Methylprednisolone treatment, immune activation, and intrathecal inflammation in multiple sclerosis

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1. INTRODUCTION

1.1 ETIOLOGY AND PATHOGENESIS OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system. The etiology of MS is unknown but accumulating evidence suggests that a T cell-mediated attack on central nervous system (CNS) myelin, initiated by environmental factors in a genetically susceptible individual, has a major pathogenic role (Sørensen and Ransohoff, 1998; Martino and Hartung, 1999; Hemmer et al, 2002). According to this model a central event in the pathogenesis is the migration of systemically activated T cells across the blood-brain barrier to the perivascular space surrounding CNS microvessels (Sørensen and Ransohoff, 1998; Martino and Hartung, 1999). Here myelin-reactive T cells are induced to produce proinflammatory T helper type 1 (Th1) cytokines which activate endothelial and glia cells, thereby initiating the migration of additional mononuclear cells from the blood to the perivascular space and into the CNS parenchyma (Ransohoff, 1999). The intrathecal inflammation induces demyelination, axonal pathology, and conduction block, i.e., the pathophysiological substrates of the symptoms and signs of MS (McDonald, 1998; Trapp et al, 1999). Proposed inflammatory effector mechanisms include the action of cytokotice substances, e.g., cytokines such as tumour necrosis factor (TNF)-α, TNF-β and interferon (IFN)-γ; nitric oxide and reactive oxygen intermediates; the action of neutral proteases including matrix metalloproteinases (MMPs); myelin-reactive autoantibodies and activation of the complement cascade; and direct interactions with inflammatory cells (Kieseier et al, 1999). This sequence of events is broadly supported by the results of studies carried out in the animal model experimental autoimmune encephalomyelitis (EAE), a T cell-mediated autoimmune disease with many similarities to human MS (Hemmer et al, 2002).

1.2 PATHOLOGY OF MULTIPLE SCLEROSIS

The histopathology hallmark of MS, i.e., demyelinated plaques in the white matter of the CNS, was initially described in the 1830’s and 1840’s by Carswell and Cruvelhier. From the late 1860’s onwards Charcot and others provided detailed descriptions of the disease, and by the beginning of the 20th century the main histopathology characteristics of the disease, i.e., focal inflammation, demyelination, and axonal loss were all recognized (Compston, 1998a; Hickey, 1999; McDonald, 1998; Trapp et al, 1999).

The results of recent histopathology studies suggest that there may be considerable heterogeneity in the pathogenesis of MS as evidenced by the identification of four distinct subtypes of MS, all characterized by perivascular and parenchymal T cell and macrophage inflammation (Lucchinetti et al, 2000). In type I lesions there is substantial T cell and macrophage inflammation, type II lesions are characterized by the additional deposition of immunoglobulin and complement. Oligodendrocytes are relatively preserved in type I and type II lesions, and remyelination is observed. Type III lesions are characterized by a diffuse loss of oligodendrocytes and a preferential loss of the minor myelin protein myelin-associated glycoprotein. In type IV lesions there is a more localized loss of oligodendrocytes with a uniform loss of minor and major myelin proteins. With the exception of an association of type IV lesions with primary progressive MS (PPMS, see below), none of these patterns correlate strictly with clinically recognized MS subtypes but type III lesions are more common in patients with acute MS (Lucchinetti et al, 2000).

1.3 EPIDEMIOLOGY AND GENETICS OF MULTIPLE SCLEROSIS

The prevalence of MS varies in different parts of the world with the highest prevalence being observed in Northern Europe and North America (Compston, 1998b). In Denmark the incidence of MS is approximately 4.5/100,000 per year (Koch-Henriksen et al, 1992). MS is a major cause of disability in young adults, and life expectancy in patients with MS is about 10 years lower than in the background population (Brønnum-Hansen et al, 1994).

Family studies have shown that genetic factors confer susceptibility to MS (Compston, 1998c; Oksenberg et al, 2001). The concordance rate for MS is approximately 30% in monozygotic twins and only 3-5% in dizygotic twins, roughly comparable to the rate in non-twin siblings. In Northern Europeans and in populations of Northern European ancestry the human leukocyte antigen (HLA) haplotype DRB1*1501 – DRB5*0101 – DQA1*0102 – DQB1*0602 (which encodes a split product of the DR2 and Dw2 specificities) confers an approximately four-fold increase in the risk of developing MS, but genome screening studies have suggested that a large number of genes influence susceptibility to develop MS (Compston, 1998b; Oksenberg et al, 2001).

1.4 CLINICAL CHARACTERISTICS AND DIAGNOSIS

Most patients initially have relapsing-remitting MS (RRMS) (Lublin and Reingold, 1996). This stage is characterized by recurrent attacks, i.e., “... a symptom or symptoms of neurological dysfunction with or without objective confirmation, lasting more than 24 hours ...” (Poser et al, 1983; McDonald et al, 2001). The onset of RRMS is most common from 20 to 40 years of age. The overall attack rate in patients with RRMS is approximately one per year, decreasing over time (Allen, 1991). Approximately two thirds of patients with RRMS later develop secondary progressive MS (SPMS), characterized by a gradual increase in disability evolving over years with or without superimposed attacks. Approximately 10-20% of patients with MS have PPMS, i.e., slowly progressive disease without an initial RRMS phase (Lublin and Reingold, 1996; Mathews, 1998; McDonald and Hawkins, 2002).

Acute optic neuritis (ON) is an inflammatory, demyelinating disease of the optic nerve (Perkin and Rose, 1979). More than 50% of patients presenting with monosymptomatic ON as a possible onset symptom of MS (POSMS) will develop other symptoms and signs of CNS disease, signifying the development of clinically definite MS (CDMS) (Perkin and Rose, 1979; Frederiksen, 1999). In Denmark ON is the onset symptom in 18-19% of patients with MS, other common onset manifestations being sensory symptoms (32%), motor symptoms (24%), cerebellar symptoms (16%), and diplopia (8%) (Brønnum-Hansen et al, 1994; Sørensen et al, 1995a). In general ON has a favourable prognosis with onset of recovery within a few weeks in the majority of patients (Pose, 1995).
This feature of ON is shared with other onset symptoms as recovery is generally better after a first attack of MS than after subsequent attacks (Möller, 1949; McAlpine and Compston, 1952; Thygesen, 1953).

The diagnosis MS depends on the demonstration of temporally and spatially disseminated lesions in the white matter of the CNS. The diagnostic criteria of Poser and coworkers, a refinement of the previous criteria of Schumacher and coworkers, grade disease in different categories according to the level of diagnostic certainty, and according to whether the diagnosis is supported by laboratory evidence of intrathecal immunoglobulin synthesis (Schumacher et al, 1985; Poser et al, 1983). The Poser criteria were recently revised by McDonald and coworkers. These criteria clarify in detail how magnetic resonance imaging (MRI) studies can facilitate an early diagnosis in suspected MS, and provide a detailed scheme for the diagnosis of POMS (McDonald et al, 2001; Dalton et al, 2002).

Hypointense white matter lesions on T2-weighted MRI are seen in almost all patients with MS and the detection of such lesions is important in the diagnosis of MS. Such lesions are, however, histopathologically heterogenous, ranging from normally myelinated areas with edema to areas with marked axonal loss (Barnes et al, 1991; Newcombe et al, 1991). Focal disruption of the blood-brain barrier is common in MS and is associated with macrophage infiltration of the parenchyma (Estes et al, 1990; Nesbit et al, 1991; Katz et al, 1993; Brück et al, 1997). This can be detected as high-signal areas on T1-weighted MRI after administration of the paramagnetic agent Gadolinium diamino-tetraethyl-pentaacetic acid (Gd-DTPA) which does not cross the intact blood-brain barrier (Rovaris and Filippi, 1999).

In POSTMS patients the risk of developing CDMS can be estimated by several methods. Patients with clinically silent lesions in the CNS as assessed by T2-weighted magnetic resonance imaging (MRI) have an increased risk of future conversion to CDMS, i.e., recurrent disease activity (Rovaris and Filippi, 1999). Intrathecal synthesis of IgG, a characteristic finding in MS, also implies an increased risk of future conversion to CDMS. The detection of intrathecal synthesis of IgG oligoclonal bands by isoelectric focusing and immunoblotting has a very high sensitivity even in POSTMS, and this method was recommended as the most important cerebrospinal fluid (CSF) analysis in a European consensus report (Andersson et al, 1994). In studies comparing the detection of disseminated brain lesions on T2-weighted MRI with the detection of intrathecal synthesis of IgG oligoclonal bands the former measure was somewhat more specific, depending on the diagnostic criteria applied, whereas the latter was more sensitive for the detection of patients who developed MS after a POSTMS episode (Sharief and Thompson, 1991; Paolino et al, 1996; Söderström et al, 1998; Turmani et al, 1998). T2-weighted MRI may provide additional prognostic information as the lesion load at the initial POSTMS correlates with the level of disability even after follow-up for more than 10 years (Brex et al, 2002).

The clinical severity of MS is commonly measured on the Kurtzke Expanded Disability Status Scale (EDSS), a measure combining features of neurological impairment, ambulatory function and general function on a 20-step ordinal scale ranging from 0 (no impairment) to 10 (death from MS) (Kurtzke, 1983). The Scripps Neurological Rating Scale (NRS) which quantitates impairment on a standard neurological examination is also used for studies of MS (Spe et al, 1984). On the NRS the maximum score (100 points) corresponds to a normal neurological examination, and maximal impairment on all components corresponds to an NRS score of –10 points. Both scales are commonly used in MS research even though both are relatively insensitive measures of clinical change (Sharrack et al, 1999).

1.5 AIM OF THE STUDIES

The presumed role of immunoinflammatory processes in the pathogenesis of MS has led to many attempts to treat the disease by immunosuppressive and immunomodulating drugs, and the recent introduction of IFN-β and other disease-modifying agents has changed the approach to treatment of patients with RRMS immensely (Paty et al, 1999; Chofflon, 2000). Glucocorticoids have anti-inflammatory and immunosuppressive effects, and treatment with glucocorticoids has a long history in MS (Andersson and Goodkin, 1998; Compston, 1998d). In a review published in 1991 the benefit of glucocorticoid treatment in MS was, however, questioned (Goodin, 1991). The aim of the studies in the present thesis was: to assess the clinical efficacy of oral high-dose methylprednisolone treatment in patients with ON and MS; to study measures of immune activation and intrathecal inflammation and their correlation with measures of disease activity in MS; and to study the effects of oral high-dose methylprednisolone treatment on immune activation and intrathecal inflammation.

2. CLINICAL EFFECTS OF GLUCOCORTICOID TREATMENT

2.1 HIGH-DOSE METHYLPREDNISOLONE IN ATTACKS OF MS

Early studies suggested that treatment with adrenocorticotropic hormone (ACTH) or glucocorticoids is efficacious in relapses of MS, but the individual response to treatment varied widely and no persistent treatment effect was detected (Rose et al, 1970; Compston, 1998d; Andersson and Goodkin, 1998). An initial uncontrolled study suggested intravenous high-dose methylprednisolone treatment to be highly efficacious in attacks of MS, the Guillain-Barré syndrome, and transverse myelitis (Dowling et al, 1980). A subsequent randomized, controlled trial failed to confirm the efficacy of methylprednisolone in the Guillain-Barré syndrome (Hughes et al, 1993). However, two trials including 22 and 23 patients with RRMS, respectively, provided evidence of faster recovery after high-dose methylprednisolone treatment than after treatment with placebo for a follow-up period of 15 days to four weeks (Durrell et al, 1986; Milligan et al, 1987).

In a trial comparing oral high-dose methylprednisolone (500 mg daily for five days followed by a ten days taper) and placebo in 51 patients with attacks of MS we found that improvement in the NRS and EDSS scores was significantly better after methylprednisolone treatment than after placebo for at least eight weeks (1). Subjective improvement on a global symptoms visual analog scale (VAS) was also significantly better after methylprednisolone treatment. Some indication of a persistent effect after one year was obtained. This was not observed for all efficacy measures, but the study was not powered to detect a long term treatment effect.

In the two early trials of intravenous high-dose methylprednisolone treatment of attacks of MS an impressive treatment response was observed with a mean improvement after two to four weeks of approximately 2 EDSS points (Durrell et al, 1986; Milligan et al, 1987). We observed a median improvement of 1.0 EDSS points (95% confidence interval, CI, 0.5 to 1.5) eight weeks after beginning treatment with oral high-dose methylprednisolone (I). This difference could reflect differences in the efficacy of oral and intravenous treatment, but other trials comparing oral and intravenous methylprednisolone treatment failed to demonstrate a major difference in the treatment response (Alam et al, 1993; Barnes et al, 1997). In a meta-analysis of the placebo-groups of several large multi-centre randomized, controlled trials the median change during an attack in patients with RRMS was 1.0 EDSS point. Median improvement after a mean of 45 days was 0.5 EDSS point, corresponding to a residual impairment of 0.5 EDSS points (Lublin et al, 2000). The improvement observed after an attack of MS in the placebo group in our study was comparable to these figures as the 95% CI for the median change after eight weeks was -0.2 to 1.0 EDSS point (median 0 EDSS points). The mean improvements of 2.0 EDSS points after intravenous methylprednisolone treatment observed in the initial placebo-controlled trials of intravenous methylprednisolone suggests that the patients included in these studies may have suffered from unusually severe attacks (Durrell et al, 1986; Milligan et al, 1987). Indeed, in a more recent, uncontrolled study, much less impressive...
responses were observed after intravenous methylprednisolone treatment of MS attacks (Patzold et al., 2002).

2.2 ORAL HIGH-DOSE METHYLPREDNISOLONE IN ON

Early studies provided no conclusive evidence that ACTH treatment improved recovery in patients with ON whereas high-dose methylprednisolone treatment was highly efficacious in an uncontrolled series (Rawson et al., 1966; Rawson and Liversedge, 1969; Bowden et al., 1974; Spoor and Rockwell, 1988; Compton, 1998d). The efficacy of glucocorticoid treatment was subsequently addressed in the North American Optic Neuritis Treatment Trial (ONTT). In this multicentre trial 151 patients were randomized to treatment with high-dose intravenous methylprednisolone for three days followed by oral prednisone for 11 days, 156 patients received oral prednisone treatment for 14 days, and 150 patients received oral placebo (Beck et al., 1992). A major concern when interpreting the results of the ONTT is that patients treated with intravenous methylprednisolone were not blinded to their treatment allocation. This may have introduced a bias in favour of methylprednisolone treatment. Nevertheless, although patients initially treated with methylprednisolone in the ONTT recovered faster than did patients treated with placebo, visual function in all three treatment arms was comparable after one year (Beck et al., 1992; Beck and Cleary, 1993). Oral prednisone treatment did not improve recovery from ON in neither the ONTT nor a Danish trial comparing treatment with oral prednisone and placebo in 128 patients with ON (Beck et al., 1992; Frederiksen et al., unpublished observations). Two other placebo-controlled trials also failed in detecting a persisting effect after treatment with different doses of methylprednisolone (Trauzettel-Klosinski et al., 1993; Kapoor et al., 1998).

In our trial 30 patients with ON were randomized to treatment with oral high-dose methylprednisolone and 30 patients received placebo (II). Patients treated with methylprednisolone had significantly better improvement in spatial visual function, colour vision, and VAS scores of visual symptoms for the first three weeks than did patients treated with placebo. After eight weeks these differences were no longer statistically significant. Post hoc analyses suggested a more pronounced response to treatment in patients treated early and in patients with more severe visual impairment both in the ONTT and in our trial (II; Beck et al., 1992).

2.3 METHYLPREDNISOLONE TREATMENT AND RECURRENT DISEASE ACTIVITY

An interesting finding in the ONTT was that high-dose methylprednisolone treatment was associated with a lower risk of future development of CDMS whereas oral prednisone treatment was associated with a higher risk of recurrent ON (Beck et al., 1993). The risk of recurrent disease activity was a secondary efficacy measure in the ONTT, and a type I statistical error is possible since there was no correction factor for multiple comparisons in the statistical analysis. The lack of blinding in the methylprednisolone treatment arm is another potential source of bias in the ONTT, and additional methodological problems have been identified in this study (Goodin, 1999). Indeed, the findings have not been confirmed in other studies, and it has been suggested that high-dose methylprednisolone treatment could even increase the risk of developing CDMS (Herishanu et al., 1989). We observed no difference in the risk of recurrent disease activity (recurrent ON, other attacks, or conversion to SPM or ON) in MS or ON attack patients treated with oral high-dose methylprednisolone or placebo (I, II).

2.4 PARACLINICAL MEASURES OF DISEASE ACTIVITY

The characteristic side effects of methylprednisolone treatment may compromise blinding, but the clinical efficacy of methylprednisolone treatment is supported by paraclinical studies. Uncontrolled studies suggested that intravenous high-dose methylprednisolone treatment suppressed Gd-enhanced MRI disease activity in the brain (Troiano et al., 1984; Burnham et al., 1991; Barkhof et al., 1991, 1992, 1994; Miller et al., 1992; Gasperini et al., 1997). We serially studied the effect of oral high-dose methylprednisolone on Gd-enhancement in 29 patients with Gd-enhancing lesions on MRI prior to treatment (III). In the methylprednisolone group enhancement was strongly suppressed after one week and three weeks compared to pre-treatment and compared to placebo-treated patients. The effect of oral high-dose treatment on Gd-enhancing lesions was comparable to the effect observed after intravenous treatment in previous studies.

A subgroup analysis of the treatment response to oral high-dose methylprednisolone showed that patients with Gd-enhancing lesions on brain MRI at baseline had a treatment response that was maintained for at least eight weeks whereas only a short-term treatment response was observed in patients with a normal Gd-enhanced MRI (III). This was not merely due to an association between the presence of enhancing lesions and a diagnosis of CDMS, as a clinical response to treatment after eight weeks was observed even when the analysis was restricted to CDMS patients with enhancing lesions on MRI but not in the entire group of patients with CDMS.

The CSF concentration of myelin basic protein (MBP) is commonly used as a measure of demyelination in MS (Cohen et al., 1976; Whitaker, 1977; Sellesjerg et al., 1998a; Lamers et al., 1998). High-dose intravenous methylprednisolone treatment was suggested in previous, uncontrolled trials to lower the CSF concentration of MBP (Barkhof et al., 1992; Frequin et al., 1992). This effect was confirmed in a serial lumbar puncture study of 50 of the patients included in our trials of oral high-dose methylprednisolone in ON or MS (IV). We used a radioimmunoassay, a standard method for measuring CSF concentrations of MBP. There was a highly significant decrease in the CSF concentration of MBP after treatment with oral high-dose methylprednisolone but no significant change in the placebo group. In addition, patients with high CSF concentrations of MBP appeared to respond somewhat better to oral high-dose methylprednisolone treatment, confirming a previous report of a more pronounced response to intravenous high-dose treatment in patients with increased CSF concentrations of MBP (Whitaker et al., 1993). In the placebo group changes in the CSF concentration of MBP correlated with changes in the Kurtzke EDSS score, supporting the relevance of MBP measurements as a surrogate measure of disease activity (IV).

2.5 CONCLUSIONS

Our results are consistent with a beneficial effect of high-dose methylprednisolone on recovery from attacks of MS, and suggest that oral treatment may be a useful alternative to intravenous treatment. We also found a short-term treatment effect of oral high-dose methylprednisolone on recovery after ON. An effect of methylprednisolone treatment on the risk of recurrent disease activity was observed in the North American ONTT but this finding remains controversial, and we found no impact of oral high-dose methylprednisolone treatment on the subsequent attack risk. Improvement in blood-brain barrier function as assessed by Gd-enhanced MRI appears to be a major effect of oral high-dose methylprednisolone treatment, and patients with enhancing lesions appear to respond better to treatment. Treatment was also associated with suppression of demyelination as assessed by the CSF concentration of MBP.

3. T CELLS IN MULTIPLE SCLEROSIS

3.1 T CELL ACTIVATION IN MS

Autoreactive T cells have a central role in autoimmune models of the pathogenesis of MS. T cells from MS patients have been studied by the analysis of antigen-specific T cell clones or T cell lines using proliferation and cytokine production as a read-out, or by immunospot and in situ hybridization assays for the enumeration of cytokine expressing cells. These studies have provided strong evidence that myelin-reactive Th1 cells are activated in the blood, CSF and brain.
of patients with MS (Navikas and Link, 1996; Wekerle and Lassmann, 1998; M artino and Hartung, 1999). Furthermore, the potential of a MBP-reactive T cell receptor derived from a patient with MS to initiate autoimmune demyelination has been demonstrated in transgenic mice (Madsen et al, 1999). We used flow cytometry to study the expression of activation-related molecules on the surface of freshly isolated T cells from patients with POSMS or CDMS in order to assess CD4 T cell activation status in MS in general. Flow cytometry is a standard method for analysing T cell activation in immunological research. The method is based on the use of fluorochrome conjugated antibodies, and modern flow cytometers readily allow the analysis of the expression of different molecules on the surface of single leukocytes.

3.2 CD26 EXPRESSION ON CD4 T CELLS

The CD26 molecule, dipeptidyl peptidase IV, is widely expressed on various cell types (Fleischer, 1994). CD26 occurs in multiple isoforms, and isoforms recognized by some monoclonal antibodies, including Ta1 and L272, are preferentially expressed on T cells involved in delayed type hypersensitivity reactions and on Th1 cells (Torimoto et al, 1992; Kahne et al, 1996; Seitzer et al, 1998). In one previous study an increased percentage of peripheral blood lymphocytes from patients with SPM S bound the anti-CD26 antibody Ta1 (Haffer et al, 1985), but this was not confirmed in another study (Crockard et al, 1988).

Increases in the percentage of CD26+ CD4 T cells were associated with clinical and MRI disease activity in MS in a recent study (Khoury et al, 2000), and we observed an increased percentage of blood CD26+ CD4 T cells in POSMS and CDMS when compared to neurological control subjects (IV, V). In both studies the Ta1 antibody was used. We have recently confirmed that there is an increase in CD26 expression on CD4 T cells from patients with active MS using the anti-CD26 antibody L272. These CD26+ CD4 T cells produce IFN-γ and TNF-α, and the percentage of CD26+ CD4 T cells in blood correlates with disease activity both in POSMS and RRMS (Jensen et al, submitted).

CD26 expression on CD4 T cells was not increased in a recent study of peripheral blood T cells from patients with active RRMS (Strunk et al, 2000). The CD26 antibody used in that study did, however, not recognize an isoform of CD26 preferentially expressed on Th1 cells as CD26 positive cells were found to produce more interleukin (IL)-2 but less IFN-γ and TNF-α than did CD26 negative T cells. CD26 expression was not increased in RRMS in other studies, possibly because expression was studied on lymphocytes in general and not specifically on T cells (Haffer et al, 1985; Crockard et al, 1988), or because the patients under study did not have active disease (Svenningsson et al, 1993; Rep et al, 1994).

3.3 CD25 AND HLA-DR EXPRESSION ON CD4 T CELLS

Expression of CD25, the a-chain of the IL-2 receptor complex, is induced by T cell activation but CD25 is also expressed by other activated leukocytes (Minami et al, 1992; Teramoto et al, 1993). CD25 was detected in active MS lesions and on CSF lymphocytes from patients with active MS (Bellamy et al, 1985; Hofman et al, 1986; Woodroofe et al, 1986; Traugott and Lebon, 1988), but this was not confirmed in other studies (Haffer et al, 1985; Hayashi et al, 1988). Studies of MBP-reactive T cells from patients with MS show them to express functional IL-2 receptors, suggesting in vivo activation (Zhang et al, 1994; Illes et al, 1999). However, in one study MBP-reactive T cells expressing CD25 underwent apoptosis after antigen stimulation in the absence of exogenous IL-2 (Bieganowska et al, 1997). This is consistent with studies showing that at least a subset of CD25+ CD4 T cells are anergic to T cell receptor stimulation and highly susceptible to activation-induced apoptosis (Taams et al, 2003). Indeed, CD25+ CD4 Treg cells co-express high levels of CD25 and cytotoxic T lymphocyte antigen (CTLA)-4, a negative regulator of T cell activation (Baecher-Allan et al, 2001; Dieckmann et al, 2001; Jonuleit et al, 2001; Le vings et al, 2001).

Only few studies have specifically addressed CD25 expression on CD4 T cells from patients with MS, and in these studies no major differences were observed between patients with MS and healthy controls (Khoury et al, 2000; Wu et al, 2000). We found a significantly lower expression of CD25 on CD4 T cells from the CSF of patients with POSMS or HLA-DR whereas there was no significant difference in the percentage of HLA-DR- CD25+ CD4 T cells between patients with POSMS, RRMS, and controls (VI).

A decrease in the expression of HLA-DR on T cells in peripheral blood was recently found to precede clinical and MRI disease activity in MS whereas there was no concomitant decrease in CD25 expression (Khoury et al, 2000). The decrease in HLA-DR expression in blood does not appear to reflect the migration of activated, HLA-DR positive cells to the brain as we and others found that even in CSF a lower percentage of T cells from patients with active MS express HLA-DR (VI, Fredriksson et al, 1987a; Konttinen et al, 1987; Oksaranta et al, 1995), and T cells in MS plaques do not express HLA-DR (Hayashi et al, 1988).

CD25 and HLA-DR expression is often considered to reflect T cell activation, but there is no simple relationship between CD4 T cell expression of CD25 and HLA-DR. The kinetics of HLA-DR expression on stimulated human T cells in vivo differs from that of CD25 expression, and HLA-DR expression after in vitro T cell activation is a late phenomenon which is temporally associated with decreasing levels of T cell proliferation (Yachie et al, 1993; Ko et al, 1979). As many CD25+ CD4 Treg cells coexpress HLA-DR, we suggest that there could be an insufficient recruitment of CD4 Treg cells to the CNS in MS (Baecher-Allan et al, 2001; Dieckmann et al, 2001; Jonuleit et al, 2001; Levings et al, 2001).

3.4 GENETIC CONTROL OF T CELL ACTIVATION

The HLA haplotype associated with the DRB1*1501 allele confers susceptibility to MS. The DRB1*1501 molecule has a high affinity for myelin peptides, the DRB1*1501 allele is associated with T cell reactivity to myelin proteins, and transgenic mice expressing a human MBP-specific T cell receptor, human CD4, and DRB1*1501 are highly susceptible to EAE (Ota et al, 1990; Valli et al, 1993; Wallström et al, 1998; Madsen et al, 1999). These findings suggest that the peptide binding characteristics of DRB1*1501 could directly influence susceptibility to develop MS. Other studies have, however, suggested that the closely linked DQB1*0602 is a susceptibility allele independent of DRB1*1501 (Serjan et al, 1992; Spurkland et al, 1997; Caballero et al, 1999). Furthermore, it has been reported that T cell clones from DRB1*1501 positive subjects have increased production of TNF-α regardless of the HLA restriction element used in peptide recognition (Zipp et al, 1995; Vandeveer et al, 1998).

We found lower expression of HLA-DR molecules on CD4 T cells from patients carrying DRB1*1501 (VI). All patients had a lower percentage of CD4 T cells coexpressing CD25 and HLA-DR than did neurological control subjects. Carriers of DRB1*1501 did, however, have a further loss of HLA-DR+ CD4 T cells not expressing CD25. Although patients carrying DRB1*1501 also had somewhat higher levels of intrathecal IgG synthesis and CSF activity of MPP-9, the latter differences were not statistically significant after correction for multiple comparisons. These findings suggest that alterations in T
cell activation, i.e., a lower level of HLA-DR on CD4 T cells is associated with the increased risk of developing MS conferred by the DRB1*1501 allele.

3.5 METHYLPREDNISOLONE TREATMENT AND T CELL ACTIVATION

Methylprednisolone treatment resulted in an increase in CD25 and HLA-DR expression on CD4 T cells in CSF (IV). As CD4 Treg cells from healthy volunteers express CD25 and HLA-DR, this could reflect a treatment-induced increase in the activity of Treg cells. A glucocorticoid-induced TNF-receptor (GITR) is over-expressed in regulatory T cells, and modulation of GITR activity is associated with changes in suppressive activity, suggesting that this molecule could be involved in glucocorticoid-induced generation of Treg activity (Gavin et al., 2002; McHugh et al., 2002; Shimizu et al., 2002). It is, however, also possible that the increase in CD25 expression after methylprednisolone treatment reflects a general increase in the expression of cytokine receptors, a recognized effect of glucocorticoids (Almawi et al., 1996).

CD26 expression decreased significantly on CD4 T cells in peripheral blood and CSF after treatment with oral high-dose methylprednisolone (IV). This finding suggests that a decrease in Th1 cytokine production following glucocorticoid treatment may reflect a direct effect on CD4 T cell activation. Indeed, a decrease in CD4 T cell production of IFN-γ and decreased expression of the Th1-associated chemokine receptors CCR5 and CXCR3 was reported after methylprednisolone treatment of MS attacks in a recent study (Marinez-Caceres et al., 2002). This effect was observed one month after a three days treatment course but was not maintained after six months.

3.6 CONCLUSIONS

Several studies have shown that there is systemic and intrathecal T cell activation in MS. We extend the results of previous studies by showing that not only RRMS patients but also POSMS patients have systemic T cell activation as evidenced by increased expression of the CD25 molecule. The expression of other measures of T cell activation such as CD25 and HLA-DR was lower in patients with POSMS and CDMS than in neurological control subjects. As discussed above, the functional roles of CD25+ and HLA-DR+ CD4 T cells in the pathogenesis of MS remain to be established but in healthy humans CD4 Treg cells are characterized by coexpression of these molecules, and we hypothesize that the lower levels of CD25 and HLA-DR expression on CD4 T cells in POSMS and CDMS may reflect insufficient generation or insufficient recruitment of regulatory T cells to the CNS in MS. Conversely, increased expression of CD26 on CD4 T cells may be directly linked to the activation of pathogenetic T cells. An association of lower levels of HLA-DR expression on T cells with the MS-associated DRB1*1501 allele, and the observed increase in CD25 and HLA-DR expression and decrease in CD26 expression on CD4 T cells after methylprednisolone treatment is consistent with this hypothesis.

4. IMMUNOGLOBULINS AND AUTOANTIBODIES

4.1 INTRATHECAL IMMUNOGLOBULIN SYNTHESIS IN MS

The role of immunoglobulins in the pathogenesis of MS is controversial. Recent studies suggest that this may be due to heterogeneity in the pathogenesis of MS. Complement and antibody-mediated demyelination may be crucial in a subgroup of patients with MS whereas in other patients this mechanism may be less important (Lucchinetti et al., 2000). Current evidence also suggests a complex role of immunoglobulins in the pathogenesis of autoimmune demyelination in the EAE model. Mice genetically deficient in B cells are susceptible to EAE induced by a MBP peptide, but the disease course in such animals is highly variable, ranging from mild to more severe than in wild-type mice (Wolf et al., 1996). Indeed, anti-myelin protein antibodies aggravate T cell mediated EAE, but immunoglobulins have also been used to treat EAE (Miller et al., 1997; Litzenburger et al., 1998; Morris-Downes et al., 2002).

4.2 QUALITATIVE AND QUANTITATIVE MEASURES OF IMMUNOGLOBULIN SYNTHESIS

Intrathecal IgG synthesis can be detected in the majority of patients with MS, and even in POSMS some 70% of patients have IgG oligoclonal bands in CSF as assessed by isoelectric focusing and immuno blotting, an internationally recommended method for the analysis of IgG oligoclonal bands (VIII; Anderson et al., 1994). IgG concentrations can be readily measured by nephelometric immunoassays, and such assays are routinely used for the analysis of IgG in CSF and serum samples. Quantitative measures of intrathecal IgG synthesis are, however, considered to be of less value in diagnosing MS than oligoclonal bands. Whereas the majority of patients with CDMS have oligoclonal bands in CSF, only 70-80% of patients have evidence of intrathecal IgG synthesis as assessed by various formulae for the quantitative assessment of intrathecal IgG synthesis by comparing CSF and serum concentrations of IgG and albumin, but quantitative formulae are well suited for analysing the relationship between IgG synthesis and disease activity (Sellebjerg et al., 1996). One previous study suggested an association between intrathecal IgG synthesis and demyelination as assessed by the CSF concentration of MBP (Frequin et al., 1992), but this was not confirmed in other studies (Warren and Catz, 1985; Pavarotti et al., 1986). We used a modification of the extended immunoglobulin indices of Öhman and coworkers to estimate intrathecal synthesis of IgG, IgA, and IgM in patients with POSMS and RRMS (Öhman et al., 1989; Sellebjerg et al., 1996, 1998a). We found that impairment and disability as assessed by the Kurtzke EDSS score, and demyelination as assessed by the CSF concentration of MBP, correlated with the intrathecal IgG synthesis levels but not with the CSF leukocyte count in patients with RRMS. In contrast, in POSMS patients both measures of disease activity correlated with the CSF leukocyte count but not with intrathecal IgG synthesis (VIII). These results suggest that immunoglobulin-mediated mechanisms of tissue injury could be more important in RRMS than in POSMS.

An association between predominance of B cells in CSF and high IgG index values with a chronic progressive disease course has been observed (Cepok et al., 2001; Izquierdo et al., 2002), and we found that the CSF concentration of the terminal complement complex correlates with the Kurtzke EDSS score (Sellebjerg et al., 1998b). These results are consistent with histopathology studies suggesting antibody- and complement mediated demyelination to be of importance in a major subgroup of patients (Lucchinetti et al., 2000), and this mechanism could be especially important in later stages of the disease.

4.3 ANTI-MYELIN PROTEIN AUTOANTIBODIES IN MS

Some studies have suggested that the majority of patients with MS have anti-MBP antibodies whereas other studies suggest that these are no more prevalent in MS than in other neurological diseases. Differences in the affinity distribution of the detected autoantibodies, immune complex formation, immunoadsorption of pathogenetically relevant antibodies, and pathogenetic heterogeneity may all help explain the difficulties in assigning a definite role of anti-MBP antibodies in the pathogenesis of MS (discussed in VII and VIII). Studies of cells secreting antibodies reactive with various myelin proteins have given more consistent results, but even by these techniques it has not been possible to detect a clear relationship between disease activity and autoantibody synthesis in patients with MS.

The regulation of specific antibody responses is under genetic control, and in mice the susceptibility to develop EAE after immunization with spinal cord homogenate in complete Freund's adjuvant is associated with a genetically determined ability to develop high-affinity antibodies (Devey et al., 1990). We hypothesized that the af-
finity of anti-myelin antibodies could also be a decisive factor in MS, and modified an immunospot assay to detect cells secreting IgG anti-MBP and anti-proteolipid protein (PLP) autoantibodies of high relative avidity. Elution of low-avidity antibodies with the chaotropic ion SCN− can be used to estimate antibody affinity (Pullen et al, 1986; M dcaldonald et al, 1988), and has been used to demonstrate that the intrathecal anti-virus antibody in MS is generally of low avidity (Luxtong et al, 1995). We found that a substantial proportion of cells secreted antibodies that maintained their binding to MBP and PLP even at an SCN− concentration of 5M (VIII). Even though cells secreting high-avidity anti-MBP and anti-PLP antibodies were present at somewhat higher numbers in patients with POSM S or CDMS, such cells could also be detected in many patients with non-inflammatory neurological diseases, and we found no correlation between the number of autoantibody secreting cells and measures of total intrathecal IgG synthesis.

In an early study using the original immunospot assay, i.e., an assay for the enumeration of both high-avidity and low-avidity autoantibody secreting cells, we found that the number of cells in CSF secreting antibodies reactive with the major myelin protein PLP increased during the course of a bout of ON, suggesting that these arise as a consequence of demyelination (Sellesbjerg et al, 1994). However, using the modified immunospot assay we found no correlation between the duration of symptoms and the number of high-avidity autoantibody-secreting cells (VIII). Furthermore, in patients with cells in CSF secreting anti-myelin protein antibodies there was a correlation between autoantibody synthesis and demyelination. These findings are consistent with a role of anti-myelin antibodies in demyelination in a subgroup of patients with MS. MBP-binding activity has, indeed, been found to co-localize with immunoglobulin deposition in active MS lesions, and anti-MBP and anti-PLP antibodies have opsonising activity in vitro assays of myelin phagocytosis (Genain et al, 1999; van der Goes et al, 1999).

Interestingly, anti-PLP antibodies appeared to correlate with disease activity in POSMS patients, whereas anti-MBP antibodies appeared to be more relevant in patients with CDMS, suggesting that pathogenetically relevant B cell autoantigens could shift during the course of MS (VIII). T cell reactivity to PLP peptides was also reported to change in patients with POSMS who subsequently developed CDMS (Tuohy et al, 1999). A memory T cell response to PLP was recently reported in healthy control subjects whereas T cell responses to MBP was restricted to naive T cells in controls (Burns et al, 1999, 2001). These findings and studies of the expression of PLP isoforms in the thymus suggest that T cell tolerance to PLP may be less complete than tolerance to MBP and, hence, that PLP could be an important target in an initial immune-mediated attack on CNS myelin (Anderson et al, 2000; Klein et al, 2000). The association of an anti-MBP antibody response with disease activity in CDMS could reflect intermolecular epitope spreading from PLP to MBP, a mechanism involved in the pathogenesis of chronic progressive disease in animal models of inflammatory demyelination (Vanderlugt and Miller, 2002).

4.4 METHYLPREDNISOLONE AND IMMUNOGLOBULIN SYNTHESIS Systemic and intrathecal synthesis of IgG was suppressed after treatment with oral high-dose methylprednisolone (IV). It is tempting to speculate that the methylprednisolone-induced decrease in IgG synthesis is at least partially responsible for the clinical efficacy of oral high-dose methylprednisolone treatment. Indeed, the clinical response to methylprednisolone treatment after one week was more pronounced in patients with levels of intrathecal IgG synthesis above median (III). One should, however, consider that this association could reflect an association of IgG synthesis with underlying pathogenic processes, e.g., a correlation with gadolinium-enhancing MRI lesions, rather than a causal relationship (III).

4.5 CONCLUSIONS Whereas the detection of intrathecal IgG production has long been known to be of value in the diagnosis of MS, it is still not clear exactly what role antibodies play in the pathogenesis of MS. In our studies we found evidence of differences not only in correlations between IgG synthesis and disease activity, but also in possible targets of anti-myelin autoantibodies between POSMS patients and patients with RRMS. In POSM S the number of cells secreting anti-PLP antibodies correlated with disease activity whereas there was no clear correlation between intrathecal IgG synthesis levels and disease activity in POSMS. In contrast, in RRMS disease activity correlated with the number of cells secreting anti-MBP autoantibodies, and we also observed a correlation between intrathecal IgG synthesis levels and disease activity in RRMS. It remains to be established whether anti-MBP and anti-PLP antibodies are directly involved in the process of demyelination in vivo, or whether they serve as markers of T cell myelin reactivity. Substantial decreases in IgG synthesis after treatment with oral high-dose methylprednisolone are consistent with a role of antibodies in the pathogenesis of MS, but it is also clear that cells secreting myelin-reactive antibodies can be recruited to the subarachnoid space in patients without evidence of immunemediated demyelination. The efficacy of intravenous IgG treatment in RRMS provides further evidence that the role of IgG extends beyond a role in demyelination (Fazekas et al, 1997; Achirot et al, 1998; Særensen et al, 1998).

5. INTRATHECAL INFLAMMATION IN MS

5.1 INTRATHECAL INFLAMMATION IN THE PATHOGENESIS OF MS

As discussed in section 1.2 CNS infiltration by T cells and mononuclear phagocytes is the hallmark of active MS lesions. Only few leucocytes, mainly activated memory T cells, cross the intact blood-brain barrier where they can be detected in low numbers in normal CSF, perivascular spaces and even in the brain parenchyma (Booss et al, 1983). However, in MS and EAE the expression of proinflammatory cytokines results in the expression of molecules which direct the migration of additional leucocytes across the blood-brain barrier (Ransohoff, 1999). In EAE induced by the adoptive transfer of myelin-reactive CD4 T cells these are first detected in the subarachnoid space and the perivascular space, presumably reflecting the ability of activated T cells to cross the intact blood-brain barrier (Skundric et al, 1993; Shin et al, 1995). In the perivascular space the autoreactive T cells undergo a secondary activation step by cognate recognition of myelin antigen presented by perivascular macrophages. This is followed by the recruitment of a broader range of T cells and monocytes from the blood and their migration into the brain parenchyma (Oppen et al, 1993; Flügel et al, 2001). The re-recruitment of mononuclear phagocytes is essential as monocyte-depleted animals do not develop classical EAE in spite of persisting perivascular T cell infiltration (Broson et al, 1981; Huitinga et al, 1990, 1995; Tran et al, 1998).

Leukocyte migration is a complex, multi step process which is initiated by a weak adhesion between circulating leucocytes and endothelial cells, mediated mainly by selectins and their glycolipid ligands (Ransohoff, 1999). This allows rolling of the leukocyte along the vessel wall. If the leukocyte is activated by chemotactic factors, e.g., chemokines immobilized on the endothelium, tight adhesion between activated integrins on the leukocyte and immunoglobulin superfamily adhesion molecules expressed on the endothelium ensues. Chemokines are small chemotactic cytokines which are divided in four different families (CC, CXC, CX3C, and C chemokines) based on conserved amino acid motifs. They bind to G protein-coupled 7-transmembrane spanning receptors expressed on the surface of leucocytes (Olson and Ley, 2002). The chemokine-induced adhesion of leucocytes is followed by transmigration across the endothelium and migration into the parenchyma. The migration is directed by chemotactic cues and is facilitated by proteolytic

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enzymes, e.g., MMPs which degrade basement membrane and extracellular matrix proteins (Cuzner and Opdenakker, 1999; Ransohoff, 1999; Hartung and Kieseier, 2000).

5.2 MAGNETIC RESONANCE IMAGING OF INFLAMMATION IN MS

Gd-enhanced MRI is generally used as a measure of inflammatory activity in RMS patients. This is based partly on histopathology studies showing that Gd-enhancing lesions are inflammatory with macrophage infiltration of the parenchyma, and partly on studies showing that the development of new lesions on T2-weighted MRI is generally preceded by the occurrence of Gd-enhancing lesions on T1-weighted MRI (Estes et al, 1990; Nesbit et al, 1991; Katz et al, 1993; Brück et al, 1997; Rovaris and Filippi, 1999). In patients with RMS Gd-enhancing lesions on MRI are much more common than clinical attacks, but the presence of enhancing lesions is associated with clinical disease activity (Kappos et al, 1999).

We found that patients with POSMS had a lower prevalence of Gd-enhancing lesions in the brain than did patients with RMS (III). This is consistent with the results of a previous study showing a higher prevalence of Gd-enhancing lesions in patients with ON as a symptom of RMS than in patients with idiopathic ON (Frederiksen et al, 1997). We also addressed the relationship between Gd-enhancing lesions and spontaneous remission in placebo-treated patients from the oral high-dose methylprednisolone trials (III). An improvement of at least one point on the Kurtzke EDSS score (in patients with attacks of MS) or on the visual function system of the EDSS (in patients with ON) was taken as evidence of a clinically relevant improvement. We found that a higher proportion of patients without Gd-enhancing lesions on T1-weighted MRI improved spontaneously after a follow-up period of up to eight weeks. This does not imply that Gd-enhancement is irrelevant in patients without overt enhancement, but in such patients enhancement might be detectable only for a shorter period or only after the administration of higher doses of Gd-DTPA. Interestingly, the duration of Gd-enhancement correlates with the development of more destructive, hypointense lesions on T1-weighted MRI and more severe changes in magnetization transfer variables, and lesions that enhance only after the administration of triple doses of Gd-DTPA are active for shorter periods and may be less destructive than lesions that enhance after standard doses (Filippi et al, 1998a-b; Rovaris et al, 1998; Ciccarelli et al, 1999).

5.3 CEREBROSPINAL FLUID MEASURES OF INTRATECHAL INFLAMMATION

Several studies have addressed the association between inflammation on Gd-enhanced MRI and blood and CSF measures of inflammation. An association between Gd-enhancement and blood T cell production of IL-2 or the expression of T cell activation markers has been observed in several studies (Calabresi et al, 1998a; Khoury et al, 2000; Jensen et al, submitted). A correlation between the CSF leukocyte count and Gd-enhancement was reported in some studies but was not generally confirmed (Trojano et al, 1996; Rieckmann et al, 1997; Calabresi et al, 1998c). There are also several reports of an association between increased serum and CSF concentrations of soluble forms of adhesion molecules with Gd-enhancing lesions on MRI but again the results of different studies are not consistent (Hartung et al, 1993, 1995; Mossner et al, 1996; Trojano et al, 1996; Giovannoni et al, 1997).

The detection of an association between Gd-enhancement on MRI and CSF levels of inflammatory markers may be complicated by the fact that the distance from the active lesion to the CSF influences the possibility to detect molecules produced in the lesion (Rieckmann et al, 1997). Nevertheless, we found an association between inflammation on Gd-enhanced MRI and CSF measures of inflammation as the area of Gd-enhancement correlated with the CSF activity of MMP-9 and the CSF leukocyte count (III). Intrathecal IgG synthesis also correlated with the area of Gd-enhancement on T1-weighted MRI and with the presence of abnormalities on T2-weighted MRI, confirming a previous report from our group (III, VII; Frederiksen et al, 1996).

Neopterin is a molecule produced by human monocytes and macrophages stimulated by IFN-γ and other proinflammatory cytokines, and neopterin has been used as a surrogate measure of proinflammatory cytokine activity (Hamerlinck, 1999). We found that the CSF concentration of neopterin as assessed by a commercially available radioimmunoassay did not correlate with Gd-enhancement, and the CSF concentration of neopterin decreased spontaneously both in methylprednisolone- and placebo-treated patients (IV). This finding is consistent with an earlier study showing a spontaneous decrease in the CSF concentration of neopterin during the course of an MS attack (Fredrikson et al, 1987b). Indeed, increased systemic neopterin production was reported to be associated with the initial development of Gd-enhancing lesions rather than with the maintenance of lesion activity (Giovannoni et al, 2000). We found that patients with high baseline concentrations of neopterin in CSF were somewhat more likely to respond to oral high-dose methylprednisolone treatment after one week but not after eight weeks, consistent with an association between neopterin production and the initiation of an attack but not with the maintenance of disease activity (III).

Transforming growth factor (TGF)-β has been implicated in the pathogenesis of many neurological diseases (Patt and McPherson, 1998). In MS TGF-β expression has been associated with the control of inflammation (Beck et al, 1991; Link et al, 1993; Mokhtarian et al, 1994; Rieckmann et al, 1994, 1995; Correale et al, 1995; Carrieri et al, 1997; Bertoletto et al, 1999). Bioactive TGF-β can be measured by enzyme-linked immunosorbent assay (ELISA) using recombinant TGF-β receptor for capture and anti-TGF-β antibody for detection of bound TGF-β. By this method the total TGF-β concentration can be measured after acid activation of latent TGF-β. We found a lower CSF concentration of total TGF-β in untreated patients with attacks of MS than in neurological control subjects whereas active TGF-β1 was not detected (IV). The CSF concentration of total TGF-β1 correlated negatively with the area of Gd-enhancement on T1-weighted MRI (unpublished observations: Spearman’s r = -0.44, p = 0.03, n = 23). This finding is in agreement with a previous study showing an inverse correlation between TGF-β1 mRNA expression in blood cells and MRI disease activity in MS (Bertoletto et al, 1999). Furthermore, high levels of TGF-β mRNA expressing blood cells has been associated with low levels of disability in MS (Link et al, 1993).

MMPs may be involved in the proteolytic degradation of basement membrane and intercellular matrix proteins during leukocyte migration, in the disruption of blood-brain barrier integrity, the degradation of myelin proteins during demyelination, and the induction of axonal pathology in MS (Cuzner and Opdenakker, 1999; Hartung and Kieseier, 2000; Newman et al, 2001). We used zymography to detect MMP-9 activity as 92 kD gelatinase activity, and we found that MMP-9 activity was assessed by zymography correlated with MMP-9 concentrations measured by ELISA (IV). We found increased CSF activity of MMP-9 in CSF from patients with POSMS and CDMS. Patients with MMP-9 activity in CSF had lower levels of intrathecal IgG synthesis, higher CSF leukocyte counts, and higher CSF concentrations of MBP and neopterin (V). A correlation between MMP-9 activity and the CSF leukocyte count was not found in one previous study (Paemen et al, 1994), but in two other studies such correlations were observed (Gijbels et al, 1992; Leppert et al, 1998). In the study by Leppert and coworkers a correlation between MMP-9 activity and the IgG index was also observed. MMP-9 activity in CSF is not directly related to clinical disease activity as patients in remission and patients with relapses all have increased CSF concentrations of MMP-9 (Leppert et al, 1998). MMP-9 in CSF is, however, observed in its inactive 92 kD proform.
which requires proteolytic cleavage in complex proteolytic cascade reactions which are initiated by the activity of, e.g., membrane-type 1 MMP (MT1-MMP) or plasminogen activator proteins (Cuzner and Opdenakker, 1999; Hartung and Kieseier, 2000). Increased MT1-MMP and plasminogen activator activity has, indeed, been reported in MS (Akenani et al, 1996, 1997; Cuzner et al, 1996; Galboz et al, 2001). The lack of correlation between MMP-9 activity and clinical disease activity could also reflect subclinical disease activity in some patients without overt clinical disease activity. Indeed, we found that MMP-9 activity in CSF correlated with the area of enhancement on T1-weighted MRI, and in two other studies increases in MMP-9 activity in blood correlated with MRI disease activity (III; Lee et al, 1999; Waubant et al, 1999). We also found an association between presence of MMP-9 activity in CSF and increased risk of recurrent disease activity (V).

5.4 GENETIC CONTROL OF INTRATHECAL INFLAMMATION IN MS

We found higher CSF activity of MMP-9 and higher levels of intrathecal IgG synthesis in patients carrying the DRB1*1501-associated HLA haplotype (VI). After correction for multiple comparisons these differences were, however, not significant. As discussed in section 3.5 the mechanism underlying HLA-associations in MS is not clear but DRB1*1501 positive patients produce higher levels of TNF-α than DRB1*1501 negative patients, suggesting that increases in proinflammatory cytokine production are involved (Zipp et al, 1995; Vandevyver et al, 1998).

The chemokine receptor CCR5 has been implicated in the pathogenesis of MS as it is expressed on an increased percentage of T cells and monocytes in blood and CSF from patients with MS, and CCR5 positive T cells produce mainly Th1 cytokines upon activation (Balashov et al, 1999; Sørensen et al, 1999b; Strunk et al, 2000; Zang et al, 2000). In addition, macrophages and microglia in MS lesions express CCR5, and MS patients have increased CSF concentrations of CCR5 ligands (Miyagishi et al, 1995; Balashov et al, 1999; Sørensen et al, 1999b; Simpson et al, 2000; Trebst et al, 2001). The CCR5 Δ32 allele lacks 32 base pairs and encodes a truncated CCR5 protein not expressed at the cell surface. CCR5 Δ32 homozygotes have no immunodeficiency phenotype, but have a slightly higher blood lymphocyte count and blood pressure (Nguyen et al, 1999).

CCR5 is a coreceptor for the human immunodeficiency virus (HIV), and CCR5 Δ32 homozygotes are resistant to HIV infection (Dean et al, 1996; Liu et al, 1996; Samson et al, 1996; Fischereder et al, 2001). CCR5 Δ32 also confers some disease protection in rheumatoid arthritis, asthma, and organ transplantation (Garred et al, 1998; Gomez-Reino et al, 1999; Hall et al, 1999). CCR5 Δ32 homozygotes may develop MS, but we found that CCR5 Δ32 heterozygotes have prolonged relapse-free intervals (VII; Bennetts et al, 1997). Whether CCR5 Δ32 influences the age of onset in MS is controversial. We found a lower age of onset in CCR5 Δ32 heterozygotes, whereas in familial MS onset of disease occurred later in CCR5 Δ32 heterozygotes (VII; Barcellos et al, 2000).

The mechanism of a protective effect of CCR5 Δ32 in MS is not clear, but this allele does not appear to be associated with major changes in intrathecal inflammation in POSM S and RRM S although we observed minor changes in nitric oxide production in CCR5 Δ32 carriers (VII; Søllberg et al, 2002). Possibly CCR5 may be more important in monocyte recruitment, activation, or migration into the CNS parenchyma than in the recruitment of T cells and other leukocytes to the subarachnoid and perivascular space (Trebst et al, 2001).

5.5 METHYLPREDNISOLON TREATMENT AND INTRATHECAL INFLAMMATION

As discussed earlier high-dose methylprednisolone treatment improves blood-brain barrier function during attacks of MS (III). The CSF concentration of TGF-β1 also increased significantly after treatment with oral high-dose methylprednisolone (IV). It is possible that TGF-β may be directly involved in the resolution of lesion activity as there is a negative correlation between Gd-enhancement and CSF concentrations of TGF-β during attacks of MS, but the concomitant changes in CSF concentrations of TGF-β and Gd-enhanced MRI activity does not definitely prove this to be the case.

In a previous study suppression of MMP-9 activity was reported immediately after high-dose methylprednisolone treatment (Rosenberg et al, 1996). We did, however, not find a persisting change in the CSF activity of MMP-9 or in the CSF leucocyte count one week after completing treatment with oral high-dose methylprednisolone (IV). This suggests that suppression of leucocyte recruitment and MMP production is not as important as changes in the activation status of the recruited leukocytes or the induction of TGF-β expression after high-dose methylprednisolone treatment. Indeed, in an additional study we found no change in the CSF concentration of the chemokine CXCL10 (inflammatory protein of 10 kD, IP-10) after treatment with oral high-dose methylprednisolone (Sørensen et al, 2001). CXCL10 and its receptor CXCR3 have been implicated in the recruitment of T cells to the CSF in MS, and we found significant correlations between the CSF concentrations of CXCL10, MMP-9, and the CSF leucocyte count both before and after treatment (Balashov et al, 1999; Sørensen et al, 1999b, 2002).

5.6 CONCLUSIONS

There is compelling evidence suggesting a major role of parenchymal inflammation, as assessed by the presence of Gd-enhancing lesions on brain MRI, in the pathogenesis of attacks of MS. Gd-enhancement is temporally associated with attacks of MS and with the development of new lesions on T2-weighted MRI; patients with Gd-enhancement have prolonged attack duration; Gd-enhancement correlates with measures of intrathecal inflammation; Gd-enhancement is attenuated by methylprednisolone treatment; and patients with Gd-enhancement appear to benefit more from methylprednisolone than Gd-enhancement correlates with several measures of intrathecal inflammation prior to therapy with oral high-dose methylprednisolone. The CSF leucocyte count and MMP-9 activity in CSF remained elevated after treatment when Gd-enhancement was still strongly suppressed. This finding suggests that whereas methylprednisolone treatment results in the resolution of parenchymal inflammation, leucocytes are still actively recruited across the blood-brain barrier. CSF leucocyte counts are higher early in the course of MS, when the attack rate is high, than at later stages where CSF leucocyte counts and attack rates tend to decrease (reviewed in Walker et al, 1985; Allen, 1991). In addition, CSF leucocyte counts, chemokine concentrations, and MMP-9 activity correlates with patients with RMS, and patients with MMP-9 activity in CSF have shorter relapse-free intervals. These findings are consistent with a major role of leucocytes and MPP activity in disease activity in RMS. However, as there is no simple relationship between these measures of inflammation and clinical disease activity in MS additional stages must be involved. The migration of monocytes into the CNS parenchyma, which is associated with Gd-enhancement in histopathology studies, could be one such stage. This would explain why CCR5 Δ32 may confer some disease protection in RMS as monocytes are the major leucocyte subset expressing CCR5 in CSF from POSM S and RRM S patients.

6. DISCUSSION AND FUTURE DIRECTIONS

6.1 IMMUNE ACTIVATION AND INFLAMMATION IN MS

Histopathologically MS is characterized by demyelinated plaques in the white matter of the CNS. Demyelination has long been thought to explain the conduction changes and ensuing symptoms and signs observed in MS. An axonopathy and axonal loss is also present in MS, and the latter is now thought to be a major determinant of irreversible impairment and disability (Ferguson et al, 1997; Trapp et al, 1998, 1999; Evangelou et al, 2000). The etiology of MS remains ill
defined but is thought to be multifactorial with an interaction between genetic susceptibility and environmental factors. As discussed in section 3 and 5, there is good evidence that myelin-reactive T cells and intrathecal inflammation are critical in the pathogenesis of MS, and inflammation can induce demyelination and axonal pathology compared to that observed in MS (McDonald, 1998; Trapp et al., 1999; Korniek et al., 2000; Danekar et al., 2001). In addition, inflammation can induce a reversible conduction block, an effect to which demyelinated axons are especially sensitive (Redford et al., 1997; Coles et al., 1999).

Studies of Gd-enhancement on T1-weighted MRI show that focal disruption of the blood-brain barrier is associated with clinical attacks of disease but also indicate a high level of subclinical disease activity in many patients with MS. Patients with ON as POSMS have less enhancing lesions than do patients with ON in CDMS, and enhancing lesions are less common in patients with benign MS (Thompson et al., 1992; Kidd et al., 1994; Frederiksen et al., 1997; Losseff et al., 2001). As Gd-enhancement correlates with macrophage infiltration, these findings are consistent with a major role of parenchymal inflammation, i.e., the presence of activated macrophages, in the pathogenesis of MS (Estes et al., 1990; Nesbit et al., 1991; Katz et al., 1993; Brück et al., 1997). Parenchymal inflammation appears to represent the end stage of a multi step process which is initiated by systemic T cell activation followed by the migration of activated T cells across the blood-brain barrier; secondary activation of myelin-reactive T cells within the CNS; and recruitment of additional mononuclear cells and migration of these into the CNS parenchyma. Autoantibodies appear to be involved in the pathogenesis in a subgroup of MS patients. Data obtained in patients with RRMS, including the results of the present studies, and results obtained in EAE generally support this model. Immune reactivity within the CNS may, however, also have beneficial effects and there is still much to be learned about the precise pathogenic mechanisms in MS (Schwartz and Moalem, 2001).

Histopathology studies have shown that inflammation is a constant feature in active MS plaques, but the role of inflammation in chronic progressive disease is controversial. Patients with SPM S have an altered and to some extent more pronounced immune activation than do patients with RRMS (Balashov et al., 1997; Windhagen et al., 1998; van Boxel-Dezaire et al., 1999; Jensen et al., 2001; Sørensen and Sellebjerg, 2001). Indeed, the development of atrophy and SPM S is preceded by inflammatory activity as assessed by Gd-enhanced MRI, and patients with progressive disease have more widespread changes in blood-brain barrier permeability than do patients with RRMS (McLean et al., 1993; Miller et al., 2000a; Silver et al., 2001; Casanova et al., 2002).

Patients with more pronounced attack activity may be at greater risk of converting to an SPM S disease course, but this has not been observed in all studies (Confavreux et al., 1980; Weinschenker et al., 1991; Runmarker and Andersen, 1993; Trojano et al., 1995). Once the transition to SPM S has occurred attacks do not appear to have much impact on the disease course. Patients with PPMS and patients with SPM S without superimposed relapses have little disease activity on Gd-enhanced MRI, but progression rates are similar in patients with or without superimposed attacks (Thompson et al., 1993; Kidd et al., 1996; Confavreux et al., 2000). It is possible that axonal loss and irreversible disability in MS may arise by different mechanisms. Immunoinflammatory changes may directly lead to axonal pathology as suggested by the correlation between inflammation and axonal loss observed in histopathology studies (Trapp et al., 1998; Korniek et al., 2000; Bitsch et al., 2000). Axonal loss in the absence of ongoing inflammation might be caused by the long term effects of demyelination and could resemble the axonal degeneration observed in patients with mutations in the gene encoding PLP. These patients develop a length-dependent axonal loss and in some cases a slowly progressive myelopathy resembling PPM S (Garbern et al., 2002). Elucidating the role of intrathecal inflammation in the pathogenesis of SPM S and PPMS is an important area in future MS research since it is in these stages that there is most development of severe disability.

In EAE susceptibility to autoimmune demyelination is under genetic control, and different genetic loci control susceptibility, chronicity and other disease characteristics (Butterfield et al., 2000). Loci involved in EAE are also involved in the pathogenesis of virus-induced demyelination and animal models of rheumatoid arthritis, and loci involved in the pathogenesis of animal models of autoimmune disease overlap with loci involved in human autoimmune diseases (Teuscher et al., 1997; Becker et al., 1998; Bergsteindottir et al., 2000). In a recent study it was found that there is an increased prevalence of autoimmune disease among relatives of patients with MS (Broadley et al., 2000). Furthermore, the prevalence of autoimmune diseases was higher in multiplex families than in families with single members affected with MS. These findings are consistent with a multifactorial model where loci that influence the general risk of autoimmune disease interact with loci that are involved in the pathogenesis of specific autoimmune diseases and are modified by environmental factors (Mackay, 2001; Wandstrat and Waldkend, 2001).

Understanding how distinct genes influence the immunoinflammatory processes in MS is at an early stage, and the association with the DRB1*1501-associated HLA haplotype is incompletely understood at the functional level (discussed in section 3). Similarly, the protection conferred by CCR5 A32 in different immunoinflammatory diseases is not understood in detail (discussed in section 5). Advances in the fields of genetic epidemiology, immunogenetics, neurobiology, and immunology are expected to improve our understanding of the immunopathogenesis of MS, but may well be complicated by heterogeneity in disease processes. Indeed, in the United Kingdom genome screening study differences in genetic linkage were observed between families where disease was associated with the M S-associated HLA haplotype and families where this did not appear to be the case (Coraddu et al., 1998).

6.2 IMMUNOREGULATION AND GLUCOCORTICOID IN MS

There is good evidence that myelin-reactive T cells are of pathogenic significance at least in RRMS but myelin-reactive T cells can also be detected in blood from healthy volunteers and patients with non-inflammatory neurological diseases, suggesting that additional factors are pathogenetically important. T cell tolerance to self antigens is maintained at several different levels. Negative selection in the thymus accounts for the deletion of some T cells with high affinity for self antigens but is insufficient to ensure deletion of all myelin-reactive T cells (Wekerle et al., 1996; Klein et al., 2000). Peripheral deletion of self-reactive T cells and the induction of anergy to T cell receptor-mediated activation are additional mechanisms by which harmful self-reactivity is avoided (Walker and Abbas, 2002). Peripheral tolerance mechanisms are defective in patients with SPM S, possibly explaining the changes in systemic immune activation observed in SPM S (Correale et al., 1996; Balashov et al., 2000). The activation of myelin-reactive T cells is also subject to control by regulatory T cells (Furtado et al., 2001; Maloy and Powrie, 2001).

Pharmacological treatment with glucocorticoids is efficacious in MS, but endogenous glucocorticoids also appear to be involved in the natural regulation of disease activity. In a recent study cortisol release induced by a dexamethasone-corticoteropin-releasing hormone stimulation test correlated negatively with the presence of Gd-enhanced MRI lesions, and endogenous glucocorticoid production is strongly involved in the regulation of autoimmunity in animal models (Tonelli et al., 2001; Schumann et al., 2002). Oral high-dose methylprednisolone treatment suppressed disease activity on Gd-enhanced MRI, and the clinical response to therapy was more pronounced in patients with enhancing lesions on the baseline MRI. Hypothetically this could be due to an insufficient induction of endogenous glucocorticoids in patients with prolonged attack dura-
tion and a more clear response to methylprednisolone treatment.

Glucocorticoids have multiple effects in the immune system. These include short-lasting effects on leukocyte recirculation patterns which, however, are unlikely to account for the efficacy of glucocorticoid treatment in MS as treatment with oral high-dose methylprednisolone has no persistent effect on the CSF leukocyte count, the CSF activity of MMP-9, and concentrations of CXCL10. Instead alterations in T cell activation and IgG and TGF-β1 synthesis may be involved. Glucocorticoids interact negatively with transcription factors involved in T cell activation and may induce apoptosis of activated T cells (Boscher et al, 2000; Laethem et al, 2001; Leussink et al, 2001). These effects may contribute to the observed changes in CD26 expression on CD4 T cells after oral high-dose methylprednisolone. The functional relevance of the increase in CD4 T cells co-expressing CD25 and HLA-DR after oral high-dose methylprednisolone treatment remains to be determined, but we hypothesize that it could reflect an increase in regulatory T cell activity, possibly related to the induction of TGF-β which induces the differentiation of regulatory CD25+ CD4 T cells in humans (Yamagawa et al, 2001).

Several studies have shown that whereas glucocorticoids suppress the expression of proinflammatory cytokines they induce TGF-β expression, and this may be an important effect of oral high-dose methylprednisolone treatment of MS. TGF-β inhibits the production of pro-inflammatory cytokines by effector T cells but may also promote the differentiation and survival of T cells by inhibiting the induction of apoptosis (Cerwenka et al, 1994; Link et al, 1995; Genestier et al, 1999; Goralik et al, 2002). We found that patients with high serum concentrations of IgA had somewhat higher IgG indices, CSF leukocyte counts, prolonged attack duration, and a more pronounced response to oral high-dose methylprednisolone treatment (III). As TGF-β is an isotype shift factor for IgA these results suggest that the role of this cytokine in the pathogenesis of MS may extend beyond a presumed protective effect, but further studies of the relationship between TGF-β and IgA production in MS are needed to substantiate this hypothesis.

Systemic TGF-β2 treatment did not attenuate disease activity in a previous phase 1 clinical trial in SPM S but this could be due to a loss of sensitivity to TGF-β in SPM S (Correalet al, 1996; Calabresi et al, 1998b). However, the induction by oral tolerance of myelin-reactive Th3 secreting TGF-β was not protective in a major clinical trial in patients with MS (Fukaura et al, 1996). Interestingly, the effect of TGF-β on T cell development differs in different inbred mouse strains, and in MS a protective effect of TGF-β on T cell activation may be restricted to DRBl*1501 positive patients suggesting that genetic heterogeneity in the response to TGF-β may be operative in MS (Hoehn et al, 1995; Soderstrom et al, 1995).

6.3 STRATEGIES FOR GLUCOCORTICOID TREATMENT IN MS

The definite aim of MS therapy is to inhibit the development of irreversible disability and associated handicap. Methylprednisolone treatment can increase the speed of recovery after an attack of MS, and two recent meta-analyses conclude that there is good evidence supporting the use of glucocorticoid treatment in attacks of MS but no evidence that the final outcome is better after methylprednisolone treatment. (Brusafar and Cande1se, 2000; Miller et al, 2000b). This does not rule out that such an effect could exist because studies of sufficient size and duration have not been conducted. The lack of a persistent effect of methylprednisolone in optic neuritis does not exclude a persistent effect in MS because spontaneous improvement is much more pronounced in ON than in attacks of RRM S. In a recent MSI study it was reported that methylprednisolone treatment results in the development of less severe changes in magnetization transfer ratios (Richert et al, 2001). This finding and a more pronounced clinical response to treatment observed in patients with active Gd-enhanced MRI suggests that treatment with high-dose methylprednisolone could prevent the development of more destructive lesions in patients with active Gd-enhanced MRI, and it was recently reported that treatment with intravenous high-dose methylprednisolone at 4 months intervals slowed the development of hypointense lesions on T1-weighted MRI and persistent disability in RRM S (Zivadinov et al, 2001). The role of glucocorticoids in chronic progressive MS is not clear. The role of inflammation in the pathogenesis of PPM S and SPM S is controversial, and myelin-reactive T cells from patients with SPM S were reported to have an impaired sensitivity to glucocorticoid-induced apoptosis (Corrreale et al, 2000). Nevertheless some clinical benefit has been observed after treatment of these patient categories with high-dose methylprednisolone, suggesting some contribution of glucocorticoid-responsive inflammatory responses to the pathogenesis in PPM S and SPM S (Mfilligan et al, 1987; Cazzato et al, 1999; Goodkin et al, 1998).

There is a need for studies establishing optimal treatment regimens for glucocorticoid treatment in MS. Unresolved questions relate to the optimal dosage, whether tapering is beneficial, and possible differences in the efficacy of different glucocorticoids and inter-patient differences in the biological response to glucocorticoids. It has been suggested that higher doses of methylprednisolone are associated with more pronounced improvement in paraclinical measures of disease activity than are the commonly used doses of 500-1000 mg daily, but it is not clear if these differences translate into meaningful differences in clinical efficacy (Oliveri et al, 1998; Fierro et al, 2002). It is commonly assumed that different glucocorticoids have similar biological effects, but there is evidence that this is not the case. The induction of responses such as the induction of gene expression and apoptosis, represssion of the transcription factor NF-κB, and inhibition of lymphocyte proliferation in vitro varies widely between different glucocorticoids, but it is not known if these differences are important in mediating in vivo effects of glucocorticoids in inflammatory disease states (Fauci, 1976; Langholf and Ladefoed, 1983; Hofmann et al, 1998). In addition, the response of inflammatory cells to glucocorticoid treatment is not constant but is modulated by the activation status of the cell (Heijnen and Kavelaars, 1999). There are no major changes in glucocorticoid binding to glucocorticoid receptors in mononuclear cells from patients with MS, but it has been reported that the response to glucocorticoid treatment is impaired in patients who have MBP-reactive T cells in peripheral blood that are non-responsive to glucocorticoid suppres- sion of antigen-induced proliferation (Webb et al, 1974; Bergh et al, 1999).

Currently available disease modifying drugs have only limited efficacy in RRMS and there is clearly a need for better treatments. The role of glucocorticoids in combination therapy with disease modifying drugs is an active research area. A temporary suppression of Gd-enhanced MRI disease activity is observed after treatment with methylprednisolone whereas treatment with IFN-β and gla- tiramer acetate results in long term suppression of Gd-enhancement. However, all three drugs promote recovery of MRI lesions as assessed by T1 hypointensity or magnetization transfer (Comi et al, 2001; Filippi et al, 2001; Richert et al, 2001). The suppression of Gd-enhanced MRI disease activity by methylprednisolone is more pronounced and of a longer duration in IFN-β-treated patients, suggesting that the combination of IFN-β therapy and regular treatment with high-dose methylprednisolone could be of benefit in pa- tients with RRMS (Gasperini et al, 1998). Glucocorticoids and IFN-β could have synergistic effects due to different effects on immuno-pathogenetic mechanisms, and it has been suggested that glucocorticoids could lower the prevalence of neutralizing antibodies to IFN-β (Pozzilli et al, 2002). Definite clinical trials are, however, needed to establish the role of glucocorticoids in combination therapy.

A major challenge in MS research is understanding the impact of histopathology and immunogenetic heterogeneity on the pathogenesis of different subtypes of MS. Parallel studies of immune ac-
tivation, immune regulation, immunogenetics and histopathology together with studies addressing phenotypic differences in subgroups of patients with MS are expected to lead to an improved insight into the pathogenesis of MS. Such studies will help in defining the role of glucocorticoid treatment given alone or in combination with other drugs in the future management of patients with MS.

7. SUMMARY

Multiple sclerosis (MS) is a major cause of disability and handicap in young adults. The etiology of MS is still unknown, but an immune-mediated attack on myelinated axons caused by environmental factors in a genetically susceptible individual is considered to be of pathogenetic importance. In the majority of patients the disease course is initially characterized by the occurrence of attacks of disease such as acute optic neuritis (ON) and other isolated syndromes. In this stage, termed relapsing-remitting MS (RRMS) there is often good recovery but in most patients incomplete recovery or the conversion to a secondary progressive disease course eventually leads to the development of irreversible impairment. The presumed role of immune mechanisms has led to many attempts to treat MS with immunomodulatory drugs, including glucocorticoids. However, when the studies included in the present thesis were initiated there was little evidence supporting the common use of glucocorticoids in MS. We conducted two double-blind, randomized, placebo-controlled studies of oral high-dose methylprednisolone treatment in ON and attacks of MS. These studies showed that whereas methylprednisolone had only marginal efficacy in ON, treatment resulted in better recovery after an attack of MS for a period of at least eight weeks. The lower efficacy in ON may reflect that these patients have less intrathecal inflammation, as assessed by magnetic resonance imaging using Gadolinium (Gd)-enhancement as a measure of inflammation, than do patients with RRMS. Indeed, patients with enhancing lesions on MRI had prolonged attack duration and a better response to methylprednisolone treatment compared to patients without Gd-enhancement on MRI. Oral high-dose methylprednisolone treatment also resulted in resolution of Gd-enhancement and lowering of the cerebrospinal fluid (CSF) concentration of myelin basic protein (MBP), a biochemical marker of demyelination.

Autoreactive CD4 T cells are considered to be pivotal in the pathogenesis of RRMS. We found that patients with possible onset symptoms of MS (POMS) and RRMS had evidence of systemic CD4 T cell activation as evidenced by increased expression of the CD26 molecule, a putative marker of proinflammatory T cells that secrete T helper type 1 (Th1) cytokines. Th1 cytokines are considered to be instrumental in driving intrathecal inflammation in MS. Patients with POEMS or RRMS had a lower percentage of CD4 T cells in CSF expressing the HLA-DR molecule, and this was especially pronounced in patients carrying the MS-associated DRB1*1501 HLA allele. We also found a lower percentage of CD4 T cells in CSF that co-expressed CD25 and HLA-DR in POEMS and RRMS. HLA-DR and CD25 expression has been associated with regulatory T cell activity, and we hypothesize that changes in CD4 T cell expression of these molecules could reflect alterations in the activity of regulatory T cells in MS. The expression of CD26 increased and co-expression of CD25 and HLA-DR increased after treatment with oral high-dose methylprednisolone. These changes may be related to the induction of the immunomodulatory cytokine transforming growth factor (TGF)-β after methylprednisolone treatment. Intrathecal IgG synthesis is a hallmark of MS. We found that intrathecal IgG synthesis levels correlated with demyelination as assessed by the CSF concentration of MBP in patients with RRMS. Furthermore, in RRMS the number of cells in CSF secreting anti-MBP autoantibodies of high relative affinity correlated with disease activity. In contrast, in POEMS the number of cells in CSF secreting anti-proteolipid protein antibodies correlated with disease activity. We hypothesize that these findings reflect a change in immunogenetically relevant myelin autoantigens during the course of MS. The precise role of myelin autoantibodies, as effector molecules in immune-mediated demyelination or merely as markers of pathogenetically relevant T cell reactivity, remains to be established. However, systemic and intrathecal IgG synthesis was suppressed after treatment with oral high-dose methylprednisolone, consistent with a pathogenetic role of IgG at least in some patients with MS.

The CSF leukocyte count and the CSF activity of matrix metalloproteinase (MMP)-9 correlated with Gd-enhancement in MRI studies prior to treatment with oral high-dose methylprednisolone. CSF pleocytosis and MMP-9 activity was not influenced by methylprednisolone treatment in spite of marked suppression of MRI disease activity, but patients with MMP-9 activity in CSF had shorter relapse-free periods. We hypothesize that this reflects the multi step nature of intrathecal inflammation in MS. Subarachnoid and perivascular inflammation may be necessary but not sufficient to induce parenchymal inflammation with infiltration by phagocytic macrophages and other effector cells. Indeed, we found that patients carrying an allele encoding a deficient chemokine receptor, CCR5 ∆32, which is expressed by the majority of blood-derived macrophages in MS lesions, had prolonged attack-free periods.

In conclusion our studies support the use of methylprednisolone treatment in MS, especially in patients with evidence of ongoing disease activity on Gd-enhanced MRI. The efficacy of methylprednisolone appears to relate to modulation of T cell activation, immunoglobulin synthesis, and TGF-β production rather than to a general inhibition of leukocyte recruitment and MMP-9 production in CSF. These findings have important implications for the future use of methylprednisolone treatment in the management of attacks of MS, and for the planning of future studies combining methylprednisolone treatment with other immune modulating drugs for the treatment of MS.

ABBREVIATIONS

ACTH: Adrenocorticotrophic hormone
CDn: Cluster of differentiation number n
CDMS: Clinically definite multiple sclerosis
CI: Confidence interval
CNS: Central nervous system
CTLA: Cytotoxic lymphocyte antigen
EDSS: Expanded disability status scale
ELISA: Enzyme linked immunosorbent assay
GITR: Glucocorticoid-induced TNF receptor
Gd-DTPA: Gadolinium diamino-tetraethylene-pentaacetic acid
HLA: Human leukocyte antigen
IFN: Interferon
IL: Interleukin
IQR: Inter-quartile range
MBP: Myelin basic protein
MP: Methylprednisolone
MRI: Magnetic resonance imaging
MRS: Multiple sclerosis
NRS: Neurological rating scale
ON: Acute optic neuritis
ONTT: North American Optic Neuritis Treatment Trial
PLP: Proteolipid protein
POEMS: Possible onset symptom of multiple sclerosis
PPM: Primary progressive multiple sclerosis
RRMS: Relapsing-remitting multiple sclerosis
SPM: Secondary progressive multiple sclerosis
Th1: T helper type 1
Treg: Regulatory T cell
TGF: Transforming growth factor
TNF: Tumour necrosis factor
VAS: Visual analog scale
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