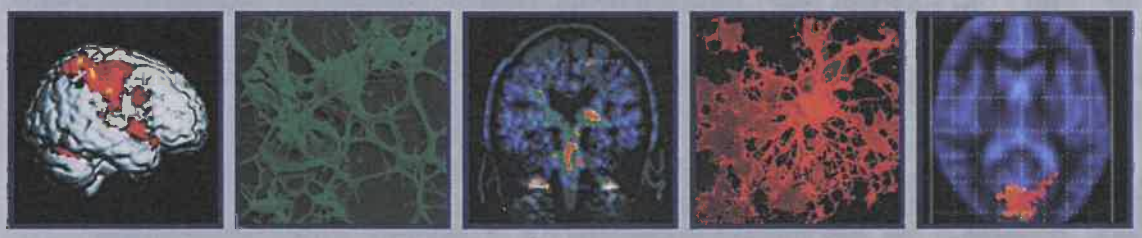
Fourth Edition

McAlpine's MULTIPLE SCLEROSIS



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McAlpine's
MULTIPLE SCLEROSIS

For NDC (1918–1986)

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McAlpine's MULTIPLE SCLEROSIS

FOURTH EDITION

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Contents

The London, Ontario, cohort was established through the multiple sclerosis clinic at the University Hospital in 1972 to provide comprehensive care for patients in the referral area of Southern Ontario (Weinshenker *et al* 1989a; 1989b; 1991a; 1991b). This cohort retains the characteristics of both a tertiary referral centre for the province of Ontario, and a geographically based clinic serving Middlesex County, where an epidemiological study on 1st January 1984 showed a prevalence of $93/10^5$ with near complete ascertainment: 91% of patients were known to be attending the clinic (Hader *et al* 1988). Those patients not registered were mainly the chronic institutionalized individuals, most of whom were already severely disabled when the clinic was established. Patients are followed annually or biennially by neurologists with a special interest in multiple sclerosis. Follow-up is maintained even after patients become institutionalized in nursing homes; and every attempt is made to determine the reason why an individual might have become 'lost to follow-up'. No specific therapies for multiple sclerosis were administered, other than corticosteroids for acute exacerbations, although the clinic has contributed to many therapeutic trials and adopted the prescribing culture now characteristic of centres in North America and Canada. Between 1979 and 1984, the authors reviewed data collected on 1099 consecutive patients evaluated between 1972 and 1984. Information on demographics, clinical course and the progress of disability as a function of time was systematically collected. Data were recorded on standardized forms and entered onto a mainframe computer. They were analysed as a total population but also in two subgroups: the Middlesex County cohort, representing a population-based group for which ascertainment was near complete; and the 'seen from onset' subgroup comprising 197 patients seen by a neurologist ≤1 year from onset. Data on this cohort have been updated to the end of 1996 and the mean duration of the disease at that time reached 24 years (D.A. Cottrell *et al* 1999a; 1999b; Kremenchutzky *et al* 1999).

THE OUTCOME LANDMARKS OF MULTIPLE SCLEROSIS: DEPENDENT VARIABLES

It has long been recognized that the course of multiple sclerosis can be described in terms of relapses, remissions and chronic progression either from onset or after a period of remissions (Charcot 1868b: 1868c; Marie 1884; McAlpine and Compston 1952). Two major outcome measures usefully describe the clinical course and prognosis: the qualitative description, an expression of the interplay between relapses and progression; and the quantitative description, which refers to the accumulation of neurological deficits and is characterized as disability, impairment or loss of social functions. Both can be used in therapeutic trials. Here, we confine our discussion to the role of clinical variables: surrogate markers are covered in Chapter 18.

Course-related dependent variables

Physicians and people with multiple sclerosis know that the cardinal features that characterize the clinical experience of this disease are:

- episodes with full recovery
- episodes with incomplete recovery
- chronic progression.

In general, these phases follow an orderly sequence; but the relationship between episodes and progression is far from straightforward, and a detailed understanding of their interplay is required in order to understand the evolution and dynamics of disability and other outcomes.

Relapses and progression

Relapses – exacerbations, attacks, bouts or episodes – are defined as the first occurrence, recurrence or worsening of symptoms representing neurological dysfunction and marked by subacute onset and a period of stability followed by partial or complete recovery – the whole process lasting ≥24 hours (see Chapter 16). On a small semantic point, it is not strictly correct to refer to the initial episode as a '*re*lapse'; although this is commonplace, we designate the first experience as the inaugural episode and everything that comes later as a relapse(s). Distinction is made between symptoms attributable only to fatigue, and those associated with fever. Events occurring within a 1-month period are considered part of the same episode (Confavreux *et al* 1992; W.I. McDonald *et al* 2001; C.M. Poser *et al* 1983; G.A. Schumacher *et al* 1965). The experienced neurologist will recognize that, despite these unambiguous definitions, it is not always easy to decide whether particular neurological symptoms do genuinely constitute a relapse. Every specialist is familiar with the difficult issue of resolving the status of worsening paraesthesia, a change in walking, or blurred vision – to name but a few of the very many challenging examples encountered in daily practice. Efforts have been made to rank the level of certainty appropriate for a putative relapse – ranging from highly suggestive symptoms with and without objective features on examination noted by the neurologist, to distinctly atypical or minimal complaints. Ranking can be based on the severity of the relapse with respect to its consequences for daily activities; the impact on objective neurological scores; the decision to administer corticosteroids and hospitalize the patient; and the distinction between new symptoms, those previously experienced and worsening of current manifestations of multiple sclerosis. Paroxysmal neurological symptoms present particular difficulties. Because very many may occur over a short period, confusion can arise as to their status – individually or collectively. Our view is that the onset of these manifestations of multiple sclerosis in isolation may constitute a new episode indicating a focal area of inflammatory demyelination resulting in ephaptic transmission. In the absence of an agreed classification for relapse assessment, it is necessary to take a pragmatic approach and adopt common definitions, both in therapeutic trials and prospective studies for which the study period lasts ≤2–3 years, using standardized clinical assessments performed at regular and close intervals by an assessor who is blinded to the therapeutic intervention and focus of interest in the study. However, this is not realistic for natural history studies where lifelong follow-up is required. In this setting, relapse ascertainment and assessment are generally less reliable, and differ for a given patient over time, and between individuals studied contemporaneously.

Perhaps no term in the lexicon of multiple sclerosis has become so confused as 'progression'. The reason is that, in modern therapeutic trials, the word is used merely to describe a worsening of neurological disability with reference to the baseline. Progression is said to be sustained if confirmed at clinic

visits, 3–6 months apart. However, disability worsening, even when sustained at 6 months, does not necessarily equate to an irreversible increase in disability (see below; C. Liu and Blumhardt 2000). Originally, the term was used to define steady worsening of symptoms and signs over ≥6 months (Confavreux *et al* 1992; C.M. Poser *et al* 1983; G.A. Schumacher *et al* 1965), or ≥12 months according to more recent criteria (W.I. McDonald *et al* 2001; A.J. Thompson *et al* 1997). By that definition, once started, progression continues throughout the disease although occasional plateaus and minor temporary improvements may be observed (Lublin and Reingold 1996). The date at which progression starts is invariably assigned in retrospect, once the required 6- or 12-month duration of continuous neurological worsening is confirmed. Herein lies the uncertainty. Relapses can be superimposed on progression, whenever that first manifests (primary and secondary progressive multiple sclerosis). Therefore, it is not helpful to use the word 'progression' both to characterize the worsening of neurological disability attributable to step changes in disability that follow a nasty relapse, and situations in which disability increases systematically over time, even when interspersed with periods of relative stability. For us, this latter is the correct and preferred usage of the term.

The phases of multiple sclerosis

The usual course of multiple sclerosis is characterized by repeated relapses associated, for the majority of patients, with the eventual onset of disease progression. The initial pattern is so characteristic that diagnostic criteria are dependent on the demonstration of dissemination in time. Consequently, it has become commonplace to speak of 'conversion to multiple sclerosis' once the inaugural neurological episode has been followed by a first relapse. By definition, ≥2 distinct neurological episodes must be documented in the course of that patient's illness, the events separated by ≥30 days (McAlpine 1961; W.I. McDonald *et al* 2001; C.M. Poser *et al* 1983). Taken with the phase of secondary progression, this establishes three distinct clinical situations qualifying for the dissemination in time criterion (Figure 4.6). In the relapsing–remitting phase, relapses alternate with periods of clinical inactivity and may or may not be marked by sequelae depending on the presence of neurological deficits between episodes. By definition, periods between relapses during the relapsing–remitting phase are clinically stable. The progressive phase of multiple sclerosis is characterized by a steady increase in deficits, as defined above and either from onset or after a period of episodes, but this designation does not preclude the further occurrence of new relapses. Thus, a full understanding of the natural history requires more than just the two basic contexts of clinical activity to be considered.

The several forms of the clinical course

Patients do not necessarily convert from the relapsing–remitting to the progressive phase: but if they do, the migration is irreversible even though the transition can initially be hard to recognize, especially when the early secondary progressive phase is characterized by continuing relapses. From the first clinical descriptions of multiple sclerosis, it was recognized that the disease may also follow a progressive course from clinical onset.

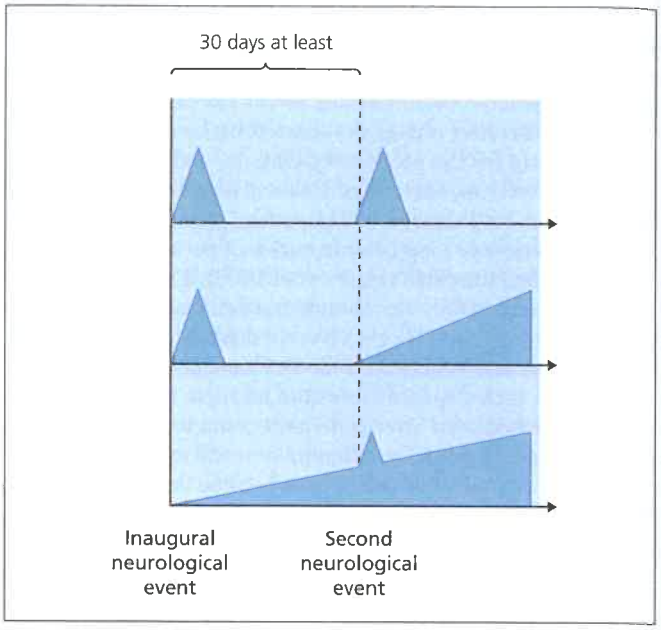


Figure 4.6 Three major patterns of dissemination in time during the course of multiple sclerosis. Top: two consecutive distinct relapses. Middle: inaugural relapse followed by the onset of the progressive phase. Bottom: onset of the progressive phase followed by a superimposed relapse. In these three instances, the time interval required between any two neurological events is ≥ 30 days.

Given this matrix, for many years classification of the clinical course in patients with multiple sclerosis distinguished three categories: relapsing–remitting; relapsing progressive, describing the situation of a relapsing–remitting phase followed by progression; and progressive multiple sclerosis, to cover the eventuality of a progressive course from onset with or without superimposed relapses (Broman *et al* 1981; Confavreux 1977; Confavreux *et al* 1980; Fog and Linnemann 1970; Leibowitz and Alter 1970; 1973; Leibowitz *et al* 1964a; 1964b; McAlpine and Compston 1952; D.H. Miller *et al* 1992a; Phadke 1987; 1990; S. Poser 1978; S. Poser *et al* 1982a; Runmarker and Andersen 1993; Trojano *et al* 1995; Weinshenker *et al* 1989a). At that time, a specific terminology was used by some authors to make the distinction between primary progressive forms with superimposed relapses (the so-called 'relapsing progressive' or 'progressive relapsing' forms, depending on preference) and primary progressive multiple sclerosis without superimposed relapses (the so-called 'chronic progressive' forms). To standardize the terminology used in the description of the pattern and course of multiple sclerosis, and to avoid confusion in communication, an international survey of clinicians involved in multiple sclerosis was performed under the auspices of the National Multiple Sclerosis Society of the USA (Lublin and Reingold 1996). The consensus intended to classify the disease course in four different categories (we regret the use of abbreviations but retain these for clarity of identification):

- *Relapsing–remitting MS (RR-MS)*: 'clearly defined relapses with full recovery or with sequelae and residual deficit upon recovery; periods between disease relapses characterized by a lack of disease progression'.

- *Secondary progressive MS (SP-MS)*: 'initial relapsing–remitting disease course followed by progression with or without occasional relapses, minor remissions, and plateaus'.
- *Primary progressive MS (PP-MS)*: 'disease progression from onset with occasional plateaus and temporary minor improvements allowed'.
- *Progressive relapsing MS (PR-MS)*: 'progressive disease from onset, with clear acute relapses, with or without full recovery; periods between relapses characterized by continuing progression'.

It must be noted that in this classification the presence of superimposed relapses is allowed in cases of secondary progressive multiple sclerosis, whereas primary progressive cases with superimposed episodes are segregated from primary progressive cases without relapses (PR-MS vs. PP-MS). Furthermore, the term 'relapsing progressive multiple sclerosis' is abandoned because the participating clinicians did not agree on its definition and the proposed definitions overlap with other categories. This classification is illustrated in Figure 4.7. Some authors add 'transitional progressive multiple sclerosis' (TP-MS) to this list, in order to identify the few patients with a course that is progressive except for a single relapse at some time (Filippi *et al* 1995b; Gayou *et al* 1997; Stevenson *et al* 1999; 2000). Some authors reserve this term only for cases with a progressive course devoid of superimposed relapses beginning many years after an isolated episode (Gayou *et al* 1997), whereas others allow the single attack before or after the onset of disease progression (Stevenson *et al* 1999; 2000). Because there is no consensus amongst these authors, and the efforts of the National Multiple Sclerosis Society international survey towards standardization and rationalization are sound and deserving of support, our position is that the few cases of transitional progressive multiple sclerosis can easily be accommodated within the recommended classification, assignment to the categories of primary or secondary progressive multiple sclerosis being determined by when the single episode occurs (Lublin and Reingold 1996). But we recognize that this can prove confusing to patients seeking not to be classified as having progressive multiple sclerosis when negotiating guidelines for the use of disease modifying therapies that are only prescribed and reimbursed for individuals with relapsing–remitting multiple sclerosis.

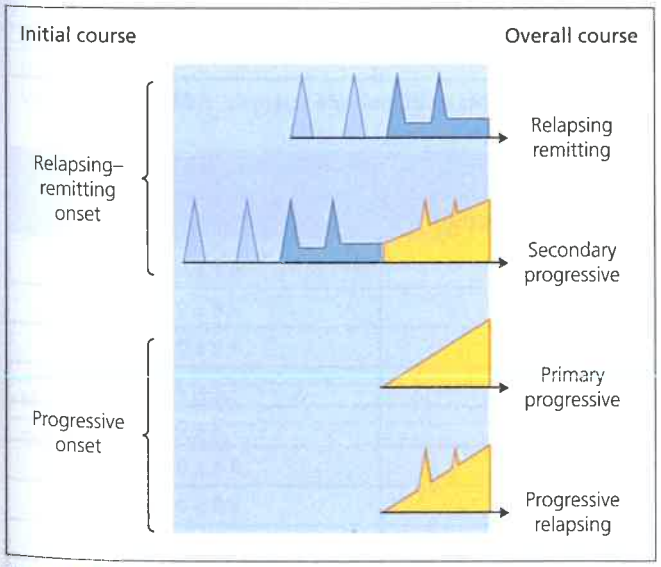


Figure 4.7 Classification of the course of multiple sclerosis. Adapted from Lublin and Reingold (1996). © 1996, reprinted with permission of Lippincott Williams & Wilkins (lww.com).

Prognosis-related dependent variables

The second dimension in the history of multiple sclerosis is the appearance of disability. This is quantitative and may prove to be transient, partially reversible, or definitely irreversible. A way of describing the natural outcome of multiple sclerosis is therefore to assess the time course to accumulation of disability. We discuss schemes that directly address the rate of progression in Chapter 6; these depend on two closely related scales used in the vast majority of studies that describe the natural history of multiple sclerosis – the DSS (Kurtzke 1961; 1965a) and its more detailed version, the EDSS (Kurtzke 1983a). Until the mid-20th century, standards used to assess the degree of disablement in multiple sclerosis were usually based either upon the capacity to work, or mobility. However, the former criterion is unreliable because it depends on individual fortitude, economic needs, and the nature of employment. The degree of mobility soon emerged as a better standard although it also is subject to potential confounds (McAlpine and Compston 1952). Classifications based mainly on degree of mobility have shortcomings because they do not take account of upper limb function, sensory symptoms, involvement of the bladder and bowel, defective vision, cranial nerve abnormalities, cognitive deficits, mood disorders or fatigue (McAlpine and Compston 1952; Rudick *et al* 1996a). Furthermore, the normal aging process may confound results based on these classifications, in older individuals where comorbidity with musculoskeletal, cardiovascular and respiratory disturbances may introduce complexities. That said, such classifications do reflect the global impairment caused by multiple sclerosis, first manifest as a disturbance in walking. This undoubtedly explains the popularity gained by Kurtzke's scales amongst the community of clinicians with a special interest in multiple sclerosis. Rather few other systems proposed for use in multiple sclerosis have gained acceptance; and, to date, no one fulfils requirements of the international multiple sclerosis community (Hobart *et al* 1996; 2001; Sharrack *et al* 1999). Although new, more sensitive and multidimensional measures have been proposed, particularly for use in clinical trials (Rudick *et al* 1996a; 1997), Kurtzke's scales are not displaced and remain, so far, the 'gold standards' for grading clinical impairment and disability in multiple sclerosis; *de facto*, they now represent reference criteria for any novel system that challenges their status and seeks to remove John Kurtzke from the podium of international approval built on familiarity and usage despite much criticism and exposition of the deficiencies. Of the two, the EDSS is now more commonly used than the DSS, especially in clinical trials (but see also below).

The limitations of the DSS are that the scale is unresponsive, combines impairment and disability, has often been shown to have only moderate inter-rater reliability, is not entirely objective,

and is heavily weighted towards ambulation (Amato *et al* 1988; 2004; D.A. Francis *et al* 1991; Goodkin *et al* 1992; Hobart *et al* 1996; 2001; Noseworthy *et al* 1990; Rudick *et al* 1996a; 1997; Willoughby and Paty 1988). It was precisely in order to improve responsiveness that the 'expanded' disability scale was elaborated. However, it soon appeared that what could be gained in precision was lost in reliability. Furthermore, the EDSS was found to provide a ranking that proved too discrete with respect to the well-demonstrated daily fluctuations in neurological signs and symptoms that characterize the course of multiple sclerosis. Administration of the EDSS often proves too complex and time consuming for physicians who do not specialize in multiple sclerosis, and even more so for epidemiological purposes requiring long-term follow-up of very many patients. Therefore, the DSS is often preferred in such settings: for example, only well-identified steps that are easily assessed even in retrospect, such as DSS 4 (limited ambulation but without aid) or DSS 6 (walking with uni- or bilateral support), are important in many epidemiological studies. It is for this reason that the EDMUS Steering Committee decided to design a simplified version of the original DSS allowing similar grading but with more rapid administration and focus on essential points reported directly by the patient, each level having a short, precise and unambiguous description (Confavreux *et al* 1992). In a European multicentre collaborative study involving six centres and 180 patients with multiple sclerosis, agreement was greater for the EDMUS Grading Scale (EGS) than for the EDSS at all intervals (Amato *et al* 2004).

It must be realized that the EDSS is ordinal and categorical but neither quantitative nor continuous. The assumption that disability naturally continues to progress at a similar rate throughout the course of the disease is clearly contradicted by observations made on different samples: the distribution of patients according to DSS score at the last follow-up is bimodal with distinct peaks at DSS 1–2, and DSS 6–7 (Table 4.3) (D.H. Miller *et al* 1992a; Minderhoud *et al* 1988; Weinshenker *et al* 1989a). It follows that the length of time spent by patients at each level of the DSS scale is uneven, being longer for DSS 1–2, and DSS 6–7 (Table 4.4) (Weinshenker *et al* 1991b). Therefore, the progression from one level to the next on the DSS scale cannot be predicted or considered as equivalent. This means that change in the mean DSS, which has often been used in studies on natural history or in therapeutic trials in multiple sclerosis, is not a valid strategy for describing change or comparing groups. Self-evidently, this confusion would not have arisen if letters instead of figures had been proposed to rank the DSS scale. Differences in the proportion of patients changing by a given degree of disability, and the period over which this occurs, are methodologically more acceptable. Ideally, patients might also be stratified by baseline DSS at inclusion (Weinshenker *et al* 1991b). Our position is that, using classifications such as the Kurtzke scales, survival techniques are currently the best means of assessing the time to reach a selected level of disability.

Table 4.3 **Distribution (%) of patients in relation to disability status scale at last follow-up examination: data from the literature**

Disability status score	Weinshenker *et al* 1989a: n = 1099	Miller *et al* 1992a n = 209
0	–	1
1	17	28
2	14	17
3	11	14
4	6	10
5	3	3
6	19	7
7	18	11
8	8	6
9	2	3
10	1	–

Table 4.4 **Time spent at each level of the disability status scale, among 1099 patients with multiple sclerosis. Adapted from Weinshenker *et al* (1991b)**

Disability status scale	Patients entering a given disability status score grade (number)	Patients worsening (%)[a]	Time spent at disability status scale grade (mean number of years ± SEM)
1	1037	82	4.1 ± 0.2
2	829	81	2.8 ± 0.1
3	662	82	1.9 ± 0.1
4	536	88	1.2 ± 0.1
5	475	94	1.2 ± 0.1
6	489	60	3.1 ± 0.2
7	306	37	3.8 ± 0.3
8	114	28	2.4 ± 0.4
9	34	41	2.5 ± 0.6

a Percentage of patients who have reached a given disability status scale grade and progressed to the next level of disability during the study period.

THE ONSET OF MULTIPLE SCLEROSIS

The many series that report the natural history of multiple sclerosis provide an excellent basis for describing demographic and disease-related characteristics at the onset of multiple sclerosis, and thereafter. These are summarized in Table 4.5. The reader may (correctly) detect some familiarity in the structure of our accounts on factors detectable early in the illness that correlate with the later course, severity and survival in multiple sclerosis. The influences of gender, age and symptoms at onset on dynamics of the relapsing–remitting phase, disability and time to progression are so interwoven as to create the impression of repetition in one account. But in reality, these interactions reinforce the evidence for coherence in listing features that describe and predict the natural history of multiple sclerosis, at least amongst groups if not the individual patient.

Table 4.5 **Main series of the long-term course and prognosis of multiple sclerosis: demographic and multiple sclerosis onset characteristics**

Study	Gender: males / females (%)	Age at onset (years)	Initial symptoms of multiple sclerosis (%)		Initial course: relapsing–remitting / progressive (%)
Long-term natural history series with cross-sectional and/or some longitudinal assessment					
R. Müller 1949; 1951	44 / 56	24 (median)	Optic neuritis Brainstem Motor Sensory Sphincter	20 33 66 33 7	87 / 13
McAlpine and Compston 1952	35 / 65	29 (median)	Not available		90 / 10
Leibowitz *et al* 1964a; 1964b Leibowitz and Alter 1970; 1973	49 / 51	32.6 (mean)	Visual Brainstem / cerebellar Motor Sensory Motor and sensory Mixed	14 11 38 13 8 12	Not available
Panelius 1969	38 / 62	28.8 (mean)	Visual Brainstem Motor / coordination Sensory	21 24 33 22	90 / 10
S. Poser 1978	36 / 64	31.1 (mean)	Not available		82 / 18
S. Poser *et al* 1982a	35 / 65	30 (mean)	Not available		87 / 13
V.A. Clark *et al* 1982 Detels *et al* 1982 Visscher *et al* 1984	29 / 71	33 (mean)	Visual Diplopia Other cranial nerves Speech Motor Sensory Incoordination	20 25 20 18 63 61 58	Not available
Phadke 1987; 1990	35 / 65	30 (median)	Optic nerve Brainstem Cerebellar Spinal cord Cerebral Mixed	11 24 4 42 1 18	91 / 9
Minderhoud *et al* 1988	40 / 60	Not available	Not available		63 / 37
D.H. Miller *et al* 1992a	29 / 71	32.2 (mean)	Optic neuritis Brainstem Limb sensory Limb motor Limb motor / sensory Cerebellar Cerebral	21 23 27 14 9 2.5 3.5	95 / 5

table continued on following page

Table 4.5 **Main series of the long-term course and prognosis of multiple sclerosis: demographic and multiple sclerosis onset characteristics, cont'd**

Study	Gender: males / females (%)	Age at onset (years)	Initial symptoms of multiple sclerosis (%)		Initial course: relapsing–remitting / progressive (%)
Riise *et al* 1992	36 / 64	31.7 (mean)	Visual Brainstem Pyramidal Cerebellar Sensory	25 22 35 17 46	88 / 12
Trojano *et al* 1995	44 / 56	26 ± 8 (mean ± SD)	Not available		81 / 19
Kantarci *et al* 1998	36 / 64	27.6 ± 8.8 (mean ± SD) 27 (median)	Optic neuritis Brainstem / cerebellar Motor Sensory Sphincter	20 30 40 43 7	88 / 12
Myhr *et al* 2001	38 / 62	32.5 ± 0.6 (mean ± SEM)	Visual Brainstem / cerebellar Motor Sensory Sphincter Multiple systems involved	16 34 32 34 2 18	81 / 19
Long-term natural history cohorts with longitudinal follow-up					
United States Army Veterans World War II cohort					
Kurtzke *et al* 1968a; 1970a; 1973; 1977	Males only	25 (mean)	Visual Brainstem Motor limb Coordination limb Sensory limb Bowel / bladder Cerebral	31 40 52 44 42 14 13	Not available
Lyon, France, multiple sclerosis cohort					
Confavreux 1977 Confavreux *et al* 1980	40 / 60	31.3 ± 10.1 (mean ± SD) 30.6 (median)	Not available		82 / 18
Confavreux *et al* 2000; 2003	36 / 64	31 ± 10 (mean ± SD) 30 (median)	Isolated optic neuritis Isolated brainstem dysfunction Isolated dysfunction of long tracts Combination of symptoms	18 9 52 21	85 / 15
Gothenburg, Sweden, multiple sclerosis cohort					
Broman *et al* 1981 Runmarker and Andersen 1993 Eriksson *et al* 2003	40 / 60	Not available	Not available		83 / 17
London, Ontario, multiple sclerosis cohort					
Weinshenker *et al* 1989a; 1989b; 1991a; 1991b	34 / 66	30.5 ± 0.3 (mean ± SEM) 29 (median)	Optic neuritis Diplopia / vertigo Acute motor Insidious motor Balance / limb ataxia Sensory	17 13 6 14 13 45	66 / 34
Long-term history series from the therapeutic era					
Amato *et al* 1999 Amato and Ponziani 2000	36 / 64	29.8 ± 9.8 (mean ± SD)	Not available		85 / 15

SD = standard deviation.
SEM = standard error of the mean.

The sex ratio in multiple sclerosis

A female predominance is apparent in all representative studies (Amato and Ponziani 2000; Amato *et al* 1999; Bonduelle and Albaranès 1962; V.A. Clark *et al* 1982; Confavreux *et al* 1980; 2000; 2003; Detels *et al* 1982; Kantarci *et al* 1998; Leibowitz and Alter 1970; 1973; Leibowitz *et al* 1964a; 1964b; McAlpine 1961; McAlpine and Compston 1952; D.H. Miller *et al* 1992a; R. Müller 1949; 1951; Myhr *et al* 2001; Panelius 1969; Phadke 1987; 1990; S. Poser 1978; S. Poser *et al* 1982a; Riise *et al* 1992; Runmarker and Andersen 1993; Trojano *et al* 1995; Visscher *et al* 1984; Weinshenker *et al* 1989a; 1989b; 1991a; 1991b). The usual ratio is two females for one male (2F:M). The highest reported proportion of females is 71% (2.5F:M) in series from North America (V.A. Clark *et al* 1982; Detels *et al* 1982; Visscher *et al* 1984) and New Zealand (D.H. Miller *et al* 1992a). Similarly, of the 324 living cases in all categories of multiple sclerosis from London, Ontario, and Middlesex County on 1st January 1984, 71% (2.5F:M) were females (Hader *et al* 1988). The lowest proportion reported is 51% (1.04F:M) in Israeli series (Leibowitz *et al* 1964a; 1964b; Leibowitz and Alter 1970; 1973).

Age at onset

It is not always easy to determine the age at which symptoms of multiple sclerosis first develop. Some symptoms, such as paraesthesia, are nonspecific and often so vague as easily to be overlooked. However, there is consensus for peak onset around 30 years of age (Table 4.6 and Figure 4.8) (Amato and Ponziani

Table 4.6 **Distribution of patients with multiple sclerosis (%) by age at onset: data from the literature**

Age at onset of multiple sclerosis (years)	R. Müller 1951 n = 793	McAlpine and Compston 1952 n = 840	Leibowitz *et al* 1964a; 1964b n = 266	Panelius 1969 n = 146	Confavreux *et al* 1980 n = 349	S. Poser *et al* 1982b n = 1529	Confavreux *et al* 2000; 2003 n = 1844
<20	22	12	15	11	11	10	12
20–29	46	35	27	48	36	36	37
30–39	24	33	28	31	33	33	30
40–49	7	17	22	9	14	21	15
≥50	1	3	8	1	6		6

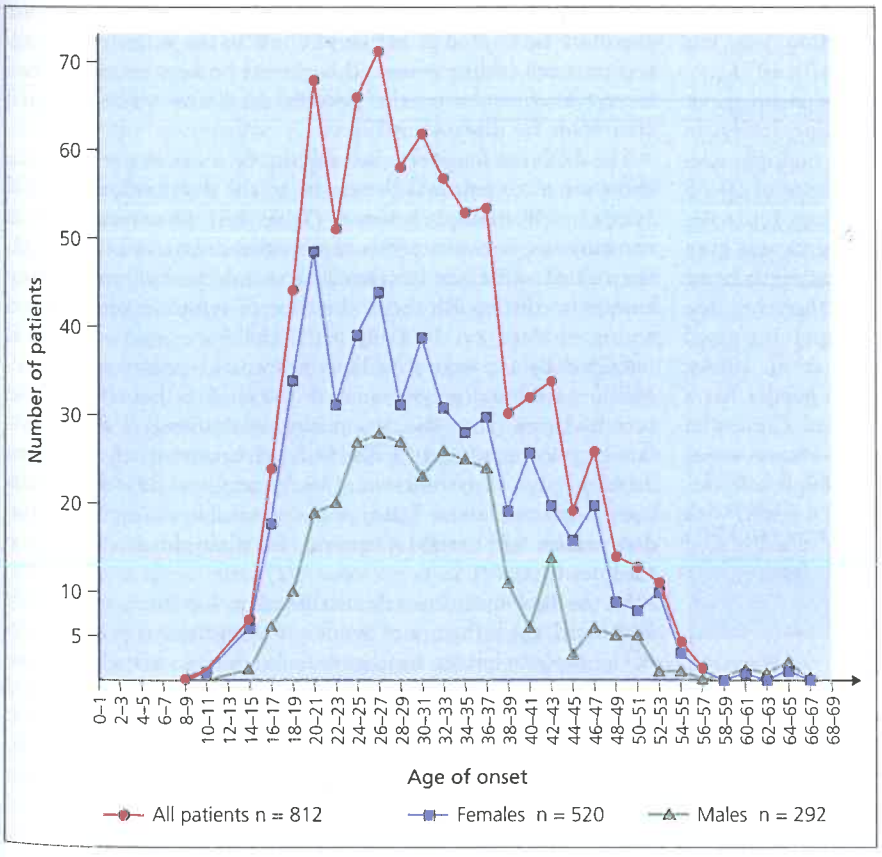


Figure 4.8 Distribution of patients by age at onset of the disease, among 812 patients with multiple sclerosis. Adapted from S. Poser (1978). © 1978, reprinted with permission of Springer-Verlag GmbH.

2000; Amato *et al* 1999; V.A. Clark *et al* 1982; Confavreux *et al* 1980; 2000; 2003; Detels *et al* 1982; Leibowitz and Alter 1970; 1973; Leibowitz *et al* 1964a; 1964b; McAlpine and Compston 1952; D.H. Miller *et al* 1992a; Myhr *et al* 2001; Panelius 1969; Phadke 1987; 1990; S. Poser 1978; S. Poser *et al* 1982a; Riise *et al* 1992; Visscher *et al* 1984; Weinshenker *et al* 1989a; 1989b; 1991a; 1991b). An earlier onset has been found in some series (Kantarci *et al* 1998; Kurtzke *et al* 1968a; 1970a; 1973; 1977; R. Müller 1949; 1951; Trojano *et al* 1995). R. Müller (1949) observed a median age at onset of 24 years in his comprehensive multicentre Swedish study: 22% of cases first experienced symptoms at <20 years. He emphasized that, for the reasons mentioned above, 'the anamnesis should be very carefully recorded in order to obtain more exact information as to the age at the outset of the disease.' This often corrects age at onset to an earlier age by comparison with the spontaneous statements of patients. R. Müller (1949) concluded: 'the explanation of the low age at the outset of the disease in this material is probably only because I devoted more attention to this point than had actually been the case.' For the United States Army Veterans cohort (Kurtzke *et al* 1968a; 1970a; 1973; 1977), the circumstances of enrolment (military service) easily account for the observed low median age at onset (25 years). For the two other series, the explanation is less straightforward (Kantarci *et al* 1998; Trojano *et al* 1995). In the majority of representative series, the distribution of patients with multiple sclerosis by age at onset is bell-shaped, with onset at ≤20 years in around 10%, at ages 20–40 years in 70%, and >40 years in 20% of cases (Bonduelle and Albaranès 1962; Confavreux *et al* 1980; 2000; McAlpine 1961; McAlpine and Compston 1952; 2003; S. Poser *et al* 1982a). In London, Ontario, onset of multiple sclerosis occurred at <20 years in 11%, and at >40 years in 20% of cases, respectively (Hader *et al* 1988). The distribution was less restricted in the study of Leibowitz and Alter (1970; 1973).

Females often appear to have a slightly younger mean age at onset than males (R. Müller 1949; 1950; McAlpine 1961). In London, Ontario, age at onset of clinically definite multiple sclerosis was 29.7 (±10.1) years for females, with a range of 10–58 years, and 31.7 (±11.8) with a range of 6–66 years for males (Hader *et al* 1988). In the Israeli series, the difference was even more marked – mean age at onset with multiple sclerosis being 31.9 years in females and 34.4 years in males. Furthermore, the F:M ratio was found to decrease as age at onset increased (Leibowitz and Alter 1970; 1973; Leibowitz *et al* 1964a; 1964b). Other authors have not considered that gender has a significant impact on age at onset (McAlpine and Compston 1952; Panelius 1969; S. Poser 1978). In the Lyon, France, series (Confavreux *et al* 1980), mean age at onset of multiple sclerosis was higher in females (32.6 years) than males (29.4 years) with a significantly greater incidence of the disease in females aged >40 years at the time of presentation ($p < 0.01$).

Symptoms at onset

At least in retrospect, symptoms can conservatively be placed in three categories: those affecting the optic nerves, the brainstem, and the long tracts – the latter designating symptoms related to motor, sensory, cerebellar or sphincter disturbances. It must be acknowledged that these categories do not strictly represent anatomical regions in the central nervous system (Broman *et al* 1981). For instance, in addition to the effects on bulbar function, eye movements and motor control, brainstem lesions may also affect the long sensory and motor tracts. Long tract symptoms cannot, in many cases, be referred to a specific part of the central nervous system. We consider it difficult, if not actually erroneous, to force too much precision onto the description and classification of inaugural symptoms and signs in multiple sclerosis, at least in the series for which there is an interval of months or years between clinical onset of the disease and first professional evaluation. For instance, cerebellar symptoms, in many cases assessed retrospectively, cannot always be distinguished from those attributable to involvement of motor or sensory tracts. It is often risky to conclude that gait disturbance is due entirely to ataxia, paraparesis or both – based merely on the interpretation of a neurological interview. Whilst acknowledging that the above classification of symptoms into three categories is imperfect and restrictive, we and others nonetheless consider it to be pragmatic, and an acceptable compromise. For instance, in their comprehensive epidemiological surveys in Norway, Riise *et al* (1988; 1992) changed the classification of initial symptoms for defined categories referable to functional systems of the DSS. For example, 'motor weakness' in the first study was subsequently changed to the 'pyramidal' category. The authors did, however, admit that 'the names used apply to the grouping of symptoms and do not necessarily mean that they can be referred to a specific location or lesion. For instance, "pyramidal function" does not mean that the signs are due only to lesions involving the pyramidal tract' (Riise *et al* 1992). We entirely endorse these conclusions. Their consequences are clear. It is risky and often erroneous to categorize initial symptoms too strictly, at least when the assessment is sometimes made years after disease onset. Data related to initial symptoms must therefore be treated as not very robust in the majority of long-term natural history series. This should be kept in mind when interpreting results on the possible predictive value of initial symptoms for disease outcome.

The different long-term natural history series in the literature show some consensus with respect to the distribution of initial symptoms in multiple sclerosis (Table 4.5). However, detailed comparisons between series are rendered impossible through the use of variations in terminology and the failure by many authors to distinguish the occurrence of symptoms in isolation and in combination. It is difficult to delineate precisely what is intended by the terms 'monosymptomatic, polysymptomatic, monoregional and polyregional' in the studies that adopt these terminologies. That said, an incidence of around 15% for isolated optic neuritis, 10% for isolated brainstem dysfunction, 50% for isolated dysfunction of long tracts, and 25% for various combinations of these features are reasonable estimates for the distribution of initial symptoms in multiple sclerosis (see Chapter 6).

In the rare instances where this issue has been specifically addressed, the influence of gender on symptoms at presentation of multiple sclerosis has been found not to exist (Panelius 1969), or to exert only a marginal effect showing a slightly greater frequency of long tract involvement in men, and of optic neuritis and diplopia in females (Leibowitz and Alter 1973; R. Müller 1949). The latter trend presumably results from the older age at onset of multiple sclerosis in males than females in these series. Indeed, the obvious influence of age at onset has

Table 4.7 **Distribution of patients (%) by initial symptoms according to age at onset of multiple sclerosis, among 1096 patients. Adapted from Weinshenker *et al* (1989a)**

Age at onset of multiple sclerosis (years)	Optic neuritis	Diplopia / vertigo	Acute motor	Insidious motor	Balance / limb ataxia	Sensory
<20	23	18	6	4	14	46
20–29	23	12	7	6	11	52
30–39	13	11	7	14	15	44
40–49	9	17	3	31	13	33
≥50	6	13	4	47	11	32

consistently been found in all studies that addressed this issue, with a higher percentage of optic neuritis and diplopia in patients with earlier age at onset, and of motor disturbances in the patients presenting later (Leibowitz and Alter 1973; Leibowitz *et al* 1964b; R. Müller 1949; 1951). Table 4.7 shows a further illustrative example from the London, Ontario, cohort.

The initial clinical course

For more than a century, all experts have agreed that multiple sclerosis usually follows an initial relapsing–remitting course, although some individuals progress from onset. Differences emerge amongst series in the literature as to the relative proportions displaying these two patterns (see Table 4.5). Indeed, the frequency of cases with progression from onset has been found to range from 5% (D.H. Miller *et al* 1992a) to 37% (Minderhoud *et al* 1988). The latter figure seems to be an outlier, and may be related to recruitment bias because this Dutch study was mainly devoted to an assessment of year at onset of the progressive phase. The estimate of 34% with primary progressive multiple sclerosis coming from the London, Ontario, cohort is, at first sight, more surprising if one considers the comprehensive sampling (Weinshenker *et al* 1989a; 1989b). Actually, among the subgroup of 197 patients seen from the onset of multiple sclerosis in this series, only 15% exhibited an initial progressive course with or without superimposed relapses, a figure similar to that of the other main long-term longitudinal natural history series (Confavreux *et al* 1980; 2000; 2003; Eriksson *et al* 2003; Runmarker and Andersen 1993). According to the Canadian authors, this disparity could reflect a tendency for patients seen for the first time at a later point in their illness to suppress or forget earlier remitting symptoms when progressive disease subsequently intervenes. It is their experience that a patient may recall a remote first relapse only after several clinic visits (Weinshenker *et al* 1989a). Moreover, when these authors updated details on their cohort in 1996, they had to reassign a significant number of patients with respect to the overall clinical course of the disease (D.A. Cottrell *et al* 1999a; 1999b; Kremenchutzky *et al* 1999). This led to a total of 216 cases with primary progressive multiple sclerosis, as defined. This represented 21% of the total cohort, a figure in agreement with that of the other main longitudinal natural history series (Confavreux *et al* 1980; 2000; 2003; Eriksson *et al* 2003; Runmarker and Andersen 1993). Noticeably, in all of these longitudinal series, the group of primary progressive multiple sclerosis encompasses cases with and without relapses superimposed on disease progression – that is, progressive relapsing and primary progressive multiple sclerosis according to current definitions (Lublin and Reingold 1996). Taken together, the initial course of multiple sclerosis can be reasonably estimated to be relapsing–remitting in 85% and progressive in 15% of cases (Table 4.5).

It has been appreciated for half a century that men more often show a progressive onset of multiple sclerosis than women (R. Müller 1949; 1951). Symptoms related to dysfunction of long tracts are relatively more frequent in males, whereas optic nerve and brainstem features occur less often in progressive onset than relapsing–remitting multiple sclerosis (Table 4.8) (Confavreux *et al* 1980; McAlpine and Compston 1952; R. Müller 1949; 1950; Riise *et al* 1992; Trojano *et al* 1995). The proportion of progressive onset cases rises steadily with age (Table 4.9) (Confavreux 1977; Leibowitz *et al* 1964a; 1964b; McAlpine and Compston 1952; R. Müller 1949; 1950; Phadke 1990; S. Poser 1978; S. Poser *et al* 1982b; Weinshenker *et al* 1989a). Gender, clinical features, age and the course at onset are interdependent and there is much potential for confounding of contributing factors in these analyses. The strongest correlation between clinical variables and the initial course of the disease is with age at onset. Thus, we can caricature the progressive onset of multiple sclerosis as a disorder of motor deficits occurring in older males. This analysis says nothing concerning

Table 4.8 **Distribution of patients (%) by initial symptoms according to the initial course of multiple sclerosis, among 574 patients. Adapted from Riise *et al* (1992)**

	Initial course of multiple sclerosis	
	Relapsing–remitting	Progressive
Pyramidal	32	54
Cerebellar	16	23
Brainstem	24	7
Sensory	48	32
Visual	26	17

Table 4.9 **Percentages of patients with a progressive initial course of multiple sclerosis according to age at onset: data from the literature**

Age at onset of multiple sclerosis (years)	McAlpine and Compston 1952 n = 414	Confavreux 1977 n = 349	S. Poser *et al* 1982b n = 1529	Weinshenker *et al* 1989a n = 1099	Phadke 1990 n = 1055
<20	0	3	9	18	3
20–29	5	10	13	19	4
30–39	14	25	27	38	5
40–49	24	24	41	63	14
≥50	29	45		74	27

the contribution to disability of manifestations that are clinically silent but discretely contribute to the accumulation of disability preceding presentation. But whatever came before, the recognition of motor symptoms attributable to multiple sclerosis at onset indicates and predicts a more advanced subsequent course of the disease.

THE OVERALL COURSE OF MULTIPLE SCLEROSIS

Most patients with multiple sclerosis experience changes in their condition that are distinct and hence recognizable – but sometimes only in retrospect – each constituting a pivotal event in the course of the illness. Easiest to recognize are the individual relapses; more elusive, but of considerable significance for the eventual level of disability, is onset of the progressive phase. In recent years, it has become commonplace to refer to a first neurological episode suggestive of multiple sclerosis as the 'clinically isolated syndrome', provided this can reasonably be attributed to the dysfunction of optic nerves, brainstem or spinal cord, with acute or subacute onset followed by recovery, and in the context of paraclinical investigations excluding an explanation other than that of suspected multiple sclerosis (Barkhof *et al* 1997a; Filippi *et al* 1994; Morrissey *et al* 1993a; O'Riordan *et al* 1998; Tintoré *et al* 2000). Some physicians restrict this term to situations in which the features are monosymptomatic, but these represent only a proportion of such episodes. Others also allow the term to indicate polysymptomatic presentations not attributable to a single central nervous system lesion, or any initial remitting episode whatever its neuroanatomical complexity. Amongst the subset of 1562 patients with an exacerbating–remitting onset of the disease in the Lyon, France, series, initial episodes were classified as monofocal or multifocal in 78% and 22% of cases, respectively. However, these monofocal initial episodes represent only 66% of the cases among the total cohort of 1844 patients with multiple sclerosis (Confavreux *et al* 2003). There is no evidence to suggest that the long-term course and prognosis of the disease are determined by the pattern of this initial episode – variously described by authors as monosymptomatic, polysymptomatic, monofocal or multifocal. Quite what they always mean is obscure and, as explained above, in order to avoid confusion, we use the term 'initial neurological episode' to cover these complexities of nomenclature.

Recovery from the initial neurological episode

On average, 85% of inaugural neurological episodes will remit, at least partially. The issue of spontaneous remission from symptoms at onset in multiple sclerosis has been studied in a series of 220 hospitalized male patients: the key predictive factor for remission was duration of the ongoing neurological episode prior to hospital admission (Kurtzke 1956). There was an inverse relationship between duration of the episode prior to admission and the probability of improvement (Table 4.10). The proportion of patients who improved decreased from 86%, when the episode lasted ≤7 days, to no improvement at all for the episodes lasting >2 years before admission. Interestingly, this decrease in the probability of improvement was steady throughout the 2-year interval prior to admission, without any discrete change allowing a recognizable frontier between exacerbation and progression to be established. The outcome of the ongoing neurological episode could not be correlated with age at onset of multiple sclerosis, duration of the disease at admission, age at admission, or symptoms, signs and severity of the neurological episode. However, conclusions regarding the possible lack of influence of age should be treated with caution due to the particular circumstances of inclusion in this Army series.

Table 4.10 **Chance of recovery (%) from the first neurological episode subsequent to hospitalization according to duration of the neurological episode, prior to admission, among 220 patients with multiple sclerosis. Adapted from Kurtzke (1956)**

Duration of the episode before admission	Probability (%) of improvement of the episode
≤7 days	86
8–14 days	64
15–31 days	38
1.1–2.0 months	18
2.1–6.0 months	14
6.1–12 months	18
1.1–2 years	7
>2 years	0

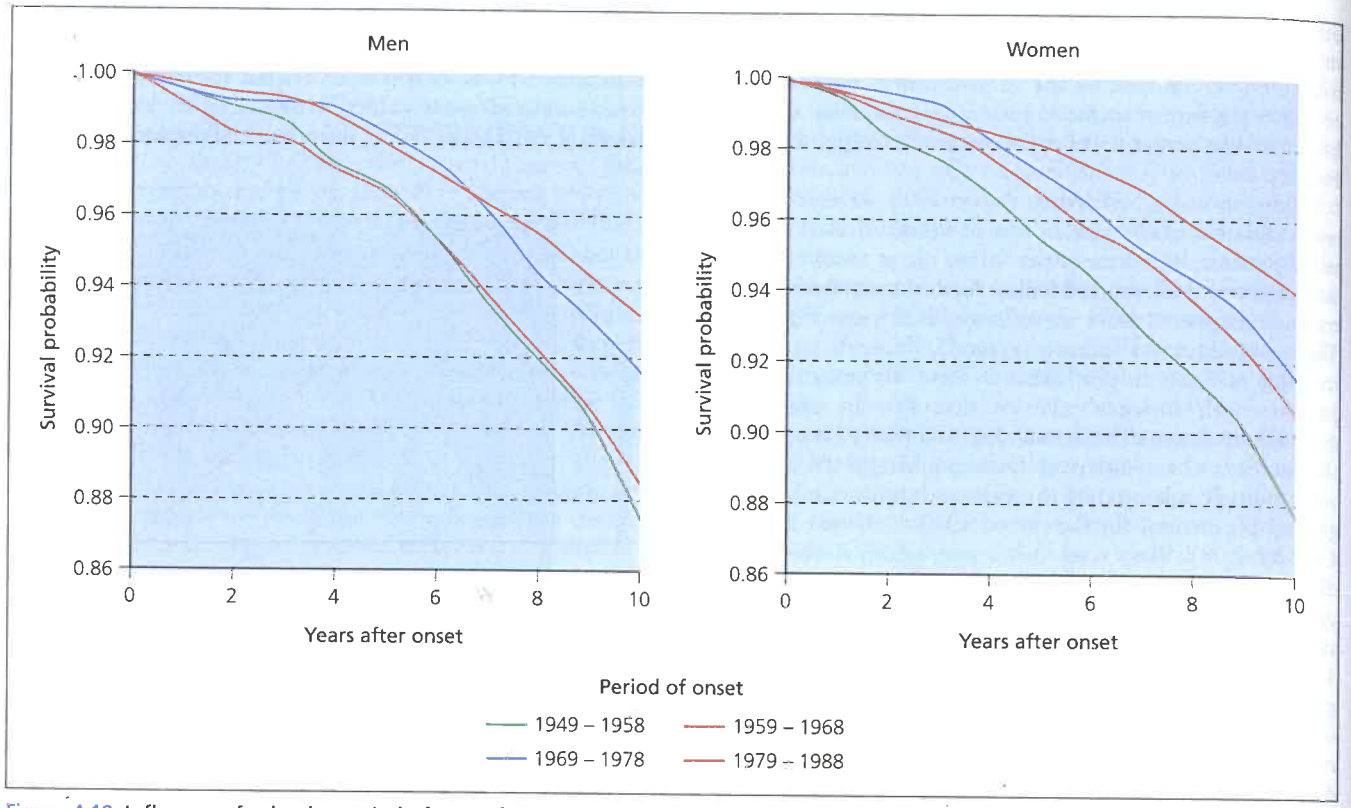


Figure 4.18 Influence of calendar period of onset for multiple sclerosis on actuarial probability of survival amongst 9881 patients with multiple sclerosis, of whom 4254 had died before the end of follow-up on 1st January 2000, and the matched general population in Denmark. Adapted from Brønnum-Hansen *et al* (2004).

(5%) and accidents and suicide (5%). Compared with the general population, there was excess mortality due to cardiovascular disease, infections, respiratory causes, accidents and suicide, but a lower risk of death from cancer.

In summary, a reasonable estimate of the median time from disease onset to death in people with multiple sclerosis is 31 years. This represents a 5–10 year reduction in life expectancy compared with the general population. It seems likely that the modest difference is progressively being eroded with advances in medical management. Female sex, younger age at disease onset, an initial exacerbating–remitting clinical course, and optic neuritis, diplopia or paraesthesiae as initial symptoms are all associated with improved survival. Death is attributable to multiple sclerosis in about two-thirds of patients. It is rare for death to result from involvement of vital centres in the central nervous system. Rather, the reduced life expectancy can be attributed to the bedridden state and its complications in chronically disabled patients. In individuals dying from causes unrelated to multiple sclerosis, the excess is due to suicide compensated by reduced mortality from cancer. Therefore, multiple sclerosis is chronic and disabling but not a fatal disease.

DISEASE MECHANISMS UNDERLYING THE CLINICAL COURSE

For the clinician, the conundrum presented by the clinical course of multiple sclerosis starts with the awareness of at least three different types of clinical evolution, and variable rates of accumulation of disability between patients. Do these patterns indicate the existence of altogether different disorders or are they merely a function of complexity (see also Chapter 14)? Understanding how these patterns come about is fundamental to a sophisticated understanding of multiple sclerosis; and, although this might be considered more the terrain of the expert in physiology or neurobiology, there is much to be learned on this topic from detailed scrutiny of the natural history. Our discussion of mechanisms underlying the disease course in multiple sclerosis necessarily first rehearses, in summary, the experimental evidence.

Inflammation and degeneration

The course of multiple sclerosis may be considered as the expression of two clinical phenomena, relapses and progression, the latter being defined as steady worsening of symptoms and signs over ≥6 months. In turn, this analysis brings into the equation the interplay between two biological activities: inflammation (focal, disseminated, acute or recurrent) and degeneration (diffuse, early, chronic and progressive) (Figure 4.19; for other versions of the same cartoon, see Figures 14.2 and 18.1). There is strong evidence that relapses are mainly the expression of acute focal inflammation occurring within the central nervous system. For each clinical episode, there is an average of ten new MRI lesions (Figure 4.20; see Chapters 7 and 13). One could say that 'multiple sclerosis never sleeps'. This is also one explanation for the strikingly loose correlations with which authorities

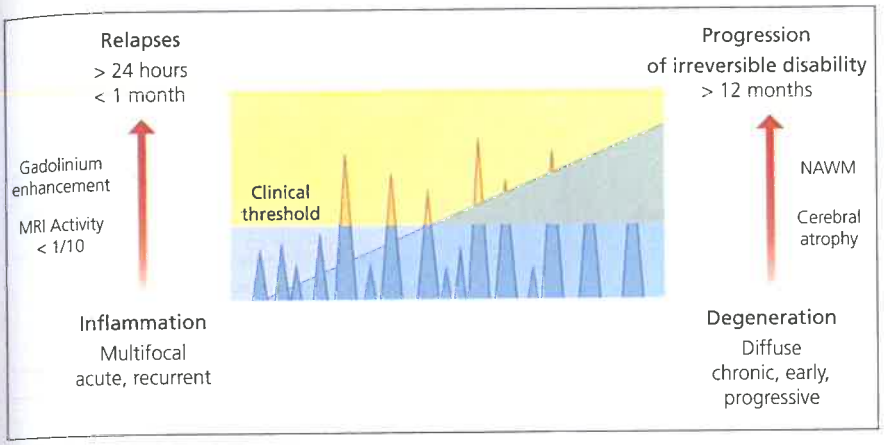


Figure 4.19 Schematic representation of the interplay between relapses and progression, and focal inflammation and diffuse degeneration in multiple sclerosis. NAWM = normal-appearing white matter.

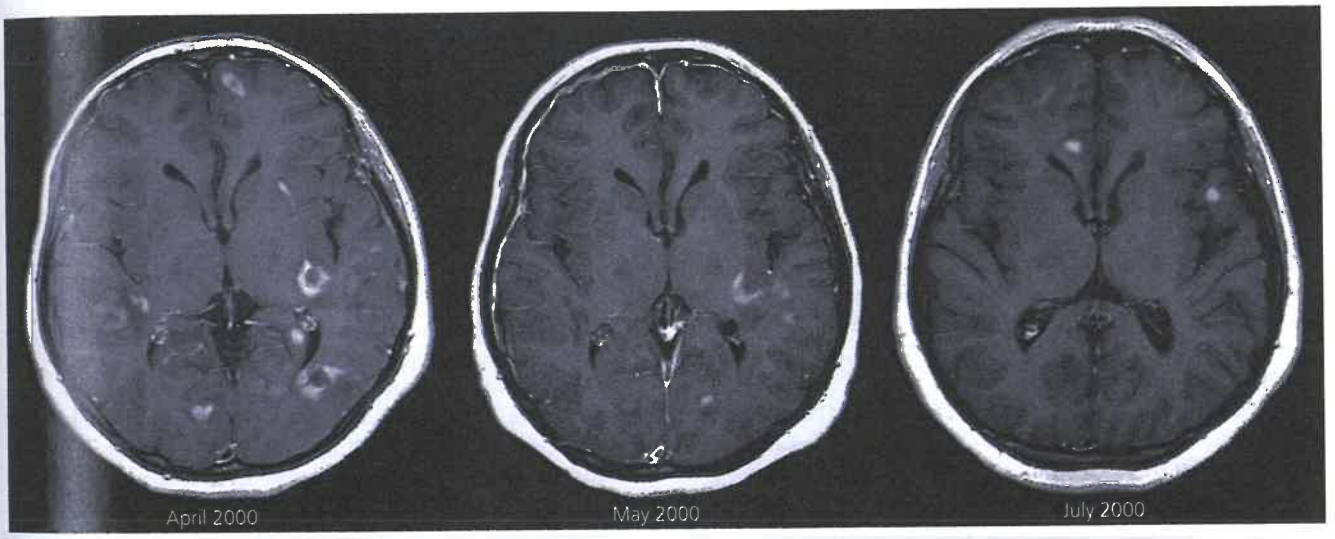


Figure 4.20 Consecutive gadolinium enhanced brain MRI scans from a patient with relapsing–remitting multiple sclerosis. The MRI activity is high despite clinical quiescence during the study period.

working in the 19th century struggled in seeking to match clinical abnormalities to the anatomical lesions observed in their pathological specimens. This so-called *dissociation anatomo-clinique* (clinico-pathological dissociation) was, for these pioneers, a hallmark of *sclérose en plaques* (multiple sclerosis). Relapses are therefore a direct but also a 'filtered' clinical expression of inflammation. This 'filtering phenomenon' may have different origins relating to the complex relationship between injury and repair, plasticity, and the presence of structural abnormality with and without functional perturbations in conduction of the nerve impulse (see Chapters 10 and 13; M. Lee *et al* 2000; Pantano *et al* 2002; H. Reddy *et al* 2000; 2002; Rocca *et al* 2002a; Staffen *et al* 2002). There is also increasing evidence that multiple sclerosis is a neurodegenerative disease, the diffuse and chronic axonal loss correlating with progression and accumulation of disability (see Chapters 1, 10, 12 and 13).

One of the central issues with respect to outcome in multiple sclerosis is the mechanism whereby irreversible disability accrues (Figure 4.21; Confavreux 2002b; Confavreux and Vukusic 2002; Confavreux *et al* 2000). From the clinical perspective, this could simply result from relapses with sequelae. Under these circumstances, the pattern of accumulation would be stepwise. Alternatively there may be a contribution from superimposed progression. Therefore, it becomes important to reconcile the relative contributions of relapses and progression, and of focal inflammation and diffuse degeneration, in the accumulation of disability. One analysis is that inflammation is directly and exclusively responsible for the initiation of degeneration. This does not necessarily mean that inflammation is also entirely responsible for the perpetuation of degeneration and progression once these have gathered their own momentum (see Chapter 10). But, according to this analysis, relapse and the underlying inflammatory component is the major cause of irreversible disability in multiple sclerosis.

At first glance this assertion is attractive. Among the 1562 patients of the Lyon Natural History Cohort with a relapsing–remitting onset of multiple sclerosis, 274 (18%) did suffer from an initial relapse with irreversible incomplete recovery as defined by a score of DSS ≥3. Among the 1288 patients making a complete recovery, as defined by a score of DSS ≤2, after the initial relapse, 391 (30%) later experienced incomplete recovery from a subsequent episode (Confavreux *et al* 2003).

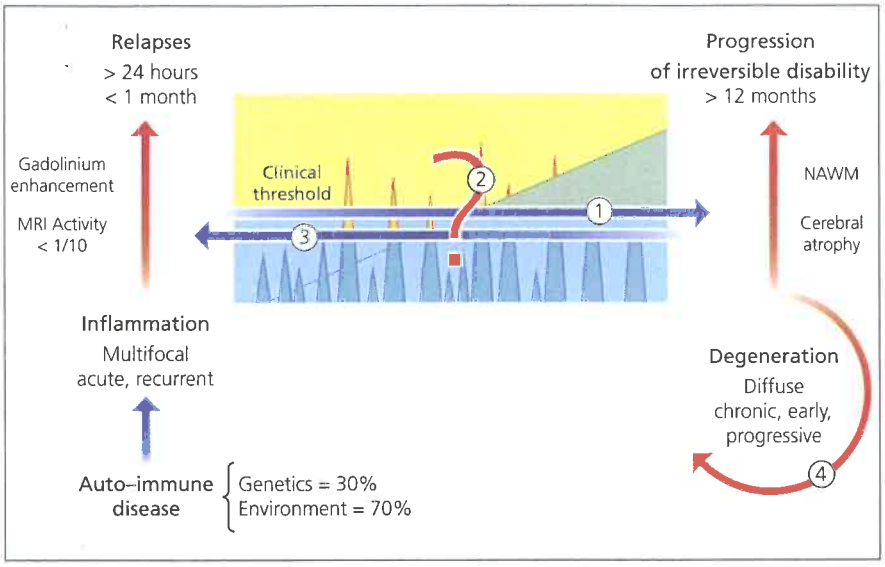


Figure 4.21 Schematic representation of the possible interplay between relapses and progression, and focal inflammation and diffuse degeneration in multiple sclerosis. 1: Relapses and focal inflammation are the major cause of irreversible disability; neurodegeneration follows the phase of active inflammation. 2: Relapses and focal inflammation are not the major cause of irreversible disability; these have independent mechanisms and proceed at different rates. 3: The initial process is neurodegenerative, and damaged tissue stimulates a secondary inflammatory reaction. 4: The initial process is autoimmune with secondary autonomous self-perpetuating neurodegeneration.

A detailed analysis of pooled data from 224 patients with relapsing–remitting multiple sclerosis enrolled in the placebo arms of several randomized clinical trials allows comparisons between EDSS assessments before, at the time of, and after a relapse (Lublin *et al* 2003). The baseline EDSS assessment is defined as the closest measurement preceding the relapse in question. Comparing post-relapse and baseline evaluations, the net increase in the EDSS score was 0.27 (±1.04). This corresponds to 42% of the patients with ≥0.5, and 28% with ≥1.0 increase in EDSS scores. However, the median time between evaluations performed during and after the relapse was only 63 (range 32–140) days.

Similarly, assessment of possible effects from the degree of recovery after the initial episode, time to the next event, and number of attacks during the first years of the disease on the disability accrual process provide consistent results in natural history cohorts. An incomplete recovery from the initial relapse, a short interval between the first two episodes, a high number of relapses overall, or a brisk relapse rate during the first years of the illness are associated with rapid accumulation of irreversible disability (Confavreux *et al* 1980; 2003; Weinshenker *et al* 1989b; 1991a).

However, the real contribution of relapses to disability accumulation is more complex. Evidence from the primary progressive form of multiple sclerosis indicates that progression of irreversible disability may occur without superimposed relapses (Lublin and Reingold 1996), or inflammation defined using standard pathological and MRI criteria. The rate of disability in these cases with progression from onset is similar to that seen in relapsing progressive forms of multiple sclerosis (Confavreux *et al* 2000; D.A. Cottrell *et al* 1999a; Kremenchutzky *et al* 1999).

Informative observations have been made on pooled data from 313 patients with relapsing–remitting multiple sclerosis enrolled in the placebo arms of two large phase III trials of interferon-β1a (PRISMS Study Group 1998) and glatiramer acetate (K.P. Johnson *et al* 1995), assessed at 3-month intervals with a 2-year follow-up (Figure 4.22: C. Liu and Blumhardt 2000). Analyses were performed on the 289 patients with complete EDSS assessments. A significant change was defined as a change of ≥1.0 EDSS points if baseline EDSS was between 0 and 5.0, or ≥0.5 EDSS point change if baseline EDSS was ≥5.5. Patients were distributed into six categories according to the observed course of EDSS scores throughout the 2 years of follow-up:

- 20% exhibited no significant change
- 37% had a fluctuating course with a significant EDSS change but not confirmed at 3 months
- 14% showed an erroneous progression as defined by a significant EDSS increase confirmed at 3 months but not sustained until the end of the observation period
- 15% had a sustained progression with a significant EDSS increase confirmed at 3 months and sustained until the end of the trial
- 8% showed an erroneous improvement with a significant EDSS decrease confirmed at 3 months but not sustained until the end of the trial
- 6% showed a sustained improvement with a significant EDSS decrease confirmed at 3 months and sustained until the end of the trial.

In these series, 29% of the patients could therefore be classified as showing progression in the trial with confirmation at 3 months but, among those who progressed, the EDSS increase was still present at conclusion of the follow-up period in about half the participants. The probability of misclassification at the end of the trial regarding the progression status was 0.52. Applying the more stringent definitions of ≥2.0 EDSS increase and/or a confirmation at 6 months led to essentially the same estimation for the probability of misclassification (range 0.33–0.47). These results clearly show that an increase in disability confirmed at 3 or even 6 months must not be considered as equivalent to an irreversible increase in disability. Interestingly, as discussed above using similar resources, Lublin *et al* (2003) also found a ≥1.0 point EDSS increase relative to baseline in 28% of patients at a median of 63 days after a relapse. This suggests that, in the available placebo cohorts of patients with relapsing–remitting multiple sclerosis, the confirmed disability increases were mainly relapse driven. It seems logical to con-

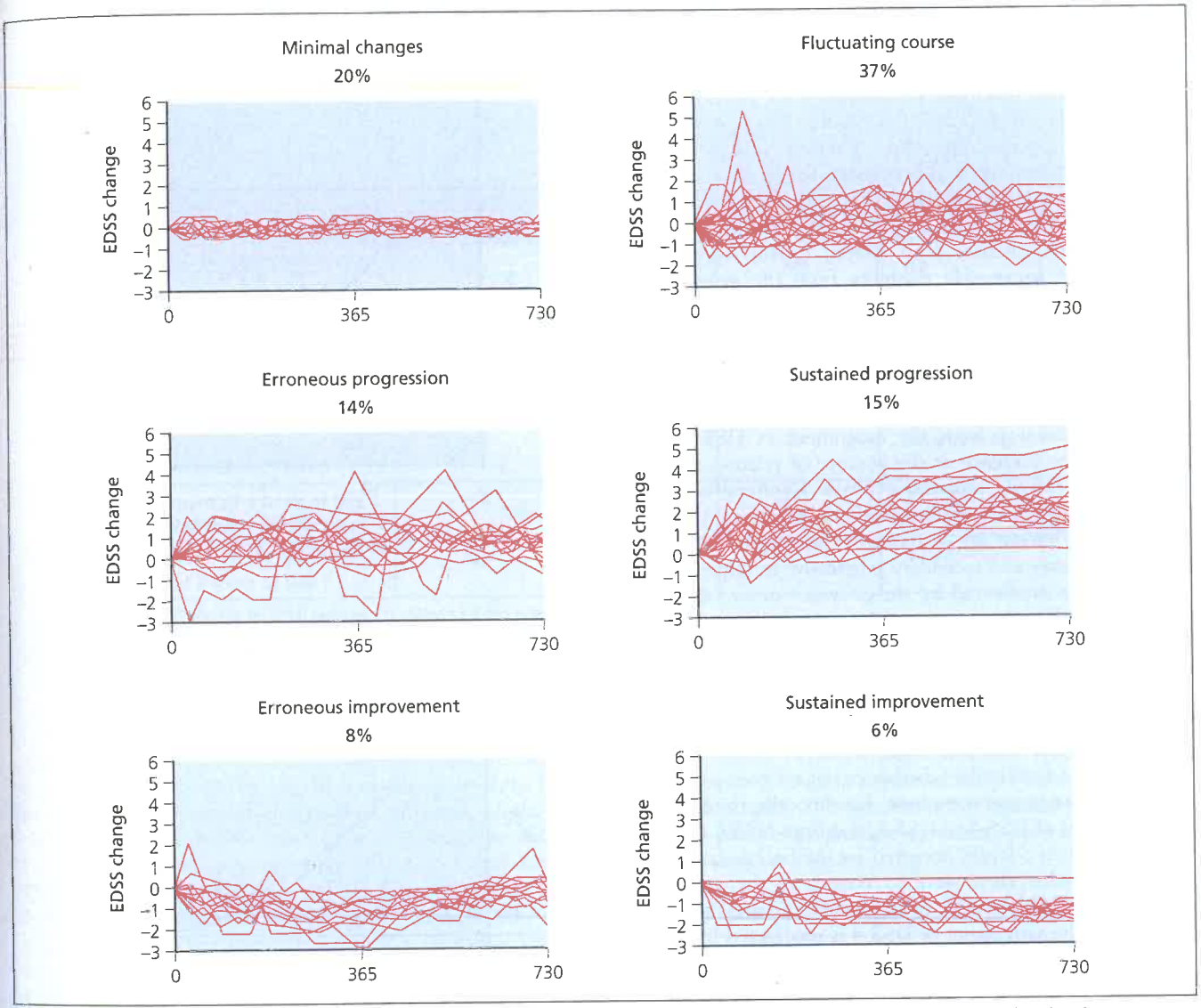


Figure 4.22 Total series of individual plots for EDSS changes from baseline versus days in study, for 289 patients with relapsing–remitting multiple sclerosis enrolled in placebo arms of two phase III trials of interferon-β1a and glatiramer acetate. A significant change is defined as ≥1.0 point EDSS change if baseline EDSS was 0–5.0, or a ≥0.5 point EDSS change if baseline EDSS was >5.5. Patients are distributed into six categories according to the observed course of their EDSS scores throughout the two years of follow-up (see text for precise definitions): minimal changes (20% of the patients); fluctuating course (37%); erroneous progression (14%); sustained progression (15%); erroneous improvement (8%) and sustained improvement (6%). Adapted from C. Liu and Blumhardt (2000). Reproduced with permission from the BMJ Publishing Group.

clude that short-term confirmed increase in disability depends primarily on relapses and is often reversible.

Totally different is the issue of long-term irreversible disability. Lessons from natural history cohorts have been instructive in this respect. For the statistical analysis of the 1844 patients of the Lyon Natural History Multiple Sclerosis Cohort, focus was placed on robust landmarks of disability that could easily be identified through successive neurological assessments as well as by retrospective interview of the patient, whenever necessary. The landmarks were:

- *DSS 4*: defined by walking without aid, although limited, but >500 metres without rest.
- *DSS 6*: walking with unilateral support and limited to ≤100 metres without rest.
- *DSS 7*: home restriction with a few steps still possible holding onto a wall or furniture but limited to ≤10 metres without rest.

Disability was defined as irreversible when one of these steps had been reached and persisted for ≥6 months, excluding any transient worsening related to relapses. This irreversibility was confirmed at any subsequent assessment during follow-up of the patient in subsequent years. From this cohort, the well-known difference between cases with a relapsing–remitting onset and those with progressive disease is again apparent: median time

from the onset of multiple sclerosis to assignment of a score of DSS 4, indicating irreversible disability, was significantly longer in the relapsing–remitting than progressive onset cases. The same observation was made for time of onset to assignment of DSS 6 or 7 (Figure 4.23 and Table 4.21). This is in agreement with former analyses of this cohort (Confavreux *et al* 1980) and with results from many other series (Eriksson *et al* 2003; Kantarci *et al* 1998; Phadke 1990; Pittock *et al* 2004b; Runmarker and Andersen 1993; Runmarker *et al* 1994b; Trojano *et al* 1995; Weinshenker *et al* 1989b; 1991a). Nevertheless, progression of irreversible disability from the assignment of DSS 4 to DSS 6 was similar in cases both with a relapsing–remitting and a progressive onset (Figure 4.23 and Table 4.21). This was also true for the progression of disability from DSS 4 to DSS 7, and from DSS 6 to DSS 7 (Confavreux *et al* 2000). This could be interpreted as follows: the rate of progression of irreversible disability from the assignment of DSS 4 is not affected by the presence or the absence of relapses preceding onset of the chronic progressive phase. Confirmation can be found by looking at the influence of current age on the course of multiple sclerosis: age at onset of the progressive phase is similar in primary and secondary progressive multiple sclerosis. It is therefore unaffected by the presence or the absence of relapses preceding disease progression.

The same material allows assessment of the possible influence of superimposed relapses during either the primary or secondary phase (Figure 4.24 and Table 4.22; Confavreux *et al* 2000). Progression of irreversible disability from the assignment of DSS 4 to DSS 6 in the cases with either a primary or secondary progressive course was similar whether or not relapses were superimposed on the progressive phase. Paradoxically, the time from the assignment of DSS 4 to DSS 7, and from DSS 6 to DSS 7, was longer when relapses occurred on the background of progression than when there were no relapses. At the very least, it appears as though the rate of irreversible progression of disability from the assignment of DSS 4 is unaffected by relapses occurring during the progressive phase. Therefore, the evidence is for dissociation between relapses and progression in multiple sclerosis. These results match and extend those from other large studies on the natural history of multiple sclerosis. Data from the London, Ontario, Multiple Sclerosis Cohort show that, by comparison with primary progressive multiple sclerosis, patients with secondary progressive disease take longer to reach end points when survival curves are drawn from the time of disease onset, but a shorter interval when these are taken from onset of the progressive phase (D.A. Cottrell *et al* 1999a; Kremenchutzky *et al* 1999). The same group also showed that the survival curves are almost identical when primary progressive forms with superimposed relapses (progressive relapsing multiple sclerosis) are compared with those without (primary progressive multiple sclerosis *sensu stricto*) with respect to the time from onset to the assignment of DSS 6, DSS 8 and death (Kremenchutzky *et al* 1999). Similar conclusions have been reached by others studying primary progressive forms of multiple sclerosis for the time to DSS 6 (Andersson *et al* 1999).

These results from the Lyon, France, cohort have been reached by dichotomizing the status of relapses as present or not. When analysing the possible influence of relapses at onset and during the early years of the disease, similar results are obtained when the degree of recovery, time to the second relapse, and the number and frequency of episodes are considered (Figure 4.25). For instance, time to a second neurological episode positively influences median times from onset of multiple sclerosis to the assignment of DSS 4, DSS 6 and DSS 7 (Confavreux *et al* 2003). Similar observations have been made in many other series (V.A. Clark *et al* 1982; Confavreux *et al* 1980; Ebers 1998; Fog and Linnemann 1970; Hyllested 1961; Kantarci *et al* 1998; Kurtzke *et al* 1977; Leibowitz and Alter 1973; McAlpine 1961; Midgard *et al* 1995; Minderhoud *et al* 1988; R. Müller 1949; Phadke 1987; 1990; S. Poser and Hauptvogel 1973; 1986; Riise *et al* 1992; Runmarker and

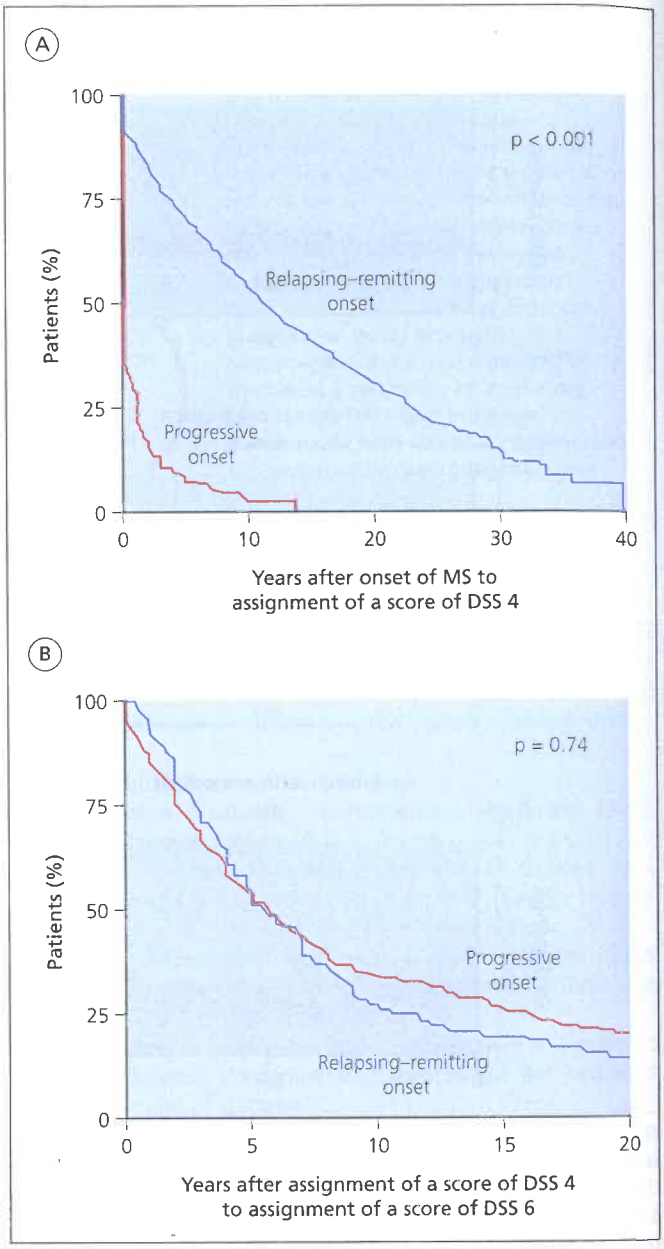


Figure 4.23 (A) Kaplan–Meier estimates for the time from onset of multiple sclerosis to the assignment of DSS 4. (B) Time from assignment of DSS 4 to DSS 6 among 1844 patients according to the initial course of the disease. Adapted from Confavreux *et al* (2000).

Table 4.21 **Kaplan–Meier estimates of the time from onset of multiple sclerosis to the onset of irreversible disability, and of the time course of irreversible disability among 1844 patients with multiple sclerosis, according to the initial course of the disease.[a] Adapted from Confavreux *et al* (2000)**

Variable	Relapsing–remitting onset		Progressive onset		p value[b]
	Number of patients (n = 1562)	Median time (95% CI) in years	Number of patients (n = 282)	Median time (95% CI) in years	
Time from onset of multiple sclerosis to assignment of a score of DSS 4	1562	11.4 (10.5–12.3)	282	0.0	<0.001
Time from onset of multiple sclerosis to assignment of a score of DSS 6	1562	23.1 (20.1–26.1)	282	7.1 (6.3–7.9)	<0.001
Time from onset of multiple sclerosis to assignment of a score of DSS 7	1562	33.1 (29.2–37.0)	282	13.4 (11.0–15.9)	<0.001
Time from assignment of a score of DSS 4 to assignment of a score of DSS 6	755	5.7 (4.9–6.4)	271	5.4 (4.3–6.6)	0.74
Time from assignment of a score of DSS 4 to assignment of a score of DSS 7	755	12.1 (10.0–14.2)	271	12.0 (10.1–13.9)	0.70
Time from assignment of a score of DSS 6 to assignment of a score of DSS 7	426	3.3 (2.8–3.9)	169	4.0 (2.9–5.1)	0.48

a Kurtzke Disability Status Scale (DSS) was used to determine the extent of disability.
b p values were calculated using the log-rank test.

Andersen 1993; Thygesen 1949; Trojano *et al* 1995; Weinshenker *et al* 1989a; 1989b; 1991a; 1991b). The originality of the French study is that it assessed the possible influence of the same clinical variables on the progression of irreversible disability from the time of assignment of DSS 4 to DSS 6, and also from DSS 4 to DSS 7 and DSS 6 to DSS 7 (Confavreux *et al* 2003). None of these variables remained predictive of the time course of disability past this point (Figure 4.25). Progression of irreversible disability is seemingly 'amnesic' with respect to the clinical characteristics of relapses that occurred during the initial stages of the disease. More generally, long-term progression of irreversible disability is mainly relapse dissociated and progression driven. These observations are reminiscent of those regarding sex and age at onset of multiple sclerosis in the Gothenburg, Sweden, series: both variables showed a correlation with prognosis when analysed from the onset of multiple sclerosis but not when the analyses were repeated taking 5 years after onset as the starting point (Runmarker and Andersen 1993).

All these observations have been collected using statistical analysis of groups of patients with multiple sclerosis. They are consistent with that claimed for individuals in the 1970s. From his prospective analysis of 73 patients, Fog concluded that the two components of the clinical course of multiple sclerosis are mutually independent. Relapses occur in an unpredictable way. Their frequency varies between individuals but also within a given individual. By contrast, the clinical progression of the neurological deficit can be subjected to mathematical analysis. In an individual patient, it is often very constant in degree and its slope decisive for prognosis. Relapses can be superimposed above the process of progression, but progression apparently pursues its course independent of the individual relapses.

> *To me at least, it seems strange that such a [steady progression] ... could be explained solely by the summation of single attacks. ... It seems therefore reasonable to believe that the phase of progression represents another biological process than the attack.*
>
> Fog and Linnemann (1970)

Course and prognosis: an age-dependent process

The influence of age on the course and prognosis of multiple sclerosis has been much studied, allowing the conclusion that patients with a late onset of disease tend to follow a primary progressive course whereas the majority of those developing symptoms earlier show an initial exacerbating–remitting pattern but with a constant rate of conversion to secondary progressive disease throughout the course of the illness (McAlpine and Compston 1952; A.R. McLean and Berkson 1951; R. Müller 1949; 1951).

More recent studies suggest that age at onset of the relapsing–remitting phase is equivalent in patients later classified either as having relapsing–remitting or secondary progressive multiple sclerosis at the time of study (Confavreux 1977; Confavreux *et al* 1980; Fog and Linnemann 1970; Leibowitz and Alter 1973; S. Poser 1978). In their 73 patients with multiple sclerosis, Fog and Linnemann (1970) found that mean age at onset of the relapsing–remitting phase was 28.5 years, and that this was similar in those remaining with relapsing–remitting multiple sclerosis (27.5 years) or converting to the secondary progressive phase (28.8 years). For onset of the progressive phase, they found a figure of 36.3 years, with no difference between

white matter (D.-L. Yao *et al* 1994). There is ambiguity concerning the nature of concentric bands of myelin – the view being expressed that these are either areas of preserved myelin (D.-L. Yao *et al* 1994) or bands of remyelination (G.R. Moore *et al* 1985). Our position, in agreement with previous studies (Courville 1970), is that the basic mechanism operating within these lesions is hypoxia-like tissue injury, the concentric bands of myelin resulting from hypoxic preconditioning of the tissue (Stadelmann *et al* 2005).

The 'preactive' lesion

The existence of preactive lesions has been postulated on the basis of studies correlating MRI appearances and tissue pathology. These observations depend largely on evidence from a single patient who died in the active stage of multiple sclerosis (De Groot *et al* 2001). There were many areas of MRI abnormality that – on the basis of pathological features – can be subsumed under one of the categories described above. Some areas of MRI abnormalities, however, revealed only inflammation, some blood–brain barrier damage and oedema, and a reduction in myelin density. It was suggested that these lesions represent stages of plaque development that precede the overt dissolution of myelin sheaths. Applying their scheme of multifactorial cluster analysis, Gay *et al* (1997) had previously reached similar conclusions. They also postulated the existence of an ultra-early stage in development of the lesion in multiple sclerosis characterized by a moderate degree of inflammation, microglia and complement activation, some reduction of myelin density, and few myelinated fibres in the initial stages of dissolution. It seems likely that such an initial stage of lesion formation does exist. It is, however, highly unlikely that all brain lesions characterized by T_2-weighted MRI abnormalities in which histological analysis reveals myelin pallor and some diffuse inflammation and oedema are preactive plaques. Most importantly, although such abnormalities are relatively frequent in brain tissue from multiple sclerosis, more or less identical changes occur in other inflammatory brain diseases and in conditions not followed by the development of demyelinated plaques. In addition, areas of Wallerian tract degeneration and remyelinated shadow plaques may show pathological features very similar to those characterized as the hallmarks of preactive multiple sclerosis plaques. Thus, the identification of a preactive lesion in multiple sclerosis requires more than MRI signal abnormality and inflammation, oedema and myelin pallor expressed as a T_2-weighted MRI signal abnormality. Identification of the earliest stages of demyelination still requires evidence for active myelin destruction and/or oligodendrocyte damage with myelin debris in macrophages.

IMMUNOPATHOLOGY OF INFLAMMATION

Having weighed the evidence, we conclude that the conduit through which all players involved in the pathogenesis of lesions in multiple sclerosis must pass is the process of inflammation. This is pivotal and the motor that drives forward the disease process. Our own view is that despite the lack of evidence implicating one or more specific candidate autoantigens or microbial triggers, that process is sustained by autoimmunity (see Chapter 11). It follows that special attention must be given to the description of cells making up the inflammatory infiltrates, the molecules on which their passage into the brain parenchyma depends, and the factors that determine their effector mechanisms and regulation.

Inflammation can be driven by an immune-mediated process or may occur as a secondary consequence of tissue injury. Immune-mediated inflammation is mediated by cells of the adaptive immune system such as T and B lymphocytes, and is thus reflected by their presence within lesions. In addition, cytokines produced by the lymphocytes stimulate effector cells such as macrophages and microglia to express and produce molecules involved in propagation and regulation of the immune response or in the induction of tissue injury. Reactive inflammation, which occurs as a response to tissue injury, is mainly reflected by activation of cells involved in innate immunity, which in the central nervous system consist mainly of macrophages, microglia and possibly dendritic cells. Activation antigens expressed under these conditions are mainly those involved in phagocytosis and antigen presentation. Recruitment of lymphocytes in such lesions is sparse or absent. It has, however, to be noted that this simple distinction between immune-driven and reactive inflammation is far from absolute and a broad overlap exists between these two conditions.

Inflammation and disease activity

Chronic persistent inflammation in the central nervous system is one of the most characteristic pathological features of multiple sclerosis. Inflammation is not restricted to the areas of demyelination but also affects large parts of the so-called normal white and grey matter and, to a lesser extent, the meninges. The density of inflammatory infiltrates in general is higher within demyelinated plaques compared with the surrounding white matter. Whether inflammation is a primary event in the evolution of demyelination and plaque formation or, conversely, merely a reaction to tissue injury resulting from alternative non-inflammatory mechanisms has been disputed for many years. Our position is that the various arguments favour the primary role of the inflammatory process, although the question has not been formally settled to the satisfaction of every commentator.

Inflammation, signified by T and B lymphocyte infiltrates, is regularly present during ongoing disease activity. Very exceptionally has active demyelination been described in the absence of inflammation (Guseo and Jellinger 1975). In such instances, lack of inflammation was defined by the absence of perivascular infiltrates. However, these sparse accounts based on morphology did not make use of immunocytochemical markers for the identification of inflammatory cells, and recent data show that diffuse parenchymal infiltration by T cells can occur in the absence of overt perivascular infiltrates (see below). In addition, those cases showing very little or no perivascular inflammatory infiltrates had all been treated with immunosuppressants. Using serial MRI, new clinical exacerbations are usually associated with focal blood–brain barrier damage (R.I. Grossman *et al* 1988; D.H. Miller *et al* 1988b). Confirmation that this is associated with brain inflammation is provided by biopsy or autopsy studies (Estes *et al* 1990; Katz *et al* 1993; Nesbit *et al* 1991).

As discussed below, the inflammatory reaction in active multiple sclerosis lesions is associated with local upregulation of immunoregulatory molecules such as histocompatibility antigens,

adhesion molecules, cytokines or chemokines. By analogy with the immunopathology of experimental models of demyelination (see Chapter 11), these findings strongly endorse our interpretation that an immunologically driven inflammatory response – at least in the majority of patients with multiple sclerosis – is the primary pathogenetic event. This view is further supported by serial brain biopsies performed in a single patient with the Marburg type of acute multiple sclerosis. Whilst the first biopsy showed a purely inflammatory disease of the white matter, a second biopsy of the same lesion performed 76 days later revealed large confluent demyelinated lesions (Bitsch *et al* 1999).

The primary nature of inflammation in the plaques of multiple sclerosis has recently been challenged in a study describing the pathological features of a brainstem lesion in a 13-year-old patient with chronic relapsing multiple sclerosis dying a few hours after onset of brainstem symptoms. In this lesion a decrease in myelin staining intensity and massive oligodendrocyte apoptosis were described, occurring in the absence of inflammation (Barnett and Prineas 2004). From these observations it was concluded that oligodendrocyte injury is the primary event in plaque formation, followed by a secondary inflammatory reaction. Although this study is based on a very careful neuropathological description, there may be alternative explanations for this finding. A role of inflammation in these lesions cannot altogether be excluded, since some perivascular inflammatory infiltrates were present and a detailed analysis of lymphocyte subsets within the lesions was not performed. In addition, the patient was treated with high-dose corticosteroids, which may downregulate the inflammatory response. Alternatively, as will be discussed below, very similar lesions with oligodendrocyte apoptosis can be found regularly in a subset of multiple sclerosis patients in the presence of inflammation (pattern III: Lucchinetti *et al* 2000). However, an identical pattern of myelin and oligodendrocyte pathology is also found in acute white matter ischaemia or hypoxia in the absence of an inflammatory response (Aboul Enein *et al* 2003). Fulminating brain diseases are frequently complicated by brainstem hypoxia, occurring either as a result of herniation damage or due to systemic preterminal complications. To us, these seem not to have been excluded in the study of Barnett and Prineas (2004).

Cellular composition of inflammatory infiltrates

As noted in the earliest studies of multiple sclerosis pathology, inflammatory infiltrates are mainly composed of mononuclear cells (Figure 12.3). In active multiple sclerosis lesions, inflammatory cells are present in the perivascular space and also dispersed throughout the central nervous system parenchyma. This holds true for lymphocytes as well as macrophages (Prineas and Wright 1978). However, B lymphocytes and plasma cells are more concentrated in the perivascular space and meninges, while parenchymal infiltration is relatively rare.

T lymphocytes

The vast majority of infiltrating lymphocytes are T cells (Nyland *et al* 1982; Traugott *et al* 1983a; 1983b). Immunocytochemical attempts to differentiate lymphocyte subsets in multiple sclerosis lesions have proved controversial. All investigators agree that both $CD4^+$ (helper) and $CD8^+$ (suppressor/cytotoxic) T lymphocytes are present in the lesions but some claim that the dominant cell population present in the active plaque is the CD8 cell (Booss *et al* 1983; Gay *et al* 1997; Hayashi *et al* 1988) and others that it is the CD4 T lymphocyte (Traugott *et al* 1983a; 1983b). The most recent studies on this topic (Babbe *et al* 2000; Gay *et al* 1997) show that T-cell infiltrates within the perivascular inflammatory infiltrates are composed of equal numbers of $CD4^+$ and $CD8^+$ cells. In contrast, the tissue infiltrates are invariably dominated by $CD8^+$ T cells, irrespective of clinical disease type (acute or chronic multiple sclerosis), or activity of the lesions. Analysing the T-cell receptor of single cells within lesions by single cell polymerase chain reaction showed that, on average, 65% of $CD8^+$ but only 25% of $CD4^+$ cells were clonally expanded (Babbe *et al* 2000), indicating that these cells may have recognized specific antigen within the lesions. In acute multiple sclerosis a high proportion of $CD8^+$ T cells also express granzyme B, indicating their activation as cytotoxic effector cells (Figure 12.3). The T-cell infiltrates in lesions are associated with expression of class I MHC molecules on infiltrating leucocytes, but also on astrocytes, oligodendrocytes and axons (Höftberger *et al* 2004). Furthermore, using confocal laser microscopy, the attachment of granzyme B-positive T cells on oligodendrocytes and axons in actively demyelinating lesions is frequently encountered. Taken together, these data suggest that activated cytotoxic class I MHC-restricted T cells play a major role in the propagation of inflammation and the induction of tissue injury in multiple sclerosis (Neumann *et al* 2002).

Cytotoxic class I MHC-restricted T cells are, however, not the only immune cells present in lesions. Clonal expansion of CD4 lymphocytes (Babbe *et al* 2000) suggests that these cells are also driven by the recognition of specific antigen. Furthermore, B lymphocytes and plasma cells accumulate and undergo clonal expansion in the perivascular and meningeal tissues in the chronic lesions of multiple sclerosis (G.P. Owens *et al* 2001). This suggests that inflammation in multiple sclerosis is driven both by class I and class II restricted T cells. In addition, there appears to be a significantly lower incidence of CD45R (putative suppressor/inducer) cells in active compared with inactive multiple sclerosis lesions or other inflammatory diseases of the nervous system (Hayashi *et al* 1988; Sobel *et al* 1988), indicating dysregulation of the immune response within the plaques.

Experimental and clinical immunological studies reveal that particular T-cell receptor subtypes may be of fundamental importance in T-cell mediated autoimmunity (for review see Lassmann and Vass 1995; Wekerle *et al* 1994). T cells carry a dimeric receptor composed either of $\alpha\beta$ or alternatively $\gamma\delta$ chains (see Chapters 3 and 11). $\alpha\beta$ T cells generally dominate in the mature immune system and characterize the vast majority of T lymphocytes in blood, lymph nodes and spleen. These cells are mainly responsible for specific immune functions involved in the elimination of pathogens. The $\gamma\delta$ T lymphocyte is mostly found in the lymphatic system of the gut and other mucosal tissues. The majority are responsive to highly conserved epitopes of cellular proteins, such as heat-shock proteins, that are homologous throughout a wide spectrum ranging from bacteria to mammalian cells. By these relatively nonspecific mechanisms, $\gamma\delta$ T cells in humans are involved in early defence against bacteria and other cellular pathogens (Kaufmann 1990).

In addition to these principal differences in T-cell receptor chain usage of different T-cell populations, recent studies suggest that, even within a given αβ or γδ T-cell population, specific subtypes of T lymphocytes may be selected in the induction of autoimmunity. Thus, most autoreactive T lymphocyte lines directed against myelin basic protein that are able to transfer autoimmune inflammation after intravenous transfer in mice and rats, express the T-cell receptor chain Vβ8.2 (Wekerle *et al* 1994). These data suggest that T-cell autoimmunity may be restricted to a small population of very specific T-cell receptor subtypes – an observation that, although as yet unproven, has nevertheless stimulated novel therapeutic approaches.

For reasons discussed above, several studies have focused on the characterization of T-cell receptors in multiple sclerosis lesions. The vast majority found in multiple sclerosis plaques carry (as expected) the αβ T-cell receptor. Some results suggest clonal expansion of certain γδ T-cell subsets in multiple sclerosis plaques (Oksenberg *et al* 1993), but this has not been confirmed by others (Birnbaum and van Ness 1992; Wucherpfennig *et al* 1992a). In addition to αβ T cells, a variable number of γδ T lymphocytes can also be found in the lesions of multiple sclerosis (Selmaj *et al* 1991a; Wucherpfennig *et al* 1992b). Because γδ T cells are capable of specifically lysing oligodendrocytes *in vitro* (M.S. Freedman *et al* 1991), their presence may be of significance in the pathogenesis of lesions in multiple sclerosis.

B lymphocytes

Besides T lymphocytes, a variable but low number of B cells are found within plaques (Prineas and Wright 1978; Figure 12.3). The number of antibody-producing cells is, in general, very low in the lesions of acute multiple sclerosis or those that arise during early bouts in the chronic phase of the disease. However, their absolute and relative numbers are much higher in the typical case of chronic multiple sclerosis (Ozawa *et al* 1994). As with all other inflammatory cells, B lymphocytes are more evident in active than inactive lesions (Esiri 1977). Relatively little is known about the antigen specificity of B lymphocytes and plasma cells in multiple sclerosis lesions. Clonal expansion of B-cell populations in the lesions is suggested by the antibody spectrum characterized in cerebrospinal fluid or the tissue itself, and some data suggest that this process may be driven by specific antigen(s) (Gilden *et al* 2001). A multiple sclerosis-specific pattern of antibody reactivity has so far not been identified, even using random screening with peptide libraries (Archelos *et al* 1998; 2000; Cortese *et al* 1998; 2001; Gilden *et al* 2001; Jolivet Reynaud *et al* 1999). However, in a single unconfirmed study, many of these B lymphocytes and plasma cells were found to produce antibodies against myelin basic protein (Gerritse *et al* 1994).

Macrophages and microglia

The vast majority of haematogenous cells within multiple sclerosis plaques are monocytes and macrophages (Adams and Poston 1990; Adams *et al* 1989; Babinski 1885b; Newcombe and Cuzner 1994; Traugott *et al* 1983a; 1983b). These cells are dispersed throughout the whole lesion and are especially prominent in actively demyelinating plaques (Figure 12.2). In fact, the presence of early myelin degradation products in macrophages is at present the most reliable marker for ongoing demyelinating activity (Brück *et al* 1994). In addition, there is extensive uptake of low-density lipoproteins in macrophages and microglia within active lesions, which is probably important for the metabolism of lipids liberated in the course of active myelin destruction (Newcombe and Cuzner 1994). Besides these myelin degradation products, the expression of activation antigens in macrophages and microglia is a good marker for actively demyelinating plaques (Brück *et al* 1995; Ozawa *et al* 1994; Ulvestad *et al* 1994). Although the majority of macrophages within multiple sclerosis plaques are thought to come from blood-borne monocytes, resident microglia may be even more important in lesion development. Ramified activated microglia that express histocompatibility antigens, adhesion molecules or markers of activated peripheral macrophages are present mainly in the white or grey matter that surrounds actively demyelinating as well as inactive lesions. Detailed quantitative analysis of the inflammatory response in lesions at different stages of development reveals that microglial activation and demyelination precede recruitment of the vast majority of haematogenous cells into the lesions (Gay *et al* 1997). Furthermore, the profiles of CCR1 and CCR5 chemokine receptor expression in the lesions of multiple sclerosis suggest that, in the earliest stages, ramified microglia mediate demyelination, digest debris and are then transformed into macrophage-like cells. Conversely, haematogenous macrophages make a small contribution to lesion formation (Trebst *et al* 2001). Irrespective of their primary origin, macrophages and microglia in multiple sclerosis lesions express a variety of molecules required for propagation and regulation of the inflammatory response, and for the induction of tissue injury. These include costimulatory molecules (Gerritse *et al* 1996; Windhagen *et al* 1995), MHC antigens (Esiri and Reading 1987), Toll-like receptors (Bsibsi *et al* 2002), macrophage colony-stimulating factor (K. Werner *et al* 2003), adhesion molecules (Peterson *et al* 2002), annexin 1 (Probst-Cousin *et al* 2002), Fc receptors (Ulvestad *et al* 1994), inducible nitric oxide synthase (J.S. Liu *et al* 2001), proteases (Anthony *et al* 1997; Cossins *et al* 1997; Hallpike *et al* 1970a; Maeda and Sobel 1996), different ion channels (Craner *et al* 2005) as well as many different cytokines (Woodroofe and Cuzner 1993) and cytokine receptors (Bonetti and Raine 1997; Hulshof *et al* 2002; Ramanathan *et al* 2001). Furthermore, MRI studies indicate the presence of free radicals in macrophages at the edge of expanding demyelinating lesions (Powell *et al* 1992). Besides these cell types characteristic of multiple sclerosis, some lesions may be infiltrated by mast cells (Olsson 1974; Toms *et al* 1990), granulocytes (Lucchinetti *et al* 2002) or cells expressing dendritic cell markers (Plumb *et al* 2003). Granulocytes and, in particular, eosinophils are mainly present in lesions with profound antibody and complement deposition and this is particularly evident in the lesions of Devic's disease (Lucchinetti *et al* 2002).

Inflammation-induced reaction of local tissue components in demyelinating plaques

A distinction can be made between those molecular signals and mediators of tissue injury and repair that are expressed constitutively in the central nervous system and others that result

from inflammation and other inaugural components of the disease process. In turn, their identification and characterization may illuminate important aspects of the pathogenesis.

Major histocompatibility complex antigens

A very characteristic feature of inflammatory lesions in the brain and other organs is the expression on local tissue components of histocompatibility antigens and adhesion molecules. T lymphocytes do not recognize soluble molecules or peptides but react to antigen, presented in the context of major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells. Thus, the distribution of MHC antigens within tissue pinpoints the possible sites where T lymphocytes are able to recognize specific antigen in propagation of the inflammatory reaction. There is now good agreement that the vast majority of cells in the central nervous system that express class II MHC antigens are resident microglia and haematogenous macrophages (Boyle and McGeer 1990; Cuzner *et al* 1988; Esiri and Reading 1987; Traugott *et al* 1983a). In addition, some immunoreactivity has been described on astrocytes and endothelial cells (S.C. Lee *et al* 1990; Ransohoff and Estes 1991; Sobel and Ames 1988; Traugott and Lebon 1988a), although this has not been reproduced by others (Bo *et al* 1994). The situation is different for class I MHC molecules. *In vitro*, their expression can be induced on virtually all cell types upon appropriate immunological stimulation (Neumann *et al* 2002). *In vivo*, class I antigens can be detected in the normal central nervous system on endothelial cells and a subpopulation of microglia. Their expression is variable in different inflammatory conditions. For example, abundant expression on neurons is seen in paraneoplastic encephalitis or Rasmussen's encephalitis (Bien *et al* 2002). We have found MHC class I mainly in active lesions of acute and, to a lesser extent, chronic multiple sclerosis diffusely expressed (as in normal brain) not only on infiltrating inflammatory cells but also endothelial cells, microglia, oligodendrocytes, astrocytes and axons (Höfteberger *et al* 2004). Thus, it seems that all cellular elements of the central nervous system have the potential to present antigen to class I MHC restricted T cells thereby exposing themselves to potential antigen-specific T-cell mediated cytotoxicity.

Antigen-specific activation of T lymphocytes requires the additional interaction of costimulatory molecules. In their absence, the interaction of T lymphocytes with antigen-presenting cells may lead to anergy and restriction of the inflammatory response rather than proliferation. In the lesions of multiple sclerosis, macrophages express CD40 and B7-2, while CD40 ligand and B7-1 are mainly present on lymphocytes (Gerritse *et al* 1996; Windhagen *et al* 1995). If the expression of these costimulatory molecules is instrumental in perpetuation of the inflammatory response, they may offer themselves as potential targets for immunosuppressive treatment.

Adhesion molecules

Because T lymphocytes can pass the intact blood–brain barrier in a non-antigen-specific manner (Hickey *et al* 1991; Wekerle *et al* 1986), the role of adhesion molecules that guide the traffic of leucocytes through body compartments and steer inflammatory cells to lesions has been much studied (Shimizu *et al* 1992). In normal brain vasculature, adhesion molecules are expressed only on endothelial cells at very low levels (Lassmann *et al* 1991b; Male *et al* 1990). Thus, selected leucocyte subsets, in particular activated T lymphocytes, appear to bind these adhesion molecules and attach to the endothelial surface before migrating across the blood–brain barrier. When an inflammatory focus is initiated, adhesion molecules on cerebral endothelial cells and local cellular constituents are upregulated and may then function as adhesion partners for a large variety of secondary effector cells (Cannella and Raine 1995).

Migration of inflammatory cells through the endothelial barrier involves a variety of different consecutive steps (Shimizu *et al* 1992). Initially, an interaction of selectins with carbohydrate moieties induces a loose interaction of leucocytes with endothelia, involving a slow rolling motion of inflammatory cells along the luminal surface of endothelial cells. Next, the interaction between adhesion molecules of the immunoglobulin supergene family with integrins results in firm binding of the inflammatory cells at the luminal surface of endothelial cells. This adhesion, together with the expression of proteolytic enzymes on the surface of inflammatory cells, allows the latter to pass either between or through channels of the endothelial cells and reach the perivascular space (Engelhardt and Wolburg 2004). However, in brain vessels, which are sealed by the blood–brain barrier, the transendothelial route appears to be preferred, while paracellular migration, associated with opening of tight junctions, is an unusual portal of entry (Engelhardt and Wolburg 2004; Raine *et al* 1990; Wisniewski and Lossinsky 1991).

Only some active adhesion partners have been identified in the lesions of multiple sclerosis (Cannella and Raine 1995). For example, E-selectin (Washington *et al* 1994), intercellular adhesion molecule 1 (ICAM-1; Sobel *et al* 1990; Tsukada *et al* 1994), vascular cell adhesion molecule 1 (VCAM-1; Dore-Duffy *et al* 1993) and unclassified vascular addressins (Raine *et al* 1990) are expressed on endothelial cells in active plaques. That said, Peterson *et al* (2002) found VCAM on activated microglia cells in close contact with oligodendrocytes rather than endothelial cells. Despite this controversy, blockade of the interaction between VCAM and its specific ligand through an antibody against α4 integrin effectively ameliorates the inflammatory reaction in the active stage of multiple sclerosis (Tubridy *et al* 1999). Other molecules that have been suggested to be involved in recruitment and activation of inflammatory cells are CD97 and its binding partner CD55. They are expressed on endothelial cells and leucocytes in the lesions of multiple sclerosis, but their functional role is so far undetermined (Visser *et al* 2002). In addition, other molecules involved in cell–cell interactions, such as fibronectin (Sobel and Mitchell 1989), urokinase, plasmin activator receptor and activated complement components, have been identified on vessels in active multiple sclerosis plaques (see below: D.A.S. Compston *et al* 1989; Washington *et al* 1994), resulting in major changes in the composition of the extracellular matrix in the lesions of multiple sclerosis in comparison with normal brain (Sobel 2001).

Chemokines

In addition to adhesion molecules, a set of newly discovered chemokines appears to be of major importance in the recruitment of inflammatory cells to brain lesions (Luster *et al* 1998).

Chemokines are small peptides liberated by inflammatory cells as well as by local parenchymal cells in response to inflammatory stimuli. Delivered into the extracellular space, they are able either directly, or by modifying the expression and affinity of adhesion molecules, to attract effector cells (in particular, monocytes, macrophages or granulocytes). These then follow the concentration gradient of secreted chemokines (Luster *et al* 1998). Indeed, abundant mRNA for chemokines has been detected in the lesions of multiple sclerosis (Schlüsener and Meyermann 1993). The more detailed analysis of chemokines and their receptors provides some interesting clues to the pathogenesis of inflammation in multiple sclerosis. The dominant T-cell population recruited into lesions is CD3/CCR5/CXCR3 positive and this profile apparently reflects a population of activated memory T cells (Goldberg *et al* 2001; J. Simpson *et al* 2000a; Sorensen *et al* 1999; 2002), whereas macrophages, recruited into the lesions from the circulation, are mainly CCR1/CCR5 positive (Sorensen *et al* 1999; Trebst *et al* 2000). A variety of different chemokines, which may interact with these receptors, has been described in the plaques of multiple sclerosis, the most important being MCP-1, MIP-1a, RANTES and IP-10 (Balashov *et al* 1999; McManus *et al* 1998; J.E. Simpson *et al* 1998; 2000b; for review see D.Huang *et al* 2000). Besides macrophages and microglia cells, astrocytes are an important source of chemokines in the central nervous system (McManus *et al* 1998).

Taken together, these data suggest that chemokines are key molecules in the process of recruiting inflammatory cells into the lesions of multiple sclerosis. Their blockade by small molecules could become a very attractive target for anti-inflammatory therapies. In addition, differences in the spectrum of chemokines expressed in lesions may reflect variation in the immunological mechanisms of inflammation and account for the detailed composition of inflammatory infiltrates between groups of patients. As an example, the high prevalence of granulocytes and eosinophils in active lesions of Devic's type of neuromyelitis optica is associated with a chemokine receptor expression, which is typical for Th2 mediated inflammatory responses (Lucchinetti *et al* 2002).

Local expression of stress proteins

Stress proteins comprise a variety of polypeptides that are synthesized by cells in response to injury. They have diverse functions, including the regulation of gene expression, intracellular traffic and delivery of proteins, and the degradation of denatured or damaged protein molecules. Inflammation is one mechanism that can be responsible for local upregulation of stress proteins (D'Souza *et al* 1994).

Since they are essential for cell survival during metabolic stress, it is not surprising that stress proteins are highly conserved in evolution and present in nearly identical forms from bacteria to mammals. The immune system has utilized this high degree of conservation for a nonspecific but effective defence mechanism (Cohen and Young 1991; Kaufmann 1990). γδ T lymphocytes use recognition of these highly conserved stress proteins to destroy microorganisms that have invaded the organism. However, immune responses directed against stress proteins may become dysregulated and augment inflammation through autoimmune mechanisms. It has been proposed that this sequence operates in the lesions of multiple sclerosis (Freedman *et al* 1991; Selmaj *et al* 1991d). γδ T lymphocytes, enriched in some active multiple sclerosis lesions, apparently by local clonal expansion (Wucherpfennig *et al* 1992b), lyse oligodendrocytes through heat-shock proteins 65 and 70 (Freedman *et al* 1991; Selmaj *et al* 1992). Another stress protein implicated in the pathogenesis of multiple sclerosis is αβ-crystallin, which appears to be the dominantly recognized autoantigen in a subgroup of patients with multiple sclerosis (Van Noort *et al* 1995). Although astrocytes express this protein within active and inactive lesions, its expression in oligodendrocytes is restricted to areas of ongoing demyelination (Bajramovic *et al* 1997).

In general, the patterns and intensity of expression appear to depend upon the type of stress protein as well as severity and stage of the inflammatory reaction. Whilst stress proteins are ubiquitously found in different cellular elements, they are not expressed to a major degree in normal tissues of the central nervous system. However, pronounced upregulation is typical of the inflammatory lesions in multiple sclerosis. Here, the pattern is of selective heat-shock protein 65 and αβ-crystallin upregulation in oligodendrocytes (Selmaj *et al* 1992; Van Noort *et al* 1995). Conversely, other molecules such as heat-shock proteins 27, 70 and 90 (Hans Lassmann, unpublished observation) or c-fos (J.S. Yu *et al* 1991) are mainly present in astrocytes and inflammatory cells. Their pattern of expression and possible release into the extracellular space are consistent with the hypothesis that stress proteins are recognized by specific T-cell mediated autoimmune reactions (Van Noort *et al* 1995). Although final proof for the involvement of such a mechanism in multiple sclerosis is still lacking, autoimmunity against these antigens could augment inflammation in established lesions and, by this mechanism, amplify the inflammatory process and its consequences. However, the upregulation of heat-shock proteins 65 and 70 in oligodendrocytes at the edge of active lesions may protect these cells against further damage in an expanding active lesion. This seems to be a major controlling mechanism in the formation of concentric demyelinating lesions in Balo's concentric sclerosis.

Cytokines in multiple sclerosis lesions

Immune-mediated inflammation, such as that present in multiple sclerosis, is influenced by a large battery of different cytokines (T. Olsson 1994). These are produced either by inflammatory cells or local tissue components. They mediate communication between inflammatory cells and local tissue constituents. Some are proinflammatory, activating certain components of the immune system and thereby propagating immune reactions and inflammation. For example, elevated levels of TNF-α in the cerebrospinal fluid of patients with multiple sclerosis correlate with disease activity and blood–brain barrier damage (Sharief and Thompson 1992). On the other hand, interleukin 4 (IL-4) and IL-10 may suppress delayed-type hypersensitivity reactions and downregulate T-cell mediated inflammation or macrophage activation (M.K. Kennedy *et al* 1992; Weinberg *et al* 1993). A similar action may also be induced by the more general immunosuppressive effect of transforming growth factor β (TGF-β; Johns *et al* 1991; Racke *et al* 1992). Furthermore, in addition to the induction of nonspecific tissue damage, molecules such as lymphotoxin (TNF-β; Selmaj *et al* 1991b), TNF-α (Selmaj and Raine 1988) and perforin

(Scolding *et al* 1990) may be directly involved in the destruction of oligodendrocytes and myelin. Conversely, cytokines – for example, insulin-like growth factor I (IGF-I) – may be essential for oligodendroglia survival and remyelination (Komoly *et al* 1992; see Chapter 9). Cytokine-like immunoreactivity has been detected in various cell types of the central nervous system. In particular, immunocytochemical analysis of inflammatory cells, astrocytes and microglia has demonstrated IL-1, IL-2, lymphotoxin, TNF-α, interferons (IFNs), IL-6 (Cannella and Raine 1995; Hofman *et al* 1989; Merrill 1992; Selmaj *et al* 1991a; Traugott and Lebon 1988b; 1988c) and IL-10, IL-12 and IL-18 (Canella and Raine 2004). In our view, immunocytochemical studies on these topics are difficult to interpret. Apart from technical problems relating to antibody specificity and avidity with possible central nervous system peptide cross-reactivity, no conclusions can be drawn about whether the cytokines are synthesized locally or taken up secondarily at the site of immunoreactivity. Polymerase chain reaction amplification reveals the presence of IL-1 in all active multiple sclerosis lesions, whereas mRNAs for IL-2, IL-4 or IL-10 are found in a minority of lesions (Schlüsener and Meyermann 1993; Wucherpfennig *et al* 1992a). Gene microarray analysis of multiple sclerosis lesions shows increased transcripts of genes encoding for IL-6, IL-13, IL-17, TNF-α and IFN-γ (Lock *et al* 2002; Mycko *et al* 2003; Tajouri *et al* 2003; Whitney *et al* 1999). Although there appears generally to be rather low expression of cytokine mRNAs, this may not be the case for chemokines such as IL-8 and MCP-1 (Schlüsener and Meyermann 1993). *In situ* hybridization studies reveal mRNAs for a variety of cytokines, including IL-1, IL-2, IL-4, IL-6 and IL-10, IFN-γ, TNF-α and TGF-β1 and -β2 (Bitsch *et al* 2000b; Werner *et al* 2002; Woodroofe and Cuzner 1993).

In summary, these studies describe the presence of many different cytokines and the expression of various cytokine receptors in the lesions of multiple sclerosis, apparently reflecting the chronic immunologically driven inflammatory process. Similar findings have been observed in other inflammatory neurological diseases (Canella and Raine 2004) and, so far, no pattern of cytokine expression specific for multiple sclerosis has been proposed. Cytokines seem to be more prominent in actively demyelinating compared with inactive lesions or 'normal' white matter, but a clear-cut differential pattern of expression, which allows different lesion stages to be distinguished, is not yet apparent.

Antibodies and complement components

As already mentioned, active lesions in multiple sclerosis are associated with pronounced damage to the blood–brain barrier, potentially allowing serum components such as antibodies or complement to enter the nervous system. In addition, B lymphocytes accumulate and may persist at the sites of active inflammation (Guseo and Jellinger 1975; Prineas and Wright 1978). These cells produce antibodies locally as reflected by the increased immunoglobulin index and oligoclonal bands in cerebrospinal fluid. IgG is mainly produced in the lesions of multiple sclerosis. Although some IgA- and IgM-producing plasma cells are also detected in lesions, they make a small contribution (Auff and Budka 1980; Bernheimer *et al* 1983; Esiri 1977; Mussini *et al* 1977). The number of B lymphocytes and plasma cells is variable and depends on stage of the disease and the activity of lesions (Prineas and Wright 1978). During lesional activity, the high number of infiltrating leucocytes includes an increased number of B lymphocytes compared with inactive lesions (Esiri 1977). However, there is also a major difference in the extent of B cell and plasma cell infiltration in lesions formed during initial bouts compared with those arising after several years of disease duration. In the former, infiltrates are mainly composed of T lymphocytes and macrophages, and only a minority (around 0.5–1%) of inflammatory cells actually produce immunoglobulins. Conversely, during late phases of the disease, the ratio of B to T lymphocytes is about 1:7 (Ozawa *et al* 1994). These stage-dependent changes in the composition of inflammatory infiltrates are also reflected in cerebrospinal fluid. Whereas in chronic multiple sclerosis, an elevated IgG index and oligoclonal pattern of immunoglobulins is typical, similar changes may be absent during the first or second bout of the disease. In active lesions, T cells and macrophages infiltrate the brain parenchyma, whereas B lymphocytes and plasma cells tend to remain in the perivascular space and meninges (Esiri *et al* 1989).

Immunoglobulin deposition, concentrated at sites of active myelin destruction and located either on activated macrophages or dressing the surface of myelin sheaths that are in the process of being destroyed, characterizes actively demyelinating plaques (Prineas 1985). Complement components are deposited in actively demyelinating lesions, mainly at the vessel walls (D.A.S. Compston *et al* 1989). In a subset of patients with multiple sclerosis, deposition of complement components, including the C9 neoantigen (a reliable marker for the lytic membrane attack complex), is present at the active edge of demyelinating plaques. In addition to lysosomal myelin degradation products in macrophages, C9 neoantigen is detected on disintegrating myelin sheaths (Lucchinetti *et al* 2000; Storch *et al* 1998a). Using biotinylated myelin oligodendrocyte glycoprotein as a probe, deposition of anti-myelin oligodendrocyte glycoprotein antibodies on disintegrating myelin has been described in lesions of acute multiple sclerosis (Genain *et al* 1999). Although complement appears mostly to enter through the damaged blood–brain barrier, additional local synthesis by macrophages and microglia is suggested. Complement inhibitory proteins are also present in multiple sclerosis lesions. For example, SP40 immunoreactivity is found in reactive astrocytes (E. Wu *et al* 1993). There is, however, no spatial relationship between the deposition of complement and the inhibitory proteins to suggest a functional role for the latter (Storch *et al* 1998a).

Blood–brain barrier alterations

Widespread blood–brain barrier damage has been demonstrated in multiple sclerosis lesions using post-mortem tracer studies (Broman 1964). Immunocytochemical studies on the distribution of serum proteins within lesions (Kwon and Prineas 1994; Tavolato 1975) indicate that the blood–brain barrier in plaques is impaired compared with the normal white and grey matter of patients with multiple sclerosis and controls. These pathological findings were not immediately consistent with some of the early MRI studies showing gadolinium-DTPA leakage restricted to active multiple sclerosis lesions (Kermode *et al* 1990; McLean *et al* 1993). As pointed out above, however, low levels of gadolinium-DTPA leakage can be detected in chronic lesions (Barnes *et al* 1991). And in experimental autoimmune encephalomyelitis,

gadolinium-DTPA is transported in such vessels in an energy-dependent manner (C.P. Hawkins *et al* 1990a; 1990b).

Infiltration of the vessel walls by inflammatory cells shows that the most intense leakage of the blood–brain barrier is in lesions with active inflammation (Gay and Esiri 1991), although intense perivascular deposition of serum proteins sometimes can also be noted around vessels that are devoid of inflammation. Whether such alterations are restricted to the vicinity of inflamed vessels is unresolved. Inflammatory infiltration of the vessel walls with perivascular accumulation of leucocytes and deposition of serum proteins is not necessarily associated with demyelination. The proposal that lesions with sparse inflammatory infiltration of the surrounding tissue and no myelin-containing macrophages represent early stages in the process of lesion formation is not fully convincing because, in experimental models of inflammatory demyelination, these features are rarely present beyond the first few hours of tissue injury. It is therefore likely that active inflammation in multiple sclerosis does not invariably result in myelin destruction. Indeed, studies of experimental autoimmune encephalomyelitis and other experimental models of inflammatory demyelination suggest that, in addition to the primary T-cell response that induces inflammation, additional immunological mechanisms are required for the establishment of demyelination (Lassmann *et al* 1988; Linington *et al* 1988).

Ultrastructural studies show increased endothelial pinocytotic vesicles, possibly reflecting enhanced permeability of the blood–brain barrier. The ultrastructure of endothelial tight junction systems has been considered normal (W.J. Brown 1978), but more detailed immunocytochemical analysis of occludin and ZO-1 proteins revealed focal discontinuities of tight junction ridges (Plumb *et al* 2002). Besides endothelial alterations, pathological changes of astrocytic foot processes are encountered (Rafalowska *et al* 1992), which may represent a footprint for the passage of inflammatory cells through the blood–brain barrier.

Vascular pathology

It is clear from the earliest histological descriptions of multiple sclerosis pathology that blood vessels play a central role in this disease. Thus, it is not surprising that interest in the pathogenesis of multiple sclerosis has often focused on possible essential vascular mechanisms (Courville 1968; Putnam 1935). These ideas eventually emerged as the concept of vasculomyelinopathy, in which a primary vascular lesion leads to myelin destruction (C.M. Poser 1987b). Vessels in active lesions may show features of vasculitis. Here, inflammation of the vessel wall is reflected by diffuse mononuclear cell infiltration, perivascular accumulation of leucocytes, leakage of serum proteins, upregulation of adhesion molecules on endothelial cells and some deposition of complement components on the luminal surface of the vessel wall. However, this accumulation of immune cells and mediators does not lead to vascular necrosis, intraluminal deposition of immune complexes and complement, or granuloma formation. Thus, it is different from the changes found in most other types of cerebral vasculitis (M.M. Brown and Swash 1989) and closely reflects the type of inflammation found in experimental models of delayed-type hypersensitivity reactions.

In rare instances, more pronounced acute vascular changes can be encountered. These consist of intraluminal platelet aggregation and thrombosis resulting in complete thrombotic occlusion of small inflamed veins and venules (Putnam 1935; Wakefield *et al* 1994). These alterations, however, are restricted to cases with extraordinarily severe inflammatory reactions. Such severe blood vessel changes may even result in blood extravasation, later marked by perivascular iron deposition within the affected tissue (Craelius *et al* 1982). That damage of cerebral endothelial cells occurs in a subset of patients with multiple sclerosis is further suggested by the presence of endothelial microparticles in plasma during active stages of the disease (Minagar *et al* 2001).

Vascular fibrosis is present in most cases of longstanding duration (Prineas and Wright 1978). Using ultrastructural criteria, many of the connective tissue channels that form in areas of vascular fibrosis show anatomical characteristics similar to those of lymphatics (Prineas 1979). Vascular fibrosis is not specific to the pathology of multiple sclerosis, but represents an alteration found in all types of chronic brain inflammation (Spielmeyer 1922). The degree of vascular fibrosis is most pronounced in the periventricular white matter and in lesions close to the pial surface of the spinal cord. Because inflammatory infiltrates are partly cleared through the cerebrospinal fluid (Cserr *et al* 1992; Weller *et al* 1992), vascular fibrosis may indeed facilitate the removal of inflammatory cells from chronically inflamed brain lesions.

Finally, inactive multiple sclerosis lesions contain a much higher density of vascular profiles compared with normal white matter. This is not only due to shrinkage and atrophy of the tissue, which by itself will result in a relative increase of vessel density, but reflects profound angiogenesis during the active phase of plaque formation (Ludwin *et al* 2001). In turn, this may result from local induction of vascular endothelial growth factor (VEGF) within active lesions (Proescholdt *et al* 2003).

Leucocyte destruction in multiple sclerosis lesions: a mechanism for clearance of inflammatory infiltrates

Experimentally, the majority of T lymphocytes invading the brain parenchyma do not leave the central nervous system during the clearance of inflammation but are destroyed *in situ* by programmed cell death (Pender *et al* 1991; Schmied *et al* 1993). Similarly, T cells with nuclear changes typical of apoptosis are found in active multiple sclerosis plaques, being most numerous in lesions from cases of acute or subacute multiple sclerosis (Figure 10.4: Ozawa *et al* 1994). However, the percentage of apoptotic T cells within multiple sclerosis lesions is much lower than that found in monophasic models of acute experimental autoimmune encephalomyelitis. This is not surprising because synchronous self-destruction of T cells cannot be expected in chronic inflammation, nor is it present in chronic models of experimental autoimmune encephalomyelitis. However, in acute disseminated leucoencephalomyelitis, very high numbers of apoptotic T lymphocytes are found within inflammatory infiltrates, comparable both in absolute and relative numbers to those found in acute experimental autoimmune encephalomyelitis (J. Bauer *et al* 1999).

We have only identified a single case – with primary progressive multiple sclerosis and receiving high-dose corticosteroid treatment despite the absence of relapses – where complete and

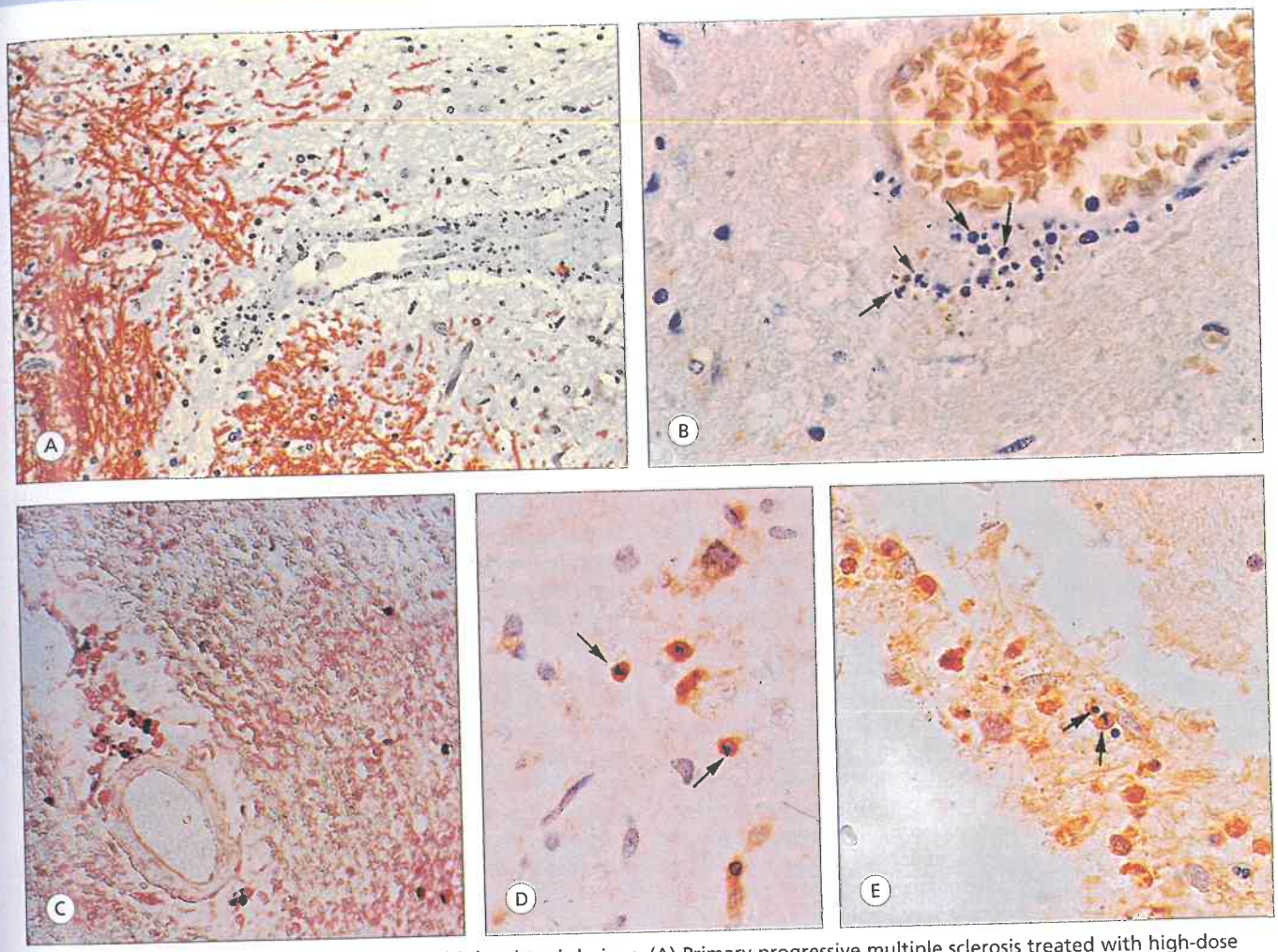


Figure 12.4 Apoptosis of T lymphocytes in multiple sclerosis lesions. (A) Primary progressive multiple sclerosis treated with high-dose steroids: immunocytochemistry for proteolipid protein. Most cells in the perivascular infiltrate at the lesional border and some in the central nervous system parenchyma (arrows) show apoptosis. ×100. (B) Typical appearances of apoptosis in perivascular inflammatory cells (arrows); ×300. (C) Chronic relapsing multiple sclerosis: *in situ* tailing for DNA fragmentation and immunocytochemistry with anti-CD3 (T lymphocytes). DNA fragmentation (black nuclei) in cells in the perivascular infiltrate and parenchyma. ×100. (D) Acute multiple sclerosis: immunocytochemistry with anti-CD3. Apoptotic cells identified by nuclear condensation (arrows) are labelled with a T-cell marker. ×300. (E) Primary progressive multiple sclerosis: immunocytochemistry with anti-CD3; perivascular infiltrate with numerous T cells (brown), some of which show nuclear condensation of apoptosis (arrows); ×300.

synchronous apoptosis of T lymphocytes was present both in the perivascular space and parenchyma (Figure 12.4). Because apoptosis of autoreactive T cells may be induced by steroids (Zettl *et al* 1995), our observation in this particular patient may reflect an effect of therapy.

Inflammatory cells in the lesions of multiple sclerosis express neurotrophins

Basic immunological studies tell us that inflammatory cells may also produce trophic cytokines, known to be essential in wound repair. Activated, and to a lesser extent resting, T and B cells and macrophages all produce neurotrophins, which stimulate neuronal survival *in vitro* (Kerschensteiner *et al* 1999; Moalem *et al* 2000). Neurotrophin expression is more pronounced in inflammatory cells within lesions than on adjacent neurons and glia. Neurotrophins may downregulate MHC expression in inflammatory lesions and thereby be involved both in downregulation of brain inflammation and the provision of immune privilege for the central nervous system (Flügel *et al* 2001b). Neurotrophins produced by activated leucocytes seem also to exert neuroprotective functions – at least in the context of brain trauma or ischaemia.

Brain-derived neurotrophic factor (BDNF) is highly expressed in lymphocytes and macrophages in actively demyelinating lesions. Receptors for this ligand are found on different cell types in the vicinity of lesions (Stadelmann *et al* 2002; Valdo *et al* 2003). Neurotrophins may be needed for remyelination, because myelin formation and repair require the local presence of macrophages (Kotter *et al* 2001). In addition, increased expression of nerve growth factor as well as its receptor, trkA, have been found in optic nerve lesions (Micera *et al* 1999).

Brain inflammation in multiple sclerosis: a summary

The following sequence is suggested as a summary of findings on the immunopathology of multiple sclerosis. The driving force for the inflammatory reaction appears to be a T-cell mediated immune reaction. The composition of leucocyte subsets is in accordance with the pattern of a delayed-type hypersensitivity reaction. Different subpopulations of T helper cells have been identified according to their cytokine profiles and functional properties (for review, see Clerici and Shearer 1993; Mosmann and Coffman 1989). Th1 cells predominantly secrete IFN-γ, IL-2 and TNF-α. Their major property is the recruitment and activation of macrophages at sites of inflammation. In contrast, Th2 cells mainly secrete IL-4 and IL-10 and their major task is to promote B-lymphocyte differentiation and antibody production. They can inhibit the Th1 reaction through the production of IL-10. In addition to these T helper subsets, the subpopulation of $CD8^+$ T lymphocytes may either be cytotoxic or mediate suppression of cellular immune reactions. Class I MHC-restricted T cells undergo a polarization into T cytotoxic (Tc1 and Tc2) cells similar to that described for $CD4^+$ T lymphocytes.

The initial event in the induction of inflammation in multiple sclerosis appears to be infiltration of the nervous system by Th1 cells and $CD8^+$ Tc1 cells, the latter dominating the T-cell infiltrates in active as well as inactive lesions. By producing their cytokine cocktail, mainly IFN-γ and TNF-α, local central nervous system elements become immunologically activated. Resident microglia express histocompatibility antigens and thereby facilitate antigen recognition by T lymphocytes in the brain. Upregulation of adhesion molecules at the blood–brain barrier together with local chemokine production adds to the recruitment of inflammatory cells (predominantly other T cells, B cells and macrophages). The antigen recognized by these Th1 and Tc1 cells in multiple sclerosis brains is not known. Autoantigens as well as foreign (virus) antigens are possible candidates. Secondary effector cells are recruited into the nascent inflammatory focus and the blood–brain barrier is compromised. This results in protein leakage into the nervous system, registered by the increased gadolinium uptake in MRI studies of active lesions. In addition, resident microglia become activated and differentiate into phagocytic effector cells. A range of effector cells including macrophages/microglia and T lymphocytes, together with humoral factors, including antibodies and/or complement components, are involved in the destruction of myelin sheaths.

Downregulation of the inflammatory process coinciding with remission may take place through various mechanisms. It has been shown in experimental models of allergic or virus-induced inflammatory disease of the nervous system, and for a low proportion of cells in the lesions of multiple sclerosis, that T lymphocytes are effectively removed from the brain parenchyma through apoptosis. That said, the significance of programmed T-lymphocyte death for the clearance of inflammation in multiple sclerosis remains to be determined. Other factors that do appear to be involved in the downregulation of brain inflammation in multiple sclerosis are the cytokines IL-10 and TGF-β, and neurotrophins. In models of experimental autoimmune encephalomyelitis, the primary encephalitogenic Th1 reaction may switch to a Th2 reaction coinciding with recovery and increased production of IL-4 and IL-10 (Issazadeh *et al* 1995a; 1995b). Both cytokines have been shown to inhibit the delayed-type hypersensitivity reactions induced by Th1 cells (Fiorentino *et al* 1989). Although the exact time course of cytokine expression in the lesions of multiple sclerosis is not yet known, the prominent IL-10 expression in some cases suggests that similar mechanisms may be operating. TGF-β, a cytokine with prominent immunosuppressive activity, has been detected in local tissue components, especially astrocytes, during the recovery from inflammation. Because intravenous administration of TGF-β may block or suppress experimental autoimmune encephalomyelitis (Johns *et al* 1991), its local production in lesions suggests a prominent role in downregulation of the inflammatory response. Systemic endocrine responses, such as glucocorticoid release, may additionally be involved in downregulation of disease activity. In experimental autoimmune encephalomyelitis, the peak systemic corticosteroid response coincides with the phase of resolving inflammation (see Chapter 13) and the susceptibility of different animal strains to experimental autoimmune encephalomyelitis partly depends upon their ability to mount a corticosteroid response in the course of immune activation. In patients with multiple sclerosis, high-dose corticosteroids stabilize blood–brain barrier dysfunction and, possibly by this mechanism, improve clinical signs and shorten the duration of relapses (Burnham *et al* 1991; D.H. Miller *et al* 1992b). In addition, steroid therapy may have a direct effect on T-cell apoptosis within lesions. But a neuroprotective role for inflammation has been shown in several models of brain disease (Moalem *et al* 1999; 2000), and inflammatory cells are an important source for neurotrophin production in the lesions of multiple sclerosis (see Chapter 10: Kerschensteiner *et al* 1999; Stadelmann *et al* 2002).

DEMYELINATION AND OLIGODENDROGLIAL DAMAGE

The demyelinated plaque is the pathological hallmark of multiple sclerosis. In its classical form, this is a sharply demarcated lesion with complete loss of myelin sheaths and demyelinated axons embedded in a dense matrix of glial scar tissue. In general, there is a sharp transition between the demyelinated plaque and adjacent normal white matter. Yet, in chronic lesions, a small rim of thinly myelinated fibres, typical of remyelination, is frequently encountered at the lesional border. The demyelinating process is primary and segmental (Figure 12.5). Thus, at the edge of the lesions, myelin sheaths terminate at the node of Ranvier, the naked axon traversing the lesion boundary.

Immunological mechanisms of demyelination

The myelin sheath is a complex structure formed through spiral ensheathment of the axon by the oligodendrocyte plasma membrane (Bunge *et al* 1962). A single oligodendrocyte can form multiple segments, yet the number of myelin sheaths provided by a single cell depends upon axon and myelin thickness (see Chapter 10; A. Peters and Proskauer 1969). Biochemically, myelin sheaths are composed of lipids and protein. Major central nervous system myelin proteins are myelin basic protein and proteolipid protein. Both apparently have important func-

THE MIDDLE STAGES OF DISEASE: MODERATE DISABILITY

For most people with multiple sclerosis, the illness brings a prolonged period during which moderate and persistent disabilities impact significantly on the extent of activity and participation in daily life. The disease-related symptoms are multiple and complex: ongoing expert care and advice are required from many health care professionals, including neurologists with experience of the disease, in their management.

Employment issues

As physical and/or cognitive impairments emerge, it may be difficult for people to maintain employment. The loss of earning potential can have serious financial implications for patients and their families, leading to loss of self-esteem and social difficulties. Understandably, many patients want to maintain employment at all costs. Neurologists may be able to assist by writing to the employer, explaining the medical problems and proposing adaptations to the workplace designed to accommodate that patient's disability, thus making it possible to continue the job. In other instances, a compromise may be appropriate, whereby there is an agreement with the employer to work part time. In some individuals, flexible working hours concentrated during the morning, if that is the time when an affected individual is least fatigued, may be helpful. For some jobs, such as those involving extensive use of computers or the internet, it may be possible for the patient to work from home, at least for a part of the week. Other individuals accept the impracticalities and strain of struggling to continue at work, and they seek early retirement on medical grounds. In such circumstances, mechanisms for continued financial support should be explored. These might involve state or government benefits, work-related pension schemes, or personal health insurance policies held by the patient. The neurologist will often be called on to write medical reports explaining the consequences of the illness and supporting the need for the patient to stop working.

Driving

Specific locomotor impairments such as ataxia, weakness, spasticity, visual loss, and – if marked – sensory loss can all cause difficulty with, or even preclude, driving. Spasticity of the lower limbs may significantly impede the rapid and precise foot controls needed for braking and acceleration, with the potential for disastrous consequences. In some cases it may be possible to continue driving by using hand controls, because upper limb function is often preserved in the context of severe lower limb spasticity.

Whilst driving regulations, and procedures for their enforcement, will vary between countries, the neurologist is frequently asked to give an opinion on the safety of the person with multiple sclerosis continuing to drive. This is likely to become an issue of major importance to patients for whom driving is a necessary part of their employment. However, despite these practical considerations, driving is a privilege, not a constitutional right, and it may reasonably be denied on health grounds even against the patient's expressed wishes. It may be difficult to judge from the neurological examination alone how safe someone will be when driving a vehicle. In forming an opinion and giving advice, there should be a consideration of the interests of the patient, on the one hand, balanced against the risks to the affected person and third parties. An independent driving assessment by an appropriate authority can be performed in some countries. This usually involves a simulated assessment of driving performance and may be very helpful in deciding how best to proceed. In the United Kingdom, the final responsibility for determining fitness to drive rests not with neurologists but with a governmental agency – the Driving Vehicle Licensing Authority.

Sporting and recreational activities

In general, people with multiple sclerosis should be encouraged to continue with normal daily activities, both work and domestic, as far as the limitations imposed by their illness allow. For most people in the early stage, leisure activities will not be affected. However, restrictions will inevitably occur with advancing disability. Issues of safety may arise even for those activities that remain accessible and a source of pleasure – such as swimming in deep water. But that said, maintaining a regular fitness programme through sports and recreational activities, or through regular exercises advised by a neurophysiotherapist, and within the constraints imposed by the disease, makes good sense.

Symptomatic therapies

The stage of moderate disability is one where symptomatic treatments are frequently used and bring most advantage to affected individuals (see Chapter 17). Both pharmacological and physical therapies will be used, sometimes together. The most tractable symptoms to manage are bladder and sexual dysfunction, neuropathic or mechanical pain syndromes, spasticity and fatigue. Often, less can be achieved to help ataxia, weakness, visual loss and cognitive impairments. Regular follow-up with a neurologist and other health care professionals will help to ensure continuity of care and adjustment of symptomatic treatments optimized to changing needs.

Physical therapies and rehabilitation

Many still regard physical therapies and rehabilitation as routine responsibilities of the physician caring for a patient. However, the specialty of neurological rehabilitation has defined a more precise role in managing both physical manifestations of multiple sclerosis and their impact on the affected individual as a person with domestic, social and professional aspirations. It is important that affected individuals benefit from all that is available for the chronic young sick. Multidisciplinary care provided within the framework of a comprehensive rehabilitation service can help to achieve this aim. The limitations provided directly by available disease-modifying or symptomatic pharmacological treatments are often apparent, and the need to help more severely affected individuals to deal with disability and handicap is all too evident in everyday neurological practice. There are prospects for neurological rehabilitation itself to progress from the present emphasis on coping or optimizing function within the constraints of disease pathology to one in which nervous systems are re-educated, realizing the full potential for new

therapies, through plasticity and restoration of structure and function – an era of biological rehabilitation.

The process of rehabilitation may not be relevant for many patients for many years, if at all. To some extent the need to consider secondary consequences of physical impairment, including contractures, urinary tract infection, osteoporosis (arising from immobility and repeated use of corticosteroids, as well as individual risk factors), and decubitus ulceration, represents failure of the more pharmacologically orientated approaches. These complications are best prevented by awareness and anticipation because, in the severely affected individual, they usually develop quickly yet take months to resolve. Maximizing activity and participation by attention to social, vocational, marital, sexual and psychological aspects of the illness are more important to most patients than drug treatment. In situations where the natural history has led to loss of mobility despite attempts at disease modification, it may be appropriate to advise the use of mechanical walking aids (foot-raising splints, walking sticks or crutches), despite the negative perceptions of dependency that some people associate with such appendages. A wheelchair may be self-propelled, electric or lightweight and can be adapted for access to and from a vehicle. In the home, it may be necessary to provide rails, transfer boards, ramps, hoists and lifts (elevators), to widen doorways and build facilities for drive-in bathing at ground floor level. Maintaining communication and outside interests for the person who is no longer able to come and go as they please can lessen frustration and boredom and home-based information technology, requiring reasonable vision but minimal hand control, can provide a welcome link with other people.

People with multiple sclerosis are aware that physiotherapy is one way to maximize the usefulness of their remaining functions and, although it is rarely necessary to provide continuous or prolonged access, the contribution of physiotherapy may be more important than rest or medication in restoring function after a temporary reduction in mobility arising from coincidental infection or recent relapse. Patients with chronic progressive multiple sclerosis, on the verge of losing their independence from impaired mobility, may be kept ambulant for a while through the use of physiotherapy, sometimes undertaken intensively during a programmed in-patient admission to a rehabilitation service lasting for several weeks. Hand function may also be amenable to physical therapy and there will be opportunities for improving quality of life through attention to speech, swallowing and the provision of low visual aids, amongst other devices.

The specialist in rehabilitation is especially alert to the possibility of depression in patients with multiple sclerosis. At diagnosis, the problems are those of facing an uncertain future. Later, the possibility of impending disability has to be confronted. Eventually, for some patients, there may be complete loss of independence. Counselling and sympathy seem to make as much sense as pharmacology but drug treatment is sometimes needed. However, antidepressants may have consequences, beneficial and adverse, for physical aspects of the disease, including bladder control. The combination of fatigue and low mood inevitably leads to poor self-esteem and tends to promote social isolation and inertia. This may aggravate physical aspects of the disease. Tackling and ameliorating both fatigue and depression are important steps towards successful outcome during a period of rehabilitation.

Managing cognitive dysfunction

Cognitive dysfunction is common and is frequently a significant problem for the person with multiple sclerosis. It can be readily overlooked in patients during a cursory neurological assessment that focuses primarily on physical aspects of the illness. It may manifest as poor coping with aspects of daily living, work and family or other responsibilities without much evidence of physical impairments to account for the apparent difficulties. Some patients report problems with memory or the organization of daily activities. Others may complain of fatigue – a common and disabling symptom in its own right. In some patients, it may be difficult to distinguish between cognitive impairment and depression. Indeed, the two commonly coexist. If cognitive impairment is suspected, formal neuropsychological assessment will help to confirm whether it is present and also to quantify its nature and severity. Where cognitive impairment is demonstrated, patients may be helped by advice on daily planning, limiting the number of tasks tackled, establishing routines and keeping written lists of what needs to be accomplished.

The role of formal cognitive therapy is unproven and no drug treatments are shown to enhance cognition, although the recent demonstration that a central-acting choline esterase inhibitor can modify the functional MRI response to a cognitive paradigm (Parry *et al* 2003) suggests the potential for pharmacotherapy favourably to influence cognitive performance. A course of antidepressant medication may be warranted if it is thought possible that cognitive performance is impaired by coexistent depression.

Disease-modifying therapy

Patients with moderate disability and frequent relapses may be eligible for existing disease-modifying treatments. Most neurologists would agree that affected individuals who are accumulating disability as a result of severe relapses with incomplete recovery are especially eligible for one of the licensed treatments. Some neurologists would opt for interferon-β rather than glatiramer acetate in patients with frequent new or gadolinium enhancing MRI lesions, given the evidence for a greater anti-inflammatory effect on MRI. In patients with clinically very active disease, more powerful forms of immunosuppression are also likely to be considered (see Chapter 18).

When moderate disability is the result of slow progression – either from onset (primary progressive) or after a relapsing–remitting phase (secondary progression) – the licensed disease-modifying treatments, and indeed more powerful immunosuppression, appear to be ineffective. Some neurologists will not use any disease-modifying treatments in this situation. Others – despite the lack of evidence (and perhaps in part as a result of pressure from patients to 'do something') – will use a variety of unproven treatments, usually exploiting an immunosuppressive or immunomodulatory mechanism. This group of patients represents an especially large area of need for therapeutic progress. Participation in well-designed, well-controlled clinical trials of promising new therapies may enable patients to feel that something is being done to address their particular needs. Participation in clinical trials also often brings more frequent contact with the clinical services, which can be helpful in its own right. The use of agents with a putative neuroprotective

mechanism is currently seen as a rational and promising strategy for tackling the progressive phase of multiple sclerosis.

Involvement with lay support groups

As the disease evolves, many patients and their carers find much benefit from involvement with lay support groups. Most countries have a National Multiple Sclerosis Society that offers a range of services to patients including information and welfare support. Some Societies are also a major source of research funds. In many countries, there will be local chapters or branches with which people can become involved, both to receive and provide support. Some people with multiple sclerosis, or their carers, derive considerable satisfaction from contributing to the organization, funding and development of their lay society. The Multiple Sclerosis International Federation provides a global framework for communication between the national societies and people with the disease (*www.msif.org*) and it is now also committed to research funding (see Chapter 18).

THE LATER STAGES OF DISEASE: SEVERE DISABILITY

For a significant number of individuals, the fears and frustrations of having multiple sclerosis do, in due course, come to pass. Whilst the balance of responsibilities may shift, and new members of the team providing comprehensive care now become more relevant, the treating neurologist still has a role to play in helping the individual patient face the daily practicalities of living with significant disabilities but nevertheless retaining participation and personal dignity consistent with advanced multiple sclerosis.

The caring physician

Dedicated neurologists have always tried to give practical help to the large number of patients with multiple sclerosis for whom they have responsibility, throughout the illness. They use the range of medications described in Chapters 16–18, adjusting thresholds to reflect the aim of improving quality of life, even in situations where there is no prospect of a cure. Neurologists advise on adaptation of the local environment for affected individuals depending on the level of disability and the impact this has on activities of daily living. Whenever possible, they negotiate the necessary financial resources available from social security or insurance schemes for domestic alterations, loss of mobility and earning potential and financial repercussions on carers, which are often the consequence of having multiple sclerosis. Physicians ensure access to physical, occupational and speech therapists, and to psychologists and social workers. They encourage patients to contact lay groups (the Multiple Sclerosis Societies) and other self-help organizations. These activities are traditionally the pastoral aspects of good doctoring and they merge imperceptibility with prescribing and the provision of accurate information on all aspects of the disease to those with a vested interest.

Symptomatic treatments and multidisciplinary care

Inevitably, as there are still only limited strategies available for disease modification, many of the patients with multiple sclerosis that a neurologist meets eventually develop severe physical and cognitive disabilities so that the role of pharmacology diminishes and the need for pragmatic measures increases with time. As these more severe manifestations develop, the requirements for provision of care become more complex, necessitating increased input from multidisciplinary and specialized teams. Now, in place of drug treatment, interventions for spasticity may include intrathecal baclofen or intrathecal phenol only available through a highly experienced service. Severe dysphagia may require percutaneous endoscopic gastrostomy, and this again should be performed in specialized centres with the close involvement of speech therapists. An active preventive programme to reduce the chances of bed sores should always be instituted in disabled patients. When decubitus ulceration does occur, management may require the use of a specialized bed and mattress, regular dressing and sometimes more aggressive surgical intervention.

Family and carer issues

The burden on family and carers is greatest when the person with multiple sclerosis has become severely disabled. The stresses and strains that are imposed on interpersonal relationships are large and it is not surprising that marriages and partnerships may break up. On many occasions, however, the commitment of the spouse, partner and other carers is sustained and profound. Professional carers need to be sensitive to these pressures and, correspondingly, to nurture loyalty and dedication to the task, providing additional support as and where possible. Periods of respite residential care enable others to take much needed holidays and thereby sustain a stable existence in the community for the affected person.

The effect is considerable on children who have a parent with multiple sclerosis. The impact of the disease on family life may restrict opportunities for a normally balanced and active childhood. Children may receive less emotional and physical support than normal and have inner anxieties about what will happen to the affected parent – or, indeed, to themselves. A study of 87 offspring from 52 families where one parent had multiple sclerosis showed that daughters cope better than sons, irrespective of which parent is affected (Steck *et al* 2001). Healthy mothers and daughters coped better with the increasing disability of a male proband.

Community support and residential care

It is often impractical for severely disabled patients to attend hospital clinics, and provision of care within the community is then more appropriate. Good liaison between hospital and community services is essential to ensure that a responsive and efficient system of care is still provided for the patient. Whereas care may have predominantly been provided in a hospital setting during the early diagnostic phase or the stage of moderate disability, the general practitioner may become more involved at this stage. Residential care may be necessary when it is no longer feasible for carers or other support services to maintain and manage the patient's needs in their own home. However, the decision on where care is best provided will depend on the nature of the available support services, and the views of all concerned in this significant decision. In many situations, the increasing difficulties and escalating demands present no

18 Disease-modifying treatments in multiple sclerosis

John Noseworthy, David Miller and Alastair Compston

THE AIMS OF DISEASE-MODIFYING TREATMENT

There have been many developments since we last reviewed the role of disease-modifying treatments in multiple sclerosis. Collectively, these represent progress but fall well short of a solution to the problem. Results of the pivotal interferon and glatiramer acetate trials led to approval of these treatments by licensing bodies throughout the world. For the first time, patients with multiple sclerosis had a treatment. This was welcome and fuelled further efforts to improve on the evidence for efficacy and indications for the timing, dose and duration of therapy. Increasingly sensitive diagnostic criteria, bolstered by serial magnetic resonance imaging (MRI) studies (W.I. McDonald *et al* 2001), now allow more rapid diagnosis and hence – in our current climate – earlier exposure to treatment. However, further work is needed on many strategic issues and points of detail:

- Will early treatment make a difference?
- Can sensitive clinical and MRI measures detect early favourable trends that predict long-term benefit?
- Might the trials be made even shorter?
- How early in any study should a monitoring committee conclude with certainty that a trial is positive and recommend early termination with generalized access to the therapy?

It is axiomatic that doctors want to make their patients better. Patients want to lead normal lives unencumbered by any physical, psychological or life-style baggage related to multiple sclerosis. As clinical scientists, we need to structure that pastoral position around concepts of the pathogenesis and strategies for what realistically can be achieved. Patients with multiple sclerosis need treatment before the onset of fixed disability. Throughout, we have argued that the clinical manifestations of multiple sclerosis can be attributed to perivascular inflammation and the tissue injury with which it is inextricably linked. Since we last reviewed the subject in 1998, the diversity of mechanisms that injure nerve fibres throughout the illness and the contribution these processes make to the clinical course have been intensively studied. Concepts have been updated and revised. Thus, whilst we remain of the view that inflammation is pivotal to the destruction in parallel of axons and oligodendroglia, the inflammatory process also triggers biological processes that increasingly contribute to tissue destruction. What position should the prescribing physician take on how and when to treat the person with multiple sclerosis? Our stance is pragmatic but informed by the neurobiology and neuroimmunology, and by the evidence from clinical trials.

We structure this discussion around the formulation that, typically, the early clinical course of multiple sclerosis is marked by relapses from which symptomatic recovery is usually complete. Inflammation drives the process. Subsequent episodes may affect the same or different myelinated pathways. Before long, clinical deficits, which correlate with abnormalities in saltatory conduction of the nerve impulse, accumulate. These reflect loss of functional reserve in the adaptive capacity of the nervous system to make best use of surviving electrical activity, and the impoverished but detectable signals that reach the cortex or distant parts of major pathways. Then, inflammation wanes (without necessarily ceasing) and the relative contribution of cumulative axonal damage, amplified by loss of trophic support, makes an impact (Figure 18.1). Initially, the clinical course is intermittent in 80% of affected individuals but a high proportion do later enter the secondary progressive phase in which impairment, loss of ability, and impact on health-related quality of life are each affected. For these patients, disability is established in 40% by 10 years, in 60% by 15 years and in 80% (that is 50% of all patients) by 25 years. It is the onset of secondary progression that gives multiple sclerosis the frightening reputation it has amongst affected individuals. Progression is the main factor distinguishing mild from severe forms of multiple sclerosis. In 20% of patients, the disease progresses slowly from onset, most typically with predominant spinal involvement, and this form of multiple sclerosis is even more predictably disabling. The analysis that fully reversible deficits mainly result from inflammation, oedema and the physiological action of cytokines whereas persistent symptoms and signs can be attributed to demyelination and the initial wave of axonal damage with failure of recovery mechanisms, and that chronic progression is attributable to cumulative axon degeneration, has obvious implications for treatment.

Immunological therapies are most likely to be effective in the inflammatory (relapsing–remitting and relapsing–persistent) phases. Conversely, it will be more difficult to influence progression with immunotherapy. Any treatment that reduces the accumulation of disability, and inhibits or delays time to onset of the progressive phase, is most likely to have a clinically useful disease-modifying effect whether or not that treatment also

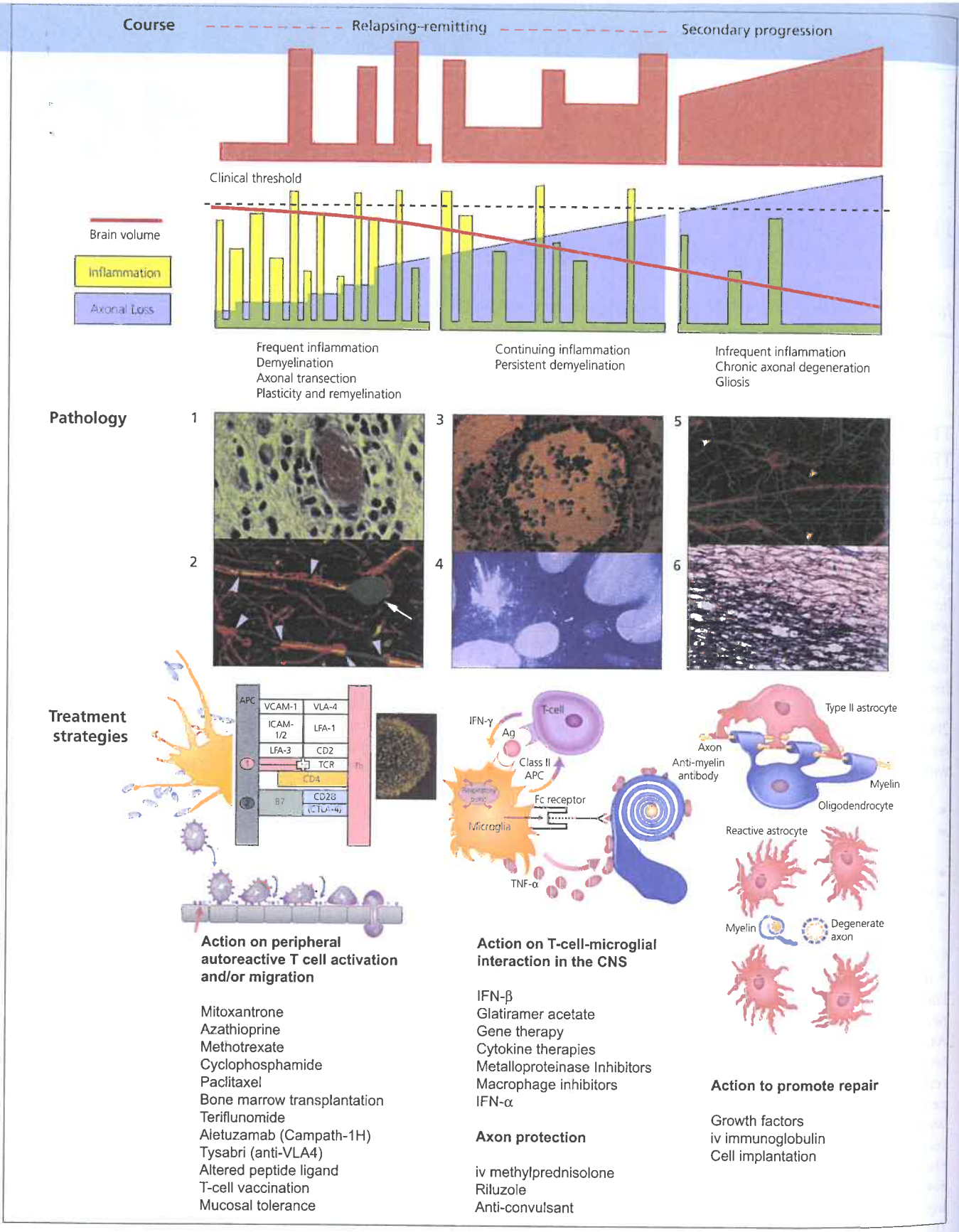


Figure 18.1 The course, pathogenesis and treatment of multiple sclerosis. *Course*: the clinical phases of relapse with recovery, relapse with persistent deficits and progression depend mainly on the effect of inflammation, demyelination and axon degeneration, respectively. Disease activity is often presymptomatic and, later, not invariably expressed clinically. As inflammation wanes, brain volume reduces with accumulated axonal loss. *Pathology*: perivascular inflammation (panel 1) causes acute axonal transection (panel 2), and microglia-mediated removal of myelin (panel 3) with persistent demyelination despite some remyelination (panel 4); chronic lesions show further axonal loss (panel 5) and gliosis (panel 6). The scheme does not depict primary progressive multiple sclerosis in which there is significant axonal degeneration with or without a preceding inflammatory phase. *Treatment strategies*: target the phase of T-cell activation in the periphery or cell migration; interactions between the activated T cell and microglia in the central nervous system; and axon protection and remyelination. Adapted from Compston and Coles (2003). © 2003, with permission from Elsevier.

affects the number of new episodes or lesions detected by brain imaging. Therefore, it makes sense to deploy strategies for treatment that address this evolution of events – choosing those interventions that preferentially tackle (or, preferably, anticipate) the individual components so as to be maximially effective. Although much contemporary research in multiple sclerosis is appropriately directed at identifying disease-modifying treatments, many patients already make clear that merely aspiring to shape the future course of the disease is not sufficient. They want to get better. If repair is a matter of restoring structure and function, it follows that dealing with the rewriting of neurological history requires treatments that enhance plasticity and reconstruct the myelinated axon in its network of connectivity. Thus, repair involves applying the lessons of neurobiology to the problems of multiple sclerosis. It remains possible that enhanced remyelination will occur in an immunologically stable environment. The experimental evidence already hints at this possibility (see Chapter 10). Remyelination may protect injured axons from further damage resulting from loss of trophic support. Conversely, optimizing their growth factor environment may reduce the extent to which axons, already insulted, are affected by further exposure to inflammatory mediators. Conversely, it is logical to assume that sophisticated repair strategies will have a low dividend for success without first having available a treatment that reliably stabilizes the disease process. Just as the dichotomy of genes versus environment is a somewhat sterile aetiological debate, so too separating inflammatory and biological mechanisms of injury to the axon–glial unit is somewhat strained.

But to go back a step, in Chapter 1 we reviewed the development of ideas concerning disease-modifying treatments in multiple sclerosis. The era prior to 1980, summarized by W.I. McDonald (1983), was empirical and largely uncluttered by serious concern about disease mechanisms. This period of intellectual freedom concerning the nature of multiple sclerosis provided ample opportunity for wild, and at times frivolous, approaches to treatment, some of which (rightly) gave the disease the bad therapeutic name from which it has not yet fully recovered. An important development in the treatment of multiple sclerosis in the 1980s was the acceptance that therapeutic claims must adopt orthodox clinical trial methodology based on blinding (single or double), use of controls (preferably placebo but sometimes receiving best existing medical practice), matching groups at entry for potentially confounding variables, setting primary outcome measures at the outset of the study and not trawling for the best result on completion, and considering power calculations during the planning stage. Working groups were convened to issue guidelines (see, for example, J.R. Brown *et al* 1979; Weiss and Dambrosia 1983). The impact of papers laboriously listing trial design tactics encouraged journal editors and referees to flex their methodological muscles – factors which undoubtedly led therapists to conform and resulted in the steady demise of therapeutic generalizations based on anecdote. The rubric 'double-blind, randomized and placebo-controlled' became commonplace. Since progress in identifying useful treatments was disappointingly slow, through no fault of those who designed the studies, separate trials of many agents proliferated and none could be regarded as definitive. Patients violated protocols and left studies for open label treatments, making it necessary to sort those who completed studies from 'intention to treat' cohorts. Commentators struggled to put their thoughts in order by cataloguing published material and seeking a best position on disparate data. Faced with too few studies involving sufficient numbers of patients from which to draw firm conclusions, the meta-analysis emerged as a device for 'seeing the wood for the trees'. Considered by some as scientific sophistry, this analytical procedure exposed the criticism of mixing chalk with cheese and creating statistical noise, not least because outcome measures in multiple sclerosis are an integral of up to three independent clinical features – acute events, persistent deficits, and progression – which contribute to impairment, loss of ability, autonomy and participation (formerly referred to as impairment, disability and handicap).

Later, came the interim analysis – often used to stop trials either on the grounds of futility or issues of patient safety. Recent examples include studies of intravenous immunoglobulin designed to measure clinical recovery in multiple sclerosis and optic neuritis; trials of altered peptide ligands; the story of agents that have an impact on tumour necrosis factor-α (TNF-α); the glatiramer acetate study in primary progressive multiple sclerosis; and the use of oral glatiramer acetate (see below for more detailed discussion). However, the interim analysis has also recently been used increasingly to stop trials early on the basis of perceived efficacy, thereby allowing active treatment to be made immediately available for all patients without the disadvantaged controls waiting for completion of the protocol. We have seen this happen repeatedly, dating from the first wave of pivotal trials in relapsing–remitting multiple sclerosis [the North American IFNβ-1a trial (Avonex)] and, subsequently, with trials in possible (CHAMPS) and secondary progressive multiple sclerosis (the European trial, SPECTRIMS) – all discussed below. More recently, trials have been reported and widely accepted as valid with as little as 6 months follow-up (for example, EVIDENCE). The lesson from the failed Mayo Clinic Canadian Sulfasalazine Trial that early benefit may wane with further blinded follow-up seems often to have been forgotten (Noseworthy *et al* 1998; Rudge 1999). Hence, we now have to provide wise counsel to a generation of patients, some treated immediately after an inaugural clinical episode (clinically isolated syndromes), others when the illness has been established for only a few years (early relapsing–remitting multiple sclerosis), and many long into the illness with advanced secondary progressive disease – despite the lack of convincing evidence for protracted benefit – aiming to steer a course between managing their expectations and not shirking our responsibilities as clinical scientists.

The concentration of clinical research on the evaluation of therapies that target the immune response in multiple sclerosis itself represents something of an advance, displacing hypotheses for the pathogenesis finding their expression in less rational clinical trials. We hope that those who contributed to these studies will accept our decision to concentrate on contemporary immunotherapy and applied neurobiology. Of course, we accept that some (or indeed many) of the agents which we have selected for detailed discussion may in time join those which we have placed on the well-stacked shelves of therapeutic history in multiple sclerosis.

By the late 1980s, Noseworthy *et al* (1989b) were able to tabulate a large number of potential therapies which experienced investigators considered to be promising options for treatment. Many are still being evaluated but some degree of consensus on

the basis for treatment in multiple sclerosis has emerged in recent years. At first, physicians were cautious when considering the use of immunotherapy for multiple sclerosis even though many of the available medications had been used successfully in other inflammatory and autoimmune diseases. This caution was appropriate since a significant proportion of affected individuals remain free from disability despite having intermittent symptoms over several decades, and it is not possible to segregate individuals destined to have benign forms of multiple sclerosis early in the course. In our opinion, however, the focus on treating secondary progressive multiple sclerosis held up progress for a generation. Since the late 1990s, that lesson has been clear. Wait until late and the contribution of anti-inflammatory therapy is so small as to not be cost effective. For many affected individuals, this is a formula for disappointment leading to cynicism that, despite intense research, no useful progress is being made in understanding the disease. It seems clear that, in the context of disease progression, the focus should now be on neuroprotective and biologically motivated approaches – alone or in combination with immunotherapy. Treat early and the dividend may be greater but still the dilemma remains. Drugs that are partially effective may not sufficiently stabilize the disease processes whereas the more actively anti-inflammatories are likely to carry nontrivial adverse-effect profiles. As we wrote in the early 1990s, the comprehensive management of multiple sclerosis is about both limiting and repairing the damage.

Progress has been made in improving outcome measures in the assessment of treatments for multiple sclerosis. Totting up the number of acute events requires them to be reliably defined, but patients will understandably assign significance to transient alterations in symptoms, perhaps having explanations other than disease activity. Conversely, motivation and the hope of a therapeutic effect will lead others to ignore clinical changes even though these are biologically meaningful. Periods of disease activity measured by high relapse rates tend to oscillate and, overall, slow with time so that a reduction in relapse rate *per se* is not necessarily impressive unless the placebo group has behaved less well and in keeping with the known natural history of the disease. The problems are even greater for the assessment of disability. There have probably been more critiques of the Expanded Disability Status Scale of Kurtzke (EDSS) (Kurtzke 1983a)and related clinical outcome measures than clinical trials in multiple sclerosis. The problems are well known. The EDSS mixes activity with disability and ignores participation. It is excessively weighted towards the motor system. It is ordinal not linear. Patients tend to cluster in the lower and higher echelons and it is insensitive in the middle range. However, it survives and despite much squabbling has yet to be replaced by a better, fully validated and universally accepted system. In this context, we welcome the deliberations of a panel convened by the United States National Multiple Sclerosis Society to make recommendations for a comprehensive clinical outcome system applied universally to treatment trials in multiple sclerosis, so allowing more meaningful comparisons between studies of the same or different agents (Rudick *et al* 1996a). The original guidelines were subsequently updated with special emphasis on the need for advisory/steering (to comment on the rationale, design, protocol, accrual and ownership of the data) and safety committees (to monitor operational aspects of trials), and the involvement of a group to supervise publication (Lublin *et al* 1997). The panel derived the Multiple Sclerosis Functional Composite scale (MSFC; G.R. Cutter *et al* 1999; Rudick *et al* 1996a; 1997) specifically to resolve these matters. The MSFC integrates scores on a timed 25 foot (7.5 m) walk (T25FW), Nine-Hole Peg Test (9HPT) of upper limb function, and Paced Auditory Serial Addition Test (PASAT). Values are reported as a Z-score, derived from comparison with an index population from the National Multiple Sclerosis Society Task Force dataset (G.R. Cutter *et* al 1999). The scale awaits validation as an accepted outcome for clinical trials and the MSFC has yet to be embraced by practising and academic neurologists. In large part, this is because, using this metric, most do not understand what is meant by changes in the Z-score, whereas few have difficulty with a single or multiple step change in the EDSS.

The introduction of novel scales has been trivial by comparison with the introduction of surrogate MRI markers as indices of therapeutic efficacy. The apparently favourable impact of approved but, in the event, partially effective treatments on relapse rates and MRI appearance solidified the sense that trials could increasingly use surrogate markers to detect a treatment effect. Clearly, reduction in the initial frequency of relapse and MRI activity may genuinely predict prolonged benefit but this requires evidence and has yet to be demonstrated. Such a study design aims to push new and existing therapies over a very high hurdle, requiring huge investments of time and money. Only recently have investigator-led studies adopted this long-term view but attention to effects that last and shape the neurological future is in the interests of patients and should be seen as motivated by the highest principles of clinical science, with a real dividend for improvement from the investment of hope in treatment. Worried by the sustained use of imperfect instruments for assessing outcome, investigators have created and partially validated increasingly sensitive measures of disability (such as the MSFC) and shown that these may detect treatment differences between treated patients with secondary progressive multiple sclerosis and controls, when standard measures (the Kurtzke EDSS) do not. Is the problem that our measures of disease progression are too insensitive to recognize a favourable response to treatment? Rather, it seems increasingly likely that existing treatments are insufficiently effective, or are deployed too late, completely to inhibit advancing disability.

The essential yet daunting task of confirming that these sensitive measures matter clinically in the long term – and are thereby predictive of a meaningful long-term benefit – has yet to be established. Here, a difference in agenda exists between physicians and the pharmaceutical industry, spawning secondary tensions between doctor and patient. The clinical scientist has a responsibility to proselytize secure knowledge even if this is gathered slowly and is disappointing in its scope. Sponsors need an early return on investment. In the context of multiple sclerosis, *Big Pharma* can be caricatured as having avoided engaging investigators in a dialogue about the importance of establishing long-term disability benefits. In turn, licensing agencies have not required that industry, assisted by teams of clinical investigators, demonstrate continued benefit for these expensive drugs as the necessary qualification for a drug licence. We have seen a proliferation of extension trials designed to demonstrate continued benefit. However, as discussed below, most are degraded by bias resulting from the recurring reality that failing patients drop out at the completion of the proper trial. Conversely, responders are

better motivated to participate in the extension limb of the study. Although re-randomized, loss of the original 'treatment failures' introduces selective sampling that subsequently haunts the trial, thereby reducing confidence that any long-term benefit claims are real. The push to earlier treatment, and acceptance by patients and physicians of the need for prolonged use of the currently available drugs (even in the face of obvious ongoing disease activity dressed up around 'perhaps the treatments are helping a bit'), is bolstered by several factors. Sponsors of the approved agents have failed to press for clinical and laboratory biomarkers that characterize responder status. As a result, far more patients are being treated than might be appropriate given the partial benefits noted in the literature.

These recent changes in the attitudes of specialists in multiple sclerosis should not be seen as mulish obstruction to the pharmaceutical agenda. Investigators and sponsors share the sense of urgency in wanting to provide patients with effective drugs as soon as possible. More than $1 billion is spent annually on these agents, in the United States alone, with no funds invested to confirm sustained benefit. This hope that treatment with existing agents will provide an extended benefit, especially for patients treated early, remains just that – an unconfirmed, elusive concept without proof for patients and physicians. Meanwhile, little (if any) progress is being seen in creating a robust strategy to validate this goal. If we appear critical, it is in the spirit of prioritizing real not virtual progress. Our attitude is in the interests of people with multiple sclerosis and the advancement of clinical science, with personal reputation and commerce well down the motivation stakes.

THE PRINCIPLES OF EVIDENCE-BASED PRESCRIBING IN MULTIPLE SCLEROSIS

As discussed in the preceding section, over the last decade clinical investigators have become increasingly familiar with the principles of clinical trial design and have adopted these structures in the evaluation of putative new treatments. Thus, practice has shifted from the extrapolation of anecdotal experience to a more evidence-based stance on prescribing (Sackett *et al* 2000). The concepts of levels of evidence and grades of recommendation are slowly becoming part of the clinical trials lexicon. The neurological community is increasingly demanding that published reports of clinical trials clearly state how each trial was conducted and wishes to know how the data were analysed and by whom. Prior to 1994 it was common to see trials that were neither randomized, blinded, nor adequately controlled. Patients with different disease courses (relapsing–remitting, and primary or secondary progressive) were often included in the same study; this latter concern is still rarely addressed. At that time, trials rarely performed an intention to treat analysis, often accounted incompletely for drop-outs, and rarely assessed the adequacy of efforts to blind patients and evaluators. Outcome measures were usually not validated (regrettably, this is still largely the case). Nor were the sensitivity and specificity of these measures provided in the reports. Sample size estimates and power calculations were often not stated, leaving open the possibility of type 2 ('false-negative') errors resulting from an underpowered sample size. Many authors did not state the predetermined primary outcome measure and post-hoc analyses were often not identified as such. Authors rarely corrected for multiple statistical comparisons (the Bonferroni correction).

With time, the clinical trials community in multiple sclerosis has become increasingly sophisticated about these essentials of trial design and conduct. Most of the design flaws already listed are now appropriately filtered during design of the protocol, and policed by the peer review process before a report is published. However, some problems remain. As emphasized repeatedly throughout this chapter, clinical trials in multiple sclerosis are rarely of sufficient duration to determine whether the intervention affects eventual outcome in terms of disability but there are many seemingly insurmountable obstacles that block the path to longer trials. These include the lack of 'equipoise' for selecting both the active treatment(s) and the control group, since many investigators have strong opinions about which treatments they consider to be superior. There is reticence by both the sponsor and patients to commit to long trials. To date, every treatment has proved incompletely effective and this regrettable truism results in an inevitable but variable degree of 'treatment failure' for most participants – most patients experiencing clinical or MRI evidence for worsening. Naturally, the disappointed patients who detect clinical worsening remain anxious to try another form of treatment and many options are now available. Whitaker (1993), on behalf of the Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis of the National Multiple Sclerosis Society (NMSS), argued against named patient prescribing (compassionate use of investigational drugs) since this bypasses or otherwise compromises the double-blind, randomized and placebo-controlled trial. His views are no less relevant today when patients have been given high expectations of drug treatment and efficacy has been proselytized through a combination of altruism and exploitation. Many doctor–patient relationships have been strained by these helter-skelter events. Agreement on trial design and protocol management and policing of methodology in treatment trials continues into the new millennium. With increasing duration, drop-outs accumulate and blinding of the patient and evaluator become increasingly difficult. Similarly, as discussed later in this chapter, extension trials are flawed by the late loss of protection from the initial randomization step that is so essential to reduce bias by balancing unknowable prognostic variables across each study group. Most reports of positive trials do not discuss the 'numbers needed to treat' analysis (see below), although independent editorials and correspondents frequently raise this matter; there remains a pressing need to establish that MRI measures can serve as reliable predictive biomarkers of disease course.

It is regrettable to acknowledge that few investigators participating in industry-sponsored trials yet have full access to raw data or the process of analysis. This continues to be a vexing problem in multiple sclerosis research despite requirement by major clinical journals for authors to confirm that an opportunity was provided to participate both in the collection of results and their analysis (Davidoff *et al* 2001). The academic community is gradually accepting the importance of so-called integrity policies that mandate full disclosure of competing interests with sponsors but greater transparency on this issue is still needed (Noseworthy *et al* 2003). The strong stance taken recently by major biomedical journals to require that trials be fully registered, if they are to be accepted for publication, may provide

much needed clarity within the clinical trials arena (De Angelis *et al* 2005).

With the proliferation of partially effective, disease-modifying treatments, fewer untreated patients are available to participate in clinical trials. In 1998, a small group of investigators decided to create a research centre independent of commercial influence and with the goal of hastening the search for therapeutic advances (Noseworthy *et al* 2003). The Sylvia Lawry Centre for Multiple Sclerosis Research at the Technical University of Munich (named in honour of the founder of the National Multiple Sclerosis Society of the United States and the International Multiple Sclerosis Society – see Chapter 1 – and directed by Albrecht Neiss and Martin Daumer) has amassed an impressive repository of data from natural history and completed clinical trials. In 2004, it had access to 43 data sets involving 14 700 cases and representing 62 000 patient years of follow-up. These data were primarily orientated towards controls since pharmaceutical sponsors had yet to donate information on individuals receiving study medications. Efforts are already under way to use this resource as the basis for understanding the contribution of demographic variables and laboratory measures (primarily MRI data) in identifying characteristic of the short- and long-term clinical course. The aim is to apply this knowledge in predicting the long-term course early in what is, for most affected individuals, almost invariably a chronic illness. Several countries (Denmark, Canada and Spain amongst others) have developed national registries to monitor the use of expensive therapies. Such databases will increasingly provide insights on long-term treatment efficacy with these drugs.

THE ROLE OF MAGNETIC RESONANCE IMAGING IN CLINICAL TRIALS

Over the last decade, the application of a range of MRI outcome measures has become a standard means of assessing therapeutic efficacy in the context of controlled clinical trials. The potential to monitor both natural history and treatment interventions was quickly recognized when MRI was introduced into clinical practice in the 1980s. As a direct and sensitive surrogate measure of the evolving disease process, it promised outcome measures that were simultaneously more objective and efficient than the cumbersome clinical markers on which clinical trials exclusively depended at that time. In the first clinical trial of interferon-β (IFN-β), culminating in a drug licence, the unequivocal evidence that new lesions could be prevented was seen as strong supporting evidence to accompany the principal clinical effect of a reduction in relapse rate (Paty *et al* 1993).

Individual magnetic resonance imaging lesions

The sensitivity of counting new MRI lesions in treatment monitoring has been amply confirmed. In relapsing–remitting or relapsing secondary progressive multiple sclerosis, serial monthly brain MRI reveals about ten new gadolinium enhancing or new T_2 lesions for every clinical relapse. It can thus be anticipated that the number of subjects and length of follow-up is reduced when using MRI lesions as the primary outcome measure (D.H. Miller *et al* 1991). Significant reduction in the number of new MRI lesions can be demonstrated in a matter of months using a relatively small number of patients and, as a result, MRI has been proposed – and is widely accepted – as the primary outcome measure in exploratory trials of potential new disease-modifying agents in relapsing multiple sclerosis (D.H. Miller *et al* 1996). This approach is biologically plausible when the treatment is intended to suppress inflammation, since gadolinium-DTPA (gadopentetate dimeglumine) enhancing lesions identify areas of active inflammation. Monthly T_2-weighted and gadolinium-DTPA enhanced (0.1 mmol/kg of a gadolinium chelate) brain MRI are usually performed in phase I/II studies. In relapsing–remitting multiple sclerosis, a parallel groups design with placebo requires about 40 patients per arm to show a 60% reduction in new enhancing lesions over 6 months (McFarland *et al* 1992; Sormani *et al* 1999; Tubridy *et al* 1998a). A single run-in scan at 1 month reduces the sample size by about 30% (Tubridy *et al* 1998a). Slightly larger numbers are needed in secondary progressive multiple sclerosis. Crossover designs are more powerful, because there is less intra- than inter-patient variability in MRI activity. A single crossover design with 6 months run-in followed by 6 months of treatment requires between 10 and 12 patients to show a 60% reduction in activity (McFarland *et al* 1992). Double crossover designs are even better, but there needs to be a wash-out period between the two phases. Both crossover designs are compromised by regression to the mean. If a safe and cheap drug shows only a moderate reduction in activity (c.50%) in a small crossover study, this might be sufficient evidence to justify going straight to a phase III trial using a clinical end point. However, if the drug has more side effects or is expensive, a parallel group design with the larger sample sizes (such as 2 groups of 40 individuals treated for 6 months) should first be undertaken to gain more certainty about the MRI effect. An important limitation of studies with this size and duration is that they will not detect infrequent, severe or delayed side effects. It is therefore still considered necessary for the definitive (phase III) trial to be longer, to involve larger cohorts and to have a primary clinical end point.

A major limitation in the interpretation of gadolinium enhancing or T_2 lesions as outcome measures in trials is that these do not strongly predict or correlate with the long-term clinical course. Although concordance of the treatment effect on MRI lesions and relapses has been observed with most (but not all) agents that have been investigated in placebo-controlled trials (Table 18.1), the magnitude of reduction on MRI has not reliably predicted the extent of any decrease in relapse rate. For example, IFN-β and glatiramer acetate both reduce relapse rate by about 30% but, whereas IFN-β reduces the new MRI lesion rate by 50–70%, glatiramer acetate is associated with only 30% reduction. More importantly, the extent of T_2 and gadolinium-DTPA enhancing lesions has consistently demonstrated little or no relationship with concurrent or future disability (Kappos *et al* 1999). This lack of a relationship may partly be the result of limited follow-up – most published studies have lasted no more than a few years and may not have allowed sufficient time for substantial changes in disability to be revealed. Two recently published cohorts of patients presenting with clinically isolated syndromes have been followed for 8.7 and 14 years, respectively. One study of 42 patients showed that infratentorial lesions at presentation are associated with greater disability after 8.7 years (Minneboo *et al* 2004). In the second, the number and

Table 18.1: **Treatment effects on active MRI lesions and relapses reported in parallel groups, placebo-controlled multiple sclerosis treatment trials**

Therapy	Sub-group	Treatment duration	Patient number	MRI effect %	Relapse effect %	Reference
Beta interferon 1b SC	RR	4 years	372	–60 to 75	–33	IFNB Study Group (1995)
Beta interferon 1a IM	RR	2 years	301	–50	–31	Jacobs *et al* (1996)
Beta interferon 1a SC	RR	2 years	560	–75	–27 to 33	PRISMS Study Group (1998)
Beta interferon 1b SC	SP	3 years	718	–65	–31	D.H. Miller *et al* (1999)
Beta interferon 1a IM	CIS	2 years	383	N/A	–44	Jacobs *et al* (2000)
Beta interferon 1a SC	CIS	2 years	308	–33	–23	Comi *et al* (2001)
Beta interferon 1a SC	SP	3 years	618	–73	–30	SPECTRIMS Study Group (2001)
Beta interferon 1a IM	SP	2 years	436	–46	–33	J.A. Cohen *et al* (2002)
Alpha interferon	RR	6 months	20	–95	None[a]	Durelli *et al* (1994)
Linomide	RR	6 months	31	–70	None[a]	Andersen *et al* (1996)
Linomide	SP	6 months	30	–55	None[a]	Karussis *et al* (1996)
Anti-CD4 antibody	RR/SP	6 months	71	None	–41	van Oosten *et al* (1996)
Mitoxantrone	RR/SP	6 months	42	–90	–77	Edan *et al* (1997)
Lenercept	RR	6 months	168	+30 to 60	+50 to 68	Lenercept MS Study Group (1999)
Tysabri	RR/SP	2 months	72	–50	None[a]	Tubridy *et al* (1999)
Cladribine	SP/PP	1 year	159	–80	None	G.P. Rice *et al* (2000)
Glatiramer acetate	RR	9 months	239	–29	–33	Comi *et al* (2001)
Mitoxantrone	RR/SP	2 years	194	–85	–60	Hartung *et al* (2002)
Oral beta interferon	RR	6 months	173	None	None	Polman *et al* (2003)
Tysabri	RR/SP	6 months	213	–90	–50	D.H. Miller *et al* (2003)

a Study too small to reliably evaluate relapses.
RR = relapsing–remitting; SP = secondary progressive; CIS = clinically isolated syndrome; PP = primary progressive.
– = decrease in activity rate treatment versus placebo.
+ = increase in activity rate treatment versus placebo.
SC = subcutaneous; IM = intramuscular.
N/A = not possible to assess because of patient censoring on developing clinically definite multiple sclerosis.

volume of T_2 lesions in 71 patients at presentation correlated modestly with EDSS after 14 years (Brex *et al* 2002). The increase in T_2 volume during the first 5 years correlated somewhat more strongly with disability at year 14 ($r = 0.61$), suggesting that early accumulation of an increased lesion load does partially relate to long-term outcome. These studies are, however, quite small and the strength of the relationship between lesions and disability remains modest, suggesting that it is not sufficient to rely on MRI lesions *per se* (or their modification by treatment) to predict long-term disability (or its prevention by treatment).

The poor predictive value for disability of T_2 and gadolinium-DTPA enhancing lesions is that they are neither specific nor sensitive to axonal loss – the major pathological substrate for irreversible disability in multiple sclerosis. These markers do not reflect axonal attrition within lesions, or the loss that occurs more widely in normal-appearing white and grey matter. As a result, increasing attention has been placed on surrogate MR measures of axonal loss to study disease progression in multiple sclerosis and its modification by treatment.

It has been suggested that axonal loss in MRI lesions may be inferred by the presence of T_1 hypointensity. Such lesions (colloquially described as T_1 black holes) account for 20–30% of all T_2 visible lesions and have been found in post-mortem studies to indicate a greater extent of axonal loss than lesions that remain T_1 hypointense (van Walderveen *et al* 1998b). However, the use of T_1 hypointense lesions as a surrogate marker for axonal loss has important limitations. First, not all such lesions are irreversible – acute enhancing lesions frequently display transient hypointensity, and their resolution with follow-up may simply imply that reversible mechanisms such as oedema contribute significantly to the appearance. Secondly, T_1 hypointensity is a subjective assessment that is less reproducible than T_2 lesion identification and is highly dependent on MR sequence parameters. Thirdly, T_1 hypointense lesions are almost never seen in the spinal cord, yet axonal loss in this location is crucially related to locomotor disability. Fourthly, being a subset of visible lesions, assessment of T_1 hypointensity provides no indication of the axonal loss occurring in normal-appearing tissues.

It has been useful in placebo-controlled clinical trials to follow the evolution of acute inflammatory gadolinium enhancing lesions through to areas of persistent T_1 hypointensity. The frequency of such an evolution is reduced in patients treated with glatiramer acetate compared to the placebo group (Filippi *et al*

2001a) and Tysabri (Dalton *et al* 2004a) but not IFN-β (Brex *et al* 2001b). This outcome could be considered as the MR equivalent of an incomplete recovery from relapses. However, given the abundant evidence for neuronal and axonal loss in the white matter and grey matter beyond MR visible lesions, attention is being focused on global MR measures as a more plausible surrogate marker of irreversible and progressive disability.

Global magnetic resonance measures of neuronal and axonal loss: atrophy

Tissue loss (atrophy) is the most widely used measure of neuroaxonal loss in treatment trials. Axons contribute 45% to white matter volume, followed by myelin (25%) and other tissue elements (glial and vascular tissues and water: D.H. Miller *et al* 2002). Neuronal cell bodies and axons constitute the bulk of grey matter volume although myelin is also present, albeit to a lesser extent than in white matter. Atrophy of white or grey matter in multiple sclerosis in large part reflects axonal and neuronal loss. In a study of the spinal cord of five people with multiple sclerosis, marked atrophy and axonal loss were both observed (Bjartmar *et al* 2000). However, neuroaxonal loss is not the only cause of atrophy. Loss of myelin, variations in glial bulk, inflammation and tissue water content also affect global or regional volume measures in multiple sclerosis. Pertinent to treatment trials, it should be noted that anti-inflammatory therapies (such as high-dose corticosteroids or IFN-β) reduce brain volume without axonal loss having occurred. We recommend that a period of 3 months should elapse after receiving such therapy before inferring that atrophy is measuring axonal loss.

The optimal technique for detecting atrophy should be reproducible, sensitive to change, accurate and pragmatic. The two distinct methodological aspects involved in measuring tissue volumes are data acquisition and data analysis. The ability to reduce partial volume errors with high resolution scans means that 3-D acquisitions are attractive, although 2-D sequences (Molyneux *et al* 2000) have also been used successfully to derive cerebral volume measures. Segmentation of the brain is necessary for whole brain atrophy measurements, and suppression of cerebrospinal fluid helps to generate a sharp distinction in signal between cerebral and extracerebral matter. The most widely used 3-D sequence is a T_1-weighted gradient echo. Specific study of white or grey matter requires good contrast at the cortical boundaries and interfaces both with cerebrospinal fluid and the individual lesions. It is aided by multiple contrast acquisitions (e.g. T_1, T_2 and proton density).

Manual outlining provides the simplest approach to measuring changes in volume and is useful in small structures or regions such as the third ventricle, where significant atrophy occurs in multiple sclerosis. Disadvantages of manual segmentation include operator bias, long analysis time and poor reproducibility when compared with automated techniques. Semi-automated methods improve speed and reproducibility. Regional segmentation algorithms are used to outline lesions, spinal cord, optic nerves and ventricles. Many automated methods exist for segmentation (and thus volume measurement) of the whole brain. Both single contrast (Chard *et al* 2002c) and multispectral data (Ge *et al* 2000) are utilized for whole brain segmentation. Usually, the difference in signal intensity between brain parenchyma and cerebrospinal fluid on a single contrast acquisition is enough to drive the segmentation process. Segmentation of grey and white matter may also be accomplished with either single contrast or multispectral data, although additional sophistication is required to separate the two tissue types. Methods include Statistical Parametric Mapping (SPM) based segmentation (Ashburner and Friston 2000) and the fuzzy C-means algorithm (Pham and Prince 2000). Masking of lesions is necessary to avoid misclassification.

Estimates of absolute volume at separate time points are not necessarily needed. Evidence for atrophy may be obtained by looking for differences between serial scans (S. Smith *et al* 2001). Nonlinear registration of such scans produces deformation fields that yield information concerning regional and global atrophy, and rigid body registration can be used to track displacement of the brain surface during atrophy (Freeborough and Fox 1997; 1998). Normalizing to head size reduces intersubject variations in brain volume. Relative volumes also remove variability due to scanner instability. The scalp, and the total intracranial capacity (determined by the sum of the volumes of grey matter, white matter and cerebrospinal fluid, or the sum of the brain and ventricular and sulcal cerebrospinal fluid) have all been used to adjust brain volumes for normalization. Atrophy is seen in both the brain and spinal cord in secondary and primary progressive multiple sclerosis. It is most marked in secondary progressive disease and correlates with disability (Kalkers *et al* 2001; Lin *et al* 2003; Losseff *et al* 1996b). In primary progressive multiple sclerosis, significant atrophy of brain and cord over 1 year was evident in a large cohort of primary progressive patients drawn from six European centres (Stevenson *et al* 2000). Change in cerebral volume over this period correlated only weakly with change in T_1 and T_2 brain load. More recently, progressive cerebral and cervical cord atrophy has been observed over a 5 year follow-up in a cohort of 41 primary progressive patients with multiple sclerosis (Ingle *et al* 2003). The rates of atrophy appeared relatively constant within individual patients but varied between subjects.

Atrophy, however, is not confined to advanced stages of the disease. Brain atrophy is also seen in established relapsing–remitting multiple sclerosis within 3 years of the onset of symptoms (Chard *et al* 2002a). Both white and grey matter atrophy are observed (Chard *et al* 2002a; de Stefano *et al* 2003). Even early follow-up of patients with clinically isolated syndromes has shown that significant brain atrophy emerges over 1–3 years in those subjects who later develop multiple sclerosis. This is most clearly seen in the grey matter and also as progressive ventricular enlargement (Dalton *et al* 2002b; 2004a). The apparent absence of progressive white matter tissue loss at this early stage of disease may reflect bulk tissue compensation by inflammation or gliosis (Fernando *et al* 2004). Possibly, grey matter atrophy will be a more sensitive measure of neuroaxonal loss because inflammation is less evident in this location (Bo *et al* 2003b; Petersen *et al* 2001). Atrophy of about 10–15% has also been observed in the optic nerve following a single attack of optic neuritis (Hickman *et al* 2001). We discuss later the evidence for atrophy as an outcome in the context of specific clinical trials.

From these data emerge a crucial lesson for the pathogenesis of multiple sclerosis and the timing of its treatment. The point is made repeatedly throughout this and other chapters. Despite effective suppression of inflammatory MRI lesions, treatments may not slow the rate of ongoing cerebral atrophy (Coles *et al*

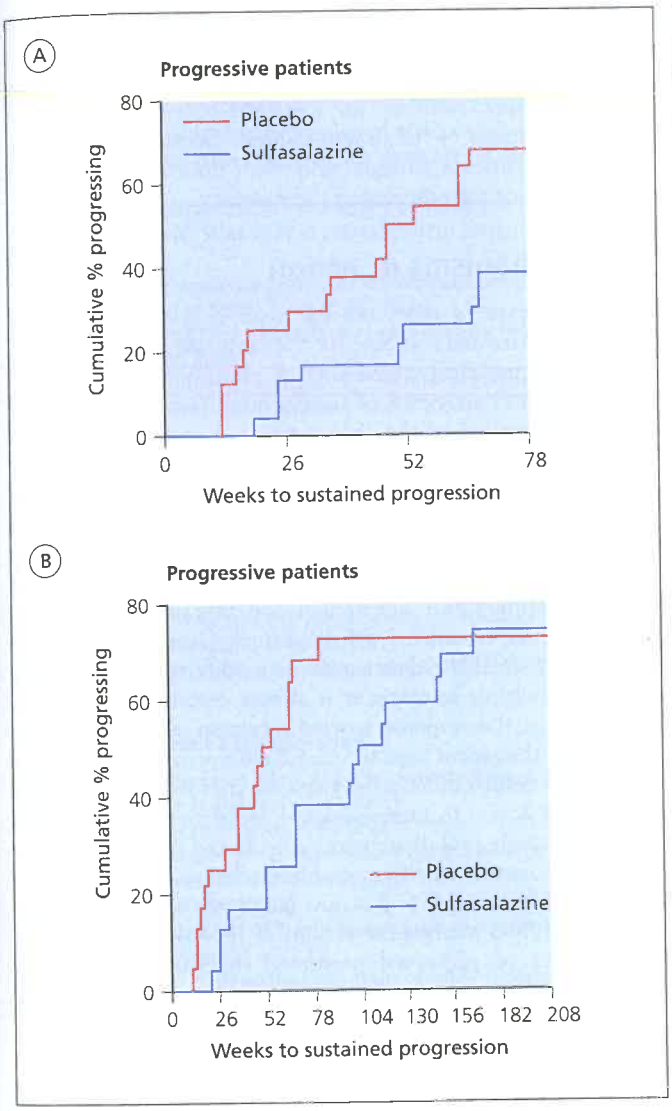


Figure 18.10 Treatment of active multiple sclerosis with sulfasalazine. (A) At the time of the interim analysis, there appeared to be an early treatment advantage for patients with progressive MS. (B) This later disappeared with prolonged follow-up. The data monitoring committee wisely did not terminate the trial early 'for apparent efficacy'. Adapted from Noseworthy *et al* (1998). © 1998, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

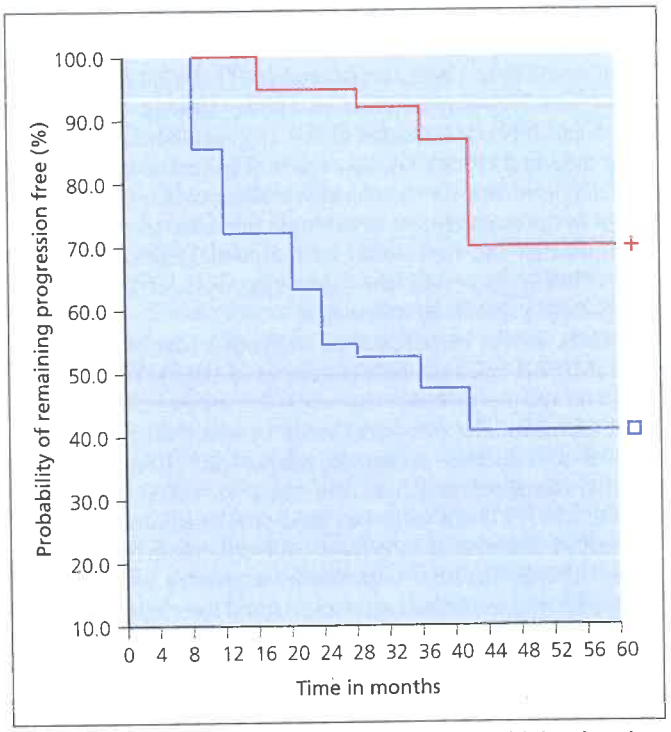


Figure 18.11 Treatment of relapsing–remitting multiple sclerosis with scheduled pulses of methylprednisolone (MP). Time survival curve to the onset of sustained EDSS score worsening. Log rank test $p < 0.001$. + = pulse MP; □ = control group. Adapted from Zivadinov *et al* (2001a). © 2001, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

differences in relapse rates, number, or time to relapse (Figure 18.11). Are these findings definitive? Regrettably not. Although certainly they are of great interest in that this is the longest trial to date (5 years) and corticosteroids are inexpensive and generally well tolerated; the putative effects on cerebral atrophy reported are, to date, unmatched so that the study needs to be repeated. The decision not to blind the evaluator was a major design flaw. This omission may have significantly biased the clinical assessments, lending a spurious credence to the reported result (Noseworthy *et al* 1994). That said, the MRI evaluations were blinded and seem robust, although cerebral atrophy measures have not been validated as definitive outcomes. Cerebral volumetric measures are clearly influenced in the short term by the use of corticosteroids, and artefacts arising from suppression of inflammation. If independently confirmed, the findings would suggest that regularly administered corticosteroids delay or prevent irreversible tissue injury. In turn, such a result would support the hypothesis that corticosteroids act to inhibit nitric oxide and excitotoxic mechanisms of neuronal and axon injury (see Chapter 10). We are not aware that a confirmatory trial is under way.

As we discuss in Chapter 4, new episodes cluster in women with relapsing–remitting multiple sclerosis during the puerperium. For this reason, De Séze *et al* (2004) treated 20 women who had recently completed a pregnancy prophylactically with methylprednisolone (1 g monthly for 6 months) and noted a lower relapse rate (0.8 ± 0.41) compared with 22 females observed expectantly a few years earlier (2 ± 0.66).

THE BETA INTERFERONS

The 1990s were dominated by the publication of large clinical trials evaluating the three brands of IFN-β and glatiramer acetate as disease-modifying drugs in multiple sclerosis, and the consequent managed introduction of these products into clinical practice. Interferons were first used in multiple sclerosis because of their antiviral activities. At first, no emphasis was placed on the type of interferon and each was assessed after administration by the systemic or intrathecal route. A series of pilot studies, mostly uncontrolled and involving small numbers of patients, was performed in the 1980s. These involved IFN-α (Camenga *et al* 1986; Knobler *et al* 1984), IFN-β given systemically

(Baumhefner *et al* 1987; M. Huber *et al* 1988; K.P. Johnson *et al* 1990; Ververken *et al* 1979) or by the intrathecal route (Confavreux *et al* 1986; Jacobs *et al* 1981; 1982; Milanese *et al* 1990), and IFN-β (Panitch *et al* 1987a; 1987b). The role of IFN-α and IFN-γ is discussed above. In general, many details of these inaugural studies are now more of historical interest than providing evidence for the clinician wishing to assess the role of IFN-β in the management of multiple sclerosis, since they have been updated and superseded by the pivotal clinical trials discussed below. However, the pioneering work of Larry Jacobs (1938–2001) should be mentioned.

Initially, Jacobs carried out an unblinded trial of intrathecal natural IFN-β in 20 patients (Jacobs *et al* 1981). There was an effect on relapse rate and this work was extended to a single-blind (sham lumbar puncture) study in which 69 patients also showed a reduction in annual relapse rate (0.8 in treated patients compared to 1.5 in controls; $p < 0.001$: Jacobs *et al* 1986b; 1987). There were practical problems associated with the use of intrathecal interferon and difficulties in obtaining natural IFN-β. Together with results suggesting an increase in disease activity in patients receiving natural interferon (Milanese *et al* 1990), no further progress was made until recombinant IFN-β was shown to be effective after parenteral administration. It was subsequently recognized that the immunological effects and physiological situations that characterize release of the naturally occurring interferons differ for IFN-α and IFN-β from IFN-γ. The results of clinical trials led to the conclusion that IFN-γ is contraindicated in multiple sclerosis and that IFN-α is not obviously effective, although its use has not altogether been abandoned (see above). However, IFN-β survived this filtration process, attracting increasing attention because of the accumulation of evidence for its partial efficacy together with vigorous marketing on a scale to which neurologists looking after people with multiple sclerosis had not previously been exposed. By comparison with azathioprine, for example, the processing of comparative clinical results attributable to IFN-β (see below) has been demonstrably less restrained.

In summary, the IFNB Multiple Sclerosis Study Group trial showed that patients receiving IFN-β1b (Betaseron 8 MIU by self-administered subcutaneous injection on alternate days) had a reduced relapse rate, although the effects on disability and disease progression did not reach conventional levels of statistical significance. Much was made of the reduction in MRI activity seen in the IFN-β1b (Betaseron) study and the extent to which this provided evidence for a disease-modifying effect. Subsequently, IFN-β1a (Avonex; 6 MIU intramuscularly on a weekly basis) was shown to reduce relapse rate and slow the rate of disability and the time to progression. The report on IFN-β1b (Betaseron) appeared in 1993, with an update in 1995. The study of IFN-β1a (Avonex) received much advance publicity but did not appear until January 1996. Sandwiched between these primary publications was a deluge of commentaries, vigorous marketing efforts by the pharmaceutical companies who stood to gain from the introduction of these products, and the jottings of several seriously ill-informed journalists. The immediate consequence was that, initially, neurologists were asked to prescribe IFN-β before it was licensed or widely available and often in settings where governments restricted its use. This complex situation subsequently evolved. Interferons became widely available for use in relapsing–remitting multiple sclerosis and, in some countries, for secondary progressive disease. Governmental efforts to ration their use still vary widely. Many countries and groups of opinion leaders have developed guidelines for prescribing these agents. Despite these efforts, however, there remains no evidence-based definition of 'responder' status or 'treatment failure'. Consequently, practitioners and patients struggle daily with decisions of when to start, change or stop the use of these agents.

The mechanisms of action

Most cells express receptors for type 1 interferons. IFN-α and IFN-β share and compete for the same site, transducing signals through protein tyrosine kinases, phosphorylation of signal transducers and activation of transcription factors (STAT1 and STAT2), formation of the IFN-stimulated gene factor 3 from the association of STAT1 and STAT2 with the p48 protein, and binding of this gene factor to promoter elements resulting in gene transcription (Karpusas 1998). IFN-γ uses a different receptor but stimulates some of the same intracellular signalling molecules. Collectively, the interferons show a variety of antiviral, antimicrobial, antitumour and immunological effects (for review, see Goodkin 1994; Weinstock-Guttman *et al* 1995; Yong 2002). If IFN-β has a role in modifying the long-term course of multiple sclerosis, it is almost certainly not through any effect on the response to viral infection, as originally suggested, and the recent logic for continued use in clinical practice rests on the results of laboratory studies that shift the emphasis on mode of action to immunological properties. T-cell-derived IFN-γ has mainly proinflammatory effects and this explains why it increases disease activity in multiple sclerosis.

The logic for using IFN-β is now based not only on the argument that IFN-β inhibits the actions of IFN-γ, but also from a wide variety of additional presumed mechanisms of action including inhibition of T-cell activation, modulation of cytokine production and reduction in T-cell migration. In this respect, IFN-β can be considered as an anti-inflammatory cytokine but it also enhances some components of the immune response. This literature is abundant and often conflicting. Research remains active and, as expected given the plethora of potential sites of activity, the story is self-evidently incomplete and not without its share of ambiguities on the specific immunological effects and their relevance for treated patients. Inevitably, individual commentators tend to focus on the mechanisms that address their preferred concepts for the pathogenesis of multiple sclerosis. We are not exempt from these accusations of parochialism. Table 18.2 provides contemporary references for recent work on the potential mechanisms of action of IFN-β, a subject comprehensively reviewed by J. Zhang *et al* (2002). Exposure of microglia to IFN-γ *in vitro* increases the expression of cell surface class II MHC antigen (Woodroofe *et al* 1989) and the constitutively expressed T helper type 1 (Th1) costimulatory molecule B7 (K.E. Williams *et al* 1994). This upregulation is inhibited by IFN-β in a dose-dependent manner. The effect is most pronounced when IFN-β is introduced *in vitro* prior to IFN-γ exposure but is still evident when IFN-β is added after exposure of microglia to IFN-γ. Since this inhibition is not associated with a decrease in class II mRNA within cells, the mechanism is considered to be post-transcriptional and, given that class II heavy chain accumulates within cells, presumably post-translational. Revel *et al* (1995) have shown that the molecular interactions of IFN-β and IFN-γ involve the STAT1 transcription

Table 18.2 Presumed mechanisms of action of the beta interferons

Inhibits T-cell costimulation and/or activiation processes

- Inhibits IFN-γ-induced expression of MHC class II molecules and other molecules required for T-cell activation (Arnason *et al* 1996)
- Modulates costimulatory molecules on dendritic and other cell types (Y.M. Huang *et al* 2001a; 2001b; 2001c; 2001d; Z. Liu *et al* 2001)
- Reduces precursor frequency of myelin-reactive T cells (Kozovska *et al* 1999; Zang *et al* 2000b)
- Treatment-induced reduction in costimulatory molecules (Shapiro *et al* 2003)
- Treatment-induced reduction in the number of antigen-presenting dendritic cells (Bergh *et al* 2004)

Modulates anti-inflammatory and proinflammatory cytokines

- Increases IL-10 and IL-4 production/expression at protein and mRNA levels (Ozenci *et al* 2000; Rep *et al* 1996; Rudick *et al* 1996b; 1998b; Tuohy *et al* 2000)
- Decreases IL-12 production (Karp *et al* 2001; McRae *et al* 1998; Tuohy *et al* 2000)
- Decreases TNF-α and IFN-γ production (Kozovska *et al* 1999; Rep *et al* 1996; Zang *et al* 2000b)
- Suppresses Th1 cells and upregulates IL-10 production (Zang *et al* 2003)

Decreases aberrant T-cell migration

- Enhanced shedding of VCAM-1 from endothelium into soluble form (Calabresi *et al* 1997c)
- Decreases T-cell migration (Prat *et al* 1999)
- Reduced integrin gene expression (Muraro *et al* 2004)
- Inhibits expression of mRNA for MIP-1α, RANTES, and CCR5 (Zang *et al* 2000a; 2001)
- Reduces migration of T cells toward the chemokines RANTES and MIP-1 (Zang *et al* 2001)
- Decreases IL-2-stimulated secretion of MMP (Leppert *et al* 1996; Lou *et al* 1999)
- Treatment-induced reduced MMP-9 levels in PPMS (Yushchenko *et al* 2003)
- Treatment-induced enhanced TIMP-1 levels in RRMS (Karabudak *et al* 2004)
- Reduces secretion of TNF-α and IL-1 (Lou *et al* 1999)

MHC = major histocompatibility complex; IL = interleukin; mRNA = messenger ribonucleic acid; TNF = tumour necrosis factor; VCAM = vascular cell adhesion molecules; MIP = macrophage inflammatory proteins; RANTES = regulated on activation, normal T-cell expressed and secreted; CCR = chemokine receptor; MMP = matrix metalloproteinases; TIMP-1 = natural tissue inhibitors of MMPs. Adapted from Zhang et al *(2002) with permission.*

factor and they propose a model involving antagonistic and synergistic actions on different genes whose products relate to cell activation. The increased class II antigen expression on microglia enables these to function as antigen-presenting cells and the proliferation of primed T cells exposed to antigen and IFN-γ activated microglia is inhibited by IFN-β (G. Hall *et al* 1997a). It is well recognized that IFN-β has an antiproliferative effect on T (and other non-immune) cells, inhibiting markers of activation such as IL-2 receptor, transferrin receptor and CD2 (A. Noronha *et al* 1993). Others have shown that the release *in vitro* of IFN-γ by mononuclear cells is reduced in patients treated with IFN-β (Petereit *et al* 1997).

Antigen-specific and IL-2-stimulated proliferation of Th1 cells are inhibited by IFN-β but without reducing their secretion of IFN-γ, TNF-α or macrophage inflammatory protein-1α. In fact, IFN-γ secretion is slightly increased, further demonstrating that the effects of IFN-β are complex and cannot simply be seen as suppression of IFN-γ-stimulated proinflammatory events (M. Pette *et al* 1997). In a comprehensive assessment, H. Jiang *et al* (1995) showed that IFN-β inhibits the ability of human antigen-presenting cells and B lymphocytes to induce T-cell proliferation. These inhibitions are associated with reduced expression of class II MHC antigens and adhesion molecules.

Taken together, these *in vitro* results suggest that IFN-β prevents the arrival of T cells and limits antigen presentation within the central nervous system, disengaging the amplification of local immune responses involving microglia and (antigen-specific) infiltrating T cells. IFN-γ also promotes the cytotoxic and phagocytic activities of microglia by increasing their respiratory burst and inducing the release of many mediators, but the interaction of IFN-γ and IFN-β on these properties is less straightforward. Rodent microglia exposed to IFN-γ increase the expression of Fc receptors and this effect is enhanced by IFN-β (G.C. Hall *et al* 1997b). IFN-β also directly stimulates the production of potentially harmful cytokines including TNF-α by microglia, further promoting their cytotoxic and phagocytic properties. TNF-α has a complementary effect on the ability of IFN-γ to increase class II antigen expression, demonstrating that intricate networks exist between pro- and anti-inflammatory cytokines. In samples obtained from patients before and during treatment, Brod *et al* (1996) showed that mitogen-induced production of cytokines (IFN-γ, IL-2, IL-6 and IL-10 but not IL-4 or TNF-α) is increased by IFN-β. Porrini *et al* (1995) took a slightly different position claiming that, *in vitro*, IFN-β induces the production of IL-10 and cytokines characterizing Th2 cells – a response not reproduced by IFN-γ. IL-10 released in response to IFN-β inhibits the production of TNF-α and IL-6 induced by IFN-γ and other macrophage activators. Others have since confirmed the antiproliferative effect of IFN-γ on human T cells *in vitro*, adding the observation that cooperation between T and B cells is also inhibited and emphasizing the anti-inflammatory consequences of the associated enhanced IL-10 production (Rep *et al* 1996; Rudick *et al* 1996b; see Chapter 11). IL-1 and IL-10 and transforming growth factor-β (TGF-β) tend to reduce class I antigen expression, providing evidence for a cascade of anti-inflammatory effects on antigen presentation in the central nervous system (Cowan *et al* 1991b; Racke *et al* 1991). IFN-β also inhibits antigen presentation of peripheral blood mononuclear cells through an effect on class II antigen expression (H. Jiang *et al* 1995). IFN-β inhibits IL-1-induced and IFN-α-induced production of nitric oxide (L.L. Hua *et al* 1998) and protects neurons from nitric oxide-mediated damage to mitochondrial complexes II/III and IV (Stewart *et al* 1998).

IFN-β and IFN-γ may therefore independently enhance the cytotoxic and phagocytic properties of microglia. At the very least, there does not appear to be complete reciprocal inhibition. Conversely, the antigen-presenting effects of IFN-γ-stimulated microglia are inhibited by IFN-β (G. Hall *et al* 1997a; 1997b). Given the part inhibitory and part complementary effects, it would be too simple to designate IFN-γ and IFN-β as entirely proinflammatory and anti-inflammatory cytokines, respectively.

The fact that IFN-β also inhibits class II expression on endothelial cells [in this situation, probably through a transcriptional mechanism (A. Miller *et al* 1996)] provides an additional potential mechanism of action through effects on cell migration across the blood–brain barrier (Huynh *et al* 1995). Further evidence is

provided by the demonstration that the IL-2-induced secretion of metalloproteinases by T cells, which normally enhances their ability to adhere and migrate through endothelial barriers, is reduced by preincubation *in vitro* with IFN-β, probably by a direct effect on IL-2 receptors (Leppert *et al* 1996). Others have assessed changes in the endothelium and conclude that IFN-β, by reducing the secretion of matrix metalloproteinases, inhibits cell migration and limits the ability of T cells and natural killer cells to cleave fibronectin on the basement membrane of endothelial cells (Stuve *et al* 1996). These studies specifically implicate matrix metalloproteinase-9. Corsini *et al* (1997) showed a reduction in adherence between mononuclear cells from patients treated for at least 6 months with IFN-β on cultured brain endothelia derived from a patient with multiple sclerosis. This was associated with reduced expression of HLA-DR on endothelial but not mononuclear cells, and with no effect on other adhesion molecules. Related work shows also that IFN-β affects the migratory activity of mononuclear cells by inhibiting their production of matrix metalloproteinase-9 (Stuve *et al* 1997). Recently, two groups have studied the effects of interferons on matrix metalloproteinase-9 and a tissue inhibitor of metalloproteinase (TIMP-1). Yushchenko *et al* (2003) showed that treatment with IFN-β1b produced reduced levels of serum matrix metalloproteinase-9 in all but one of 19 patients with primary progressive multiple sclerosis; there were no consistent changes in TIMP-1 levels. Karabudak *et al* (2004) reported that IFN-β1a treatment induced transient increases in TIMP-1 levels compared with baseline (at 3 and 6 months but not at 1 year) in a study of 16 patients with relapsing–remitting multiple sclerosis. However, no consistent changes were detected in matrix metalloproteinase-9 levels. Again, the relevance of these findings remains uncertain.

In a study of 35 patients with relapsing–remitting multiple sclerosis and 12 with secondary progressive disease, Shapiro *et al* (2003) demonstrated that treatment with IFN-β1a (Rebif) may induce changes in the ratio of costimulatory molecules (for example, suppression of CD80 and induction of CD86) detected within the first year of treatment that favour a Th2 predominance. They raise the theoretical concern that these patients could be at risk of humoral mediated autoimmunity or allergic phenomena.

An effect of IFN-β on lymphocyte migration, and hence inflammation, is also provided by the demonstration of reduced very late antigen-4 (VLA-4) expression on monocytes (Soilu-Hanninen *et al* 1995) and lymphocytes from a small group of treated patients, but this finding could not be reproduced *in vitro* (Calabresi *et al* 1997a; Muraro *et al* 2000). The interferons may increase shedding of vascular cell adhesion molecule (VCAM) and intracellular adhesion molecule-1 (ICAM-1) from endothelial cells thereby increasing circulating levels of these adhesion molecules (Calabresi *et al* 1997c). IFN-β treatment could thereby block migration of activated T cells by reducing the concentration of endothelial membrane-bound adhesion molecules. Alternatively, once shed from the cell surface, these soluble adhesion molecules may block their respective receptors on activated peripheral blood mononuclear cells [for example, soluble ICAM-1 binding to lymphocyte function associated antigen (LFA) and Mac-1; and soluble VCAM binding to VLA-1]. In a recent study of 50 patients with relapsing–remitting multiple sclerosis treated with IFN-β1a and IFN-β1b, Muraro *et al* (2004) reported that integrin gene expression of VLA-4 and LFA-1 is reduced in patients classified as 'IFN responders', raising the intriguing (but unconfirmed) suggestion that transcription of integrin genes may correlate with the treatment effect.

In summary, IFN-β probably exerts its effects through a variety of mechanisms. These include actions that reduce T-cell and monocyte activation and lymphocyte proliferation, decrease the proinflammatory cytokine bias that is thought to underlie some of the steps in tissue injury, reduce the IFN-γ upregulation of class II expression, diminish antigen presentation, and reduce T-cell migration through the blood–brain barrier. As discussed later, the putative mechanisms of action of glatiramer acetate differ from those for the interferons but with some interesting overlap and redundancy. These major differences raise a possible role for combination therapy.

The pivotal trials

The evidence that informed prescribing patterns, and led to product licences for the three brands of IFN-β, was derived from a series of pivotal studies incorporating randomized, double-blind and placebo-controlled designs carried out in the 1990s. IFN-β1b is produced by recombinant DNA technology using *Escherichia coli*. It differs from natural human and recombinant IFN-β1a (made in Chinese hamster ovary cells) in having 165 amino acids (lacking the methionine at position 1), a serine residue substituted for cysteine at position 17 to prevent incorrect disulphide bond formation, and no glycosylation of the asparagine residue at position 80. In the pivotal trials IFN-β1a (Avonex) was administered by weekly intramuscular injection (6 MIU), and IFN-β1a (Rebif; 22 or 44 μg thrice weekly) and IFN-β1b (Betaferon; 8 MIU), and as alternate day subcutaneous injections. These regimens were justified by the demonstration that serum levels of IFN-β1b peak between 8 and 24 hours and return to baseline by 48 hours (O.A. Khan *et al* 1996). There are no obvious differences between IFN-β1b and IFN-β1a in their biological activity or *in vivo* pharmacokinetics. Each is associated with the development of neutralizing antibodies. Here, we review the efficacies and adverse effects of these therapies, and the position that has emerged on the timing of treatment with respect to disease course. In turn, these inform the evidence base for the role of IFN-β in the management of multiple sclerosis at several stages of the illness. The sponsors of trials n multiple sclerosis have used an inconsistent and unhelpful format for designating doses of the interferons. To avoid controversy with regard to bio-equivalence, we refer to these studies using the doses as published. For reference, subcutaneous IFN-β1b has been tested in doses of 1.6 and 8 MIU (Betaseron) and 22 and 44 μg (Rebif). Intramuscular IFN-β1a (Avonex) has been tested predominately at 30 μg. On a mass basis, 6 MIU equates to 22 μg and 8 MIU to 44 μg.

IFN-β1b (Betaferon)

The pilot study of IFN-β1b was used to determine primary outcome measures for the definitive trial (K.P. Johnson *et al* 1990). Compared with seven controls, treatment in 24 patients using different doses of IFN-β1b showed a modest effect on relapse frequency. During treatment, patients receiving IFN-β1b had a

relapse rate of 0.7 per year compared with 0.9 per year in the placebo group; and the probability of remaining relapse free at 3 years was 83% compared with an estimated pretreatment rate of 63%. There was no effect on disability. In fact, the treated group did marginally worse.

The phase three trial was conducted simultaneously in Canada and the United States (IFNB Multiple Sclerosis Study Group 1993). It involved 372 patients, each having two relapses in the previous 2 years and with pre-entry EDSS scores <5.5 (the mean was about 3.0; Table 18.3). Treated cases were younger and had slightly longer disease duration. Corticosteroids were used during the trial period by 35% and 50% of treated and placebo cases, respectively. Those who did not complete the study (19%) were considered to have remained stable from the point at which they dropped out. The study was not therefore analysed strictly on an intention to treat basis. The results were broadly similar in the Canadian and United States groups. Most commentators consider this to have been a single trial, although attempts were made to represent these as independent and hence confirmatory studies, respectively.

In patients receiving 8 MIU of IFN-β1b, both primary outcome measures – relapse rate and number of relapse-free patients – achieved statistically significant results ($p = 0.0001$ and $p = 0.007$, respectively). Of the secondary end points, reduction in relapse rate in those who continued to relapse ($p = 0.001$), increase in time to first relapse ($p = 0.015$) and second relapse ($p = 0.007$), and reduction in the proportion of relapses judged to be moderate or severe (placebo vs. 8 MIU, $p = 0.002$) were also achieved.

The subsequent experience of these participants was later reported. The overall tone of the second publication (IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group 1995) was notably more sober than the initial paper. Participants had remained in the study for a median time of just under 4 years. Taking this entire period, the reduction in relapse rate associated with the use of IFN-β1b reported in 1993 was maintained at follow-up (8 MIU: 0.78 per year compared with 1.12 for the placebo group; $p = 0.0006$). The main effect of treatment was achieved in the first year. Although there was a reduction in relapse rate, both in treated patients and the placebo group, in each subsequent year, the cumulative reduction beyond year 1 was in fact greater as part of the untreated natural history (–0.63 between years 2 and 5 in placebo-treated patients compared with –0.39 in the treated group; Table 18.3 and Figure 18.12). However, this observation may be somewhat disingenuous since the baseline was lower at the start of year 1 in the treated group, therefore providing less room for manoeuvre in terms of further reduction in relapse rate by comparison with controls. Understandably, the authors emphasized these results as showing a continuing difference in exacerbation rates between treated and placebo groups, year on year, borrowing the substantial reduction in the first year for the subsequent cumulative reduction in

Table 18.3 **IFN-β1b: updated report of pivotal trials**

	Placebo	1.5 MIU	8 MIU
Exacerbation rates			
Enrolled	123	125	124
Number entering year 5	56	52	58
Overall exacerbation rate	1.12[a]	0.96	0.78[a]
(baseline-year 5)	(1.02–1.23)	(0.87–1.06)	(0.70–0.88)
Year-on-year exacerbation rates			
Year 1	1.44	1.22	0.96
Year 2	1.18	1.04	0.85
Year 3	0.92	0.80	0.66
Year 4	0.88	0.68	0.67
Year 5	0.81	0.66	0.57
Reduction in exacerbation rate			
Baseline-year 1	–0.36	–0.48	–0.74
Year 2–5	–0.63	–0.56	–0.39
Disability			
Enrolled	123	125	124
Number entering year 5	56	52	58
No. with EDSS >1 point	56/122 (46%)	59/125 (47%)	43/122 (35%)[b]
Baseline EDSS <3	26/58 (45%)	30/59 (51%)	20/55 (36%)
Baseline EDSS >3	30/64 (47%)	29/66 (44%)	23/67 (34%)
Median time to progression (years)	4.18	3.49	4.79[c]
MRI: lesion load			
Enrolled	73	66	78
Number entering year 5	72	61	75
Baseline MRI (median)	1503	1086	1525
Completing year 1	+6.7	+5.7	–4.9
Completing year 4	+30.2[d]	+10.6	+3.6[d]
Increase: year 2–5	+23.5	+4.9	+8.7

a $p = 0.0001$; b $p = 0.096$; c $p = 0.087$; d $p = 0.04$.

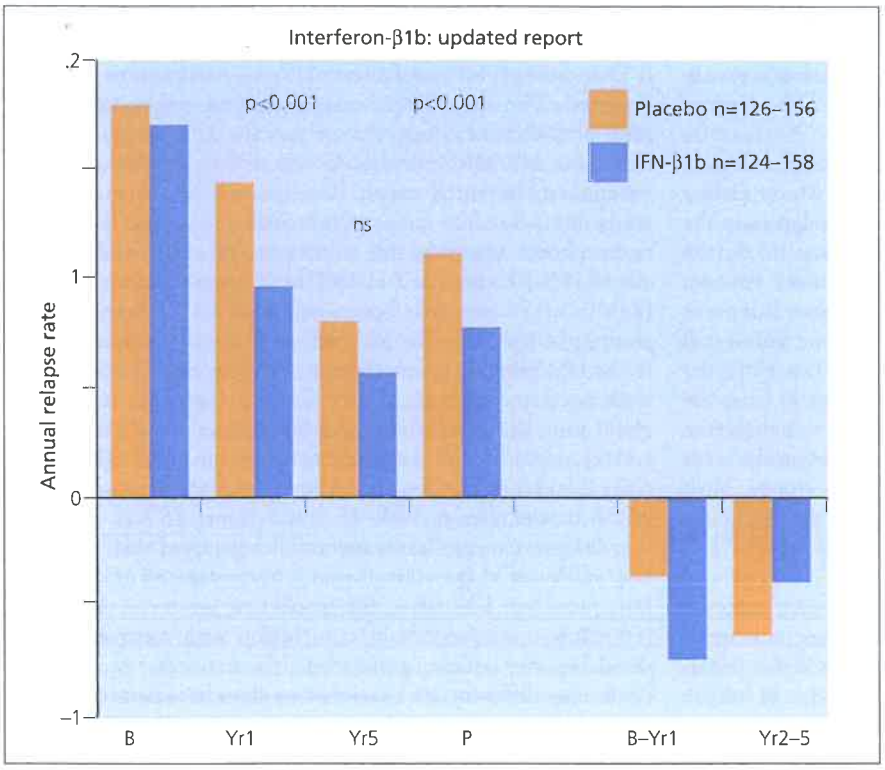


Figure 18.12 Annual relapse rates at baseline (B), baseline to year 1 (Yr1) and baseline to year 5 (Yr5) and in placebo-treated patients (P) in a trial of IFN-β1b; the rate of reduction is shown from baseline to end of year 1 (B-Yr1) and end of year 1 to end of year 5 (Yr2-5). Adapted from the IFNB Multiple Sclerosis Study Group (1993; 1995). © 1993, 1995, reproduced with permission of Lippincott Williams & Wilkins (lww. com).

relapse rate over the entire period. In this second report, once again IFN-β1b at a dose of 8 MIU reduced the proportion of patients judged to have a moderate or severe relapse compared with placebo ($p = 0.012$) although the data are not shown.

It has since been much debated whether a modest effect on relapse rate is useful for the majority of patients with multiple sclerosis. Relapses are distressing but usually self limiting, although they cause disability if recovery is poor. IFN-β1b may reduce relapse severity but the magnitude of this putative protection is somewhat unclear. In the IFN-β1b trial reports, 'moderate and severe' relapses are grouped together. The numbers of patients with each type of relapse, including those of 'unknown' severity, are not given. That said, it may be that, in protecting individuals from about one relapse every 3 years, IFN-β1b may be more likely to reduce a severe attack than a mild one. Relapses affecting the pyramidal and cerebellar systems are often relatively disabling, at least in the short term, and there were fewer of these in the treated patients. The effect on relapse rate reduced the need for hospitalization, and presumably also the impact on aspects of daily living, although this could not directly be assessed. Critics pointed out that the relapses were self reported and not universally confirmed by the attending neurologist. We understand that analysis only of those relapses that were physician confirmed was still highly significant and so take a charitable view on this design fault, accepting that there was no bias in the distribution of pseudo- and non-relapses in treated patients compared with the placebo group.

In many natural history studies, relapse frequency has not emerged as a factor which predicts disability. In recent studies from the Mayo Clinic (with a small cohort of patients followed closely for several decades), it has been difficult to confirm a close link between relapses and disability. No single demographic or disease variable (including relapse number in the first year) closely predicted prognosis (Pittock *et al* 2004a). In this series, as reported previously (Confavreux *et al* 2000), relapses did not influence further progression after reaching EDSS 3.0 (Pittock *et al* 2004b). In the large Canadian series, however, the number of relapses in the first 2 years, and time to the first relapse after presentation, did each correlate with eventual disability (Weinshenker *et al* 1991a; see also Chapter 4). This has encouraged people with multiple sclerosis that the reduction in relapse rate may have a dividend for an altered natural history of disease. It remains completely unknown, however, whether a reduction in relapse rate attributable to treatment (if this was shown to be long lasting by appropriately designed trials) shares the same good prognosis enjoyed by untreated patients experiencing a relatively relapse-free existence as part of their natural history.

Much has been made of the extent to which the MRI results influenced the overall impact of the IFN-β1b study. The IFNB Multiple Sclerosis Study Group studied a cohort of cases with serial assessments of lesion load (an indicator of the volume of affected brain), supplemented by measures of new and active lesions (Paty *et al* 1993). Comparable at entry, IFN-β1b-treated cases showed a reduction in lesion load within the first year (−4.9%) compared with the placebo group (+6.7%). These differences were maintained into the year 5 but, here too, the early effect attributed to IFN-β1b slipped marginally with time (Figure 18.13). Thus, both for the effect on relapse frequency and MRI lesion load, the experience of the first year proved crucial in this pivotal trial (Table 18.3).

In a subsequent study involving patients not recruited for the IFN-β1b (Betaseron) trial, L.A. Stone *et al* (1995) compared the contrast enhancing new lesion rate in the 7 months before and 6 months after introduction of IFN-β1b. A minimum pre-treatment rate of 0.5 lesions/month was required for entry and

13 of the 14 participants showed a reduction in active lesions. This represented an average change from 3.1/month to 0.5/month ($p = 0.002$). The number was 230 before and 20 after starting treatment with IFN-β1b, a reduction of 90%. Many patients had been studied over a longer period (up to 50 months) and the new lesion rate changed from 2.7/month to 0.2/month. These patients had eight clinical episodes in the pre-treatment period and four during treatment – a surprisingly high number given the MRI results. As an extension of this work, L.A. Stone *et al* (1997) prospectively studied 29 patients having >0.5 lesions/month during a 7 month qualification period. Eighty-six per cent of scans were active before and 33% were active during treatment with IFN-β1b. The median number of new lesions per patient per month dropped from 2.5 to 0.17 ($p < 0.0001$). Inevitably, there was variation between patients, prompting the authors to define a group of nonresponders but these did not have identifiable clinical or natural history characteristics.

A different marker of biological efficacy, measurement of urinary myelin basic protein-like material, was used for some participants in the IFN-β1b study (Whitaker *et al* 1995b). In so far as levels of this breakdown product represent a marker of disease progression and both number of lesions and total MRI lesion load, this result provided further surrogate evidence for efficacy. However, randomization bias prevented detailed analysis of the effect of IFN-β on urinary myelin basic protein.

There was no statistically significant effect of IFN-β1b on disability in these mildly affected patients participating in the North American study and that situation did not change with extension to 5 years (Figure 18.14). Thus, 43 of 122 (35%) treated patients showed a sustained (>6 months) deterioration of ≥ 1 EDSS points compared with 59 of 122 (46%) of the placebo group ($p = 0.10$). These results were uninfluenced by stratification for disability (baseline EDSS <3 and ≥ 3 at entry). It has been argued that restriction of the trial to include only stable, ambulant, relapsing–remitting patients did not give the study adequate power to assess this outcome. Thus, the results can be read as providing evidence for no effect on disability, or no evidence for an effect on disability. This difference in outcome may relate to the relative ease with which new episodes can be defined and the low stringency conditions used to assign relapses in this study, together with the insensitivity of scales routinely used to define disability. We have made the point repeatedly that inflammation and degeneration make different contributions to the pathological processes underlying relapse and progression. Saida *et al* (2005) randomized 205 patients from Japan with relapsing–remitting multiple sclerosis either to receive high- [250 μg (8 MIU)] or low-dose [50 μg (1.6 MIU)] IFN-β1b (Betaseron) three times weekly. As reported previously in the original North American trial, high doses of IFN-β1b were superior to low doses in reducing relapse rates and other measures of

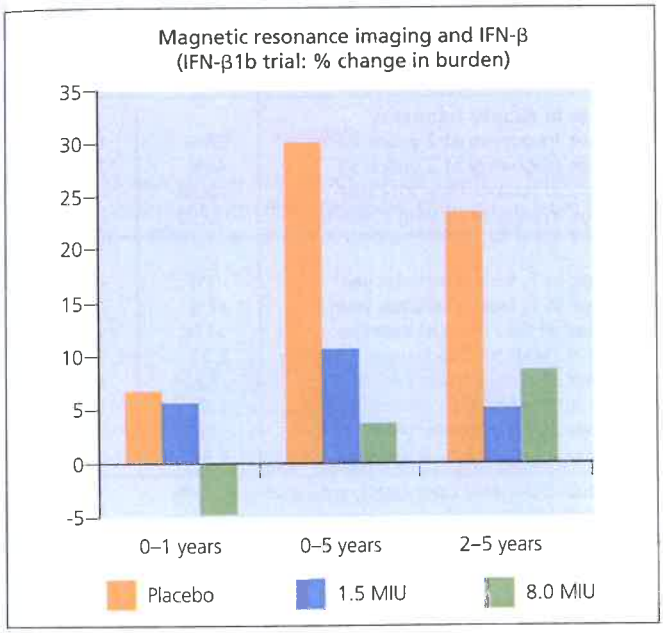


Figure 18.13 Annual change in MRI disease burden at baseline, baseline to year 1 and baseline to year 5 in patients receiving IFN-β1b; the rate of reduction is shown from baseline to end of year 1 and end of year 1 to end of year 5. Adapted from Paty *et al* (1993) and the IFNB Multiple Sclerosis Study Group (1993; 1995). © 1993; 1995, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

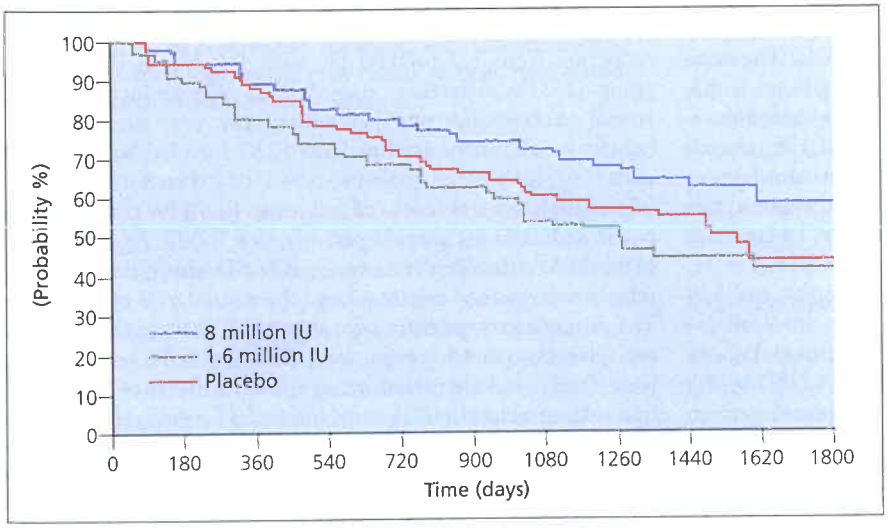


Figure 18.14: Kaplan–Meier curves showing, by treatment arm, the probability of avoiding progression of multiple sclerosis equal to at least 1 EDSS point. Adapted from the IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group (1995). © 1995, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

disease activity. This suggests that, despite some differences in phenotype, IFN-β may be no less effective in Japanese populations than elsewhere.

IFN-β1a (Avonex)

The results of a study using IFN-β1a, with disability as the primary outcome measure, were first presented to a joint meeting of the American Neurological Association and Association of British Neurologists in October 1994 and immediately published in abstract form (Anon 1994). Despite widespread distribution of fly-sheets further advertising these results at scientific meetings and a description of the methodology (Jacobs *et al* 1995), no peer-reviewed publication of the results appeared until early 1996 (Jacobs *et al* 1996) by which time the procedure for granting a product licence in the United States and Europe was well advanced. In the trial, existing symptomatic treatments were not discontinued. Relapses were treated (at the discretion of physicians) with corticosteroids and immediate adverse effects of IFN-β1a were prophylactically managed with acetaminophen. Three hundred and one patients with clinically definite multiple sclerosis in the relapsing phase (some with persistent symptoms and signs), and with EDSS scores ranging from 1 to 3.5, were treated with placebo (n = 143 at entry; n = 87 for 2 years) or IFN-β1a (n = 158 and n = 85, respectively; Table 18.4). Each had two or more physician-documented relapses in the preceding 3 years but none in the previous 2 months, and the pretreatment exacerbation rate was >0.67 per year. Compliance with the trial protocol was good, with >99% of assessments completed. There were 23 early exits from the study but assessments continued in these patients. Sample size calculations allowed for 25% of patients to discontinue treatment but remain available for analysis on an intention to treat basis and with 10% lost to follow-up. The drop-out rate was <3%. The decision was taken to restrict recruitment to 288 patients (in fact, 301 had already been enrolled) and to stop the study a year earlier than planned. In retrospect, this was unwise because it has been assumed (wrongly according to the manufacturer) that premature termination of the study was taken with reference to interim efficacy analyses. Whatever the reason, this decision left the study significantly underpowered.

Treatment with IFN-β1a was shown to be associated with a slower rate of disability (defined in advance as deterioration by ≥ 1 point on the EDSS for ≥ 6 months; Table 18.4). The decision to stop the trial early left only 172 (IFN-β1a, n = 85; placebo, n = 87) participants observed for the intended duration of the study. Two years after the start, 22% of 158 patients who had received IFN-β1a were classified as treatment failures compared with 35% of the 143 placebo cases. At this point, two patients had not completed 6 months on the study; 14 (seven in each arm) had been involved for <1 year; 67 (IFN-β1a, n = 32; placebo, n = 35) had been studied for <18 months; and 134 (IFN-β1a, n = 56; placebo, n = 73) had been involved for <2 years (Figure 18.15). The numbers of treatment failures in those who completed 2 years were 18 of 85 (21%) in the IFN-β1a-treated group and 29 of 87 (33%) in the placebo group, respectively. Using the probability of sustained progression in the first year as an outcome also revealed the modest effect on disability (22% and 16% during year one, and 12% and 11% during year two, for placebo and treated patients, respectively; p = 0.02: Figure 18.16). These proportions did not differ between those who completed a second year in the study and those who did not.

Table 18.4 IFN-β1a (Avonex): pivotal trial

	Placebo	IFN-β1a
Number enrolled	143	158
Number completing year 1	136	151
Number completing year 2	87	85
Change in disability		
Sustained progression at year 1	22%	13%
Sustained progression at year 2 (all patients)[1]	35%	22%
Sustained progression at year 1	22%	13%
Sustained progression at year 2 (patients completing 2 years on study)	33%	21%
Change in EDSS at 2 years: –<1 point	12%	19%
Change in EDSS at 2 years: +<1 point	37%	24%[a]
Change in relapse frequency		
Relapse frequency at 2 years: <2	56%	68%
Relapse frequency at 2 years: >2	44%	32%
Change in relapse rate: all cases	–0.38	–0.53[b]
Change in relapse rate: at 2 years	–0.30	–0.59[c]
MRI		
Change in T_2 lesion volume: year 1	–3%	–13%[a]
Change in T_2 lesion volume: year 2	–7%	–13%
Number of Gd+ lesions: baseline	>174	>196
Mean number of Gd+ lesions: baseline	2.32	3.17
Number of Gd+ lesions: year 1	>124	>85
Mean number of Gd+ lesions: year 1	1.59	1.04[a]
Number of Gd+ lesions: year 2	>78	>49
Mean number of Gd+ lesions: year 2	1.65	0.80[d]

a p = 0.02; b p = 0.04; c p = 0.0002; d p = 0.05.
Gd = gadolinium.

Relapse rate (each exacerbation had to last >48 hours and be confirmed by a neurologist) was a secondary outcome measure. Overall, the reduction amongst treated patients was 18%. Fewer treated patients in the cohort who completed 2 years (12 of 85, 14%) had three or more exacerbations during the study than controls (28 of 87, 32%; p = 0.03). In the group studied for 2 years, annual exacerbation rates reduced from 1.2 to 0.61 per year (–0.59) in patients receiving IFN-β1a compared with a reduction from 1.2 to 0.90 per year (–0.30) in the placebo group (a 31% difference; p = 0.002). The reduction in the annual exacerbation rate per patient per year suggested less benefit for all randomized patients (0.82 for placebo compared with 0.67 for treated patients; p = 0.04) than for the subset who completed 104 weeks of follow-up (0.90 for placebo compared with 0.61 for treated patients; p = 0.002; Figure 18.17). In marked contradistinction to the IFN-β1b study, the change in relapse rate was not apparent until the second year of the study. The proportions free from any relapse at 2 years in the IFN-β1a and placebo-treated groups were 38% and 26%, respectively (p = 0.03), and there was no significant difference in time to first relapse between the groups (36 and 47 weeks, respectively; p = 0.34; Table 18.4). Partly in response to critical comments on the IFN-β1a study, the investigators subsequently re-analysed their results using more stringent outcome measures (Rudick

correlated with changes in the proportion or specificity of T-cell clones reactive to myelin basic protein, or its encephalitogenic peptides. In other contexts, oral feeding of antigen has been shown to favour the induction of T cells which secrete IL-4, TGF-β and IL-10 at low doses and to delete both Th1 and Th2 cells at higher doses (Y. Chen *et al* 1995). Although superficially attractive, the results of the pilot study always seemed to us overstated and few were surprised by the widely publicized news that the phase three trial of oral myelin (515 patients, 14 sites) showed no clinical effect. However, this negative study remains unpublished several years after the trial was stopped and seems destined never to be reported in full.

Altered peptide ligands

Antigen-specific immunotherapy was dealt a further tough blow in 2000 when two phase two trials of altered peptide ligand therapy designed to interfere with T-cell responses were terminated early because of concerns about patient safety. In the first, use of the altered peptide ligand (CGP77116) was associated with clinical relapses and systemic hypersensitivity reactions that persisted despite dose reduction (Bielekova *et al* 2000). The trial was terminated after only eight patients were enrolled. The finding that two-thirds of patients who had clinical relapses after starting therapy were shown to have developed high T-cell precursor frequencies to the ligand and myelin basic protein peptide 83–89 suggested that the intervention incited clinical relapses. In the second study, three doses of the altered peptide ligand NBI5788 (5, 20 or 50 mg weekly by subcutaneous injection) were compared with placebo administration. The trial was stopped when nine of the 142 patients experienced hypersensitivity reactions. Immunological studies suggested that treatment induced a Th2 profile of immune response (Kappos *et al* 2000). This study was of insufficient duration to detect a clinically meaningful response but MRI monitoring suggested a possible benefit using the lowest dose of altered peptide ligand. D.E. Goodkin *et al* (2000) demonstrated that various doses of a complex of HLA-specific DR2 solubilized with the myelin basic protein peptide 84–102 (AG284) were well tolerated but the trial was not powered for an efficacy analysis.

Copolymer-1 or glatiramer acetate (Copaxone)

Following the logic that immunological damage in multiple sclerosis is mediated by antigen-specific T cells, a synthetic peptide composed of L-alanine, L-glutamic acid, L-lysine and L-tyrosine was designed specifically to mimic the structure of myelin basic protein. Copolymer 1 [Cop-1, later renamed glatiramer acetate (Copaxone) by the sponsor upon approval of this agent for use in multiple sclerosis by the FDA in North America] was neither encephalitogenic nor toxic, and was shown to suppress experimental autoimmune encephalomyelitis (perhaps by inducing antigen-specific suppressor cells). It moved into clinical practice in the early 1980s (Abramsky *et al* 1977).

Clinical studies

Bornstein *et al* (1982) first reported in detail on the therapeutic use in 16 patients with multiple sclerosis. In a subsequent blinded and placebo-controlled study of patients having two or more relapses in the previous 2 years, and EDSS scores of <6 at entry, randomization to active or placebo preparations was within EDSS bands (Bornstein *et al* 1987). Participants received subcutaneous Cop-1 for up to 2 years. A neurologist assessed disease activity, and analysis was on an intention to treat basis. Taking absence of relapse during the trial as the primary end point, a greater proportion of individuals in matched pairs randomized to Cop-1 were relapse free on completion than placebo cases (ten of 22 compared with two of 22 in whom the placebo partner but not the Cop-1-treated individual was free from relapse, and ten of 22 pairs in whom the course was concordant within individual pairs; $p = 0.039$: Figure 18.38A). There were 62 exacerbations in 23 placebo-treated patients compared with 16 amongst 25 Cop-1-treated individuals. Although the placebo group showed a reduction in relapse rate during the trial as part of the natural history, or regression to the mean, the difference between groups was 4.9 in the first year and 3.3 in the second, favouring treatment with Cop-1. Overall 14 of 25 treated patients were free from relapse compared with six of 23 in the placebo group ($p < 0.001$). An apparent difference in the rate at which Cop-1- and placebo-treated patients deteriorated (five of 25 and 11 of 23, respectively), which was especially marked in less affected individuals (EDSS <2 at entry), was not statistically significant. There was, however, a delay in time to progression by one EDSS point amongst Cop-1-treated patients (Figure 18.38B). Local injection site reactions seriously undermined blinding in this study and 29 of 40 participants (in whom information was available) correctly guessed treatment assignments (the authors suggest that unblinding was also influenced by the response to treatment). Subsequently, Bornstein *et al* (1991) completed a study of 106 patients with secondary progressive multiple sclerosis who had documented evidence for an increase in disability over the preceding 6–18 months. Treatment had no effect on the proportion of patients showing sustained progression by a further one EDSS point. Apart from local skin reactions at the injection site, Cop-1 was well tolerated.

The result of the above study was presumably influential in the decision to design a phase three trial involving patients with relapsing–remitting multiple sclerosis and using relapse rate as the primary outcome. This involved 251 patients randomized to Cop-1 (20 mg by daily subcutaneous injection for 2 years; $n = 125$) or placebo ($n = 126$; K.P. Johnson *et al* 1995). The relapse rate over 2 years in treated patients was 1.2 ± 0.1 compared with 1.7 ± 0.1 in controls (a 29% reduction giving annual rates of 0.6 and 0.8, respectively; $p < 0.007$: Figure 18.39A). More Cop-1-treated patients were free from relapse, and treatment also favoured a delay in time to relapse. With respect to disability, the proportions of patients taking Cop-1 who were unchanged, improved or worse by 1 EDSS point were 54%, 25% and 21% compared with 56%, 15% and 29%, respectively, in the placebo group (Figure 18.39B). Results of the pivotal North American trial led the FDA to approve Cop-1 for the reduction of exacerbations in patients with relapsing–remitting multiple sclerosis. (As noted above, following FDA approval for licensure the company renamed the agent glatiramer acetate or Copaxone.) A United Kingdom licence for the same indication followed in 2000 and in the rest of the European Union in 2001 (*www.tevapharm/com/copaxone/*). In the United Kingdom, the

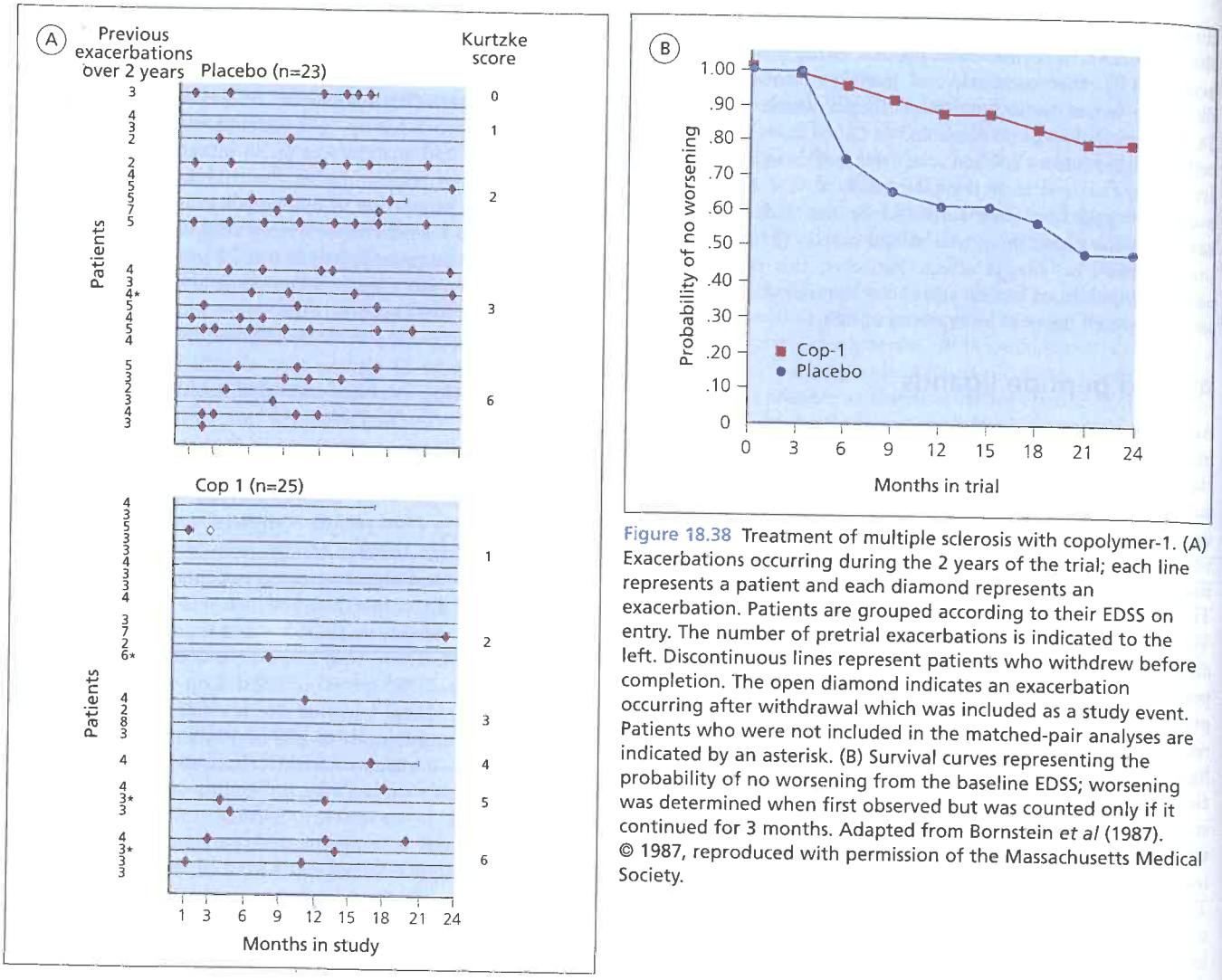


Figure 18.38 Treatment of multiple sclerosis with copolymer-1. (A) Exacerbations occurring during the 2 years of the trial; each line represents a patient and each diamond represents an exacerbation. Patients are grouped according to their EDSS on entry. The number of pretrial exacerbations is indicated to the left. Discontinuous lines represent patients who withdrew before completion. The open diamond indicates an exacerbation occurring after withdrawal which was included as a study event. Patients who were not included in the matched-pair analyses are indicated by an asterisk. (B) Survival curves representing the probability of no worsening from the baseline EDSS; worsening was determined when first observed but was counted only if it continued for 3 months. Adapted from Bornstein *et al* (1987). © 1987, reproduced with permission of the Massachusetts Medical Society.

Association of British Neurologists guidelines on eligibility criteria for glatiramer acetate are:

- ambulant patients with relapsing–remitting multiple sclerosis able to walk at least 100 m without support (EDSS ≤5.5)
- at least two clinically significant relapses in the last 2 years
- age ≥ 18 years.

The pivotal North American glatiramer acetate (or Copaxone, see above) study has been continued. In a blinded extension for up to 11 additional months, there was no loss of effect on relapse rate. Sustained disability was seen in 23% of patients receiving glatiramer acetate compared to 29% of controls (K.P. Johnson *et al* 1998). The European–Canadian glatiramer acetate MRI study was designed to evaluate the effect of treatment on MRI features of disease activity in relapsing–remitting multiple sclerosis (Comi *et al* 2001b). Two hundred and thirty-nine patients with relapsing–remitting multiple sclerosis from 29 centres in seven countries were randomized to receive daily subcutaneous injections of placebo or glatiramer acetate (20 mg). Monthly MRI studies were performed for 9 months. This study demonstrated that treatment reduced the total number of gadolinium enhancing lesions (overall reduction, 29%; p = 0.003: Figure 18.40) although a large number of enhancing lesions were still seen in treated patients. This effect was first apparent after approximately 6 months of treatment (Figure 18.41). There was no difference, however, in the proportion of patients showing MRI contrast enhancements (although the specific data were not reported). Notably, only three treated patients remained free of contrast enhancing lesions during the 9 month study. T_2 volume continued to worsen in both groups but to a lesser degree in those receiving glatiramer acetate. The change in hypodense lesion volume was not significantly different between groups. Treatment also reduced the number of relapses but not for the first 6 months. A subsequent publication from this prospective study reported that treatment with glatiramer acetate reduced the proportion of the 1722 new contrast enhancing MRI lesions that developed into persisting hypodense T_1 'black holes', at 7 months (p = 0.004) and 8 months (p = 0.0002) after they were first detected in the 239 participants (Filippi *et al* 2001a) but not at the 6 month assessment (Filippi *et al* 2002c; N.D. Richert 2002). These reports suggest

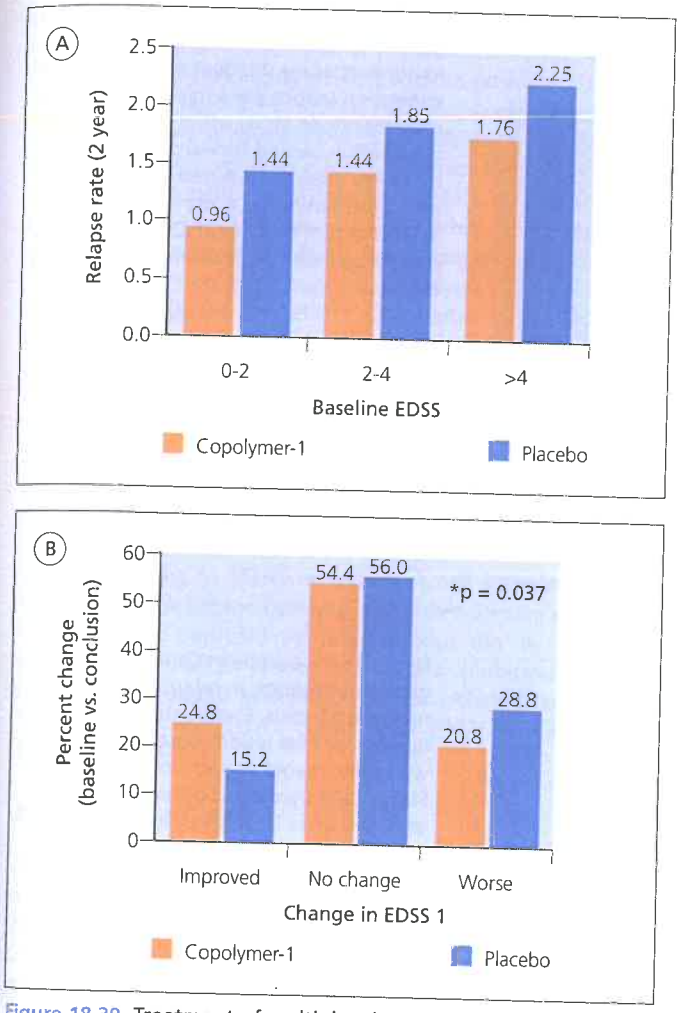


Figure 18.39 Treatment of multiple sclerosis with copolymer-1. (A) Changes in relapse rate observed over 2 years, by baseline EDSS. Numbers above each bar represent the mean 2 year relapse rate for each group. (B) Percentage of patients who improved, were unchanged, or were worse by ≥1 EDSS points between baseline and the last (24 month) measurement (repeated measures ANCOVA). Numbers above each bar represent the percentage of patients in the respective copolymer-1 or placebo group. Adapted from K.P. Johnson *et al* (1995). © 1995, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

that glatiramer acetate has less immediate impact on MRI markers of inflammatory demyelination than IFN-β but may favourably affect the proportion of MRI lesions that develop significant axonal loss.

A 9 month, placebo-controlled trial of glatiramer acetate in 239 pateints with relapsing–remitting multiple sclerosis revealed a mean 0.7–0.8% reduction in central cerebral volume with no significant differences between the patient groups (Rovaris *et al* 2001c). The study showed a weak association between enhancing lesion numbers and atrophy. Rovaris *et al* (2002b) later designed a small, prospective study of glatiramer acetate on the formation of new T_2 lesions and (new and total) contrast-enhancing MRI lesions. This cohort of 20 patients with relapsing–remitting multiple sclerosis underwent monthly MRI studies during both pretreatment and posttreatment periods of observation (two sessions of monthly scans beginning 5 months before and then restarting 90 days after treatment with daily subcutaneous injections of glatiramer acetate, 20 mg). Patients were given both a standard and triple dose of gadolinium separated by 12–24 hours. Using MRI measures of disease activity based on pretreatment behaviour, the authors concluded that treatment reduced both new T_2 and new and total gadolinium enhancing lesion formation. The benefit was detectable within 4 months of starting therapy. In addition, glatiramer acetate reduced the number of contrast enhancing lesions using all doses of contrast enhancing agent. These observations suggested that, in the context of multiple sclerosis, glatiramer acetate may reduce the number of inflammatory lesions in situations of both mild and severe blood–brain barrier disruption – although this conclusion is necessarily based on an indirect measure of blood–brain barrier integrity.

A large, phase III, randomized, double-blinded, placebo-controlled trial designed to determine whether either of two doses of daily oral glatiramer acetate (5 and 50 mg) were superior to placebo in reducing relapse rate (primary outcome), MRI activity (secondary outcome) or disability (tertiary and other end points) was terminated after an interim analysis at 14 months suggested little likelihood of a positive outcome. The results are not yet published.

The clinical relapse rates of 85 patients with relapsing–remitting multiple sclerosis who switched from IFN-β1a (6 MIU by intramuscular injection for 18–24 months) to glatiramer acetate (20 mg by subcutaneous injection daily) either for reasons of perceived lack of efficacy (62 patients) or persistent intolerance to treatment-related side effects (23 patients) were evaluated by O.A. Khan *et al* (2001b). After a further period of 18–24 months prospective follow-up, the authors concluded that glatiramer acetate administration reduces relapse rate in patients previously not fully responsive to IFN-β. The degree of reduction is no less than in patients who responded but switched because of drug intolerance.

Although this result invites the conclusion that glatiramer acetate can rescue patients who fail on treatment with IFN-β, no definitive studies comparing the relative efficacies of these drugs are available although several trials are in progress. A comparison was made by O.A. Khan *et al* (2001c) of clinical outcomes at 18 months in a group of 156 patients with relapsing–remitting multiple sclerosis followed prospectively. In this open label, non-randomized and unblinded study, patients were permitted to choose no treatment (n = 33) or standard doses of intramuscular IFN-β1a (Avonex; n = 40), subcutaneous IFN-β1b (Betaseron; n=41) or glatiramer acetate (n = 42). At 18 months, 122 patients remained in the study (18/34 drop-outs were from the 'no treatment' group). Annual relapse rates were significantly reduced only by glatiramer acetate (0.49; p = 0.001) and subcutaneous IFN-β1b (0.55; p = 0.001) but not by intramuscular IFN-β1a (0.81) compared with the 'no treatment' group (1.02). Similarly, the percentage of relapse-free patients was significant only for glatiramer acetate and subcutaneous IFN-β1b (33% for both; p = 0.05; intramuscular IFN-β1a, 12%; no treatment, 7%). Mean change in EDSS also favoured these two treatment groups (IFN-β1b: –0.25, p = 0.010; glatiramer acetate: –0.44, p = 0.003; IFN-β1a +0.19, p = 0.452 compared with untreated patients: +0.60).

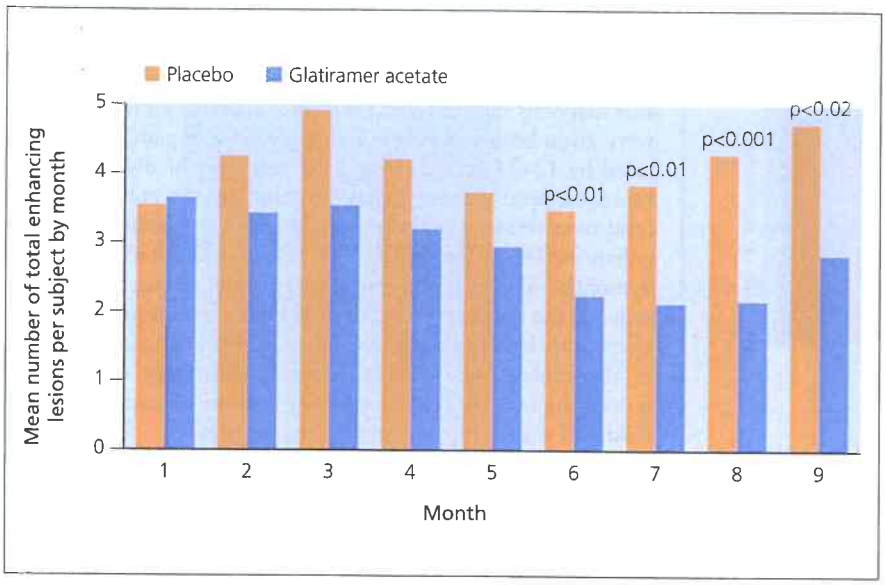


Figure 18.40 European/Canadian trial of glatiramer acetate in relapsing–remitting multiple sclerosis. Median number of total enhancing lesions per subject observed at each month on study using the last observation carried forward. Repeated measures analysis favoured a treatment effect for glatiramer acetate (p = 0.003). Adapted from Comi *et al* (2001b). © 2001, reproduced with permission of John Wiley & Sons.

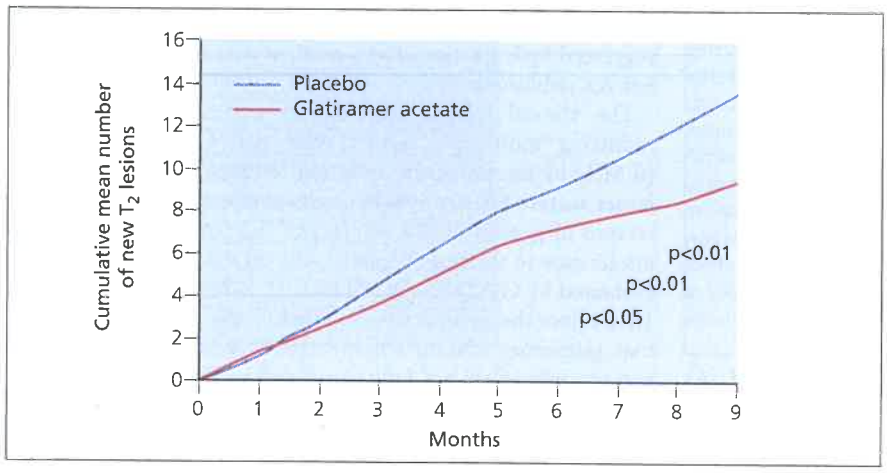


Figure 18.41 European/Canadian trial of glatiramer acetate in relapsing–remitting multiple sclerosis. Cumulative mean number of new lesions observed on the T_2-weighted images at each month on study. Statistically significant differences first emerged after 5 months on treatment. Adapted from Comi *et al* (2001b). © 2001, reproduced with permission of John Wiley & Sons.

The impact of this study is clearly reduced by the design, but nevertheless it provides some evidence that, in practice, glatiramer acetate may have similar efficacy to IFN-β1b, and both are superior to IFN-β1a. A large, National Institutes of Health funded, phase three trial comparing IFN-β1a by once weekly subcutaneous injection (Avonex) and glatiramer acetate (Copaxone) given either alone or in combination (four treatment arms) to patients with relapsing–remitting multiple sclerosis is in progress in the United States (Fred Lublin, personal communication) An open label prospective study of modest size is already reassuring with respect to safety issues (Lublin *et al* 2001; 2002). Clearly, these turf wars are not yet settled.

Munari *et al* (2004; 2004b) completed a systematic review of glatiramer acetate and concluded that there is no conclusive evidence that this agent alters relapse or progression rate in patients with multiple sclerosis. The interested reader is referred to correspondence rebutting and supporting the methodological assumptions that led to this conclusion (Caramanos and Arnold 2005; Comi *et al* 2005; deJong *et al* 2005; Munari and Filippini 2005).

PROMISE was a massive, randomized, double-blind, placebo-controlled phase three study involving 943 primary progressive patients randomized in a 2:1 ratio either to receive glatiramer acetate or placebo. It was recently terminated after an interim analysis suggested futility (Wolinsky *et al* 2004). There were no safety concerns and doubtless post-hoc analyses will add greatly to our understanding of the nuances of testing therapies in this disease category. PROMISE was not fulfilled.

Adverse effects

Glatiramer acetate is generally well tolerated. It is usually possible to initiate treatment at full strength (20 mg by subcutaneous daily injection) without dose titration. Although a daily subcutaneous injection is required, rarely do patients discontinue this drug because of intolerance. In the early reports (Bornstein *et al* 1982; 1987) and pivotal North American trial (K.P. Johnson *et al* 1995; 1998), there is comment on an unpredictable, sometimes frightening but transient and usually self-limiting systemic reaction (facial flushing, chest tightness, anxiety,

palpitations and dyspnoea) lasting for around 30 minutes immediately after an injection. This is experienced by up to 15% of patients (but not recurring in 50%) with an estimated frequency of one episode per 840 injections (K.P. Johnson *et al* 1998). It is important to warn patients but, although alarming, it is seemingly an innocent adverse effect. Approximately two-thirds of patients notice pain at the injection site. Local irritation may occur in 2–3% (K.P. Johnson *et al* 2000) but skin breakdown is very rare (Johnson *et al* 1998). Focal atrophy of subcutaneous tissue at injection sites and adjacent lymphadenopathy may develop (Windhagen *et al* 2001). Glatiramer acetate is not associated with laboratory abnormalities and routine blood studies are not needed to monitor its safety. There are no important drug interactions.

Treated patients may develop antibodies to glatiramer acetate. However, unlike the situation with neutralizing antibodies to IFN-β, experimental and clinical evidence does not suggest that these anti-glatiramer acetate antibodies reduce biological function (Teitelbaum *et al* 2003). This has been shown in assays that measure binding to MHC molecules, T-cell stimulation, interference of competition between glatiramer acetate and myelin basic protein peptide, cytokine production by glatiramer acetate-specific T-cell clones and *in vivo* inhibition of experimental autoimmune encephalomyelitis (Aharoni *et al* 1998; T. Brenner *et al* 2001; C. Farina *et al* 2002; Teitelbaum *et al* 1973; 1991; 2003).

Mechanism of action

As for IFN-β, the precise mechanism(s) whereby glatiramer acetate influences the course of multiple sclerosis continues to be discussed (Dhib-Jalbut 2002; Neuhaus *et al* 2001; Yong 2002). Table 18.8 and Figures 18.42 and 18.43 itemize many of the key findings and provide references to the supporting literature. As discussed earlier, interferon administration is generally followed within weeks by a striking reduction in MRI evidence of blood–brain barrier disruption. This effect is much less dramatic following the administration of glatiramer acetate although MRI activity reduces gradually over a period of several months (see above, Comi *et al* 2001b). This may be explained by the finding that glatiramer acetate does not significantly inhibit T-cell migration (Dufour *et al* 2000; Prat *et al* 1999).

Each drug influences T-cell function (especially CD4 cells) in ways that both overlap and are distinctive. Each agent blocks T-cell activation and promotes Th2 (IL-4, IL-5, IL-10, IL-13 and TGF-β) cytokine production. Unlike IFN-β, however, glatiramer acetate induces the production of regulatory T cells in the periphery. These glatiramer acetate reactive cells cross the blood–brain barrier, respond to central nervous system myelin antigens by secreting Th2 (M. Chen *et al* 2001; Y. Qin *et al* 2000) and Th3 cytokines (Aharoni *et al* 2003) that inhibit Th1 cells, thereby effecting so-called 'bystander suppression'. Glatiramer acetate-reactive cells may also induce anergy (Gran *et al* 2000b).

Recently, abundant evidence has accumulated that glatiramer acetate-specific cells secrete brain-derived growth factors (Aharoni *et al* 2003; Kappos and Duda 2002; Ziemssen *et al* 2002) raising the intriguing possibility that this agent may enhance repair at the site of the multiple sclerosis lesion by inducing a degree of localized 'neuroprotective autoimmunity'.

Table 18.8 Presumed mechanisms of action of glatiramer acetate (adapted from J. Zhang *et al* 2002 with permission)

Modulates T-cell activation and/or proliferation
Competes for binding sites of MHC class II antigens (MBP, PLP, MOG: Ben-Nun *et al* 1996; Fridkis-Hareli *et al* 1994; Fridkis-Hareli and Strominger 1998; Racke *et al* 1992; Teitelbaum *et al* 1996; 2003)
May modify dendritic cell costimulation processes (Hussien *et al* 2001) or act as weak/partial T-cell receptor agonist (Wiesemann *et al* 2001)
Reduces proliferation of MBP-reactive T cells (Duda *et al* 2000; Karandikar *et al* 2002; Neuhaus *et al* 2000)
Actives both Th1 and Th2 cells (Zang *et al* 2003)
Increases ratio of anti-inflammatory (Th2) to proinflammatory (Th1) cytokines
Increases IL-10, IL-4, and IL-6 production (C. Farina *et al* 2001; Hussien *et al* 2001; Neuhaus *et al* 2001a) and decreases IL-12 production (Hussien *et al* 2001)
Increases and then decreases IFN-γ secretion with repeated antigen stimulation (Aharoni *et al* 1997)
Upregulates CD8+ T-cell responses (Karandikar *et al* 2002)
Induce regulatory Th2/3 cells that penetrate the central nervous system and express their anti-inflammatory cytokines and neurotrophic factors *in situ* in animal models of multiple sclerosis (Aharoni *et al* 2003)
Induces TNF-α and IFN-γ production (C. Farina *et al* 2001; Neuhaus *et al* 2000; Zang *et al* 2003)
Enhances production of brain-derived nerve growth factor (Ziemssen *et al* 2002)
Reduces monocyte (Weber *et al* 2004) and antigen-presenting cell function (S. Jung *et al* 2004; H.J. Kim *et al* 2004)

MHC = major histocompatibility complex; IL = interleukin; ; TNF = tumour necrosis factor; MBP = myelin basic protein; PLP = proteolipid protein; MOG = myelin oligodendrocyte glycoprotein; Th = T-helper cell.

The idea that inflammation may enhance remyelination has attracted great interest in the experimental literature (Kipnis *et al* 2000; M. Rodriguez and Lennon 1990; Schori *et al* 2001; Schwartz 2001; Schwartz *et al* 1999; Schwartz and Kipnis 2001) and is discussed more fully in Chapter 10. Recently, M.S. Weber *et al* (2004) have reported that glatiramer acetate blocks monocyte reactivity *in vitro* using cells from treated patients. Both S. Jung *et al* (2004) and H.J. Kim *et al* (2004) have shown that glatiramer acetate also reduces the function of antigen-presenting cells. Together, these studies demonstrate that the mechanisms of action of glatiramer acetate extend well beyond the lymphocyte population of immune cells.

An enzyme-linked immunoadsorbent spot (ELISPOT) assay was developed by C. Farina *et al* (2002) that may correlate 'responder' status to glatiramer acetate. They created three immunological criteria (reduced proliferative response to glatiramer acetate, *in vitro* activation of IFN-γ-producing cells, and activation of IL-4-producing cells) and found that 13 of 15 clinical responders (87%) met two or all three criteria, compared with 22% of patients who appeared to be failing treatment. If correct, this assay may ultimately find more widespread use and lead to the development of other *in vitro* measures to inform treatment decisions.

Antibodies to glatiramer acetate develop within 3 months and may later diminish (T. Brenner *et al* 2001; C. Farina *et al* 2002).

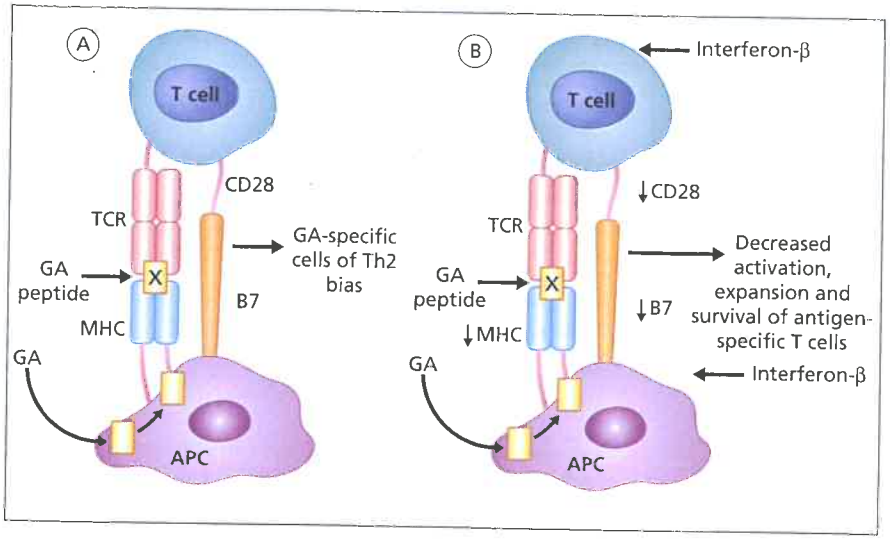


Figure 18.42 Mechanisms of action of glatiramer acetate (GA) and beta-interferons on antigen presentation. (A) The high affinity of GA for the MHC groove or the uptake of GA by an antigen-presenting cell leads to the presentation of GA as an antigen and the generation of GA-specific Th2-biased cells. (B) IFN-β acts on its receptor on T cells and antigen-presenting cells. This decreases the expression of molecules needed for antigen presentation. Together with a further activity of interferon on T-cell expansion and survival, this leads to the decreased generation of antigen-specific T cells. X refers to an antigen-siting in the MHC groove; TCR = T-cell receptor. Adapted from Yong (2002). © 2002, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

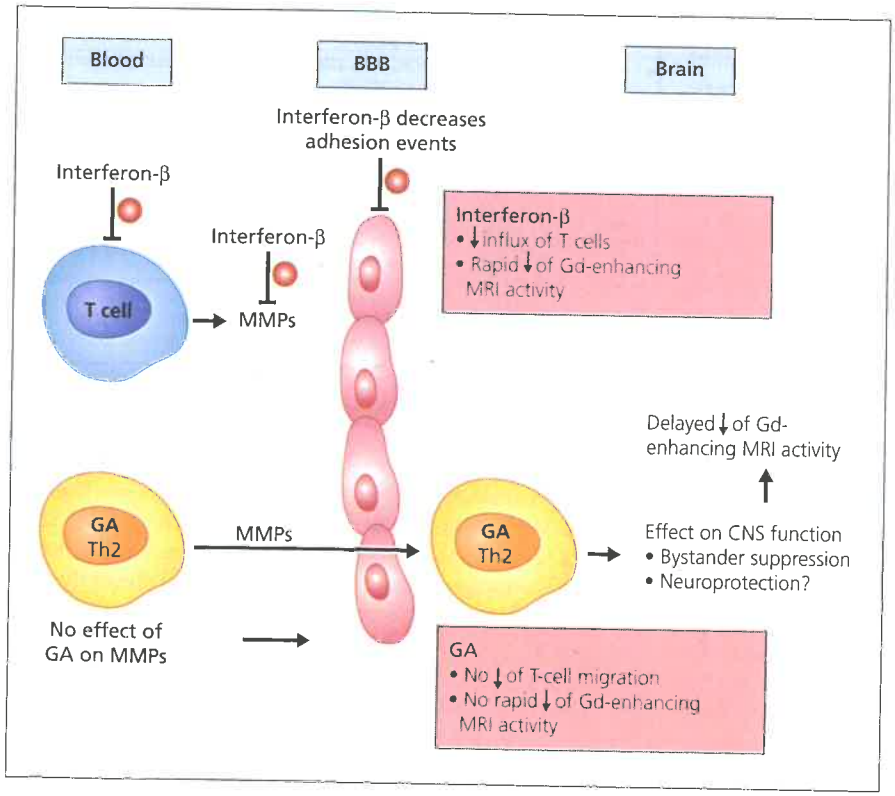


Figure 18.43 Effects of glatiramer acetate (GA) and IFN-β on the blood–brain barrier (BBB) and within the central nervous system. IFN-β reduces the production of matrix metalloproteinases (MMP) by T cells and diminishes the adhesion of T cells to endothelium. These two effects reduce the influx of T cells into the central nervous system. GA-specific Th2 cells traffic into the central nervous system to produce local bystander immune suppression and possibly exert neuroprotection. Gd = gadolinium. Adapted from Yong (2002). © 2002, reproduced with permission of Lippincott Williams & Wilkins (lww.com).

There is great interest in whether these antibodies reduce the clinical benefit of glatiramer acetate (see the discussion of the possible influence of neutralizing antibodies on clinical effects of the interferons). In a large series of *in vitro* and *in vivo* experiments, Teitelbaum *et al* (2003) reported that these antibodies do not seem to reduce activity. A subsequent study, however, reported that antibodies to glatiramer acetate reversed many of these putative activities, including the effect on T-cell proliferation and both pro- and anti-inflammatory cytokines (Salama *et al* 2003). More work is therefore needed to clarify these ambiguities.

Early work of great interest suggests that antibodies to glatiramer acetate induce remyelination in the Theiler's virus animal model of demyelinating disease (Ure and Rodriguez 2002). This finding parallels the observation that immunoglobulins directed against central nervous system antigens also induce abundant remyelination in this animal model (Bieber *et al* 2003; Ciric *et al* 2003; 2004; Mitsunaga *et al* 2002; M. Rodriguez and Lennon 1990) and raises the possibility that anti-glatiramer acetate antibodies may enhance repair of the lesion in multiple sclerosis.

In what ways might these two agents complement their mechanisms of action? Zang *et al* (2003a) showed that, when tested

together *in vitro*, IFN-β1a and glatiramer acetate act to antagonize their respective modes of action. Specifically, IFN-β1a blocked glatiramer acetate-induced T-cell proliferation and the drug-specific pattern of cytokine production was lost. Glatiramer acetate induced both Th1 (TNF-α and IFN-γ) and Th2 (IL-4 and IL-10) cytokines. Conversely, IFN-β inhibited Th1 cytokine production. Together, IFN-β reduced the number of IFN-γ-producing cells compared to glatiramer acetate alone – suggesting another type of antagonism between these two agents. Firm conclusions on the practical and immunological aspects of combination therapy seem premature.

The licence for Copaxone

In the United States, information distributed by the pharmaceutical company that markets glatiramer acetate states that this agent is

> *recommended for reduction of the frequency of relapses in patients with relapsing remitting multiple sclerosis. It is contraindicated in patients with known hypersensitivity to glatiramer acetate or mannitol and is not recommended for use in pregnancy. The safety and efficacy of glatiramer acetate is unknown in nursing women, in those with impaired renal function, in patients <18 years old, and in the elderly.*

Copaxone was approved for marketing in the United Kingdom and launched in December 2000 with the same general indications. This first approval in a major European market led to extension throughout the European Union by the end of 2001 under the European Mutual Recognition Procedure with the United Kingdom acting as reference member state.

TREATMENTS THAT TARGET T CELLS

In view of the conditional benefits of immune suppression in the treatment of patients with multiple sclerosis, attention has turned to alternative means of interfering with the sequence of events that leads to tissue injury. The theoretical basis for designing therapies, the experimental evidence that they might work, such clinical evidence as there is for efficacy, and the reasons why theory does not always translate into clinical success were admirably reviewed by R. Hohlfeld (1997). These approaches include:

- the use of monoclonal antibodies that achieve much more precise immunological effects than nonspecific immunosuppressants
- reagents which block recognition between antigen-presenting cells and responder lymphocytes
- depletion of autoreactive lymphocyte populations by T-cell vaccination
- bone marrow transplantation.

The validity of these approaches has yet to be confirmed and most are conditional on whether the central hypothesis for the role of T cells in the pathogenesis of inflammatory demyelination is correct. Most of these therapies target circulating white blood cells, in particular lymphocytes, and so qualify as potential disease-modifying treatments in multiple sclerosis. We retain a brief discussion of the earlier studies as background to the current interest in this strategy.

Anti-lymphocyte globulin

The first trial, assessing treatments designed to target circulating lymphocytes and adopting a double-blind and placebo-controlled protocol, was reported in 1982. Inevitably it was underpowered. Forty-three patients with relapsing–remitting multiple sclerosis were randomized to treatment with anti-lymphocyte globulin, azathioprine and prednisolone in combination for 1 month, followed by azathioprine (3 mg/kg) alone for a further 14 months, or placebo preparations. The reduction in relapse rate from 1.0 to 0.7 in the treated group, time to first episode and accumulation of disability all favoured an effect of treatment but the magnitude of these results did not impress the investigators, who reported their findings as offering no more than an indication for continuing to evaluate immunosuppression in the context of multiple sclerosis (Mertin *et al* 1982).

Total lymphoid irradiation

Cook *et al* (1986) first compared total lymphoid irradiation given over 5 weeks with sham irradiation in a group of 45 patients with secondary progressive multiple sclerosis. They reported a beneficial effect on time to further sustained progression, especially over the first 12 months of the study. This clinical response was anticipated by a reduction in absolute lymphocyte count to $<850/mm^3$ and only these patients showed lower functional impairment scores for up to 4 years after the start of treatment. Wiles *et al* (1994) studied 27 patients (the plan was to study 56 but recruitment proved difficult) randomized to active or sham irradiation with 1980 cGy to the lymphoid system and spleen. There was no difference between groups in the clinical course over 2 years, other than a small improvement in bladder function. However, MRI activity was reduced. Three patients died – two, sham-treated, from respiratory complications of multiple sclerosis, and one, who received total lymphoid irradiation, from cardiac failure. Although these fatalities were not related to lymphoid irradiation, the treated group experienced more adverse effects than controls. Subsequently, Cook *et al* (1995) claimed that the concomitant use of corticosteroids further improved the effects of total lymphoid irradiation, and that this additional benefit correlated with the emergence of T cells having the $CD^+/CD3^-$ or $CD8^+/CD3^-$ phenotype. Mortality after total lymphoid irradiation was 1% compared with 14% in the sham-treated group. With EDSS scores at entry of >6.5, these were moderately severely affected patients at the outset.

Monoclonal antibodies

With developments in therapeutic immunology came the opportunity to design small molecules and monoclonal antibodies targeting one component only of the immune system, and leaving the rest intact. In theory, chimaerization and humanization reduce the immunogenicity of therapeutic antibodies and allow courses of reagents having prolonged effects to be given repeatedly (Winter and Milstein 1991). A single pulse of treatment

can induce prolonged alteration in immunological behaviour long after the targeted immune population has been reconstituted (S. Qin *et al* 1993). Anti-CD6 (Hafler *et al* 1986), anti-CD2 (Hafler *et al* 1988), anti-CD3 (Weinshenker *et al* 1991b), anti-CD4 (Lindsey *et al* 1994a; 1994b; van Oosten *et al* 1997; Racadot *et al* 1993) and anti-CD52 (Moreau *et al* 1994; 1996) antibodies have all been administered to patients with multiple sclerosis. In some instances, anti-globulin responses and acute adverse effects limited the usefulness of these potential treatments and an additional problem has been modulation of the targeted lymphocyte antigen, allowing some cells to survive.

Anti-CD6

Using a murine antibody which recognizes the T12 antigen (CD6) present on most (post-thymic) T lymphocytes (but neither chimaeric nor humanized), together with corticosteroids, Hafler *et al* (1986) reported clinical stabilization in six of 12 patients with secondary progressive multiple sclerosis (severe enough to require the recent use of a wheelchair in two cases) at 6 months in an open uncontrolled study. This effect was maintained for a further 3 months in three of these responders. Human anti-mouse antibodies developed within 7 days in seven of nine patients in whom assays were performed. Recovery of circulating T12 cells was rapid and there was evidence *in vitro* for antigen modulation. Studies of cerebrospinal fluid did not suggest that antibody had entered the central nervous system.

Anti-CD3

Weinshenker *et al* (1991c) treated 16 patients, selected for recent rapid accumulation of disability or a high relapse rate, with 50 mg of an anti-CD3 monoclonal antibody (OKT3) over 10 days. Each received corticosteroids and non-steroidal anti-inflammatory drugs. One patient developed anaphylaxis within minutes of receiving the first dose of OKT3 and did not continue in the protocol. A variety of systemic symptoms occurred in all patients despite prophylactic measures (typical symptoms included hypotension, fever, nausea, vomiting, diarrhoea and skin rash). Six out of 16 deteriorated during the course of treatment but this alteration was transient in three. Two patients died from complications of severe multiple sclerosis between 9 and 12 months after enrolling in this trial. Overall, the authors were uncertain that the treatment provided any lasting benefit to this group of patients. At the 1 year follow-up examination, of the 15 patients who completed the treatment protocol, four had worsened by ≥ 1.0 EDSS points (including the two deaths), nine were unchanged (EDSS changed by ≤ 0.5 points) and two improved by ≥ 1.0 EDSS point. No conclusions could be reached in the three patients with relapses of whom one each improved, remained stable and deteriorated. A small number of serial MRI scans failed to show an effect on lesion load. Rapid but transient reductions in circulating lymphocytes and their subpopulations were observed. All patients developed high titres of human anti-mouse antibodies. The systemic manifestations of OKT3 administration are known to be cytokine mediated, to correlate with sequential release of circulating TNF-α and IFN-γ followed by IL-6, and to be suppressed with methylprednisolone (Pececs *et al* 1993). Each of the two patients studied by Weinshenker *et al* (1991c) showed a transient surge in circulating TNF-α and IFN-γ on the first day of treatment. Therapy was complicated by oral candidiasis, and two patients were thought on clinical grounds to have aseptic meningitis. Whilst not promoting the continued use of murine monoclonals in multiple sclerosis, Weinshenker *et al* (1991c) advocated the development of more specific and less toxic reagents, manipulated to restrict their immunogenicity.

Anti-CD4

The first reported study using murine anti-CD4 monoclonal antibody therapy in multiple sclerosis (Racadot *et al* 1993) included 21 patients with disease progression or frequent relapse and showed no acute effects (good or bad). Clinical stabilization was claimed for 12 of 20 patients at 3 months and eight of the 20 at 6 months. No new relapses were documented. The reductions in circulating lymphocytes had returned to normal ranges within 90 days. A transient elevation was observed in circulating TNF-α, soluble TNF receptor and IL-6 but not IFN-γ, IL-1 or soluble CD8 and CD4 antigen. Unlike most other investigators, Racadot *et al* (1993) reported a detectable rise in cerebrospinal fluid TNF-α levels.

Lindsey *et al* (1994a) treated 29 patients in an open uncontrolled study with a chimaeric anti-CD4 antibody in doses ranging from 10 to 200 mg given as a single infusion, or over 3 days. The reduction in total circulating lymphocytes was partial and recovered within 6 months. The same pattern was observed for CD4 cells but without complete return to the normal range. Five patients developed anti-murine antibodies. Small improvements were noted in three of 26 patients undergoing clinical evaluation, but the majority remained unchanged (16 patients) or deteriorated (seven patients). Fourteen of 25 patients in whom serial scans were obtained had enhancing lesions on baseline MRI. One hundred enhancing lesions were seen on 91 scans obtained during follow-up, and 17 of 25 patients showed an increase in T_2-weighted lesions. The patients reported minor systemic symptoms and there was an increase in infections requiring treatment.

Most of these patients were subsequently considered for re-treatment on the basis that their CD4 count returned to >300 cells/mm^3 (Lindsey *et al* 1994b). Several were withdrawn or elected not to continue and one died suddenly after aspiration whilst eating. Twenty-one of the original cohort received up to three further treatments (a total of 36 courses were administered), responding with a drop in CD4 count on each occasion and sometimes showing prolonged lymphopenia. There seemed to be no increase in the development of anti-idiotype antibodies with this second exposure. One patient improved, three worsened, 16 remained unchanged and one was lost to follow-up. MRI activity was seen on ten of 16 scans before treatment and on 12 of 26 scans (from 16 patients) at follow-up (six of 17 in the subgroup with persistently low CD4 counts). Other than minor infections, some requiring treatment, and one episode of herpes zoster, there were few complications of repeated treatment. On the basis of these preliminary results, van Oosten *et al* (1997) randomized 71 patients, most with clinical and radiological evidence for disease activity, to treatment with chimaeric anti-CD4 or placebo under double-blind conditions.

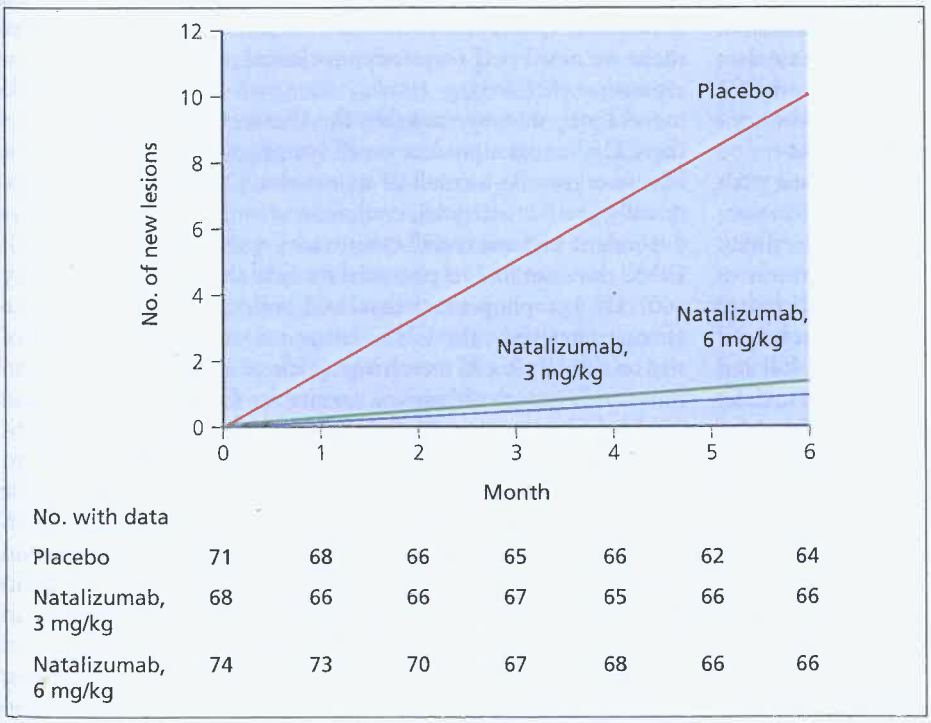
Although circulating CD4 counts were reduced, both groups showed persistent radiological activity (at around 1.5 new lesions per patient per month) although the number of clinical exacerbations was lower, by 41%, in the treated than placebo group. A mild cytokine release syndrome was apparent in these patients leading to withdrawal from the study in a few instances. Serial immunological observations in a subgroup of participants showed, as expected, reduced numbers of $CD4^+$ naive memory cells (which persisted for 12 months after treatment) but there was no effect on serum levels or on mitogen-stimulated release of TNF-α (Llewellyn-Smith *et al* 1997). These blood markers did not correlate with MRI activity.

Natalizumab (anti-VLA4)

Lymphocytes and monocytes express $\alpha_4\beta_1$ integrin on their cell surface. This glycoprotein binds the endothelial VCAM-1 and thereby mediates cell adhesion and transendothelial migration. Natalizumab (initially marketed as Antegren, Elan Pharmaceuticals and Biogen) is a humanized monoclonal antibody that blocks the α_4 integrin adhesion molecule and so reduces cell migration across the blood–brain barrier. Since serial gadolinium-DTPA enhanced MRI scans indicate breakdown of the blood–brain barrier as a consistent feature of new lesions (Kermode *et al* 1990), these properties suggested a potential therapeutic role during the active inflammatory stage of active multiple sclerosis. Data from the experimental autoimmune encephalomyelitis model of multiple sclerosis (Engelhardt *et al* 1998; van der Laan *et al* 2002) indicated that adhesion molecule inhibition might have therapeutic effects in inflammatory brain disease independent of the effect on cell migration. Natalizumab administration reduced new MRI activity in a placebo-controlled pilot study where two intravenous doses were given 1 month apart (Tubridy *et al* 1999; and see Schwid and Noseworthy 1999).

Against this background, D.H. Miller *et al* (2003a) reported a phase two randomized, double-blind, placebo-controlled study comparing two doses of natalizumab (3 and 6 mg/kg) administered intravenously once monthly for 6 months in 213 patients with relapsing–remitting multiple sclerosis. Both doses favourably influenced the primary end point (number of new MR lesions as determined by monthly scanning). Significantly fewer patients reported clinical relapses in the active treatment arms at 6 months (relapse-free: placebo, 62%; both active groups, 81%; p = 0.02: Figure 18.44). However, the apparent benefit of these treatments was not prolonged beyond the period of treatment. During the subsequent 6 months of follow-up, during which patients were untreated, those previously randomized both to the placebo and natalizumab groups, had essentially identical numbers of relapses and amounts of MRI activity. Treatment was well tolerated although there were a few allergic responses (including one episode of anaphylaxis causing bronchospasm and urticaria that responded quickly to emergency treatment with antihistamines), and perhaps a minor trend suggesting increased risk of infection (pharyngitis) in treated subjects. In a follow-up report, Dalton *et al* (2004b) demonstrated that gadolinium enhancing MRI lesions developing in the natalizumab-treated patients were less likely to develop into T_1 hypointense lesions (T_1 black holes) at 1 year than those present in the placebo patients. This report suggests that even a relatively limited period of treatment with this agent might have a degree of prolonged benefit on MRI behaviour. The clinical relevance of this finding remains to be determined.

These encouraging early findings led to further evaluation in two large phase III trials. Nine hundred and forty-two patients with relapsing–remitting multiple sclerosis, who had not received any other drug treatment for at least 6 months, were randomized to treatment with either natalizumab (300 mg) or placebo intravenously every 4 weeks for 28 months. A second placebo-controlled trial was designed to determine whether



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Placebo	71	68	66	65	66	62	64
Natalizumab, 3 mg/kg	68	66	66	67	65	66	66
Natalizumab, 6 mg/kg	74	73	70	67	68	66	66

Figure 18.44 Treatment of relapsing–remitting multiple sclerosis with intravenous natalizumab (a humanized monoclonal antibody against anti-α_4 integrin) at 3 mg/kg (blue line) and 6 mg/kg (green line). Cumulative mean number of new gadolinium-enhancing lesions on MRI while on treatment. However, in the subsequent 6 months, upon discontinuing treatment, the patients originally treated with the active antibody had equal numbers of relapses and MRI markers of disease activity to those originally randomized to placebo. Adapted from D.H. Miller *et al* (2003a). © 2003, reproduced with permission of the Massachusetts Medical Society.

prolonged administration of natalizumab provides additional benefit to weekly IFN-β1a in 1171 individuals who have already experienced at least one episode whilst on IFN-β1a. Preliminary data on safety, immunogenicity, pharmacokinetics and pharmacodynamics indicated that there were no unfavourable interactions between these therapeutic agents (Vollmer *et al* 2004a). In a similar but smaller trial, investigators randomized 110 patients treated with glatiramer acetate to monthly doses either of natalizumab or placebo for a period of 6 months.

Preliminary results of the first two studies described above, after patients had received treatment for a median duration of 13 months, led the Food and Drug Administration (*http://www.fda.gov/*) to license natalizumab (Tysabri) 300 mg by intravenous infusion every 4 weeks, late in 2004 for the 'reduction of clinical exacerbations in patients with relapsing forms of multiple sclerosis'. The advice on use in pregnancy is ambiguous ('only if clearly needed'); administration to individuals aged under 18 years is contraindicated; advice on the upper age limit is noncommittal. Although experience beyond 13 months was limited at that time, the only adverse effects of note were headache, arthralgia, infections and hypersensitivity reactions but these were infrequent and rarely limiting. In study 1, the annualized relapse rate was 0.25 in individuals receiving natalizumab compared to 0.74 in the placebo group; the percentages of patients remaining relapse free were 76% and 53%, respectively. In study 2, annualized relapse rate was 0.36 in patients receiving natalizumab compared to 0.78 (the same as placebo-treated cases from study 1) in those only being treated with IFN-β1a; the percentages of patients remaining relapse free were 67% and 46%, respectively – even less than the placebo rate for these individuals receiving IFN-β1a. As expected, the clinical results were matched by comparable reductions in disease activity using imaging surrogates. Again, there was no difference in the proportion or number of cases showing activity comparing the placebo group of study 1 and the IFN-β1a-only group of study 2 but this may reflect differences in the study populations. About 6% of patients developed a persistent antibody response to natalizumab that was associated with an apparent loss of clinical effectiveness. Clearly, these data do not provide support that IFN-β1a is effective in reducing clinical or MRI evidence of disease activity in patients who continue to report clinical relapses while receiving this agent.

The patients involved in the 2 year placebo-controlled trials are being followed in an extension study while on natalizumab; in order to obtain long-term data on effectiveness, tolerability and safety in addition to immunogenicity. The intention was to follow what happens to these patients in the 6–12 months after infusions cease, so as to determine whether this more prolonged antibody administration proves more durable against clinical and MRI indicators of disease activity than the results reported to date.

On 28th February 2005 the sponsors of Tysabri (Biogen Idec and Elan Pharmaceuticals) voluntarily removed this agent from clinical and research trial use because progressive multifocal leukoencephalopathy was reported in two patients treated with the combination of Tysabri and IFN-β1a (Avonex) for more than 2 years. A third case was soon reported in a patient treated with Tysabri for Crohn's disease (eight doses over a period of 18 months), again in the context of a randomized trial; the patient died from what initially was thought to have been a fatal glioma and later confirmed to be progressive multifocal leukoencephalopathy. This patient had been refractory to corticosteroids, azathioprine and other immunosuppressants – perhaps contributing to the risk of additional immunosuppression with this experimental agent. Two additional unconfirmed cases have been subsequently reported in the context of clinical trials for multiple sclerosis but, at the time of writing, the details are scarce. Tysabri-treated patients from these trials are now under close scrutiny to detect progressive multifocal leukoencephalopathy as early as possible and investigators are reviewing the available data to understand the mechanism(s) of presumed reactivation of the JC virus in this setting. As highlighted earlier, these events remind us of the potential risks inherent in clinical trials .(Drazen 2005). It remains uncertain that this once-approved therapeutic agent will resurface for use in multiple sclerosis and other inflammatory disorders.

Anti-Vβ5.2/5.3$^+$ T cells

In a study designed to determine whether administration of the humanized monoclonal antibody ATM-027 (with specificity for Vβ5.2/5.3$^+$ T cells) would reduce MRI measures of disease activity, Killestein *et al* (2002b) stratified relapsing–remitting patients by HLA-DR2 status to receive monthly intravenous infusions either of the antibody (n = 47) or placebo (n = 12). The dose was titrated to deplete the target T cells. Treatment successfully suppressed the Vβ5.2/5.3$^+$ T-cell population and was well tolerated. There was a trend suggesting a reduction in MRI activity (lesion count, volume of enhancing lesions) but this result did not achieve the goal of the study.

Anti-CD52

Campath-1H may emerge as an important treatment for multiple sclerosis. From the clinical science perspective, its credentials are already established. Clinical observations provided the stimulus for basic research that has illuminated key aspects of the pathogenesis (Coles *et al* 1999a; Moreau *et al* 1996; Redford *et al* 1997; K.J. Smith *et al* 2001; Wilkins *et al* 2003). In turn, these are now being recycled into clinical practice and with provisionally encouraging results. Campath-1H is a humanized monoclonal antibody suitable for therapeutic use that targets the CD52 antigen present on all lymphocytes and a proportion of monocytes. As a result of its isotype, Campath-1H is exceptionally good at activating complement and mediating antibody-dependent cell-mediated cytotoxicity (M.Q. Xia *et al* 1993). CD52 does not lose its potential for lysis through modulation by antibody. Lymphopenia is rapid and prolonged following a pulse of treatment since the CD52 antigen is expressed in high density on the target cell membrane (Hale *et al* 1990). The median times to recovery of baseline counts for CD3, CD4, CD8 and total lymphocytes are 51, 61, 30 and 66 months, respectively. Conversely, B-cell numbers return more rapidly and tend to overshoot above baseline but rarely rise above the upper limit of the normal range. Although a single treatment does not elicit an anti-globulin response, this may not be the case if repeated courses of antibody are given. Campath-1H has been studied in three cohorts of patients with multiple sclerosis treated on an open label basis.

The change in MRI evidence for disease activity following treatment with Campath-1H established that a reduction in the