

## MULTIPLE SCLEROSIS: NEW PATHOPHYSIOLOGICAL AND THERAPEUTIC CONCEPTS

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### SUMMARY

The development of putative disease-modifying therapies in multiple sclerosis (MS) has depended upon an increase in the knowledge of pathogenetic mechanisms of the disease process. In turn, results of several therapeutic trials may influence concepts regarding the role of inflammation and other mechanisms in the development of long-term disability in this condition.

Consistent evidence supports therapies that reduce the rate and severity of clinical relapses, while the evidence that these influence long-term outcome is less certain. However, it was recently suggested that inflammation may condition the development of axonal loss, a cause of chronic functional impairment. Thus, disease-modifying agents may be more effective in influencing prognosis if given before the consequences of inflammation are irreversibly established, and would support the concept of early treatment with such agents.

This review will cover the role of MRI in diagnosis and prediction of prognosis, current immunopathogenetic concepts in MS, and will concentrate on therapeutic developments of potential disease-modifying agents in MS. A number of detailed and comprehensive books offer the interested reader further information on various aspects of this review.<sup>1-4</sup> These texts also cover symptomatic and multi-disciplinary management issues.

### BACKGROUND

MS is the most common progressive neurological disorder in young adults and usually manifests in the third and fourth decades, with a prevalence of approximately 1 in 1,000. It has been estimated to have a total lifetime cost per case of \$2.2 million.<sup>5</sup> MS is more common in Caucasians, females (female/male ratio of 2/3:1) and in northern latitudes. It typically presents with a 'relapsing-remitting' course (episodic deterioration in function with full or partial recovery). It then enters a 'secondary progressive' phase (a progressive deterioration in function occurring between relapses) after about ten years. Around 20% of MS patients may experience little cumulative disability after 15 years and this observation has led to the term 'benign MS' in this group. Approximately 10% of patients have a 'primary progressive' disease course, with progression from the onset without relapses. A longitudinal study of 1,099 consecutive MS patients referred to MS clinics in Ontario<sup>6</sup> found that the median time to reach the stage at which walking assistance is needed was 9.4 years in a sub-group seen from onset of disease.

The aetiology of MS is unknown, though it is considered likely that an environmental trigger precipitates MS in genetically predisposed individuals, and the disease state then persists as an autoimmune condition, and this has prompted use of generalised immunosuppression and other immunomodulatory therapies. Historically, the disease

has been considered as being primarily an inflammatory demyelinating disease of the central nervous system, although a degree of axonal loss was also apparent even in the first pathological reports. More recently, accumulating evidence suggests axonal loss may be an important component of the disease process; it has been found to occur early in disease evolution and before disability is clinically apparent. Subsequent disability has also been found to correlate with markers of axonal loss.

TABLE 1  
Factors associated with a good prognosis in MS.

- Female
- Young age at onset
- Relapsing-remitting disease
- Sensory deficit presentation
- Long inter-attack interval
- Low initial relapse rate
- Fewer lesions on baseline MRI

### MAGNETIC RESONANCE IMAGING (MRI) IN DIAGNOSIS AND OUTCOME

MS remains a predominantly clinical diagnosis. However, since the discovery that many clinically silent lesions occur before the first symptoms, MRI has been used in diagnosis as a predictive test and as a research tool investigating the pathology of the condition. At present the diagnostic criteria of Poser<sup>7</sup> are the most widely used, which allow for paraclinical evidence from oligoclonal bands, visual evoked potentials and MRI to be used to aid diagnosis.

MRI is of prognostic value in patients with clinically isolated syndromes of the CNS. A recent ten year follow-up study has found that 83% of such patients with an abnormal MRI at presentation subsequently developed MS, while only 11% with a normal MRI progressed to MS.<sup>8</sup> In patients with established MS, up to 99% of patients with clinically definite MS have been found to have at least one focal white matter lesion (plaque).<sup>9</sup> Total lesion volume on T2 weighted scans correlates both with subsequent total lesion volume and disability after ten years of follow-up.<sup>10</sup> Other factors associated with prognosis are shown in Table 1.

Several new putative MR techniques correlate more closely with clinical disability than the less specific MRI markers of inflammation such as T2 scans. Such putative markers include hypointense lesions on T1 weighted MRI, measures of cerebral and spinal cord atrophy, low magnetisation transfer ratios on MT imaging and magnetic resonance spectroscopy. These imaging techniques may thus reflect pathological changes (such as axonal loss) considered to cause chronic functional impairment.

## IMMUNOPATHOGENESIS

Accumulated evidence indicates that MS is an autoimmune disease<sup>11</sup> and that autoreactive T-cells initiate the process of CNS myelin damage. The presence in perivenous inflammatory infiltrates of CD4+ and CD8+ lymphocytes,<sup>12, 13</sup> plasma cells<sup>14</sup> and macrophages<sup>13</sup> suggests that these cell types also contribute to myelin damage in MS lesions.

Molecular mimicry with activation of myelin specific T-cells by viral peptides would provide one possible mechanism for an infective cause to induce autoimmunity and precipitate MS in a susceptible individual.<sup>15</sup> Other putative, though non-exclusive, mechanisms include activation of myelin specific T-cells with release of pro-inflammatory cytokines, and stimulation of autoreactive T-cells by a viral superantigen. A number of environmental factors (such as viruses) have the potential to trigger disease, and epidemiological studies support the concept that exposure to environmental agents early in life may be important. Although many studies have implicated various infective agents, such reports are difficult to substantiate.

Current evidence supports MS as a primarily cell-mediated disorder. The MHC (or human leukocyte antigen (HLA) cluster) on chromosome 6 is involved in the presentation of peptides to T-cells. In MS the class II HLA antigen plays the pivotal role in antigen presentation. Of the many genetically determined variants of the HLA class II, the subtype HLA-DR2 appears more likely than others to present antigen in an orientation which mimics an 'MS self antigen', and set off the release of several proinflammatory cytokines into the CNS.

In addition to the antigen/MHC complex, a number of T-cell co-receptors are essential to activate a T-cell. These include:

- i) adhesion factors on the surface of antigen-presenting cells (APC), such as intercellular adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM); chemokines (small peptides liberated by inflammatory cells as well as by local parenchymal cells) may modify the expression and affinity of adhesion molecules;
- ii) adhesion factors on the surface of the T-cell, such as the integrin family, e.g. leucocyte functional antigen (LFA) and very late antigen (VLA-4), and selectins;
- iii) co-stimulatory factors on both APC and T-cells, such as cytokine receptors, and cytotoxic T-lymphocyte antigen-4 (CTLA-4);
- iv) CD4 or CD8 molecules of the T-cell.

Other factors implicated in the immunopathogenesis include oxidative stress,<sup>16, 17</sup> chemokines<sup>18, 19</sup> and the matrix metalloproteinases.<sup>20-22</sup>

Cytotoxic T-cells are characterised by the CD8 molecule, and helper T-cells express the CD4 molecule. CD4 cells may be sub-divided according to their cytokine release profiles into T helper-1 (Th-1) cells (interferon-gamma (IFNG), IL-2, (TNF-beta) and T helper-2 (Th-2) cells (IL-3, IL-4, IL-5, IL-10, IL-13).<sup>23</sup> Such differential Th responses depend upon the local environment and may be altered by cytokines secreted by a particular antigen-presenting cell and also by co-stimulatory factors. The antigenic peptide itself may also determine Th-1/Th-2 responses. Th-2 cells may have an important role in allergic responses,<sup>24</sup> while Th-1 cells have been found to be

involved in autoimmune diseases<sup>25</sup> and are presumed to be the dominant factor in MS.

The initiating antigen(s) responsible for the production of autoreactive T-cells are not yet identified; most studies have been performed using *in vitro* models, the animal model of MS (i.e. experimental allergic encephalomyelitis (EAE)) or biochemical studies of cerebrospinal fluid and peripheral blood in MS. Direct evidence is generally lacking, although several putative autoantigens have been implicated,<sup>26, 27</sup> including the myelin proteins.

- i) Myelin basic protein (MBP) has proven encephalitogenic potential.<sup>28</sup> Glatiramer acetate, an artificial polypeptide mixture designed to mimic MBP, has been found to decrease autoreactive T-cells in an animal model, suppresses EAE, and is of benefit in MS.<sup>29, 30</sup>
- ii) Proteolipid protein (PLP) specific T-cells secrete cytokines mainly in a Th-1 like fashion.<sup>31</sup> Anti-PLP and anti-MBP have been found in CSF and tissue samples of patients with MS and optic neuritis.<sup>32</sup>
- iii) Myelin associated glycoprotein (MAG). Cells secreting antibodies against MAG and MBP have been found in the CSF of MS patients.<sup>33, 34</sup> However, myelination proceeds essentially normally in transgenic mice deficient in the gene for MAG.<sup>35</sup>
- iv) Myelin oligodendrocyte glycoprotein (MOG): MOG may generate an encephalitogenic T-cell response and an autoantibody response in Lewis rats.<sup>36</sup> Peripheral blood lymphocytes from patients with MS respond predominantly to MOG rather than to PLP, and glatiramer acetate inhibits the binding of MOG peptides to MHC molecules, as well as the proliferation of MOG reactive T-cells, in a dose dependent manner.<sup>37</sup>
- v) Myelin oligodendrocytic basic protein (MOBP): a synthetic peptide representing a predicted T-cell epitope on MOBP was found to induce experimental autoimmune encephalomyelitis,<sup>38</sup> and is associated with MS.<sup>39</sup>

The route through which the immune system encounters such putative autoantigens may be important in relation to their clinical effect. Oral tolerance is a well known mechanism that downregulates the immune response. It does so by inducing suppressive agents such as cytokines TGF-beta and IL-4 which suppress the disease process in an antigen non-specific fashion (termed 'bystander suppression'). This concept has been explored in the animal model and orally administered autoantigens have been found to suppress disease activity in several experimental autoimmune models, including EAE.<sup>39</sup> Unfortunately however, a large clinical trial of oral myelin in 515 patients with relapsing-remitting MS was ineffective.

Although MS is generally thought to be a T-cell driven autoimmune disease, B lymphocytes can also be involved in brain autoimmune responses via secreted autoantibodies. For example, MBP specific B lymphocytes and autoantibodies have been reported in the CSF of patients with MS.<sup>40-42</sup> A variable, albeit low, number of B-cells can be found within MS plaques,<sup>43, 44</sup> and an unconfirmed study has found lymphocytes and plasma cells producing antibodies against MBP in tissue sections of five out of 12 patients.<sup>45</sup> In addition, immunoglobulin bound to vesiculated myelin networks has been demonstrated in marmosets immunised with MOG and in three cases of

MS,<sup>46</sup> while autoantibodies to disintegrating myelin have been found in MS lesions.<sup>47</sup> A transgenic mouse with astrocyte targeted production of a soluble inhibitor of complement activation has been found to be resistant to developing EAE,<sup>39</sup> suggesting that complement mediated events may occur early in the course of EAE. Finally, in therapeutic terms, PE has been found to lead to functionally important recovery in eight out of 19 patients with steroid resistant relapse of idiopathic inflammatory demyelinating disease,<sup>48</sup> further supporting a role for antibody in myelin damage.

#### PATHOGENIC FACTORS

MS is pathologically heterogenous. This heterogeneity may be reflected by the degree of clinical variability. A recent pathological study suggests MS cases could be divided into four broad types, two that resemble autoimmune diseases and two in which viruses or toxins may be to blame.<sup>49</sup> Inflammation, demyelination and a variable degree of axonal loss characterise MS lesions. A T-cell mediated immune response is a relatively characteristic feature and may mediate inflammation, an early and important component of the disorder.

##### a) *Inflammation and blood-brain barrier breakdown*

Clinico-pathological studies indicate that in both MS and EAE an increase in blood-brain barrier (BBB) permeability occurs in association with acute inflammation,<sup>50, 51</sup> as evidenced by gadolinium enhancement. Serial MRI studies of new and evolving MS lesions during an MS relapse<sup>52</sup> suggest that an increase in blood-brain permeability is one of the earliest detectable changes, gadolinium enhancement being noted to generally occur within a week and to be relatively short lived (six to eight weeks). The amount of inflammation and number of inflammatory episodes show a modest correlation with clinical outcome later on in the disease.<sup>53</sup>

MRI studies using magnetic resonance spectroscopy demonstrate myelin breakdown product during the enhancing phase of a lesion,<sup>54</sup> and significant delays in VEP latency (suggesting demyelination) are noticed early in optic neuritis.<sup>55</sup> Thus, the onset of demyelination may occur at around the same time as inflammation. Demyelination is not necessarily a prerequisite for inflammation,<sup>56</sup> and myelin breakdown product has also been observed to occur independently of inflammation.<sup>57</sup>

##### b) *Demyelination*

Humoral elements, e.g. autoantibodies, may act as amplification factors on a background of a T-cell mediated inflammatory response; complement attack may also contribute.<sup>58</sup> Possible mechanisms of demyelination include T-cell mediated destruction of myelin and oligodendrocytes, macrophage mediated demyelination, and demyelination by metabolic instability of oligodendrocytes.<sup>59</sup> However, it has also been suggested that CNS demyelination may not be dependent on a continued presence of autoreactive T-cells and, rather, that microglial activation might be central to the initiation of demyelination,<sup>60</sup> the target being the oligodendrocyte-myelin unit.

Myelin damage may be reversible. The potential of the adult CNS for remyelination is well established and is a

particular feature of early MS lesions.<sup>61</sup> Remyelinating oligodendrocytes may arise from proliferation of oligodendrocyte progenitors, and such progenitors were recently identified in cultures prepared from human brain tissue.<sup>62</sup> Myelin repair is thus of potential therapeutic importance,<sup>63</sup> although repeated episodes of demyelination may impair remyelination by depleting progenitors.<sup>64</sup> This would imply that a remyelinating strategy should ideally be started early in the disease process, particularly as remyelination may also protect against axonal loss, which is thought to be a major determinant of disability in MS.

Whatever the mechanisms involved in demyelination, the ability of demyelinated CNS fibres to transmit high frequency impulses is impaired and may contribute to functional deficit, and particularly to temporary dysfunction, e.g. during relapses. Demyelinated fibres are particularly vulnerable to their milieu. For example, a transient impairment in function occurs with a rise in temperature (Uhthoff's phenomenon) and is related to an intermittent conduction block. Similarly, the functional deterioration that occurs in association with an infective illness could also be related to a temperature increase, although inflammatory cytokines may also contribute. Conduction block has also been noted in demyelinated axons at normal body temperature, which would suggest that persistent conduction block may also be a feature.<sup>65</sup> Functional deficit can be related to inflammation and conduction block, and may improve or resolve without remyelination. The mechanism for this could be resolution of inflammation and the formation of new sodium channels into the demyelinated portions of the axon, as a large increase in sodium channel density has been found in studies of demyelinated axons obtained at autopsy.

Although functional recovery following relapse is a common feature early in the course of MS, progressive and irreversible disability tends to develop later in almost all patients. This is likely to be related to axonal loss.

##### c) *Axonal loss*

Axonal loss has been described in the earliest pathological investigations.<sup>66</sup> As the component of myelin damage was noted to be relatively more pronounced, historically studies did not tend to concentrate on axonal pathology.

However, in recent years axonal loss has emerged as a potentially important factor.<sup>67</sup> It has been suggested that axonal loss is related to irreversible disability, may occur relatively early in the disease process and may be conditioned by inflammatory processes. These findings have potentially important therapeutic implications.

Broadly speaking, there are two lines of evidence:

##### 1) *Pathological studies.*

Immunocytochemical studies have demonstrated evidence of axonal degeneration and axonal transection in MS lesions. Accumulation of amyloid precursor protein (a marker of axonal damage) has been noted within active MS lesions and at the border of active chronic MS lesions.<sup>68</sup> Axonal transection has been found to be a consistent feature of MS lesions, and the observation that the frequency of transected axons is related to the degree of inflammation implies that axonal transection may occur early in the disease.<sup>69</sup> A recent study has suggested that smaller axons may be preferentially affected.<sup>70</sup> Acute axonal

damage, measured by the accumulation of amyloid precursor protein, has been found in remyelinating, inactive demyelinated lesions as well as actively demyelinating lesions.<sup>71</sup> Demyelinating activity does not therefore appear to be a prerequisite for acute axonal loss.

## 2) *Imaging studies.*

Proton magnetic resonance spectroscopy (MRS) measures the relative resonance intensity of choline, creatine/phosphocreatine (Cr), and N-acetyl-aspartate (NAA). NAA is specifically enriched in axons and neurons in the mature brain while creatine is more evenly distributed. A decrease in the NAA ratio has been observed in MS lesions and has been interpreted as reflecting axonal/neuronal damage.<sup>72</sup>

The decrease in NAA per lesion volume is greater in patients with secondary progressive MS, compared with those who have relapsing-remitting disease.<sup>73</sup> MRI investigations also suggest that axonal loss may occur early in the disease.<sup>68,74-76</sup> Axonal loss may be an extensive process, as NAA levels have been found to be decreased beyond the inflammatory lesions of MS<sup>77</sup> and are low in the normal appearing white matter (NAWM).<sup>78</sup> Moreover, alterations of the NAA/creatine ratio in the NAWM have been found to correlate with changes in disability.<sup>79,80</sup> Accordingly, whole brain NAA assessment has been proposed as a measure of disease progression.<sup>81</sup>

Brain and spinal cord atrophy occur in MS and may be due to both axonal degeneration and demyelination, which may occur together in chronic MS lesions.<sup>82</sup> The potential for remyelination within a lesion may, at least in part, be dependent on the degree of axonal loss that has occurred. Disability in MS patients has been found to correlate with atrophy in the spinal cord,<sup>83,84</sup> cerebral cortex<sup>85</sup> and cerebellum.<sup>86</sup> Moreover, progressive cerebral atrophy has been found to correlate with worsening disability.<sup>85</sup> Progression of cerebral atrophy may occur over short periods of time (one year).<sup>87</sup> Furthermore, as progressive enlargement of the third ventricle (a measure of atrophy) has been found to occur more commonly in patients with prior gadolinium-enhancing lesions,<sup>87</sup> tissue loss may occur preferentially in patients with active inflammatory disease. In support of this is the observation that axonal degeneration on MRS has been found to correlate with prior inflammatory disease activity,<sup>88</sup> and that rates of whole brain atrophy may be slowed in patients receiving interferon-beta-1a.<sup>89</sup> These data suggest that suppression of inflammation may confer long-term benefit. On the basis of observations that axonal loss proceeds despite continued inflammatory suppression,<sup>88</sup> it has been suggested that axonal degeneration is conditioned by prior inflammation and supports the view that treatment in MS should be given before the consequences of inflammation are fully established. In the pivotal North American trial of interferon-beta-1b in relapsing-remitting disease, although there was suppression of inflammatory disease activity by 80% on MRI, overall there was no major change in the progression of neurological disability after five years in the extended follow-up study.<sup>90,91</sup>

This raises the question of whether additional factors are involved in the development of disability. Other potential mechanisms include damage to myelin and axons from products of oxidative stress or from cytokines. Recovery of function due to resolution of inflammation

and the formation of new sodium channels in the axon has been discussed. However, other mechanisms may also be important. Decreased NAA in lesions can be partially reversible<sup>54</sup> and may be related to resolution of interstitial and intracellular oedema, although reparative processes such as remyelination may also play a part. Adaptive mechanisms contribute to functional recovery after stroke,<sup>92</sup> and a change in patterns of cortical activation has been noted after optic neuritis, suggesting that adaptive mechanisms may also be important in recovery from MS relapses.<sup>93</sup>

## THERAPEUTIC AGENTS

### *Steroids and relapse*

The use of steroids in treating acute relapses is well established. The first form of steroid therapy to enjoy widespread use in MS was adrenocorticotrophic hormone (ACTH). Corticotrophin was shown to hasten the recovery of a relapse of multiple sclerosis,<sup>94</sup> and both corticotrophin or intravenous methyl-prednisolone are superior to placebo.<sup>95,96</sup> ACTH as therapy caused unselected release of steroids from the adrenals in unpredictable quantities and was responsible for other side effects, e.g. hypomanic reactions.<sup>97</sup>

The usefulness of steroids for MS can be summarised as shortening the recovery time of relapses without influencing the ultimate degree of recovery or influencing the longer term natural history of the disease. It has been suggested that patients with optic neuritis with long lesions on MRI of the optic nerve, and those with intra-canalicular disease, may have a poorer visual prognosis, though a study designed to test this hypothesis found no significant effect on outcome or on MRI.<sup>98</sup>

Short courses of intravenous methyl-prednisolone are equal to, or more effective than, corticotrophin in accelerating recovery from a relapse.<sup>94,99,100</sup> Steroids stabilise the damaged (BBB) in MS. MRI also shows reduced gadolinium enhancement with intravenous methyl-prednisolone.<sup>101-104</sup> This indicates an improvement in BBB integrity, and correlates with clinical recovery, though the study at the National Hospital, London<sup>102</sup> indicated that many lesions re-enhance within days of stopping intravenous steroids, in spite of clinical improvement. The effect of steroids on the BBB cannot therefore be the sole mechanism whereby they bring about recovery from relapses, particularly as longer steroid courses (e.g. three weeks) are not significantly more effective than shorter ones (three days).

Some controversy exists regarding the most appropriate route for steroid treatment – oral or intravenous – in relapse. A large influential multi-centre acute optic neuritis trial<sup>105</sup> compared placebo with either reducing dose oral prednisolone or intravenous methyl-prednisolone followed by reducing dose oral prednisolone. The rate of recovery of normal visual fields and contrast sensitivity, the two main outcome criteria, were significantly greater in the group that had received intravenous steroids. The rate of progression to MS after two years was halved in the group given intravenous methyl-prednisolone.<sup>106</sup> An unexpected finding was that patients treated with oral prednisolone alone had significantly more new episodes of optic neuritis during the next two years than the placebo group.<sup>105</sup> This would seem to favour the intravenous route of steroid delivery, although how far the results of these trials can be extrapolated in relation to MS relapses is perhaps debatable.

However, with the later publication of the three year follow-up data, any observed difference seemed to have disappeared.<sup>107</sup> Moreover, a randomised trial directly comparing oral and intravenous methyl-prednisolone for acute MS relapses<sup>108</sup> showed no significant differences, and the optimum manner of steroid administration remains questionable. The suggestion has been made that intravenous, followed by oral, tapering might be the preferential option.<sup>109</sup> At present, however, the most frequently used steroid regimen in the UK is IVMP 1 g daily for three days.

Although steroids are given to shorten the duration of a relapse, no convincing evidence exists that they alter the eventual outcome of a relapse, or the subsequent course of the disease. There has therefore been considerable interest in possible 'disease-modifying' agents.

#### INTERFERON BETA THERAPY

The interferons are intercellular messengers or cytokines, and are of two broad types; type I, which includes interferons alpha and beta, and type II, known as interferon gamma. The mechanism of action of interferon beta is uncertain; it may have immunomodulatory activity, for example on cytokines such as interferon gamma, may alter the Th1/Th2 balance, and influence the expression of adhesion molecules, chemokines, and matrix metallo-proteinases.<sup>110</sup>

#### RELAPSING-REMITTING DISEASE

*Interferon-beta-1b (Betaseron<sup>®</sup>, Schering)*<sup>111, 112</sup>  
Results of the first full scale multi-centre, randomised, double blind, placebo controlled trial of interferon-beta-1b were published in 1993.<sup>12</sup> Entry criteria included patients aged 18–50 with at least two exacerbations in the two years prior to enrolment, and mild to moderate disease severity (EDSS ≤5.5). Two doses of the drug were used: a low dose of 1.6 MIU, and a high dose of 8.0 MIU subcutaneously every other day. The primary endpoints were the annual exacerbation rate and the proportion of patients remaining exacerbation-free. Secondary endpoints included time to the first exacerbation, confirmed change in EDSS and the effect on MRI measures.

The annual exacerbation rate was reduced by approximately one third ( $p = 0.001$ ) and the proportion of patients remaining exacerbation free during the first two years was 16% in the placebo group and 31% in the high dose treated group ( $p = 0.007$ ). The time to the first exacerbation was doubled ( $p = 0.015$ ) and the time to subsequent exacerbations was significantly prolonged. The number of patients hospitalised for multiple sclerosis and its complications ( $p = 0.046$ ) was also reduced. Treatment had a significant effect on MRI burden of disease (BOD). The MRI detected BOD was stabilised over three years in the high dose treated group. The increase in BOD in the placebo group over the first two years was 16.5%, and -0.8% in the high dose treated group ( $p = 0.001$ ). These differences continued to be significant in the third year of double blind therapy. The randomised study was extended to a median of 45 months (3.75 years) of double blind follow-up.<sup>111</sup> The exacerbation rate for each year continued to be reduced by one third in the 8 MIU arm compared to placebo. MRI data continued to show significant reductions in extent and activity due to drug throughout the study.

#### *Interferon-beta-1a*

##### 1. *Multiple Sclerosis Collaborative Research Group Trial (MSCRG) (Avonex<sup>®</sup>, Biogen)*<sup>113, 114</sup>

The MSCRG trial of rIFNB-1a involved 301 patients with relatively early RRMS who were mildly impaired (EDSS range 1.0–3.5) and specifically excluded patients with secondary progression. The patients were aged 18–55 years with at least two documented attacks (with or without complete remission) in the previous three years. The actual pre-treatment attack rate was approximately 0.7/annum. Patients were required to be free of attacks in the two months prior to trial entry. The patients were randomised to receive either 30 mcg of rIFNB-1a, or placebo, by once weekly intramuscular injection. Initially, 143 patients were included in the placebo group and 158 in the active treatment group. Forty-two per cent of patients completed less than the originally planned 24 months.

The main finding was a significant ( $p = 0.02$ ) delay in the 'progression of disability' (the primary endpoint) as defined by a deterioration of at least one point on the EDSS 'confirmed' six months later. Of those completing two years in the study, 29/87 (33%) on placebo and 18/85 (21%) of those actively treated developed 'progression'. Most treatment failures occurred in the first year. Active treatment with 30 mcg of rIFNB-1a weekly did not significantly reduce the relapse rate in the first year of the MSCRG trial. By two years, there was an overall 18% reduction of relapses ( $p < 0.05$ ), but neither the proportion of patients rendered free of relapses, nor the time to the first in-trial exacerbation, were significantly reduced. Nevertheless, the annual relapse rate fell from 0.90/patient/year in the placebo group to 0.61/patient/year in the active treatment group ( $p = 0.002$ ) and high relapse rates (three or more exacerbations) occurred in 12/85 (14%) of treated patients compared with 28/87 (32%) of controls ( $p = 0.03$ ). Neither the proportion of active scans at the end of year one (30% on treatment compared with 42% on placebo), nor year two, attained significance. The proportion of patients free of activity on their MRI scans at two years was not significantly increased. However, the number and volume of Gd lesions was reduced for both year one and year two ( $p < 0.05$ ). The percentage change in T2 lesion volume (BOD), while significant in favour of treatment with rIFNB-1a in the first year (-13.1% on active therapy versus -3.3% on placebo,  $p = 0.02$ ), was not significantly different by the end of year two. This was probably due to the paradoxical reduction of T2 lesion volume in the placebo group (-13.2% for rIFNB-1a and -6.5% for placebo,  $p = 0.36$ ). *Post hoc* analysis<sup>115</sup> using cox proportional hazard models demonstrated that the only baseline characteristic strongly associated with a longer time to disease progression was IFN-beta-1a treatment.

A *post hoc* analysis of the two year MRI data from this trial has shown significant reductions in the number of new ( $p = 0.006$ ) and enlarging T2 lesions ( $p = 0.024$ ) with treatment, with differences particularly for those patients whose scans showed Gd lesions at baseline.<sup>116</sup>

##### 2. *The prevention of relapses and disability by interferon-beta-1a (Rebif<sup>®</sup>, Ares-Serono) sub-cutaneously in MS (PRISMS)*<sup>117</sup>

The PRISMS trial involved 560 patients (389 females, 171 males) from 22 centres and studied the safety and efficacy of rIFNB-1a given in two relatively high doses (22 mcg and 44 mcg, three times weekly by subcutaneous injection)

to patients with at least two attacks in the last two years. The inclusion criteria required an EDSS of 0–5.0 and no attacks in the two months prior to study entry. The primary efficacy endpoint was the number of relapses per patient. Secondary endpoints included the duration and severity of exacerbations, time to first exacerbation, progression of disability on the EDSS, the need for hospitalisation, intravenous steroids, and disease activity and burden of disease on MRI. Ten per cent of the patients (58/560) failed to complete the planned two years of treatment.

Significant beneficial effects were observed in all three major disease domains (relapses, disability and MRI) for all primary and secondary outcomes, generally favouring the high dose. A highly significant reduction of relapses occurred after both one and two years, and an increased proportion of patients was free of attacks. At two years, the likelihood of freedom from attacks was increased by 69% with the weekly dose of 66 mcg, and by 119% with 132 mcg (significant in favour of the high dose). Also, there was a significant increase in the time to the first exacerbation (prolonged by 69% with 66 mcg and 113% with 132 mcg) and to the second exacerbation (prolonged by 56% with 66 mcg and not reached at two years with 132 mcg). In addition, the numbers of moderate and severe attacks were significantly decreased by both doses; accordingly, hospitalisation (48% lower than placebo with the high dose) and steroid use (30% and 46% lower than placebo for low and high doses, respectively) were reduced by rIFNB-1a.

The time to confirmed progression of the EDSS (one point confirmed at three months) was 11.9 months in the placebo group, 18.5 months in the group treated with 66 mcg weekly and 21 months in those treated with 132 mcg. These differences were significant.

The effect on MRI measures was more pronounced than clinical effects. At 24 months the median increase in BOD in the placebo group was 10.9% compared with -1.2% and -3.8% respectively for those actively treated with 66 mcg and 132 mcg weekly ( $p < 0.0001$  for both arms versus placebo and  $p = 0.0537$  for 66 mcg versus 132 mcg). In addition, the number of active scans (T2 weighted scans with new or enlarging lesions) was reduced from 75% to 50% and 25% respectively, by the low and high doses ( $p < 0.0001$  and for 66 mcg vs. 132 mcg,  $p = 0.0002$ ). The proportion of patients who had no T2 activity throughout the study was 8% on placebo, 19% on 66 mcg ( $p < 0.0001$ ) and 31% on 132 mcg ( $p < 0.0001$ ) (66 mcg vs. 132 mcg,  $p < 0.009$ ). The median number of new enhancing lesions was reduced from 8.0 in the placebo group to 1.4 and 1.3 respectively for the 66 mcg and 132 mcg doses (both  $p < 0.0001$  vs. placebo). This treatment effect was seen within two months of treatment initiation and was persistent. A sub-cohort of 205 patients from seven centres had monthly PD/T2 and Gd enhanced T1 MRI at baseline (one month before and one day before treatment started) and then monthly for nine months. Of these, 198/205 data sets were available for analysis. Combined unique activity was significantly reduced by rIFNB-1a (as reflected by both T2 active lesions and Gd lesions), by 89% and 98% for 66 mcg and 132 mcg respectively, compared with placebo. This treatment effect commenced early and persisted throughout the study period. In addition, the percentage of patients with no T2 or T1 Gd activity was

increased from 11% in the placebo group to 31% and 41% respectively by the low and high doses.

*The Once-Weekly Interferon for MS Trial (OWIMS) (Rebif®, Ares-Serono)*<sup>118</sup>

The two interferon-beta-1a agents (Avonex® and Rebif®) are administered at different doses and frequency, and by different routes. As uncertainty exists regarding optimal dosing regimens, a trial investigated the effects of once weekly subcutaneous injections of two relatively low doses of rIFNB-1a (Rebif) on monthly MRI to partially address these issues. The results support a dose response relationship for MRI and clinical variables.

Two hundred and ninety-three patients with RRMS were randomised to receive either 22 mcg or 44 mcg weekly for one year. Inclusion criteria were similar to the PRISMS study, except that patients with one relapse in the last two years were included, a pre-study MRI was required to show at least three lesions consistent with MS and neurological stability was required for 21–35 days prior to study entry.

The primary outcome measure was the number of 'combined active lesions' (new/enlarging lesions on PD/T2 images and/or Gd lesions on T1 weighted images) on monthly MRI during the first 24 weeks of treatment. The secondary measures included: numbers of active PD/T2 lesions; the percentage of scans with combined active lesions; the change in the burden of disease (total area of lesions on PD/T2 scans); the relapse count; time to first relapse; percentage of relapse free patients; and the need for steroids and hospitalisation. The study was not powered to detect clinical endpoints.

The 44 mcg/week (-53.5%,  $p < 0.01$ ), but not the 22 mcg/week, dose (-29.6%) resulted in a significant reduction in combined active MRI lesions. In addition, the median percentage of MRI scans showing combined active lesions was reduced, compared to placebo, 50% to 33% ( $p < 0.05$ ) with 44 mcg, and to 45% with 22 mcg once weekly (not significant (NS)). The median percentage change in the burden of disease was reduced, compared with placebo, 5.9% to -2.0% ( $p < 0.005$ ) and -1.4% ( $p < 0.01$ ) by the 22 mcg and 44 mcg doses, respectively. The 22 mcg dose had no effect on relapse rates when compared with placebo, whereas there was a mean reduction of 19% for the 44 mcg dose (NS).

#### SECONDARY PROGRESSIVE MS

The effects of interferon beta have recently been studied in secondary progressive forms of MS. Such patients show a progressive deterioration between relapses, tend to have a longer disease duration and are more disabled.

*Interferon-beta-1b (Betaseron®, Schering)*

Seven hundred and eighteen patients in 32 centres were studied using a double blind randomised placebo controlled design of 8 MIU given subcutaneously on alternate days.<sup>119</sup> Patients had either two or more clearly identified relapses in the previous two years or had at least one EDSS point (or 0.5 points between EDSS scores of 6.0–7.0) worsening over the preceding 24 months. EDSS at entry was between 3.0 and 6.5.

The primary outcome measure was time to confirmed neurological deterioration (defined as progression in one point on the EDSS scale or 0.5 point for EDSS 6.0 at

entry, present for at least three months). The secondary outcome measures were time to becoming wheelchair bound (EDSS 7.0), annual relapse rate, MRI lesion volume (lesion load or BOD) with annual MRI scans in all patients and the number of newly active MRI lesions in a frequent MRI scanning sub-group (N = 124).

After 24 months, the independent advisory board recommended termination of the study because of a significant treatment effect on the time to confirmed neurological disability ( $p = 0.0008$ ). An intent to treat analysis showed that there were significant treatment effects in all primary and secondary outcome measures. Relapse rate was reduced by 31% ( $p = 0.0002$ ). As in the relapsing and remitting study, the number of hospitalisations due to MS was significantly reduced, as was the number of courses of corticosteroids required. The MRI endpoints were also significant for a treatment effect. In the complete cohort the lesion load (BOD measure) increased by 8% in two years in the placebo group, with a decrease of 5% in the treated group ( $p = 0.0001$ ). In the sub-group having frequent MRI scans there was a marked and significant reduction in the number of new lesions at one to six months and 19–24 months ( $p = 0.0001$ ).

#### *Interferon-beta-1a (Rebif<sup>®</sup>, Ares-Serono)*

A phase III trial of rIFNB-1a (Rebif<sup>®</sup>, Ares-Serono) 66 mcg and 132 mcg weekly in secondary progressive MS was reported at the European Neurological Society meeting in Milan, Italy, in 1999, though it has not yet been published. This was of longer duration than the terminated study of Betaseron. Six hundred and eighteen patients with clinically definite SPMS were studied over three years. Effects on disability progression appeared less than for the PRISMS study, with significant effects resulting only when multiple covariants were taken into account ( $p < 0.046$ ). An unexpected finding was an effect of gender, with significant beneficial effects on disability progression for females at both doses, but no significant effects for males. As in studies of relapsing-remitting patients, both doses (66 mcg and 132 mcg weekly) resulted in a significant reduction in relapse rate, use of steroids, disease activity and change in the burden of disease on MRI.

#### *Other beta-interferon issues*

##### *(i) Safety.*

Adverse events of IFNB are noted mainly within the first few weeks of treatment and reported to be mostly self-limiting or controlled by paracetamol without the need for termination of treatment or dose alteration. The most common adverse events were flu-like symptoms, fever, injection site reactions and myalgia. The most frequent laboratory abnormality was lymphopenia and, less commonly, mild leukopenia, neutropenia or mildly elevated hepatic transaminases. Side-effects of rIFNB-1a and IFNB-1b are similar. IFNB-1a has been well tolerated in all recent major phase III trials with few serious adverse events. In the MSCRG trial, the relatively low dose intramuscular rIFNB-1a was associated with a significant incidence of flu-like symptoms, muscle aches and chills but no significant laboratory abnormalities were reported.

Skin necrosis has been reported rarely. For example, in the PRISMS trial only eight instances of skin necroses occurred in more than 150,000 injections. Each was a single event which healed spontaneously, requiring no

specific treatment or dose change and with no subsequent recurrences. Despite the reduction of white cells by rIFNB-1a, no evidence of an increased risk of infection was found. Infections in the PRISMS trial were invariably less frequent in the active treatment arms (with a consistent trend in favour of high dose).

##### *(ii) Neutralising antibodies (NABs).*

Neutralising antibodies to IFNs typically appear several months after starting treatment and initially their prevalence generally increases with time, although their detection in different trials varies with the route of administration of the treatment, type of assay and definitions.

However, long-term follow-up of NABs in these same patients has shown that the appearance of anti-interferon antibodies is often transient even at high titres. Although anti-interferon alpha and anti-interferon beta antibodies may be associated with loss of efficacy under certain circumstances in some patients, there is no clear indication or consistent correlation between the appearance of these antibodies and the clinical course of MS in individual patients. For example, in the PRISMS study, no significant relationship was found between NABs and relapse frequency. Moreover, the higher dose of interferon was paradoxically associated with a lower NAB rate than the lower dose.

##### *(iii) Dose response effects.*

Many pharmacodynamic effects and immunomodulatory actions of IFNB which may be clinically relevant have been shown to be dose dependent *in vitro*. Early dose ranging studies of IFNB demonstrated greater efficacy of 8 MIU and 16 MIU of IFNB-1b on annualised relapse rates compared with 0.8 MIU and 4 MIU.<sup>120</sup> In addition, the pivotal phase III trials of interferon-beta-1b showed dose response effects on clinical and MRI outcome measures.<sup>91, 112</sup> Clinical and MRI related dose response effects of rIFNB-1a have also been noted.

A meta-analysis of the individual data from the OWIMS and PRISMS trials suggests that the dose response relationship for rIFNB-1a approximates reasonably to a linear model; predicting for each additional 22 mcg increment of rIFNB-1a, there is an approximately 10% improvement for clinical outcome measures and 20% for MRI measures.<sup>121</sup> Overall, there are clear and significant treatment advantages in favour of the higher weekly dose regimen (132 mcg) for many endpoints and, as yet, no significant downside in terms of increased adverse events. The risk/benefit ratio therefore clearly favours the maximum dose for all RRMS patients and particularly for the high risk, high EDSS cohort where it is essential to obtain equivalent benefits to those achieved in patients with less marked disability or mainly impairment.<sup>122</sup>

##### *(iv) Clinical relevance of rIFNB therapy for long-term disability.*

Data from the trials of relapsing-remitting and secondary progressive patients show that treatment with interferon beta reduces the number and severity of relapses. This is important because relapses are unpredictable and have significant social and employment impact.

Clinical observations, epidemiological<sup>123</sup> and MRI<sup>8, 124</sup> studies all testify to the relationship between the levels of disease activity at or near disease onset and the disability reached five to ten years later. Recurrent episodes of

inflammation are more likely to be followed by permanent disability as damage accumulates and repair mechanisms become increasingly compromised. As clinical relapses represent only one tenth to one thirtieth<sup>125</sup> of the underlying, ongoing, acute disease activity on MRI, the major anti-inflammatory effects of the higher doses of interferon beta can be expected to be relevant also for long-term disability well beyond the durations of recent trials. Natural history studies show that it takes some 15 years or more on average for 50% of patient cohorts to reach firm endpoints such as an EDSS of 6.0.<sup>126</sup> The appropriate use of such clinical endpoints requires much larger and/or longer trials than those performed to date.

#### GLATIRAMER ACETATE (COPAXONE®; FORMERLY COPOLYMER 1, TEVA)

In the animal model for MS, experimental allergic encephalomyelitis (EAE) can be induced by immunisation using myelin basic protein (MBP). This led to the hypothesis that a polypeptide of similar structure might inhibit the immune response to MBP and thus block its adverse action. Glatiramer acetate is such a polypeptide and it suppresses EAE in several animal models without evident toxicity.<sup>127,128</sup>

The first placebo controlled clinical trial in humans<sup>129</sup> with relapsing-remitting MS demonstrated that patients treated with subcutaneous glatiramer acetate had lower annual exacerbation rates (0.6) than those on placebo (2.7). Those with minor disability at entry (EDSS of 0–2) had less progression, though no benefit was noted in those with more severe disability at study entry. A phase 3 multi-centre trial was thus performed. This influential study was reported in 1995.<sup>130</sup> Two hundred and fifty-one relapsing-remitting patients were involved, and the primary outcome measure was a difference in the MS relapse rate. Patients were randomised to receive 20 mg of subcutaneous glatiramer acetate or placebo each day for two years. The final two year relapse rate was 1.19 for patients on glatiramer acetate and 1.68 for those on placebo, a 29% reduction favouring glatiramer acetate ( $p = 0.007$ ). Significantly, more patients on glatiramer acetate improved and more receiving placebo worsened, as measured by a change of point on the EDSS scale. This study was extended double blind for a mean of around five months.<sup>131</sup> The effect of glatiramer acetate on relapse was maintained. More patients on glatiramer were relapse-free over the entire study than those on placebo (33.6% vs. 24.6%,  $p = 0.035$ ), and multiple relapses were more frequent among the placebo treated group ( $p = 0.008$ ). A Kaplan-Meier analysis of time to progression of either 1.0 or a more stringent 1.5 points on the EDSS in the absence of a recent exacerbation-associated deterioration favoured glatiramer ( $p = 0.008$  and  $p = 0.004$  respectively).

The treatment was well tolerated. Mild injection site reactions were noted, and a transient self-limiting reaction of chest tightness with anxiety, palpitations, or dyspnoea was more common in the glatiramer acetate group. Many patients chronically injected with glatiramer acetate develop antibodies that peak at three to six months and then decline. However, there is no convincing evidence that antibodies compromise the effects of treatment.

In order to conclusively address whether glatiramer has any measurable effect on MRI monitored activity, a randomised, placebo controlled and blinded, multi-centred

European and Canadian study was undertaken.<sup>132</sup> In glatiramer-treated patients the mean number of accumulated lesions per patient was reduced by 32% and the relapse rate was reduced by 33%.

A single placebo controlled trial of glatiramer in chronic progressive MS patients with moderately severe disability has been conducted at two centres.<sup>133</sup> One hundred and six patients were randomised either to treatment with 15 mg glatiramer administered subcutaneously twice daily, or to placebo injections. Fewer patients progressed in the glatiramer treated arm with a 24 month probability of progression of 20.4% for glatiramer and a 29.5% probability for the control group ( $p = 0.09$ ). However, when a proportional hazards model was applied to correct baseline characteristics of the subjects, no statistically significant differences were found.

#### OTHER THERAPEUTIC AGENTS

##### *Azathioprine*

One of the simplest and relatively less toxic immunosuppressants is azathioprine, which is metabolised to 6-mercaptopurine and suppresses a wide variety of T- and B-cell functions.

A meta-analysis of all the blind randomised trials<sup>134</sup> indicated a significant improvement in relapse rate over three years. The relative probability of remaining exacerbation free for three years while taking azathioprine compared to placebo was 1.9. A minimal benefit on progression, albeit only by 0.2 Kurtzke EDSS grades, was also demonstrated. This required two to three years to become apparent. Many individual trials, including the largest multi-centre study of 354 patients,<sup>135</sup> have not shown this agent to have a statistically significant effect on progression.

Common side effects include macrocytosis, increased transaminase levels, leucopenia, anaemia, hepatotoxicity, alopecia and pancreatitis. The risk of neoplastic development is uncertain. In a retrospective study of the Lyon database an increased risk was suggested only after about ten years of continuous treatment.<sup>136</sup> The Florence group found a lower incidence of cancer in the treated group compared to controls.<sup>137</sup>

A comparison between trials of 'newer' treatments<sup>138</sup> and the azathioprine meta-analysis<sup>134</sup> showed azathioprine to be as effective in increasing the proportion of patients who were relapse free at two years as glatiramer acetate, interferon-beta-1a, or interferon-beta-1b. Although it is considerably cheaper than these agents, azathioprine has only a modest impact on disease progression. It may be that azathioprine use will decline, because many newer agents are arguably more effective in this respect and azathioprine is more toxic.

##### *Methotrexate*

Two pilot studies have suggested efficacy. An 18 month trial suggested benefit in relapsing-remitting, but not progressive, disease.<sup>139</sup> In a larger two year study in chronic progressive disease using low dose oral methotrexate (7.5mg/week), patients were one third less likely to have progression in a composite score of EDSS, ambulation index, box and block and nine hole peg tests.<sup>140</sup> Particular benefit was suggested for upper limb function. MRI demonstrated less change in T2 signals in the treated group and this correlated with upper extremity performance.<sup>141</sup>

*Intravenous immunoglobulins (IVIg)*

IVIg has been successful in other autoimmune neurological disorders, e.g. Guillain Barre syndrome. Several open label studies have reported a beneficial effect of IVIg on the course of MS.<sup>142-145</sup> Experiments using the mouse model have shown that IVIg may promote remyelination within lesions induced by Theiler's virus.<sup>146</sup> A small study of patients with MS and stable optic neuritis demonstrated that IVIg treatment led to a prolonged improvement in visual acuity and colour vision from one to two months onwards.<sup>147</sup> This would suggest that IVIg might induce clinical improvement. A two year randomised double blind study involving 148 relapsing-remitting patients (baseline EDSS of 1-6.0) showed the number of confirmed relapses in the monthly IVIg treated group to be more than half that of the placebo group, an annual relapse rate reduction of 59%. In addition, a small decrease of 0.23 from baseline EDSS (3.33) was noted in the IVIg treated group, as compared with an increase of 0.12 among controls.<sup>148</sup> The clinical improvement occurred within the first six months and was sustained over the 18 months of follow-up.<sup>149</sup> A further study of 40 patients over two years also found intravenous immunoglobulin to have a significant effect on the relapse rate<sup>150</sup> and a significant reduction in active lesions on MRI has also been noted.<sup>151</sup> However, in progressive MS, a combination of steroids and IVIg was found to be no more effective than steroids alone in preventing exacerbations.<sup>152</sup>

*Mitoxantrone (Mx)*

Reports regarding this mitoxantrone in phase 1 and II studies are conflicting. Eight out of ten patients with a rapidly deteriorating disease profile treated with mitoxantrone showed clinical improvement at one year; this correlated with a decrease in gadolinium enhancing lesions by 95%.<sup>153</sup> These findings were supported by a two year follow-up study which also demonstrated that change in MRI seemed more marked than clinical improvement.<sup>154</sup> However, a small open label pilot trial<sup>155</sup> suggested no benefit. Toxic side effects included myelosuppression and amenorrhea. A more recent two year randomised controlled trial of 51 relapsing-remitting patients showed a significant reduction in exacerbations in the treatment group and a trend towards reduction of lesions on T2 weighted images.<sup>156</sup> No statistically significant effect on mean EDSS progression over two years was observed. Larger clinical trials have extended knowledge of this agent in MS.

*The French and British multi-centre controlled trial.*<sup>157</sup>

This randomised, MRI controlled (but clinically unblinded and not placebo controlled) trial evaluated the efficacy of mitoxantrone (Mx) over six months in a group of 42 patients with aggressively active clinical and radiological disease. All patients received monthly injections of Methylprednisolone and were randomised to a six month treatment period with intravenous mitoxantrone (Mx 20 mg/month plus 1 g Methylprednisolone/month) or 1 g Methylprednisolone/month alone. Over a period of six months, a significant improvement in MRI (90% of patients with no enhancing lesions in Mx group vs. 31% in placebo group) and clinical indices (seven relapses versus 31) was noted. In addition, a mean improvement of one EDSS point was noted in the Mx group, while the placebo group showed a mean deterioration of 0.3.

*The Italian multi-centre controlled trial.*<sup>156</sup>

A placebo controlled trial evaluated the efficacy of mitoxantrone over two years in a group of 51 relapsing-remitting MS patients.

Significantly fewer exacerbations were observed in the treated group and the proportion of patients with confirmed disease progression (one point on the EDSS scale) was also significantly reduced in the treated group after two years.

*The controlled phase III MIMS trial.*<sup>158, 159</sup>

Data from this randomised, placebo controlled, observer blind study was presented in September 1998 at the 14<sup>th</sup> congress of ECTRIMS.<sup>158, 159</sup> One hundred and ninety-nine patients with relapsing-progressing or secondary progressive MS were randomised to 12 mg/m<sup>2</sup> Mx, 5 mg/m<sup>2</sup> Mx or placebo (i.v. Methylene Blue). The regimen was given every three months for two years.

Five primary endpoints of the study were: change from baseline of EDSS; ambulation index (AI) and standard neurological status (SNS); the number of treated relapses; and the time to first treated relapse. After 24 months a significant difference favouring the Mx groups was noted in all five primary endpoints. For example, change in EDSS was +0.23 in the placebo group and -0.13 and -0.23 in the treated groups (high and low doses respectively); change in AI was 0.8 in placebo and -0.3 and -0.4 in the treated groups (high and low doses respectively); and the number of treated relapses in two years was 1.2 in placebo and 0.4 and 0.7 in the treated groups (high and low doses respectively). The secondary outcome criteria were one point confirmed EDSS progression and MRI evaluation. In the 12 mg Mx group, 7% had a three months confirmed one point EDSS progression compared with 19% in the placebo group ( $p = 0.03$ ). On MRI, there was a significant reduction of the percentage of active scans at months 12 and 24 from baseline with both doses (86% and 75% reduction in the 12 mg Mx group and the 5 mg Mx group respectively), whereas the percentage of active scans remained stable in the placebo group. There was almost no progression of MRI lesion load detectable in the treatment groups, whereas in the placebo group a significant increase was found at months 12 and 24 ( $p < 0.05$ ).

*Adverse events.*

Mx has a high potential for toxicity and its widespread clinical use may therefore be limited. Bone marrow depression is its principal dose limiting toxic side-effect of mitoxantrone, predominantly granulocytopenia which develops ten days after a single large dose and persists for four to seven days. Cardiotoxicity may also be related to Mx. Previous studies suggest that the risk of Mx cardiac effects was low when it was given in patients with no previous cardiotoxic therapies or pre-existing heart disease and at a cumulative dose lower than 160 mg/m<sup>2</sup>.<sup>160</sup> In order to prevent cardiotoxicity it is mandatory to monitor the left ventricular ejection fraction (LVEF) by either performing an echocardiogram or a radionuclide ventriculogram. The recommendation is to obtain a baseline echocardiogram before treatment, and to repeat the examination once the cumulative dose exceeds half of the nominal limit (i.e. 80 mg/m<sup>2</sup>) prior to each alternate dose. The administration of Mx should be discontinued if the LVEF is reduced by more than 10% between two readings

or if the LVEF is less than 50% at any time. Respecting these guidelines, no symptomatic cardiotoxicity was seen in the three controlled trials using Mx in MS at a cumulative dose between 70 and 96 mg/m<sup>2</sup>. However, drug trials are of short duration and longer term problems cannot yet be excluded. As with many chemotherapeutic agents, fertility problems can also be a delayed side effect. Other acute side effects include nausea or vomiting and alopecia.

Given the evidence for a beneficial effect on disease activity, albeit with the potential for significant side effects, a limited role for Mx exists. For example, Mx may be an option in patients with rapidly progressive or aggressive MS, particularly those patients who do not respond to interferon beta, or glatiramer acetate. In addition, the concept of induction treatment followed by a consolidation treatment combining several drugs has been proven effective in infectious diseases and in oncology, but has been little studied in MS.

#### *Plasma exchange*

Plasma exchange (PE) is effective in various immune mediated disorders, e.g. myasthenia gravis and Guillain Barre syndrome. Although it is widely accepted that MS is predominantly a cell mediated disorder, a role for autoantibodies or other proteinaceous substances has not been entirely ruled out, and this explains in part the rationale behind various studies of PE in MS.

The first double blind study of PE<sup>161</sup> involved 54 chronic progressive patients receiving oral prednisolone and cyclophosphamide, plus either active or 'sham' PE. After 20 weeks, EDSS showed either a significant improvement or stabilisation in the active PE group. A longitudinal study<sup>162</sup> of 200 chronic progressive patients on low dose immunosuppression showed a significant stabilisation rate of EDSS following the addition of PE treatment at six years of follow-up. However, there was no control group and interpretation is difficult; furthermore, the Canadian cooperative MS study group<sup>163</sup> reported no benefit in a similar population.

Certain studies<sup>164</sup> have demonstrated a short-term benefit in facilitating recovery from acute relapses, including severe steroid unresponsive demyelination.<sup>165</sup> This is supported by a recent randomised study which demonstrated a functionally important recovery in eight out of 19 such patients,<sup>48</sup> and would thus support a role for auto-antibodies in at least a proportion of patients with MS.

#### FAILED THERAPIES

Many other drugs that initially showed promise have fallen by the wayside. For the purpose of completeness, some of these are listed below.

#### *Cyclosporin*

Cyclosporin appeared to reduce the proportion of progressive MS patients confined to a wheelchair, but has no effect on time to sustained progression<sup>166</sup> or the number of MRI lesions.<sup>167</sup> Side-effects of hypertension, renal toxicity, and hypertrichosis are a major problem.<sup>166, 168</sup> No significant difference with (the less toxic) azathioprine was noted in a direct comparison.<sup>168</sup> The only study which demonstrated (at 7.2 mg/kg, but not at 5 mg/kg) a slight benefit in terms of the number and time to first relapse reluctantly concluded that toxic effects preclude its use in a high enough dose to influence the course of disease.<sup>169</sup>

#### *Cyclophosphamide*

A prospective trial suggested a benefit during the first year with pulsed intravenous cyclophosphamide plus ACTH when compared to low dose oral cyclophosphamide with ACTH and PE, or ACTH alone.<sup>170</sup> An open label trial<sup>171</sup> of cyclophosphamide and ACTH showed 81% of the chronically progressive patients treated with this combination had improved or 'stabilised' at one year, although 69% subsequently re-progressed after a mean time of 17.6 months. However, a large study by the Canadian cooperative MS group, comparing cyclophosphamide combined with oral steroids and PE, failed to show any significant impact on progression when compared to placebo<sup>163</sup> over a two year period. The lack of unequivocal benefit, together with toxic side effects, has significantly limited further interest in this drug.

#### *Total lymphoid irradiation*

Two studies have demonstrated long-term suppression of circulating lymphocytes in patients with progressive MS and a decrease in the likelihood of progression.<sup>172, 173</sup> Patients in whom circulating lymphocytes were less than  $0.9 \times 10^9$  during the first three months after irradiation were two to three times less likely to have progression of disability at 18–24 months.<sup>173</sup> The addition of prednisolone further reduced progression after three to four years.<sup>172</sup> Adverse effects included amenorrhoea, nausea, fatigue, depression and fractures. This approach to treatment has not been widely accepted.

#### *Lenercept*

This agent is a recombinant soluble fusion protein consisting of a TNF-alpha receptor and an Ig Fc receptor, thus conferring an anti-TNF effect. Although effective in the animal model, a placebo controlled trial in MS patients was terminated early because of an increased relapse rate in the treated group.<sup>174</sup>

#### *Sulphasalazine*

An effective agent in inflammatory bowel disease, trials in MS found sulphasalazine to be of no significant benefit.<sup>175</sup>

#### *Linomide*

Linomide is an immunomodulatory compound that increases natural killer cell activity. It suppresses disease in the chronic EAE model. A trial with 2.5 mg orally each day decreased enhancing MRI lesions by 70% in relapsing-remitting MS.<sup>176</sup> A similar radiological response was noted in secondary progressive MS.<sup>177</sup> In addition, this study showed a favourable effect on disease progression at two years. A large randomised controlled trial was planned. Unfortunately, this was terminated in April 1997 because of unanticipated complications of myocardial infarction in treated patients.

#### CONCLUSION

Although symptomatic treatments remain important, the management of MS has now entered the era of disease modification. Therapies such as beta-interferon and glatiramer acetate are now established as efficacious with few major side-effects. Other treatments such as immunoglobulin, azathioprine, PE and methotrexate have shown evidence of benefit. Experience with chemotherapeutic agents such as mitoxanthrone is growing; this drug may

have a future role in the management of patients with recent onset, severe and rapidly progressive disease. Studies of combination therapies are in progress.

Axonal loss appears to be an important cause of long-term disability. The degree of axonal loss may be conditioned by prior inflammation but may then proceed independently of it. This suggests that treatment with disease-modifying agents will be most effective if started early in the disease process, before the consequences of inflammation are irreversibly established.

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