cells that predominantly use the Vδ1 and Vδ2 gene segments in acute MS lesions. They concluded that $\gamma\delta$ T cells appeared to have undergone clonal expansion following recognition of a specific CNS ligand, possibly hsp. Hvas *et al.* (1993) found that the majority of $\gamma\delta$ T cells in chronic MS lesions express the Vγ2 and Vδ2 chains, but in a clonality assessment of brain samples from two patients did not find evidence of an MS-specific expansion of clones using particular types of $\gamma\delta$ TCR.

Immunological findings in the peripheral blood

Non-specific T cell findings

CD4 and CD8 expression

In the peripheral blood of MS patients, particularly those with chronic progressive MS, the $CD8^+$ T cell subset is decreased and the $CD4^+/CD8^+$ ratio is increased (Brinkman, Nillesen & Hommes, 1983; Hughes, Kirk & Compston, 1989; Trotter *et al.*, 1989; Ilonen *et al.*, 1990). In one study the $CD11b^+CD8^+$ subset (reportedly suppressor cells) (Hughes *et al.*, 1989) was found to be reduced but in another study the $CD11b^-CD8^+$ subset (reportedly cytotoxic) showed the more marked decrease (Ilonen *et al.*, 1990). CD8 and CD4 are released in soluble form upon T cell activation. In one study, soluble CD8 but not soluble CD4 was found to be significantly increased in the peripheral blood of MS patients, with the soluble CD8 level being higher in exacerbation than in remission (Tsukada *et al.*, 1991); however, in another study the soluble CD8 level was not elevated (Maimone & Reder, 1991). Munschauer *et al.* (1993) found that MS patients have a significantly greater population of circulating $CD3^+CD4^+CD8^+$ T cells than do healthy controls. The significance of these changes in CD4 and CD8 expression in the peripheral blood of MS patients is unknown.

Expression of T cell activation markers

CD45RA, the high molecular weight isoform of leukocyte common antigen, is expressed on naive T cells but not memory T cells. Patients with clinically

active MS have generally been found to exhibit a selective decrease in the $CD4^+CD45RA^+$ subset in the peripheral blood compared with patients with clinically inactive MS and controls (Rose *et al.*, 1985, 1988; Morimoto *et al.*, 1987; Zaffaroni *et al.*, 1990; Porrini, Gambi & Malatesta, 1992; Eoli *et al.*, 1993). Serial studies on the same MS patients have shown that the peripheral blood $CD4^+CD45RA^-/CD4^+CD45RA^+$ ratio increases at the time of relapse (Rose *et al.*, 1988; Corrigan, Hutchinson & Feighery, 1990): in one study this increase usually resulted from a simultaneous decrease in $CD4^+CD45RA^+$ cells and increase in $CD4^+CD45RA^-$ cells (Rose *et al.*, 1988), whereas in another study there was no significant alteration in the $CD4^+CD45RA^+$ population but an increase in the $CD4^+CD45RA^-$ population (Corrigan *et al.*, 1990). These findings suggest that clinical disease activity is accompanied by a conversion of naive T cells to memory T cells (Corrigan *et al.*, 1990; Zaffaroni *et al.*, 1990).

$CD4^+CD29^+$ T cells (reportedly memory cells) have been found to be increased in the peripheral blood of MS patients (Gambi *et al.*, 1991). This was associated with an increase in circulating $CD4^+CD45RA^-$ cells and a decrease in $CD4^+CD29^-$ cells and hypothesized to be related to B cell activation (Gambi *et al.*, 1991). IL-2R (CD25) expression is a marker of T cell activation. Several studies have reported an increased proportion of $IL\text{-}2R^+$ cells in the peripheral blood of patients with MS (Bellamy *et al.*, 1985; Selmaj *et al.*, 1986; Konttinen *et al.*, 1987; Porrini *et al.*, 1992; Scolozzi *et al.*, 1992), but other studies have not found such an increase (Hafler *et al.*, 1985*b*; Crockard *et al.*, 1988). CD44 (Ta1) is also a marker of T cell activation. An increase in the proportion of $CD44^+$ cells in the peripheral blood of MS patients has been reported (Hafler *et al.*, 1985*b*) but this was not confirmed in another study (Crockard *et al.*, 1988).

Suppressor cell function

Non-specific suppressor cell function has been assessed in MS by determining the ability of peripheral blood mononuclear cells, after activation by concanavalin A and treatment with mitomycin C, to suppress the proliferative response of autologous cells to concanavalin A (Antel, Arnason & Medof, 1979). Antel *et al.* (1979) have shown that such activated suppressor cell function is reduced in patients with clinically active MS compared with patients with clinically stable MS, patients recovering from an exacerbation and normal controls. It is significantly higher in patients with progressive MS and severe disability than in those with progressive MS and moderate disability (Antel *et al.*, 1989). The functional suppressor deficit involves the $CD8^+$ T cell subset (J.P. Antel *et al.*, 1986*a*) and is also exhibited by $CD8^+$ T cell lines derived from the peripheral blood of patients with progressive MS and, to a lesser degree, stable MS (J. Antel *et al.*, 1986, 1988). *In vitro*

pokeweed mitogen-induced IgG secretion by peripheral blood mononuclear cells (used as an indirect measure of $CD8^+$ T cell suppressor function) is increased in progressive MS, whereas alloantigen-directed cytotoxicity (a predominantly $CD8^+$ T cell function) is normal, suggesting a selective defect of suppressor cell function in MS rather than a generalized dysfunction of $CD8^+$ T cells (J.P. Antel *et al.*, 1986*b*). Other groups have confirmed the defect of peripheral blood suppressor cell function in active MS (Morimoto *et al.*, 1987; Chofflon *et al.*, 1988; O'Gorman, Aziz & Oger, 1989; Trotter *et al.*, 1989; Baxevanis, Reclos & Papamichail, 1990). Chofflon *et al.* (1988) found that the decrease in functional suppression in MS is linked to the decrease in circulating $CD4^+CD45RA^+$ T cells (previously called 'suppressor–inducer' cells); however, Baxevanis *et al.* (1990) concluded that it is due to the deficient expression of DR antigen on monocytes.

Autologous mixed lymphocyte reaction

The autologous mixed lymphocyte reaction (AMLR), which measures the T cell proliferative response to antigens on the surface of autologous non-T cells, is reduced in patients with MS compared to controls (Hafler, Buchsbaum & Weiner, 1985*a*; Hirsch, 1986). $CD4^+$ T cells from MS patients also exhibit a decreased AMLR (Baxevanis *et al.*, 1988; Hafler *et al.*, 1991). Hirsch (1986) attributed the decreased AMLR to a functional defect in a subpopulation of $CD4^+$ T cells, and Chofflon *et al.* (1988) concluded that both the decrease in the AMLR and the decrease in functional suppression are tightly linked to decreases in the $CD4^+CD45RA^+$ cells. However, Baxevanis *et al.* (1988) have provided evidence that the decreased AMLR is due to a monocyte functional (stimulatory) defect. Decreased secretion of IL-1, which is produced by monocytes as well as by other cells, has also been implicated in the decreased AMLR by the finding that IL-1 corrects the defective AMLR in MS patients but has no effect on the AMLR in controls (Hafler *et al.*, 1991). Moreover, the magnitude of the AMLR corresponded to the level of IL-1 secretion induced by lipopolysaccharide in the non-T-cell population (Hafler *et al.*, 1991).

β-adrenergic receptor expression

The density of high-affinity β-adrenergic receptors on $CD8^+CD28^-$ (reportedly suppressor cells) T cells is increased in progressive MS (Karaszewski *et al.*, 1990, 1991, 1993). Basal and isoproterenol-stimulated cyclic AMP levels in $CD8^+$ cells are also increased in patients with progressive MS (Karaszewski *et al.*, 1993). Karaszewski *et al.* (1990) have suggested that the increased β-adrenergic receptor density and the decreased suppressor cell function may be due to reduced sympathetic nervous system activity as a

result of lesions in progressive MS. However, Zoukos *et al.* (1992) have found an increased density of β-adrenergic receptors on peripheral blood mononuclear cells from patients with chronic active rheumatoid arthritis as well as from patients with MS, indicating that the receptor upregulation can occur in the absence of nervous system disease. A possible role for cortisol and IL-1 was suggested by the finding that hydrocortisone or IL-1 upregulated β-adrenergic receptors on peripheral blood mononuclear cells from normal controls but not from patients with MS (Zoukos *et al.*, 1992).

Specific T cell findings

T cell reactivity to myelin basic protein

As MBP is encephalitogenic in laboratory animals (see Chapter 3), it has been proposed that it may be a target antigen in MS. Standard T cell proliferation assays have demonstrated MBP-reactive T cells in the peripheral blood of a minority of MS patients and also occasionally in healthy controls and patients with other neurological diseases (Lisak & Zweiman, 1977; Brinkman *et al.*, 1982; Johnson *et al.*, 1986; Vandenbark *et al.*, 1989; Trotter *et al.*, 1991; Kerlero de Rosbo *et al.*, 1993; Y. Zhang *et al.*, 1993). MBP reactivity appears to be more common in patients with clinically active MS than in those with clinically stable MS (Johnson *et al.*, 1986). In some studies but not others, group analysis has shown that the reactivity to MBP is significantly greater in MS patients than in normal controls or patients with other neurological diseases. Baxevanis *et al.* (1989*b*) found that all patients with severe progressive MS had significant proliferation of peripheral blood T cells in response to peptide fragment 45–89 of human MBP and also to synthetic peptides 15–31, 75–96 and 83–96 but not to 131–141. Normal controls and patients with other neurological diseases only occasionally showed significant proliferation in response to these peptides. The responding T cells from MS patients were $CD4^+$ and were dependent on monocytes and HLA-DR molecules for their activation (Baxevanis *et al.*, 1989*b*). Frick (1989) has reported increased $CD8^+$ T cell cytotoxicity towards cells coated with bovine MBP or human MBP peptide 114–122 in patients with MS. The results of Baxevanis *et al.* and of Frick require confirmation.

On the basis that mutant T cells represent a population enriched with dividing cells, Allegretta *et al.* (1990) isolated hypoxanthine guanine phosphoribosyltransferase-mutant T cell clones from the peripheral blood of patients with chronic progressive MS to determine their reactivity to MBP. Eleven of 258 mutant T cell clones from five of six MS patients proliferated in response to human MBP without prior *in vitro* exposure to this antigen, but no wild-type clones from these patients nor any mutant or wild-type clones from three normal controls responded to MBP. These

results indicate that there are circulating activated MBP-specific T cells in patients with MS. A similar conclusion was reached by Ofosu Appiah *et al.* (1991) who used the limiting dilution technique to generate clones from *in vivo*-activated IL-2-responsive T cells in the peripheral blood of MS patients. Seven (three $CD4^+$ and four $CD8^+$) of 20 clones from ten MS patients but none of eight clones from five normal controls proliferated specifically in response to MBP. Using the limiting dilution assay, Chou *et al.* (1992) found an increased frequency of MBP-reactive T cells in the peripheral blood of MS patients compared with normal subjects and patients with other neurological diseases. In contrast, Zhang *et al.* (1992*a*) found no significant difference in the precursor frequency of MBP-reactive T cells in the peripheral blood of MS patients and normal controls; however, after primary culture with IL-2 the frequency of MBP-reactive T cells was significantly higher in MS patients than in normal individuals (Zhang *et al.*, 1994). Increased frequencies of T cells reactive to MBP and MBP peptides have been found in the peripheral blood of MS patients by counting the number of cells secreting interferon-γ (IFN-γ) in response to antigen in short-term cultures (Olsson *et al.*, 1990*b*, 1992); however, these results are difficult to interpret, because of the high background response. Using *in situ* hybridization with radiolabelled complementary DNA oligonucleotide probes, Link *et al.* (1994*a*,*b*) have demonstrated that, compared with patients with other neurological diseases, MS patients have increased numbers of peripheral blood mononuclear cells expressing IFN-γ, IL-4 and transforming growth factor-β mRNA after short-term culture in the presence of MBP.

A number of laboratories have isolated MBP-specific T cell lines or clones from the peripheral blood of MS patients and controls (Weber & Buurman, 1988; Vandenbark *et al.*, 1989; Martin *et al.*, 1990; Ota *et al.*, 1990; Pette *et al.*, 1990*a*; Liblau *et al.*, 1991; Burns *et al.*, 1991). Generally the MBP-specific T cell lines and clones are $CD4^+$ and restricted by HLA-DR molecules. The majority of the long-term lines and clones have been cytotoxic towards MBP-coated target cells (Weber & Buurman, 1988; Martin *et al.*, 1990; Zhang *et al.*, 1990) and have secreted substantial amounts of IFN-γ (Martin *et al.*, 1990). Multiple immunogenic regions of the MBP molecule have been identified by this approach but two regions are immunodominant, one in the middle of the molecule (87–106) (Martin *et al.*, 1990; Ota *et al.*, 1990; Zhang *et al.*, 1992*a*), and the other at the C-terminal region (154–172) (Martin *et al.*, 1990; Ota *et al.*, 1990; Zhang *et al.*, 1990, 1992*a*; Liblau *et al.*, 1991). Within the 87–106 region there are several nested immunogenic epitopes (Martin *et al.*, 1992). It is important to note that the 87–106 region includes peptides encephalitogenic in the SJL/J mouse (Sakai *et al.*, 1988) and in the Lewis and Buffalo rats (Offner *et al.*, 1989; Jones *et al.*, 1992), and that the 154–172 sequence includes the region that is

encephalitogenic in monkeys (Karkhanis *et al.*, 1975). Ota *et al.* (1990) found that the proportion of MBP-specific T cell lines reacting with peptide 84–102 was higher in MS patients than in controls. Voskuhl *et al.* (1993*b*) reported that MS patients have a higher frequency of T cell lines specific for epitopes within isoforms of MBP expressed mainly during myelination, raising the possibility that the epitopes could be targeted during the remyelination that commonly occurs in MS.

Martin *et al.* (1990, 1991) found that the 87–106 peptide is recognized by cytotoxic T cells in the context of DR2, DR4 and DR6 and the 154–172 peptide is recognized in the context of DR1, DR4 and DR6. Furthermore, the DR2 molecule is capable of restricting T cell responses to multiple MBP epitopes (Chou *et al.*, 1989, 1991; Martin *et al.*, 1990; Jaraquemada *et al.*, 1990; Pette *et al.*, 1990*b*). In $DR2^+$ MS patients, both the DR2a and DR2b products function as restriction elements for MBP (Jaraquemada *et al.*, 1990; Pette *et al.*, 1990*b*). Valli *et al.* (1993) determined the binding of synthetic peptides spanning the entire human MBP sequence to ten purified HLA-DR molecules. All the peptides tested showed binding affinity for at least one of the DR molecules analysed, but three peptides (included in sequences 13–32, 84–103 and 144–163) were capable of binding to three or more DR molecules. Peptide 84–103 was the most degenerate in binding, in that it bound to eight out of the ten DR molecules tested. Notably it bound with highest affinity to DRB1*1501 and DRB1*0401 molecules. As DRB1*1501 is associated with an increased susceptibility to MS, Valli *et al.* concluded that their findings were consistent with a role for the 84–103 MBP peptide in the pathogenesis of MS. To correlate the binding pattern of MBP peptides to DR molecules with their recognition by T cells, they established MBP-specific T cell lines from the peripheral blood of MS patients, who were homozygous, heterozygous or negative for DRB1*1501. There was a good correlation between the binding data and T cell proliferation to MBP peptides. Although virtually all MBP peptides tested could be recognized by at least one T cell line from MS patients, there were three immunodominant epitopes, corresponding exactly to the peptides capable of binding to several DR molecules. These immunodominant epitopes correspond to the two demonstrated in earlier studies (see above) and a third previously suggested but undefined epitope in the N-terminal region (Martin *et al.*, 1990). No major difference was detected in the recognition of immunodominant MBP peptides by the lines from DRB1*1501 positive or negative MS patients (Valli *et al.*, 1993). Wucherpfennig *et al.* (1994) found that the 84–102 MBP peptide binds with high affinity to the DRB1*1501 and the DRB5*0101 molecules of the DRw15 haplotype, but that only DRB1 molecules served as restriction elements for a panel of T cell clones from two MS patients, suggesting that the complex of the 84–102 MBP peptide and DRB1 molecules is more immunogenic for MBP-reactive T cells. In a study on a

multiplex family with MS, Voskuhl, Martin & McFarland (1993*a*) found no difference in the estimated precursor frequencies of MBP-specific T cell lines or peptide specificity of T cell lines when affected and unaffected siblings were compared. However, MBP-specific T cell lines from affected siblings were restricted to DRw15/DQw6 significantly more frequently than were those from unaffected siblings. A study of monozygotic twins discordant for MS revealed no significant differences in the frequency or HLA restriction patterns of MBP-specific T cells in affected and normal individuals but showed some differences in peptide specificity, indicating that, despite genetic identity, the MBP-specific T cell repertoire may be shaped differently (Martin *et al.*, 1993).

The finding of restricted TCR Vβ gene usage by encephalitogenic MBP-specific T cells in EAE (see Chapter 3) prompted studies to determine whether there was a similar restricted usage by MBP-specific T cells in MS, which could be exploited by selective anti-TCR therapy. Conflicting results have been obtained by different laboratories. Wucherpfennig *et al.* (1990) found that Vβ17 and to a lesser extent Vβ12 were frequently used by T cell lines reactive with the 84–102 peptide in different individuals, while Kotzin *et al.* (1991) reported a biased usage of Vβ5.2 and to a lesser extent Vβ6.1 by MBP-specific clones from MS patients but not controls. On the other hand, Ben Nun *et al.* (1991) demonstrated heterogeneous TCR Vβ gene usage among MBP-specific T cell clones from different individuals but a restricted usage among MBP-specific T cell clones of the same individual. Other studies have reported that the TCR Vα and Vβ gene usage by MBP-specific T cells in humans is highly heterogeneous, even among T cells that recognize the same region of MBP in association with the same DR molecule in the same individual (Richert *et al.*, 1991; Martin *et al.*, 1992; Giegerich *et al.*, 1992). An interesting recent finding is that identical twins discordant for MS use different Vα chains in the T cell recognition of MBP or tetanus toxoid, whereas twins concordant for MS and control twin sets use similar Vα chains (Utz *et al.*, 1993). The different Vα chain usage in twins discordant for MS was not due to a gap in the T cell repertoire, but could be due to skewing of the repertoire by either an environmental factor or the disease itself. As only two twin sets in each category were examined, further studies on other monozygotic twins will be needed to determine whether this is generally true.

In conclusion there is an increased frequency of activated MBP-specific T cells in the peripheral blood of MS patients. It is unknown whether these T cells are pathogenic, although the high-affinity binding of the immunodominant 84–102 MBP peptide to the MS-associated HLA-DRB1*1501 molecule supports a role for MBP-specific T cells in the pathogenesis of MS.

T cell reactivity to myelin proteolipid protein

As PLP is also encephalitogenic in laboratory animals (see Chapter 3), studies have been undertaken to determine whether autoreactivity to PLP contributes to the pathogenesis of MS. Trotter *et al.* (1991) have demonstrated significant T cell proliferative responses to PLP in the peripheral blood of six of 16 patients with rapid chronic progressive MS, three of 15 patients with clinically stable relapsing–remitting MS, none of 12 normal controls and one of ten patients with other neurological disease. T cells from the MS patients with positive responses to the whole protein also proliferated significantly in response to one or more of the PLP peptides 88–108, 103–116 and 139–154, which correspond to regions encephalitogenic in the rabbit (Linington, Gunn & Lassmann, 1990), SWR mouse (Tuohy *et al.*, 1988) and SJL/J mouse (Tuohy *et al.*, 1989). The findings of Trotter *et al.* (1991) are in contrast to those obtained in an earlier study, which demonstrated no significant T cell proliferative response to PLP in patients with active MS or normal controls, but significant responses in six of 16 patients with other neurological disease (Johnson *et al.*, 1986). Kerlero de Rosbo *et al.* (1993) did not find a significant increase in the T cell proliferative response to PLP in the peripheral blood of MS patients.

Using the limiting dilution assay, Chou *et al.* (1992) found no significant increase in the frequency of T cells reactive to PLP peptide 139–151 in the peripheral blood of MS patients. However, Zhang *et al.* (1994) demonstrated that after primary culture with IL-2 the frequency of PLP-reactive T cells was significantly higher in MS patients than in normal individuals, indicating that MS patients have an increased frequency of circulating *in vivo*-activated PLP-specific T cells. An increased frequency of T cells secreting IFN-γ in response to PLP has been found in the peripheral blood of MS patients compared to normal controls; however, these results are difficult to interpret, because of the relatively high background response and because no significant difference was found between MS patients and patients with other neurological diseases (J.B. Sun *et al.*, 1991). Using *in situ* hybridization with radiolabelled complementary DNA oligonucleotide probes, Link *et al.* (1994*a*,*b*) have demonstrated that, compared with patients with other neurological diseases, MS patients have increased numbers of peripheral blood mononuclear cells expressing IFN-γ, IL-4 and transforming growth factor-β mRNA after short-term culture in the presence of PLP. Pelfrey *et al.* (1993) used synthetic PLP peptides to generate T cell lines from the peripheral blood of MS patients. The lines were predominantly specific for the 40–60 PLP peptide and were $CD4^+$, cytotoxic and restricted by class II MHC molecules.

In conclusion, there is some evidence of increased T cell reactivity to PLP

in the peripheral blood of MS patients, but further studies, particularly with synthetic peptides, are needed.

T cell reactivity to myelin/oligodendrocyte glycoprotein

The findings that antibodies against MOG augment demyelination in EAE, and that EAE can be transferred by a combination of MOG-specific T cells and MOG-specific antibodies (see Chapter 3) raise the possibility that MOG may be a target antigen in MS. J. Sun *et al.* (1991) found an increased frequency of T cells secreting IFN-γ in response to MOG in the peripheral blood of MS patients compared to controls. Kerlero de Rosbo *et al.* (1993) reported that the T cell proliferative response to MOG, but not to MBP, PLP or MAG, was significantly increased in the peripheral blood of MS patients compared to controls. Further studies are required to determine the role of MOG-specific T cells in the pathogenesis of MS.

T cell reactivity to myelin-associated glycoprotein

Johnson *et al.* (1986) demonstrated increased T cell proliferative responses to MAG in the peripheral blood of nine of 30 patients with active MS, two of ten patients with stable MS, one of seven patients with other neurological diseases and none of ten normal controls. Y. Zhang *et al.* (1993) found increased T cell proliferative responses to MAG in the peripheral blood of seven of 11 patients with MS and none of ten normal controls. In contrast, Kerlero de Rosbo *et al.* (1993) found no evidence of increased T cell proliferative responses to MAG in the peripheral blood of MS patients. Link *et al.* (1992) found a significantly increased frequency of peripheral blood T cells secreting IFN-γ in response to MAG in patients with MS compared to those with other neurological diseases but not compared to patients with tension headache. Further studies are needed to establish whether MAG-specific T cells have a role in the pathogenesis of MS.

T cell reactivity to other autoantigens

Cell-mediated immunity to human brain gangliosides as determined by the leukocyte migration inhibition test is significantly increased in the peripheral blood of patients with attacks of MS as compared to clinically stable MS patients, patients with other neurological diseases and normal controls (Beraud *et al.*, 1990). Increased $CD8^+$ T cell cytotoxicity towards cells coated with bovine brain gangliosides or cerebrosides has also been observed in patients with active MS compared to those with inactive MS (Frick, 1989).

Heat shock proteins are potential autoantigens because of their evolution-

ary conservation and immunogenicity. Peripheral blood T cell proliferative responses to mycobacterial hsp70, but not hsp65, are significantly more frequent in patients with MS than in patients with other neurological diseases or normal subjects (Salvetti *et al.*, 1992). Furthermore, the proportion of purified protein derivative-specific T cell lines that proliferate in response to hsp70 was found to be significantly higher in MS patients than in normal controls. $\gamma\delta$ T cells formed only a minority in nearly all the lines.

Specific suppressor or regulatory T cells

Zhang *et al.* (1992*b*) have generated, from MS patients, suppressor T cell lines specific for MBP-specific helper T cell clones. Most of the suppressor T cell lines were $CD4^+$ but one was $CD8^+$. The lines exhibited potent antigen-specific suppressor activity on the proliferation of MBP-specific T helper clones but not on T cell lines with other antigen specificity. The suppressor lines were weakly responsive to MBP and required the presence of autologous peripheral blood mononuclear cells for proliferation: the proliferation of $CD4^+$ suppressor lines was restricted by HLA-DR molecules, whereas that of the $CD8^+$ line was restricted by HLA class I molecules (Zhang *et al.*, 1992*b*). Further studies are required to determine whether such specific suppressor T cell activity differs in MS patients and controls. Anti-clonotypic cytotoxic $CD8^+$ T cells specific for MBP-reactive T cells have been isolated from the peripheral blood of MS patients vaccinated with irradiated autologous MBP-reactive T cells, but not from the blood of non-vaccinated MS patients (J. Zhang *et al.*, 1993). Furthermore, cytotoxic $CD4^+$ T cells specific for the TCR β chain of an autologous MBP-reactive T cell clone have been isolated from a normal subject (Saruhan Direskeneli *et al.*, 1993). Further studies are required to determine what function specific regulatory T cells have *in vivo*. Specific suppressor or regulatory T cells have also been isolated from rats recovering from EAE or protected against EAE by T cell vaccination or oral tolerance (see Chapter 3).

Antibody/B cell findings

Using a nitrocellulose immunospot assay, Olsson *et al.* (1990*a*) found no B cells producing antibodies against myelin or MBP in the peripheral blood of MS patients, although such cells were found in the CSF. With a different technique, Zhang *et al.* (1991) also found that the frequency of B cells producing anti-MBP antibodies was not increased in MS patients, although the frequency of B cells producing antibodies against measles virus was significantly increased. Patients with MS have a significantly higher frequency of peripheral blood cells producing anti-PLP IgG antibodies in the nitrocellulose immunospot assay compared to normal controls but not

patients with other neurological diseases (J.B. Sun *et al.*, 1991). This assay has also shown an increased frequency of cells producing anti-MOG IgG antibodies in the peripheral blood of MS patients compared to controls (J. Sun *et al.*, 1991). Anti-MOG IgG antibodies have not been detected by enzyme-linked immunosorbent assay in the plasma of MS patients, although they are present in the CSF of some patients (Xiao, Linington & Link, 1991). Cells secreting IgG antibodies against MAG have been found in the peripheral blood of 20% of MS patients and only occasionally in controls (Baig *et al.*, 1991). With a sensitive solid-phase radioimmunoassay, Moller *et al.* (1989) could not detect an increase in anti-MAG antibodies in the sera of MS patients, although they found elevated levels in the CSF. Using an indirect immunofluorescence assay, Henneberg, Mayle & Kornhuber (1991) found antibodies to brain white matter in the sera of 33% of MS patients (73% of patients with active chronic progressive MS) and 3% of controls; however, the specific antigen(s) recognized by these antibodies was not determined. As mentioned earlier, circulating antibodies to the brain protein, β-arrestin 1, have been found in patients with MS, but not in controls (Ohguro *et al.*, 1993). Increased serum levels of IgG antibodies against endothelial cells have also been demonstrated in patients with MS, especially during an exacerbation (Tanaka *et al.*, 1987). Evidence for a more general systemic B cell activation in MS has been provided by the finding that patients without known intercurrent infection have higher numbers of antibody-secreting cells in both the bone marrow and the peripheral blood compared to normal controls (Fredrikson, Baig & Link, 1991).

Immune complexes

Serum immune complexes are increased in patient with MS, especially in those with active disease (Tanaka *et al.*, 1987; Procaccia *et al.*, 1988). The complexes have been found to contain IgG, IgM, IgA, complement components, β_2-microglobulin, anti-viral antibodies and sometimes viral antigens, and antibodies reactive to galactocerebroside and ganglioside (Coyle & Procyk Dougherty, 1984; Procaccia *et al.*, 1988). MBP or anti-MBP antibodies were found in the serum immune complexes of some MS patients in one study (Coyle & Procyk Dougherty, 1984), but MBP was not found in another study (Geffard, Boullerne & Brochet, 1993).

Monocytes

Baxevanis *et al.* (1989*a*) have found reduced HLA-DR antigen expression on peripheral blood monocytes from MS patients, especially those with active disease, and have concluded that this is responsible for the reduced AMLR (Baxevanis *et al.*, 1988) and reduced suppressor T cell activity

(Baxevanis *et al.*, 1990). In contrast, Armstrong *et al.* (1991) found normal HLA-DR antigen expression and increased HLA-DP and HLA-DQ antigen density on monocytes from patients with active MS. Increased HLA-DR expression has been demonstrated on blood monocytes from patients experiencing an increased frequency of exacerbations after intravenous administration of IFN-γ (Panitch *et al.*, 1987*b*). Other reported abnormalities of blood monocytes from patients with active MS include: an increased production of prostaglandin E in tissue culture (Dore Duffy *et al.*, 1986); increased levels of cellular cyclic AMP and reduced sensitivity to agents that stimulate prostaglandin E synthesis (Dore Duffy & Donovan, 1991); increased expression of the monocyte activation antigen Mo3 without increased HLA-DR expression (Dore Duffy, Donovan & Todd, 1992); increased spontaneous IL-6 secretion and intracellular IL-1β synthesis, and increased secretion of IL-1β after stimulation with T-cell-derived cytokines (Maimone, Reder & Gregory, 1993); and increased production of TNF-α, IL-1α, IL-1β and IL-6 after stimulation with lipopolysaccharide or phorbol ester (Imamura *et al.*, 1993). Reder *et al.* (1991) have suggested that prostaglandins secreted by monocytes may be responsible for the impairment of function of CD2 (the sheep red blood cell receptor) in peripheral blood T cells from MS patients. It is unclear whether the above changes in monocyte function are secondary to specific T cell activation or whether they are due to a primary abnormality of the monocyte.

Cytokines and adhesion molecules

Serum IL-2 levels are increased in patients with active MS, indicating systemic T cell activation (Gallo *et al.*, 1988, 1989*a*; Trotter *et al.*, 1988; Adachi, Kumamoto & Araki, 1989; Trotter, van der Veen & Clifford, 1990). However, serial studies on individual patients have shown no correlation between the level of serum IL-2 and clinical disease activity (Gallo *et al.*, 1991). Periodic bursts of increased serum IL-2 levels have been observed in patients with chronic progressive MS without associated sudden clinical worsening (Trotter *et al.*, 1990). Soluble IL-2R is released when T cells are activated and can be used as an index of T cell activation. Serum levels of soluble IL-2R are increased in patients with active MS (Adachi *et al.*, 1989; Gallo *et al.*, 1989*a*; Adachi, Kumamoto & Araki, 1990; Hartung *et al.*, 1990; Weller *et al.*, 1991; Chalon, Sindic & Laterre, 1993). However, serial studies on individual patients have shown no correlation between the serum level and clinical disease activity (Gallo *et al.*, 1991).

IL-6, a cytokine that promotes differentiation of B cells to antibody-secreting cells, is elevated in the sera of patients with MS, indicating systemic B cell activation (Frei *et al.*, 1991; Weller *et al.*, 1991; Shimada, Koh & Yanagisawa, 1993). Serum levels of soluble ICAM-1 are increased in

MS patients with clinically active disease or enhancing lesions on MRI, supporting a role for this adhesion molecule in the pathogenesis of MS (Hartung *et al.*, 1993; Tsukada *et al.*, 1993). Furthermore, in patients with an exacerbation of MS there is a positive correlation between serum soluble ICAM-1 and serum TNF-α levels (Tsukada *et al.*, 1993).

Immunological findings in the CSF

Non-specific T cell findings

A mild to moderate mononuclear pleocytosis is often present in the CSF in MS. The majority (80–90%) of cells are T lymphocytes (Brinkman *et al.*, 1983; Hauser *et al.*, 1983*b*). The proportion of T cells in the CSF is slightly increased compared to that in the peripheral blood, as it also is in normal controls (Hedlund, Sandberg Wollheim & Sjogren, 1989).

CD4 and CD8 expression

The $CD4^+$:$CD8^+$ ratio in the CSF in MS patients is about 2:1 (Brinkman *et al.*, 1983; Hauser *et al.*, 1983*b*). The proportion of $CD4^+$ T cells is increased and the proportion of $CD8^+$ T cells is decreased in the CSF compared to the peripheral blood (Antonen *et al.*, 1987; Matsui *et al.*, 1988; Hedlund *et al.*, 1989; Salmaggi *et al.*, 1989; Mix *et al.*, 1990; Scolozzi *et al.*, 1992). It appears that a similar difference in the proportions of $CD4^+$ T cells in the CSF and peripheral blood occurs in normal controls, but it is less clear whether this also applies to the difference in the proportions of $CD8^+$ T cells (Hedlund *et al.*, 1989). It is apparent that the decline in $CD8^+$ T cells in the peripheral blood (see above) is not accompanied by a sequestration of these cells in the CSF (Hauser *et al.*, 1983*b*). It has also been found that the proportion of $CD8^+$ T cells that are $CD11b^+$ (reportedly suppressor cells) is reduced in the CSF compared to the peripheral blood in active MS and non-inflammatory neurological diseases and compared to the CSF in other inflammatory neurological diseases (Salonen *et al.*, 1989; Matsui, Mori & Saida, 1990). Most of the $CD8^+$ T cells in the CSF in active MS are $CD11b^-$ (reportedly cytotoxic cells) (Salonen *et al.*, 1989). Soluble CD8 levels in the CSF are increased in MS compared to non-inflammatory neurological diseases, and the amount of soluble CD8 per CSF leukocyte is higher in MS than in other inflammatory neurological diseases (Maimone & Reder, 1991).

Expression of T cell activation markers

The proportion of $CD4^+$ cells that are $CD45RA^+$ (naive cells) is reduced in the CSF compared to the peripheral blood in patients with MS (Chofflon *et*

al., 1989; Hedlund *et al.*, 1989; Salonen *et al.*, 1989; Matsui *et al.*, 1990; Zaffaroni *et al.*, 1991); however, this CSF/peripheral blood differential is also found in patients with other neurological diseases and normal controls (Hedlund *et al.*, 1989; Salonen *et al.*, 1989; Matsui *et al.*, 1990). The fall in the proportion of $CD4^+CD45RA^+$ cells occurs in parallel with an increase in the proportion of $CD4^+CD45RO^+$ (memory) cells in the CSF compared with the peripheral blood (Hedlund *et al.*, 1989). Indeed, the majority of T cells in the CSF in MS patients, aseptic meningitis patients and healthy subjects are $CD45RO^+$ (Svenningsson *et al.*, 1993). An enrichment of memory cells has also been found in the CNS parenchyma in MS (Sobel *et al.*, 1988) and in EAE (see Chapter 3). T lymphocytes move rapidly from the peripheral blood into the CSF in progressive MS, as shown by the finding that 70% of T cells in the CSF are labelled by anti-CD2 monoclonal antibody 72–96 h after *in vivo* labelling of peripheral blood T cells with this antibody (Hafler & Weiner, 1987).

An increase in the proportion of $CD4^+CD29^+$ cells (reportedly memory cells) in the CSF compared to the peripheral blood has been found in parallel with the decrease in the proportion of $CD4^+CD45RA^+$ cells in the CSF compared to the peripheral blood in patients with MS and in normal controls (Chofflon *et al.*, 1989; Hedlund *et al.*, 1989). However, in one study it was found that there were decreases in the proportions of both $CD4^+CD29^+$ cells and $CD4^+CD45RA^+$ cells in the CSF in patients having exacerbations of MS compared to those with stable MS or non-inflammatory neurological disease (Marrosu, 1991).

Using flow cytometry to assess cell-cycle phase, Noronha *et al.* (1980, 1985) demonstrated activated cells and in particular activated $CD4^+$ T cells in the CSF in MS. Moreover, $IL\text{-}2R^+$ cells are enriched in the CSF compared to the peripheral blood (Bellamy *et al.*, 1985; Tournier Lasserve *et al.*, 1987; Scolozzi *et al.*, 1992). The proportion of T cells expressing HLA-DR molecules (a marker of T cell activation) is increased in the CSF compared to the peripheral blood in MS patients and normal controls (with tension headache) (Mix *et al.*, 1990). CSF T cells also express higher levels of very late activation antigens 3–6, LFA-1, LFA-3, CD2, CD26 and CD44 than do T cells in the peripheral blood in MS patients, aseptic meningitis patients and normal subjects, indicating that activated T cells selectively migrate to the CSF under both pathological and normal conditions (Svenningsson *et al.*, 1993).

Oligoclonal T cells (including $\gamma\delta$ cells)

Analysis of the rearranged TCR β chain and γ chain genes of T cells cloned from the CSF before *in vitro* expansion has shown oligoclonal T cells in some but not all patients with MS, but not in any patients with other neurological

diseases (Hafler *et al.*, 1988*a*; Lee *et al.*, 1991). There was common usage of the TCR Vβ12 gene segment among four oligoclonal T cell populations derived from three patients with MS, suggesting that oligoclonal T cells might share similar specificities and that clonal expansion might have resulted from specific stimulation by an antigen. Furthermore, identical clones were found in the blood and CSF in three of nine patients (Lee *et al.*, 1991). Shimonkevitz *et al.* (1993) found clonal expansion of oligoclonal $\gamma\delta$ T cells in the CSF of patients with recent-onset MS, but not of patients with chronic MS or other neurological diseases.

Specific T cell findings

T cell reactivity to MBP

The proliferative response of CSF lymphocytes to MBP is increased in patients with clinically active MS compared to those with stable MS or patients with other neurological diseases (Lisak & Zweiman, 1977). Interestingly, the response of CSF lymphocytes to MBP is greater than that of peripheral blood lymphocytes in patients with clinically active MS, but not in patients with acute disseminated encephalomyelitis (Lisak & Zweiman, 1977). Chou *et al.* (1992) have found that 24% of IL-2/IL-4-reactive T cell isolates from the CSF of MS patients are MBP-specific compared to 3% of the corresponding isolates of patients with other neurological diseases. They also found that the frequency of MBP-reactive T cells in the CSF of MS patients is much higher than in the peripheral blood. Using limiting dilution analysis the same group found that, in contrast to the reactivity to intact MBP, the frequency in the CSF of T cells reactive to 'cryptic' epitopes of MBP is similar in MS and other neurological diseases (Satyanarayana *et al.*, 1993). Zhang *et al.* (1994) found that after culture with IL-2 the frequency of MBP-reactive T cells in the CSF of MS patients was more than tenfold higher than in the peripheral blood of the same patients. MBP-reactive T cells accounted for 7% of the IL-2-responsive cells in the CSF of MS patients but could not be detected among the IL-2-responsive cells in the CSF of patients with other neurological diseases (Zhang *et al.*, 1994). These T cells predominantly recognized MBP peptides 84–102 and 143–168. Increased frequencies of T cells secreting IFN-γ in response to MBP and MBP peptides have been found in the CSF of MS patients compared to the peripheral blood of MS patients and compared to the CSF of controls (Olsson *et al.*, 1990*b*; Soderstrom *et al.*, 1993); however, these results are confounded by the high background response. Cells expressing IFN-γ, IL-4 and transforming growth factor-β mRNA after short-term culture in the presence of MBP were found to be enriched in the CSF compared to the peripheral blood of MS patients; however, no comparison was made with CSF cells from controls (Link *et al.*, 1994*a*,*b*).

In conclusion, *in vivo*-activated MBP-specific T cells are enriched in the CSF of MS patients and occur at a substantially higher frequency than in patients with other neurological diseases. These findings are highly suggestive of a role for MBP-specific T cells in the pathogenesis of MS.

T cell reactivity to PLP

Chou *et al.* (1992) found that 13% of IL-2/IL-4-reactive T cell isolates from the CSF of MS patients recognized the PLP peptide 139–151 compared to 2% of the corresponding isolates of patients with other neurological diseases. They also found that the frequency of these T cells in the CSF of MS patients was much higher than in the peripheral blood. J.B. Sun *et al.* (1991) found an increased frequency of T cells secreting IFN-γ in response to PLP in the CSF of MS patients compared to the CSF of controls and compared to the peripheral blood of MS patients, but the high background response renders interpretation difficult. Cells expressing IFN-γ, IL-4 and transforming growth factor-β mRNA after short-term culture in the presence of PLP were found to be enriched in the CSF compared to the peripheral blood of MS patients; however, no comparison was made with CSF cells from controls (Link *et al.*, 1994*a*,*b*). These findings are suggestive of a role for PLP-reactive T cells in the pathogenesis of MS, but further studies are needed to establish this.

T cell reactivity to MOG, MAG and mycobacterial antigens

By counting cells secreting IFN-γ in response to antigen in short-term cultures, increased frequencies of MOG-reactive T cells and MAG-reactive T cells have been found in the CSF of MS patients compared to controls and compared to the peripheral blood of MS patients (J. Sun *et al.*, 1991; Link *et al.*, 1992). T cells proliferating in response to mycobacterial antigens are also enriched in the CSF of patients with MS, particularly those with disease of recent onset (Birnbaum, Kotilinek & Albrecht, 1993).

Non-specific antibody/B cell findings

A classical finding in the CSF in MS is the presence of oligoclonal IgG bands, which are not present in the serum (Link & Müller, 1971). This also occurs in other inflammatory diseases of the nervous system and indicates intrathecal synthesis of IgG. Intrathecal synthesis of IgG has also been demonstrated by calculating quantitative indices based on CSF and serum levels of albumin and IgG, but the most sensitive and specific method is isoelectric focusing, which detects oligoclonal IgG bands in 95% of cases of clinically definite MS (McLean *et al.*, 1990). Serial studies have indicated that the oligoclonal

banding pattern in the CSF in MS remains stable over long periods (Walsh & Tourtellotte, 1986). The oligoclonal IgG is predominantly of the IgG1 subclass, but may also be of the IgG3, IgG2 and IgG4 subclasses in order of decreasing frequency (Losy, Mehta & Wisniewski, 1990). Intrathecal production of IgA and IgM also occurs in MS, as demonstrated by quantitative studies or by the detection of oligoclonal bands (Grimaldi *et al.*, 1985; Lolli, Halawa & Link, 1989; Sharief, Keir & Thompson, 1990; Sindic *et al.*, 1994). Oligoclonal IgM bands are more reliable than quantitative indices for detecting intrathecal production of IgM (Sharief *et al.*, 1990). Intrathecal synthesis of IgD has also been demonstrated in MS by calculation of index values (Lolli *et al.*, 1989; Sharief & Hentges, 1991*a*). The intrathecal synthesis of IgM and that of IgD have been found to correlate positively with MS relapse activity, CSF pleocytosis, and CSF/serum ratios of IL-2 and of soluble IL-2R (Sharief & Thompson, 1991; Sharief & Hentges, 1991*a*; Sharief, Hentges & Thompson, 1991). Furthermore, oligoclonal free kappa and free lambda light chains can be detected in the CSF by isoelectric focusing and immunoblotting in the majority of patients with MS and other inflammatory neurological disorders (Gallo *et al.*, 1989*b*; Sindic & Laterre, 1991).

The specificity of the major portion of the oligoclonal IgG in the CSF in MS has not been determined. In chronic relapsing EAE, oligoclonal IgG bands are present in the CSF; however, in contrast to the usual situation in MS, identical oligoclonal IgG band patterns are also found in the serum (see Chapter 3). This difference may be due to a more severe breakdown of the blood–brain barrier in EAE. In chronic relapsing EAE the predominant reactivity of the oligoclonal IgG is against CNS antigens, particularly MBP, whereas in MS there is little or no reactivity of oligoclonal IgG to CNS antigens (Mehta *et al.*, 1987; Cruz *et al.*, 1987).

The proportion of B cells that are $CD5^+$ (reportedly activated B cells) is significantly increased in the CSF of patients with relapsing–remitting MS compared to patients with chronic progressive MS and to patients with tension headache, but not compared to those with aseptic meningitis (Correale *et al.*, 1991). This proportion is higher in the CSF than in the peripheral blood of MS patients. It has been suggested that $CD5^+$ B cells in the CSF are responsible for the production of autoantibodies (Correale *et al.*, 1991).

Specific antibody/B cell findings

B cell reactivity to MBP

Cruz *et al.* (1987) found oligoclonal IgG antibody bands against MBP in the CSF of 32% of MS patients but not in the CSF of patients with other

neurological diseases. Warren *et al.* (1994) detected elevated CSF anti-MBP antibodies in the vast majority of MS patients with clinically active disease and in a minority of MS patients in clinical remission. They also found anti-MBP antibodies in extracts from MS cerebral tissue and concluded that the most likely epitope of anti-MBP antibodies is located between residues 84 and 95 of human MBP (Warren & Catz, 1993).

However, studies of antibody levels in biological fluids, such as the CSF, may not accurately reflect a B cell response, as autoantibodies may bind to their target antigens, and catabolism *in vivo* may limit their detection. A new approach to studying the B cell response in MS has been provided by the use of the nitrocellulose immunospot assay. With this technique Olsson *et al.* (1990*a*) found that 79% of MS patients had CSF cells producing IgG antibodies against myelin, and 57% had CSF cells producing IgG antibodies against MBP. These cells comprised a large proportion of the total IgG-producing cells but were not detected in the peripheral blood. Cells producing IgG antibodies against myelin and MBP occurred at significantly lower frequencies in the CSF of patients with aseptic meningoencephalitis. The same group found a significantly higher frequency of cells secreting IgG antibodies against guinea pig MBP peptide 70–89, but not against three other MBP peptides or (in contrast to their earlier study) myelin, in the CSF of MS patients compared to patients with other neurological diseases, and concluded that the 70–89 peptide is an immunodominant B cell epitope in MS (Martino *et al.*, 1991). Cash *et al.* (1992) reported that CSF mononuclear cells from five of 11 patients with acute exacerbations of MS produced anti-MBP antibodies *in vitro* after stimulation with pokeweed mitogen, but did not find such reactivity in 20 patients with other neurological diseases.

Overall, these findings suggest that B cells producing anti-MBP antibodies in the CNS may play a role in the pathogenesis of MS.

B cell reactivity to PLP

Warren *et al.* (1994) found that a small percentage of patients with clinically active MS have an increase in anti-PLP antibodies, but not anti-MBP antibodies, in the CSF. J.B. Sun *et al.* (1991) found cells secreting IgG antibodies against PLP in the CSF of 82% of patients with MS. The frequency of these cells was significantly lower in patients with aseptic meningitis and other neurological diseases. In MS patients the cells were highly enriched in the CSF compared to the peripheral blood.

B cell reactivity to MOG

Anti-MOG IgG antibodies have been detected by enzyme-linked immunosorbent assay in the CSF (but not the plasma) of some patients with MS and

less frequently in the CSF of patients with other neurological diseases (Xiao *et al.*, 1991). J. Sun *et al.* (1991) found cells secreting IgG antibodies against MOG in the CSF of eight of ten patients with MS. These cells occurred at a significantly higher frequency than in the CSF of controls. In MS patients they were highly enriched in the CSF compared to the peripheral blood.

B cell reactivity to MAG

Moller *et al.* (1989) observed a significant elevation of anti-MAG antibodies in the CSF, but not the serum, of patients with MS compared to patients with other neurological diseases and normal controls. Baig *et al.* (1991) found cells secreting IgG antibodies against MAG in the CSF of 48% of patients with MS. The frequency of these cells in the CSF in MS was higher than in other inflammatory and non-inflammatory neurological diseases and was higher than in the peripheral blood of MS patients. In the CSF from two of ten MS patients, anti-MAG and anti-MBP IgG-secreting cells were present concurrently (Baig *et al.*, 1991).

Antibodies to other autoantigens

Elevated levels of anti-galactocerebroside antibodies have been found in the CSF of 70% of MS patients and 50% of patients with other neurological diseases (Ichioka *et al.*, 1988). Zanetta *et al.* (1990) detected antibodies to the endogenous mannose-binding protein, cerebellar soluble lectin, in the CSF of 92% of MS patients and 16% of patients with other neurological diseases. Elevated levels of antibodies against many autoantigens expressed in non-neural tissues have also been found in the CSF of MS patients compared with normal controls and patients with other neurological diseases (Matsiota *et al.*, 1988).

Complement

Morgan, Campbell & Compston (1984) found a significant reduction in the level of C9 (terminal component of complement) in the CSF of patients with MS compared to controls with other neurological diseases, and concluded that this indicates intrathecal consumption of C9 due to formation of membrane attack complexes, which could contribute to CNS tissue damage in MS. In contrast, another study, which calculated the C9 index ([CSF C9/plasma C9] : [CSF albumin/plasma albumin]), concluded that there was intrathecal consumption of C9 in aseptic meningitis but not in MS (Halawa, Lolli & Link, 1989). Sanders *et al.* (1986) detected fluid-phase complement C5b–9 complexes in the CSF of 16 of 21 patients with MS and 13 of 14 patients with the Guillain–Barré syndrome and, at low concentrations, in the CSF of three of 11 patients with non-inflammatory CNS diseases. They

suggested that terminal complement components may participate in nervous tissue damage in MS and the Guillain–Barré syndrome.

Cytokines

CSF levels of IL-2 are increased in patients with acute exacerbations of MS, compared to patients in remission, patients with chronic progressive MS and normal controls (Gallo *et al.*, 1988, 1989*a*; Sharief *et al.*, 1991; Sharief & Thompson, 1993). In patients with acute exacerbations of MS, the level of IL-2 is significantly higher in the CSF than in the serum, indicating intrathecal production (Sharief *et al.*, 1991). CSF/serum ratios of IL-2 correlate with intrathecal synthesis of IgM and that of IgD but not with that of IgG or IgA (Sharief *et al.*, 1991). There is conflicting evidence concerning the level of soluble IL-2R in the CSF in MS, with some groups reporting an increase, particularly in patients with acute exacerbations (Adachi *et al.*, 1990; Kittur *et al.*, 1990; Sharief *et al.*, 1991; Sharief & Thompson, 1993), and others finding it normal in all or nearly all patients (Gallo *et al.*, 1989*a*, 1991; Peter, Boctor & Tourtellotte, 1991; Fesenmeier *et al.*, 1991; Weller *et al.*, 1991; Chalon *et al.*, 1993). There are also conflicting reports regarding the level of IL-1β in the CSF, with one group detecting it in 53% of cases of active MS (Hauser *et al.*, 1990) and others finding it rarely or not at all (Maimone *et al.*, 1991; Peter *et al.*, 1991). CSF IL-6 levels are significantly higher in patients with MS than in normal controls and patients with non-inflammatory neurological diseases, but not than in patients with other inflammatory neurological diseases (Weller *et al.*, 1991; Maimone *et al.*, 1991; Frei *et al.*, 1991; Shimada *et al.*, 1993). Interestingly, Frei *et al.* (1991) found that MS patients had much higher levels of IL-6 in the plasma than in the CSF, but that patients with acute meningoencephalitis had much higher levels in the CSF than in the plasma.

TNF is increased in the CSF in MS compared to non-inflammatory neurological diseases (Hauser *et al.*, 1990; Maimone *et al.*, 1991; Sharief & Hentges, 1991*b*). The CSF level of TNF-α is significantly higher in chronic progressive MS than in stable MS (Sharief & Hentges, 1991*b*). In chronic progressive MS it is also significantly higher than the corresponding serum level, and correlates with the degree of disability and the rate of clinical progression (Sharief & Hentges, 1991*b*). These findings suggest that TNF-α is produced in the CNS in MS and that it may contribute to CNS tissue damage. TNF^+ cells have been detected in MS brain but not in normal brain (Hofman *et al.*, 1989).

In conclusion, IL-2 and TNF are likely to have important roles in promoting inflammation in MS, as is the case in EAE (see Chapter 3). The increased levels of IL-6 are consistent with the increased antibody production in MS.

Myelin basic protein

Antigenic material that is cross-reactive with MBP can be detected by radioimmunoassay in the CSF of patients with active myelin destruction caused by MS or other processes, such as CNS infarction (Cohen, Herndon & McKhann, 1976; Whitaker, 1977). MS patients with acute exacerbations have the highest levels, those with chronic progressive MS have slightly increased or normal levels, and clinically stable patients have normal levels (Cohen *et al.*, 1976; Whitaker, 1977; Whitaker & Herman, 1988). As the level of immunoreactive MBP in the CSF is a reliable indicator of active demyelination in MS, it may be used to monitor response to therapy. The sensitivity of the radioimmunoassay has been improved by using human MBP synthetic peptide 69–89 as a radioligand (Whitaker & Herman, 1988). An epitope in peptide 80–89 that shares a conformation with intact MBP appears to be a dominant epitope of MBP-like material in the CSF after CNS myelin injury (Whitaker & Herman, 1988). MBP-like material is also increased in the CSF during attacks of EAE (Rauch *et al.*, 1987). As MBP is also expressed in the PNS, the spinal root demyelination that commonly occurs in EAE (see Chapter 3) may contribute to this increase.

Transfer of neurological signs and CNS lesions to severe combined immunodeficiency mice

Saeki *et al.* (1992) transferred a disease characterized by paralysis, ataxia and inflammatory necrotic CNS lesions into severe combined immunodeficiency mice by the intracisternal injection of CSF cells from MS patients during exacerbation but not from MS patients during remission or from patients with cervical spondylosis. However, Hao *et al.* (1994) were unable to confirm this finding.

The role of viral and bacterial infection

For many years viruses have been incriminated in the pathogenesis of MS. No virus has been consistently isolated from the CNS of patients with MS and there is no convincing evidence that viral infection of the CNS itself plays a role in the development of MS. However, viral infection outside the nervous system might have a pathogenic role in MS by leading to the polyclonal activation of autoreactive T and/or B cells or, through molecular mimicry, to cross-reactivity against CNS autoantigens. Sibley, Bamford & Clark (1985) found that the exacerbation rate of MS was almost threefold higher at the time of common viral infections (two weeks before the onset of infection until five weeks afterwards) than at other times. This finding

suggests that viral infections may trigger attacks of MS. Increased immune responses to a number of viruses have been reported in MS.

Measles virus

Anti-measles virus antibodies are produced intrathecally in MS (Norrby, 1978; Salmi *et al.*, 1983; Felgenhauer *et al.*, 1985; Dhib Jalbut *et al.*, 1990; Schadlich *et al.*, 1990). The intrathecal anti-viral response is not restricted to measles virus but is also directed against other viruses, including rubella, herpes zoster, parainfluenza, influenza, mumps and respiratory syncytial viruses (Norrby, 1978; Salmi *et al.*, 1983; Felgenhauer *et al.*, 1985; Schadlich *et al.*, 1990). Using the nitrocellulose immunospot assay, Baig *et al.* (1989) found cells secreting anti-measles virus IgG in the CSF of 88% of MS patients. They found a similar incidence and frequency of cells secreting IgG against herpes simplex virus in the CSF, but could not detect any cells secreting antibodies against these two viruses in the peripheral blood. However, using a different techique, another group found an increased frequency of peripheral blood B cells producing antibodies against measles virus in patients with MS (Zhang *et al.*, 1991). Dhib Jalbut *et al.* (1990) studied the antibody reactivity to purified measles virus polypeptides and concluded that the results were consistent with polyclonal B cell activation within the CNS, although a heightened response to the fusion polypeptide might also reflect cross-reactivity with a CNS autoantigen.

An unexplained finding in MS is the decreased generation, from the peripheral blood, of measles virus-specific and herpes simplex virus-specific cytotoxic T cells, which are predominantly restricted by HLA class II molecules (Jacobson, Flerlage & McFarland, 1985; de Silva & McFarland, 1991). In contrast, the generation of influenza virus-specific and mumps virus-specific cytotoxic T cell responses, which have large HLA class I-restricted components, is normal in MS (Jacobson *et al.*, 1985; Goodman, Jacobson & McFarland, 1989). Increased numbers of T cells secreting IFN-γ in response to measles virus and mumps virus have been found in the CSF, but not the blood, in MS compared to other neurological diseases (Link *et al.*, 1992); however, because of the high background response, these results are difficult to interpret.

Compston *et al.* (1986) reported that patients with inflammatory demyelinating diseases of the CNS had measles at a later age than HLA-DR matched normal controls, but the significance of this finding is unclear. Using the nested reverse transcription polymerase chain reaction, Godec *et al.* (1992) did not find measles virus genomic sequences in the brain of any of 19 MS patients. Another study using the polymerase chain reaction failed to detect measles virus genomic sequences in the peripheral blood lymphocytes of patients with MS (Bates *et al.*, 1993).

Epstein–Barr virus

The seropositivity rate and the titre of serum antibodies to Epstein–Barr virus (EBV) antigens is significantly higher in MS patients than in controls (Bray *et al.*, 1983; Larsen, Bloomer & Bray, 1985; Sumaya *et al.*, 1985). Larsen *et al.* (1985) found that the seropositivity rate was 100% in MS patients compared to 84% in controls. Furthermore, 85% of MS patients had CSF antibodies against EBV nuclear antigen-1 compared to 13% of EBV-seropositive controls (Bray *et al.*, 1992). A search of a protein sequence database revealed two pentapeptide identities between EBV nuclear antigen-1 and MBP; none of more than 32 000 other proteins in the database contained both pentapeptides (Bray *et al.*, 1992). This raises the possibility that EBV-specific T cells and antibodies might cross-react with MBP and contribute to the CNS tissue damage in MS. In a case–control study of 214 MS patients, recall of infectious mononucleosis in subjects seropositive for EBV capsid antigen was associated with a relative risk of 2.9 (Martyn, Cruddas & Compston, 1993). Those who reported having infectious mononucleosis before the age of 18 years had a relative risk of MS of 7.9. These epidemiological findings suggest that an age-dependent host response to EBV infection may have a role in the pathogenesis of MS.

Rubella virus

Anti-rubella virus antibodies are produced intrathecally in patients with MS (Norrby, 1978; Salmi *et al.*, 1983; Felgenhauer *et al.*, 1985; Schadlich *et al.*, 1990). As in the case of intrathecally produced anti-measles virus antibodies, this most probably represents polyclonal B cell activation within the CNS. However, Nath & Wolinsky (1990) found a relatively decreased IgG response to the rubella virus surface glycoprotein E1 and a relatively increased response to the surface glycoprotein E2 in the sera of MS patients compared to controls, and concluded that the response in MS is not simply due to polyclonal B cell activation. Patients with inflammatory CNS demyelinating disease were found to have had rubella at a later age than HLA-DR matched controls (Compston *et al.*, 1986), but the significance of this is unclear. Using the nested reverse transcription polymerase chain reaction, Godec *et al.* (1992) did not detect rubella viral genomic sequences in the brain of any of 19 MS patients.

Other viruses and bacteria

Koprowski *et al.* (1985) incriminated a retrovirus related to the human T cell lymphotropic viruses in the pathogenesis of MS. However, subsequent

studies have found no evidence for a role of such a retrovirus in MS (Nishimura *et al.*, 1990; Ehrlich *et al.*, 1991). Although antibodies to human T cell lymphotropic virus-1 are slightly elevated in the sera of some patients with MS, this occurs in the absence of viral antigen and thus appears to be due to cross-reactivity (Shirazian *et al.*, 1993). A significant proportion of MS patients have CSF antibodies to the paramyxovirus, simian virus 5, but this is not specific for MS, as similar reactivity occurs in other neurological diseases where CSF oligoclonal banding is present (Goswami *et al.*, 1987; McLean & Thompson, 1989). Antibodies to human herpesvirus 6 are elevated in the sera of patients with MS, but viral DNA is rarely detected (Sola *et al.*, 1993; Wilborn *et al.*, 1994). Murray *et al.* (1992) detected coronavirus RNA by *in situ* hybridization in 12 of 22 MS brain samples and found coronavirus antigen by immunohistochemistry in two patients with rapidly progressive MS. However, the number of sections that were positive for coronavirus RNA was low (11%) and coronavirus RNA was also found in two of 21 controls. Further studies will be needed to confirm their findings and to determine how specific they are for MS.

Bacterial infections may also have a role in the pathogenesis of MS. Bacterial superantigens bind to certain TCR Vβ chains and MHC molecules and can thereby activate T cells using the fitting Vβ chains. Burns *et al.* (1992) showed that superantigenic staphylococcal toxins can activate human MBP-specific T cells and PLP-specific T cells, and suggested that toxins produced during bacterial infections may thereby contribute to the induction or exacerbation of MS. Staphylococcal superantigens can trigger relapses of EAE by activating MBP-specific T cells (see Chapter 3).

In conclusion, there is epidemiological evidence that viral infections may contribute to the pathogenesis of MS; however, there is no convincing evidence that viral infection of the CNS itself is involved. The elevation of anti-viral antibody levels in the sera or CSF appears to be mainly due to polyclonal activation resulting from the MS disease process or perhaps to an underlying disorder of immunoregulation. Viral infections may induce anti-viral immune responses that cross-react with myelin antigens, but the extent to which this contributes to the pathogenesis of MS is unclear. Conversely, some apparent anti-viral responses may actually represent cross-reactive responses driven by myelin antigens. Viral infections may trigger attacks of MS by non-specifically activating the immune system or by interfering with immunoregulation, but there is no direct evidence to support these hypotheses. An interesting possibility requiring further study is that bacterial infections may trigger attacks of MS through superantigenic activation of autoreactive T cells.

Therapy

Therapy in MS may be divided into (1) therapy of the disease process and (2) symptomatic therapy. Symptomatic therapy has an important role in the management of patients with MS and entails the use of drugs for the treatment of such problems as spasticity, pain, paroxysmal phenomena, tremor and urinary difficulties (Pender, 1992). It will not be discussed further here. Therapy of the disease process is directed at inhibiting the immune attack on the nervous system, and embraces a range of different approaches which generally have been inspired by research findings in EAE.

Oral administration of myelin

As the oral administration of MBP or myelin prevents EAE (oral tolerance) (see Chapter 3), Weiner *et al.* (1993) conducted a double-blind pilot study of oral myelin therapy in relapsing–remitting MS. The proportion of patients having exacerbations was lower in the myelin-treated group than in the placebo-treated group. However, in view of the small number of patients studied, conclusions about efficacy cannot be drawn from these data, and a more extensive clinical trial will be required to evaluate this treatment.

Vaccination with T cells, and anti-TCR therapy

As vaccination with attenuated MBP-specific T cells protects animals against EAE (see Chapter 3), preliminary studies of this therapy have been conducted in patients with MS. Subcutaneous inoculation of MS patients with irradiated autologous MBP-reactive T cells was found to induce a proliferative T cell response to the inoculates and a correlated decrease in the frequency of MBP-reactive T cells (J. Zhang *et al.*, 1993). T cells that specifically inhibited the proliferative response of the inoculates to MBP could be detected in the vaccinated MS patients but not in non-vaccinated ones. The majority of T cell lines responding to the inoculates were $CD8^+$, with a minority being $CD4^+$. The $CD8^+$ lines were specifically cytotoxic for the inoculates in an HLA class I-restricted manner. J. Zhang *et al.* (1993) concluded that clonotypic interactions regulating autoreactive T cells can be induced in humans by T cell vaccination. It will be important to determine whether this therapy can inhibit clinical disease activity in MS.

The observation of restricted TCR Vβ gene usage by MBP-specific T cells in mice and rats led to the finding that anti-Vβ8 monoclonal antibodies or immunization with a synthetic TCR Vβ8 peptide can inhibit EAE (see Chapter 3). On the basis of the observation that there is a preferential usage of TCR Vβ5.2 and Vβ6.1 genes by MBP-reactive T cells in some patients

with MS, MS patients have been immunized with synthetic peptides encompassing the second complementarity-determining regions of Vβ5.2 and Vβ6.1 (Bourdette *et al.*, 1994; Chou *et al.*, 1994). Some of the inoculated patients developed a T cell response to the TCR peptides. Further studies will be needed to determine whether this therapy has any effect on disease activity. Potential limitations of this approach are suggested by the generally heterogeneous TCR Vβ gene usage by human MBP-specific T cells (see above) and the finding that TCR peptide therapy can also aggravate EAE (Desquenne Clark *et al.*, 1991; Sun, 1992).

Anti-CD4 antibody

As anti-CD4 antibody therapy inhibits EAE (see Chapter 3), preliminary studies of this therapy have been conducted in MS (Hafler *et al.*, 1988*b*). Anti-CD4 or anti-CD2 murine monoclonal antibody infusions were found to inhibit *in vitro* immune responses; however, repeated infusions induced anti-mouse antibodies with anti-idiotypic-like activity that could block binding of the anti-T-cell monoclonal antibody to the T cell surface (Hafler *et al.*, 1988*b*).

Cop 1

Cop 1 is a synthetic basic random copolymer of L-alanine, L-glutamic acid, L-lysine and L-tyrosine with a molecular weight of 21 000 and with immunological cross-reactivity with MBP (Teitelbaum *et al.*, 1991). As it inhibits EAE (see Chapter 3), it has been suggested as a possible therapy for MS. In a double-blind, randomized, placebo-controlled pilot trial, Bornstein *et al.* (1987) observed that subcutaneous cop 1 reduced the number of exacerbations in relapsing–remitting MS. A more extensive clinical trial is in progress. Cop 1 has been observed to inhibit the responses of MBP-specific human T cell lines and clones to MBP, suggesting that it can compete with MBP for the binding to human HLA molecules (Teitelbaum *et al.*, 1992; Racke *et al.*, 1992); however, in another study it had no such effect (Burns & Littlefield, 1991).

ACTH and corticosteroids

In 1950 Moyer *et al.* found that adrenocorticotrophic hormone (ACTH) prevented acute EAE when administered after inoculation and before the onset of neurological signs. The corticosteroid, methylprednisolone has a similar effect (Kibler, 1965). Furthermore, ACTH and methylprednisolone each reverse the neurological signs of EAE when administered after the onset of signs (Gammon & Dilworth, 1953; Vogel, Paty & Kibler, 1972).

Moyer *et al.* (1950) suggested that ACTH or a corticosteroid might have a beneficial effect in the human diseases, post-vaccination encephalitis and acute MS. It was subsequently shown that, compared with placebo, intramuscular ACTH hastens neurological improvement after a relapse of MS (Rose *et al.*, 1970). High-dose intravenous methylprednisolone therapy accelerates recovery from relapses (Durelli *et al.*, 1986; Milligan, Newcombe & Compston, 1987) and is as effective as intramuscular ACTH (Thompson *et al.*, 1989). Although oral corticosteroids are often used in clinical practice to treat attacks of MS, they have not been demonstrated by placebo-controlled trials to be effective. Indeed, in acute optic neuritis, oral prednisone therapy was found to have no beneficial effect and appeared to increase the risk of new episodes of optic neuritis when compared to placebo, whereas high-dose intravenous methylprednisolone followed by a short course of oral prednisone accelerated recovery, resulted in slightly better vision six months later and had no effect on the recurrence of optic neuritis (Beck *et al.*, 1992). Interestingly, high-dose intravenous methylprednisolone therapy followed by a short course of oral prednisone for acute optic neuritis was also found to reduce the rate of development of MS over a two-year period (Beck *et al.*, 1993). Further studies are needed to determine whether this important observation can be confirmed. Long-term treatment with ACTH or corticosteroids has not been shown to have a beneficial effect on the course of MS.

High-dose intravenous methylprednisolone therapy reduces intrathecal IgG synthesis, the level of MBP in the CSF, and gadolinium enhancement of MRI brain lesions, but has no effect on the oligoclonal IgG pattern in the CSF (Durelli *et al.*, 1986; Warren *et al.*, 1986; Wajgt *et al.*, 1989; Burnham *et al.*, 1991; Barkhof *et al.*, 1992; Frequin *et al.*, 1992). As the MRI appearance of increased water content in normal-appearing white matter is also reduced by this therapy, it has been suggested that the clinical improvement is due to resolution of oedema (Kesselring *et al.*, 1989). However, an alternative explanation for the beneficial clinical effect is inhibition of immune-mediated demyelination (Pender, 1992), as indicated by the reduction in the level of MBP in the CSF.

Immunosuppressants

Cyclophosphamide

Treatment with high-dose intravenous cyclophosphamide plus ACTH has been reported to stabilize or improve progressive MS (Hauser *et al.*, 1983*a*), although a randomized, placebo-controlled, single-masked trial found that therapy with intravenous cyclophosphamide plus oral prednisone had no such effect (Canadian Cooperative Multiple Sclerosis Study Group, 1991).

Intensive immunosuppression with cyclophosphamide in combination with prednisone has been reported to decrease the level of MBP in the CSF in chronic progressive MS, indicating that it may inhibit demyelination (Lamers *et al.*, 1988). This therapy or high-dose cyclophosphamide alone was also found to decrease intrathecal IgG synthesis (Lamers *et al.*, 1988; Wajgt *et al.*, 1989). As cyclophosphamide can aggravate EAE as well as inhibit it (see Chapter 3), it is possible that cyclophosphamide may aggravate MS in some patients.

Cyclosporin A

Long-term cyclosporin A therapy has been found to have a modest effect in delaying disease progression in patients with moderately severe progressive MS (Multiple Sclerosis Study Group, 1990). However, this therapy has a high incidence of severe adverse effects, particularly renal impairment and hypertension, and its use requires close supervision. As low-dose cyclosporin A therapy converts acute EAE into chronic relapsing EAE (Polman *et al.*, 1988; Pender *et al.*, 1990), the possibility that cyclosporin A may aggravate MS in some patients needs to be considered (Pender, 1991).

Azathioprine

Long-term azathioprine therapy appears to have a small beneficial effect on MS, but the effect is so small that adverse effects preclude its routine use (British and Dutch Multiple Sclerosis Azathioprine Trial Group, 1988).

Total lymphoid irradiation

In a randomized double-blind study, patients with chronic progressive MS treated with total lymphoid irradiation (1980 cGy) had significantly less functional decline than those receiving sham-irradiation (Cook *et al.*, 1986). There was a significant relationship between the absolute blood lymphocyte count in the first year after total lymphoid irradiation and the subsequent course, patients with higher lymphocyte counts generally having a worse prognosis.

Interferon-γ

Intravenous IFN-γ therapy increases the exacerbation rate in MS and is therefore unsuitable for the treatment of this disease (Panitch *et al.*, 1987*a*). The number of circulating monocytes expressing HLA-DR molecules increased during therapy, particularly in those patients who had exacerbations. In contrast to MS, EAE is inhibited by IFN-γ and aggravated by anti-

IFN-γ therapy (see Chapter 3). Why IFN-γ has different effects on MS and EAE is unknown.

Interferon-β

In a randomized, double-blind, placebo-controlled trial, long-term subcutaneous IFN-β therapy significantly reduced the exacerbation rate in relapsing–remitting MS compared with placebo (IFNB Multiple Sclerosis Study Group, 1993). As there was little change in disability from baseline in both the placebo and treatment arms of the trial, it could not be determined whether IFN-β therapy had any effect on disability. A concomitant study found a significant reduction in disease activity as determined by MRI and a significant reduction in MRI-detected burden of disease in the patients receiving IFN-β compared to those receiving placebo (Paty *et al.*, 1993). Further studies are required to determine whether IFN-β therapy has any effect on clinical disability in relapsing–remitting MS and whether it has any beneficial effect on chronic progressive MS. IFN-β significantly augments *in vitro* non-specific suppressor cell function in progressive MS and in normal subjects (Noronha, Toscas & Jensen, 1990, 1992). IFN-α has a similar effect, whereas IFN-γ has no effect (Noronha *et al.*, 1992). IFN-β has also been reported to inhibit IFN-γ-induced HLA-DR gene transcription in a human astrocytoma cell line, but not to inhibit IFN-γ-induced HLA-DR expression in human monocytes (Ransohoff *et al.*, 1991). Furthermore, *in vitro* IFN-β inhibits mitogen-induced proliferation, IL-2R expression and IFN-γ production by peripheral blood mononuclear cells of MS patients and normal controls (Noronha, Toscas & Jensen, 1993; Rudick *et al.*, 1993). In a pilot study it was found that mitogen-driven IL-2R expression on peripheral blood T cells was reduced in patients with relapsing–remitting MS after IFN-β therapy but not after placebo (Rudick *et al.*, 1993). These actions of IFN-β may account for the beneficial clinical effect in relapsing–remitting MS. Alternatively, the anti-viral action of IFN-β may be responsible for the beneficial effect, as viral infections may trigger attacks of MS (Sibley *et al.*, 1985).

Conclusions

There is now convincing evidence that MS is an autoimmune disease. It has been clearly demonstrated by twin studies that there is a major genetic contribution to MS susceptibility, although at present the only confirmed genetic factor predisposing to MS is the HLA-DR-DQ haplotype DRw15,DQw6,Dw2 (DRB1*1501-DQA1*0102-DQB1*0602). The increased association of MS with other autoimmune diseases in the same

individual and in family members suggests that a primary autoimmune gene(s) may also be involved, but further studies are needed to determine this. The CNS lesions of MS are characterized by primary demyelination and infiltration by T cells, macrophages and B cells, as is the case in EAE. As MBP, PLP and MOG are target antigens in EAE, immune responses to these antigens have been studied in patients with MS. There is good evidence that the frequency of *in vivo*-activated MBP-specific T cells is increased in both the peripheral blood and CSF and that MBP-specific B cell reactivity is increased in the CSF of MS patients. However, it is unknown whether these increased immune responses are pathogenic. There is also some evidence of increased T cell and B cell reactivity to PLP, MOG and MAG. A major question is whether the target antigen in MS is the same in all patients and at all stages of disease. It is possible that the initial target antigen may differ among patients and that additional antigens may be targeted in the same patient as the disease progresses. If the autoimmune process in MS is driven by a single antigen, it may be possible to treat the disease by tolerization with the appropriate antigen. However, at present there is no therapy that has been proven to prevent the progression of disability in MS. Further advances in the understanding of the pathogenesis of MS and autoimmunity in general may lead to the development of such a therapy.

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