



Review

The Immunological Basis of Current and Novel Therapies of Multiple Sclerosis

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Abstract. The etiology and the pathogenesis of multiple sclerosis are not yet known. There might be a role for genetic susceptibility, for environmental factors and for inflammatory and immunological changes. Most of the actual therapies are based on the latter two phenomena. We review here corticosteroids, interferon β and copolymer 1 as the current drugs of choice and compare schematically the immunological basis of the mechanisms of action of these three substances with those of experimental or other treatments.

Key words: multiple sclerosis; therapy; immunological network; interferon β ; copolymer 1; corticosteroids.

Multiple sclerosis (MS) is one of the most frequent and best studied neurological diseases in many countries²². In spite of this, the etiology and the pathogenesis of this acquired, inflammatory and demyelinating central nervous system disease still remain an enigma.

Multifocal white matter lesions are the histologic and radiologic (magnetic resonance imaging, MRI) hallmarks of this disease. The white matter foci are caused by perivascular lymphocytic infiltrations. CD4⁺ T cells and macrophages⁶⁴ predominate in the lesions and these inflammatory cells invade the surrounding parenchyma and lead to demyelination. In the acute stage of the disease, inflammation with destruction of the blood brain barrier plays a central role⁵⁸, in the more chronic stages there is also axonopathy.

The current hypothesis about the pathogenesis of MS is that an external factor, such as a viral infection, induces an abnormal immune reaction in persons who

are genetically predisposed. This immune reaction¹⁰⁷ leads to chemical and enzyme-mediated damage (e.g. extracellular proteolysis), or vice versa, and results in demyelination and axonal loss⁷⁵.

Most of the actual therapies are focussed on the prevention or suppression of inflammation and demyelination (Fig. 1). More experimental strategies try to promote remyelination and to prevent axonal damage. Nevertheless, progress with novel treatment strategies is slow and this may be attributed to different factors. The variability of the first clinical symptoms and the lack of a diagnostic test make an early diagnosis nearly impossible. Moreover, the course of the disease is not predictable, with exacerbations and remissions, rapid or slow progression and all combinations of these. We also lack valid and simple parameters to follow the disease activity. The exacerbation frequency, the expanded disability status score (EDSS)

Abbreviations used: ACTH – adrenocorticotrope hormone, EAE – experimental autoimmune encephalomyelitis, EDSS – expanded disability status score, HLA – human leukocyte antigen, IFN – interferon, I.M. – intramuscular, I.V. – intravenous, MBP – myelin basic protein, MIU – million international units, MRI – magnetic resonance imaging, MS – multiple sclerosis, P.O. – per os, TNF – tumor necrosis factor.

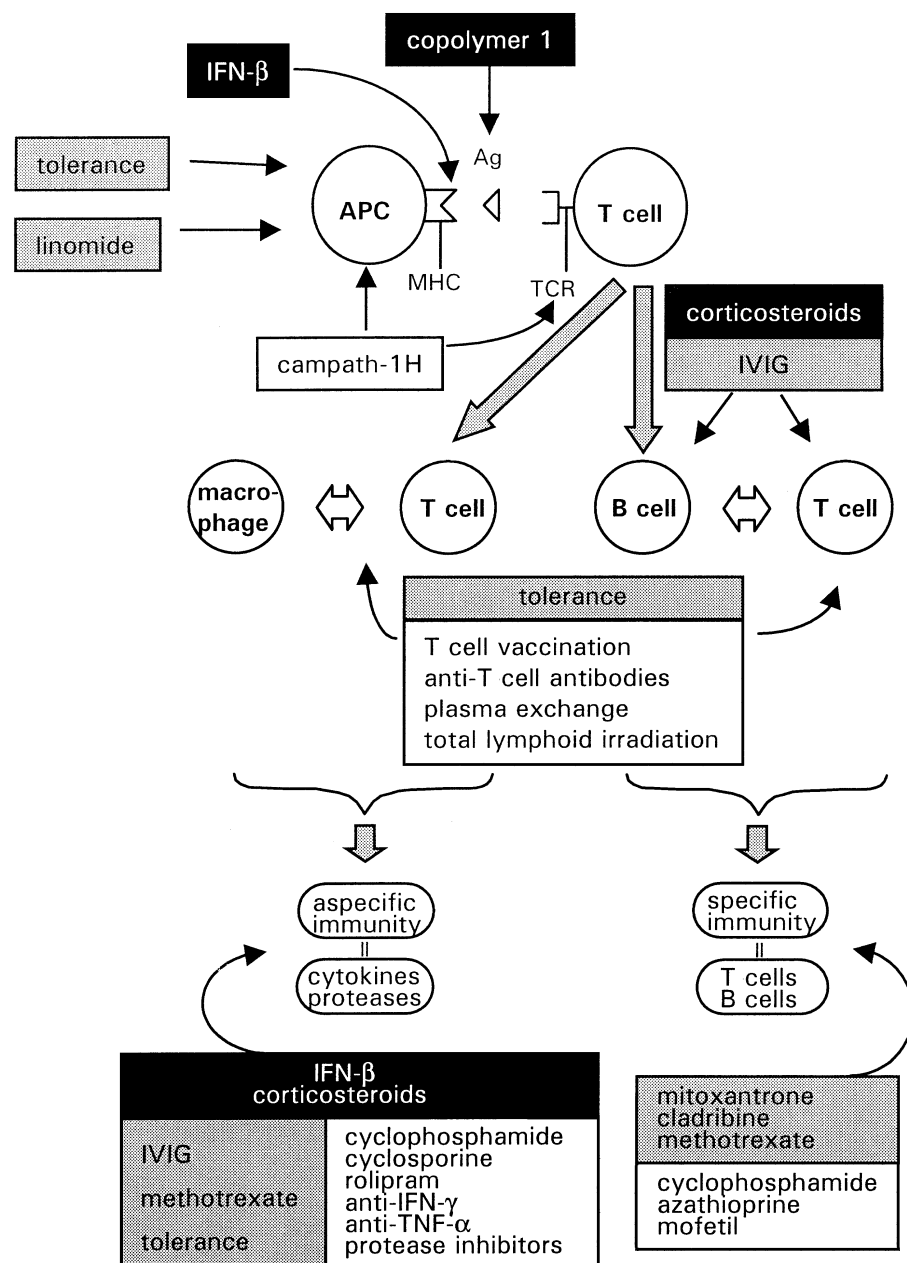


Fig. 1. Multiple sclerosis treatment within the frame of inflammatory and immunological networks. The proven useful therapies appear on a black background. Effective therapies, but requiring further studies, are shown on a grey background, contested and experimental therapies on a white one. Black arrows indicate the targets of the treatment, open arrows indicate the cellular interactions in aspecific and specific immunity. The mechanisms of action are discussed in the text. APC – antigen presenting cell; MHC – major histocompatibility complex; TCR – T cell receptor; IVIG – intravenous immunoglobulin

and MRI analysis are used as surrogate markers and all these parameters have serious limitations. Exacerbation frequency is not convincingly correlated with the final evolution and decreases with the duration of disease. The EDSS is not reliable in patients who experience little neurologic impairment. In more severe cases it is limited to the evaluation of the ambulation problems of

the patient. Moreover, in chronic progressive MS, this scale is not sensitive. Finally, the MRI offers the possibility to trace more disease activity than a clinical evaluation. This is useful to select patients in the active state of the disease and also to evaluate the short term effect of new therapies. Moreover, the hypointense lesions on MRI correlate rather well with the EDSS¹⁰¹. It

is therefore understandable that clinical trials in MS do not always lead to unequivocal conclusions^{65, 70}. In addition, placebo treatment leads to improvement in one third of the MS-patients, to stabilization and to worsening in two other thirds⁸⁹.

We here review the treatments with proven efficiency in large, randomized, placebo-controlled studies and those with more equivocal results. Next, the therapies with a suggested beneficial effect in small studies, that still need confirmation, are compared. Finally, we discuss some experimental treatments in which the positive effects are restricted to animal models. For all treatment strategies, the mechanism of action within inflammatory or immunological networks is illustrated (Fig. 1).

Proven Useful Treatments

Corticosteroids as treatment of an exacerbation

There exists a considerable literature on exacerbations, treated by corticosteroids^{3, 6, 9, 10, 18, 26, 46, 68, 69, 99, 100, 105, 110}. The exact mechanism of action of corticosteroids in MS is not known but these substances reduce the inflammation and the oedema in acute demyelinating lesions. Corticosteroids also downregulate the intrathecal IgG synthesis and the number of T cells in the cerebrospinal fluid.

Previously, intramuscular (I.M.) adrenocorticotrope hormone (ACTH) was used to accelerate remission after an exacerbation. The disadvantage of this therapy was the unpredictability of the evoked corticosteroid production. Nowadays, intravenous (I.V.) methylprednisolone or peroral (P.O.) prednisolone are mostly used. Most frequently, one uses I.V. methylprednisolone (in a dose of 500–1000 mg/day during 3 to 5 days). This may be followed by using decreasing doses of P.O. prednisolone during a couple of days. Probably the effect of a higher total dose is more pronounced, as is seen by the reduction of the number of exacerbations during the year following treatment with a higher dose of methylprednisolone⁵⁹. Another study³ did not document a significant difference between 500 mg I.V. or P.O. but the number of included patients was too low to exclude a difference.

As was already mentioned, corticosteroids accelerate the repair after an exacerbation. However, they do not prevent new attacks and do not influence the disease progression.

The Optic Neuritis Treatment Trial^{11, 12, 100} showed that a monosymptomatic optic neuritis progresses two times less to MS (evaluated after two years) if P.O. prednisone is preceded by a high daily dose of methyl-

prednisolone during some days. This effect was most marked for those patients who, at the time of the optic neuritis, had an MRI that was suggestive for MS. Prednisolone P.O. alone – in a dose of 1 mg/kg/day during 14 days – yielded more episodes of optic neuritis than in the placebo group and resulted in a minimal reduction of the MS incidence during the two years of the study.

The influence of corticosteroids in chronic progressive MS is not pronounced. The most remarkable observation is a short term reduction of spasticity^{18, 68}, which is independent of inflammation.

The monthly (or another interval) use of high dose corticosteroid therapy is based on the knowledge that chronic progression is associated with the appearance of new acute lesions. However, the clinical efficiency has never been well controlled.

The use of corticosteroids in MS has little side-effects⁶⁹. Most frequently, transient mental changes, gastric ulcers and urinary or respiratory infections are observed. The higher incidence of osteoporosis in MS patients is more correlated with the reduced mobility than with the short use of high doses corticosteroids. The long term use of low doses, however, has to be avoided because of no beneficial effect on the number of exacerbations and disease progression and because of the occurrence of side-effects.

Interferons as prevention of exacerbations

Recent studies have confirmed the partial effect of interferon β (IFN- β) on reducing exacerbations.

The interferons that are used in the treatment of MS are produced by genetic engineering. Besides their known antiviral activity, interferons^{4, 6, 7, 9, 19, 30, 31, 33, 35, 46, 48, 49, 62, 67, 76, 77, 80, 83, 85, 97, 99, 105, 110, 113} possess other biological effects, e.g. inhibition of cell growth, immunomodulation, enhancement of the functions of macrophages, natural killer and killer cells and also neutrophils. They also induce cell differentiation and may possess adjuvant antitumoral activity.

IFN- γ

In contrast with the effect of IFN- γ on experimental autoimmune encephalomyelitis (EAE), in man IFN- γ ^{76, 77} increases the number of exacerbations. In the future, novel molecules with an inhibitory effect on IFN- γ production (e.g. IL-10) or a blocking effect on IFN- γ (receptors) may be used in the treatment of MS.

IFN- α -2A (Roferon-A®)

The first studies with IFN- α were rather disappointing. However, the use of higher doses^{33, 46} (9 million international units (MIU) every 2 days during 6 months)

resulted in a beneficial clinical and radiological effect in 20 relapsing-remitting MS patients. After the treatment, the original disease process was restored³³. Larger studies on this interferon are needed.

IFN- β

In a small study⁶⁷ of 16 patients an increase of the number of exacerbations was seen with the intrathecal administration of natural IFN- β during 6 months while the disability was not influenced. The purpose of the intrathecal way is mainly local delivery in the central nervous system.

IFN- β is a cytokine of 166 amino acids. There exist two forms⁴⁹ of recombinant IFN- β , IFN- β -1a (Avonex[®], Rebif[®]) is expressed in a mammalian cell line and is glycosylated. IFN- β -1b (Betaseron[®]) is expressed in bacteria, is not glycosylated, misses the aminoterminal methionine and has a serine substitution for cysteine on position 17.

In relapsing-remitting MS the subcutaneous administration of IFN- β -1b leads to a reduction of 1/3 in the exacerbations and 50% in severe exacerbations. The number of exacerbation-free patients increases during the first 3 years. The total lesion load on MRI and the number of new lesions is smaller in IFN- β -treated patients^{63, 97}. All these effects persisted during a 5-year follow-up³⁰, except the reduction in the number of exacerbation-free patients. The latter did not show a significant difference anymore after two years. There exists also a clear dose effect: 8 MIU every two days is more effective than 1.6 MIU. In the latter patient group there was no marked effect of IFN- β -1b on disease progression.

A subcutaneous dose of 3 resp. 9 MIU IFN- β -1a⁸³ three times a week during 6 months, leads to a reduction of the number of gadolinium enhancing lesions (gadolinium enhancement means a disruption of the blood-brain barrier) with 40 resp. 64% and of the number of exacerbations with 1/3. Six MIU once a week intramuscularly⁴⁸ has a similar effect and also delays disease progression. However, the patients included in this study were part of a functionally better group and there was no influence on the total lesion burden on MRI. IFN- β -1a, subcutaneously and 3 times a week, at dosages of 6 MIU and 12 MIU has significant effects on the 3 major categories of outcome measures in MS: progression in disability, exacerbations, and MRI disease burden and activity²³. Both IFN- β -1a and -1b⁸⁰ are currently being tested in chronic progressive MS. The preliminary results show encouraging effects.

The most important side-effects of IFN- β are red-

ness and pain at the injection sites, flu-like symptoms, rarely lymphopenia and liver dysfunction. Mostly these side-effects are transient. More important is the development of neutralizing antibodies in approximately 1/3 of the treated patients. Possibly this goes along with the disappearance of the beneficial effect^{32, 78}.

Additional studies are needed to determine the most beneficial administration way, the optimal dose and the long term effect on exacerbations and progression.

Copolymer 1 as prevention of exacerbations

This synthetic mixture of oligopeptides^{6, 8, 9, 13, 14, 46, 47, 50, 51, 82, 95, 99, 115} consists of 4 amino acids (L-lysine, L-tyrosine, L-alanine and L-glutamic acid). It was synthesized as an analogue of myelin basic protein to induce EAE in animals. However, it was found to protect the animals from developing EAE, presumably by binding to antigen presenting cells and consequently blocking the activation of T cells.

Copolymer 1 reduces the number of exacerbations with 29–32% in 2 years if one starts the therapy early in the disease process⁵¹. The number of exacerbation-free patients is increased. Although a similar number of patients deteriorate, the progression of disability in relapsing remitting patients is less pronounced with copolymer 1 therapy⁵¹. The influence on the MRI lesions is not as convincing as with IFN- β . However, this examination was limited to a small number of patients²⁰. A new study to evaluate the effect on MRI has been started. In chronic progressive MS so far, there is little effect on evolution^{13, 15}.

Copolymer 1 has to be injected every day subcutaneously in a dose of 20 mg/day. Besides some local reactions on the injection sites there are little side-effects. Some patients experience flushing, dyspnoe and palpitations. The latter last for 30 s to 30 min but usually occur just once in a patient. The elicited antibodies against copolymer 1 do not have clinical consequences⁵².

Contested Therapies

Plasma exchange

Studies in which plasma exchange was combined with I.M., ACTH or I.V. methylprednisolone and cyclophosphamide showed that remission after an exacerbation was more rapid and longer lasting if plasma exchange was added to therapy^{9, 72, 99, 105, 109, 110}. However, in this setting it is difficult to evaluate the effect of plasma exchange alone and moreover the results were dependent on the used statistical method. The Canadian Cooperative Trial showed no significant ef-

fect of plasma exchange on disability in chronic progressive MS⁷².

Azathioprine

The purine antagonist azathioprine^{9, 46, 105, 110} exerts its effect on rapidly dividing cells such as lymphocytes. A meta-analysis¹¹⁶ of some controlled studies^{16, 34, 54, 79} demonstrated a small effect of azathioprine. This favourable effect was primarily a reduction in relapse rate. After 2 to 3 years there is also a minimal beneficial effect on disability. The question remains whether this counterbalances the side-effects.

Cyclophosphamide

This alkylating drug^{9, 41, 46, 105, 110, 112} influences essentially proliferating cells. T cells produce less IL-2 and this has again a negative feedback on their proliferation. The effect of cyclophosphamide has been evaluated in several studies^{41, 44, 45, 60, 77} but remains questionable. The drug can cause serious adverse effects⁴⁴.

Cyclosporine

Cyclosporine is a potent immunosuppressant by inhibition of T helper cells (via inhibition of cytokine synthesis)^{9, 54, 87, 110}. In a large American study⁸⁷ cyclosporine postponed wheelchair dependency and upper extremity impairment but this had no influence on long term progression nor on MRI.

Total body irradiation

The toxic effect of total body irradiation on DNA replication is responsible for its potent immunosuppressive effect^{9, 17, 24, 25, 99, 110, 114}. In combination with low doses prednisone²⁴, total body irradiation resulted in delayed disease progression. Despite of the absence of significant clinical effects of total body irradiation alone^{17, 110}, there was a reduced accumulation of lesions on MRI¹¹⁰. This does not counterbalance the serious risks of this therapy.

Effective Therapies Requiring Further Study

Intravenous immunoglobulins

The exact action mechanism of immunoglobulins is not yet known. Possible explanations are interactions with T cells, downregulation of cytokine production (TNF- α), neutralization of cytokines, modulation of Fc-receptors on macrophages and of anti-idiotypic networks^{1, 2, 9, 46, 56, 73, 92, 99, 103}. Furthermore, immunoglobulins might serve as receptor for activated comple-

ment. This prevents the adhesion of complement to oligodendrocytes and myelin proteins. Finally, they are able to promote remyelination.

In relapsing-remitting MS, the relapse rate and the number of gadolinium-enhancing lesions is reduced^{2, 93}. The neurological disability is influenced in a beneficial way³⁶.

Mitoxanthrone

This anthracycline inhibits the relaxation of supercoiled DNA by electrostatic cross-binding, leading to a block of the cell cycle. It is an immunomodulator that reduces the number of T cells, suppresses humoral immunity and inhibits T cell function^{9, 28, 46, 99, 105}. It has a therapeutic effect in very active MS both on relapse rate and MRI^{40, 53} and is currently being investigated in secondary progressive MS. Its use is limited by its cardiotoxicity.

Oral tolerance

When intact peptides pass through the gastrointestinal mucosa a functional T cell tolerance arises against these proteins^{6, 9, 94, 99, 106, 111}. Antigen specific regulatory cells start looking for a protein that is similar to the perorally ingested one and then exhibit a suppressor effect by secreting antiinflammatory cytokines e.g. TGF- β . There is also a decrease of the number of myelin basic protein (MBP) reactive T cells.

Thirty relapsing remitting MS patients were administered 300 mg bovine myelin perorally¹¹⁰. There was a reduction of 50% in the exacerbations in patients who were not HLA-DR2-positive. Statistic significance was not reached and, moreover, the HLA-DR2-genotype is well represented in MS. A recent trial in the United States showed no influence on the exacerbation frequency nor the disease progression.

Cladribine

The purine-analogue 2-chloro-deoxyadenosine mimicks the accumulation of deoxynucleotides in adenosine-deaminase deficiency^{6, 9, 46, 81, 90, 99, 108}. It is frequently used in the treatment of hairy cell leukemia and lymphomas. The consequent effect on DNA synthesis and cellular metabolism influences resting as well as dividing cells.

A small trial⁹⁰ in chronic progressive MS demonstrated clinical stability in patients treated with cladribine, whereas the others deteriorated significantly. Unfortunately, these clinical findings could not be reproduced in a larger placebo-controlled trial although MRI data from this trial show a favourable effect.

Linomide

Linomide^{5, 55, 99} is a synthetic immunomodulator with effects on antigen presentation and cytokine secretion.

Linomide showed encouraging effects in relapsing remitting as well as in chronic progressive MS. Unfortunately, a larger trial had to be interrupted because of severe side-effects, particularly an increased risk of myocardial infarction.

Methotrexate

This drug stimulates suppressor activity, production of inhibitory cytokines and downregulation of pro-inflammatory cytokines and proteolytic enzymes^{27, 42, 43, 46}.

In chronic progressive MS there is less progression in upper extremity impairment, but this is not reflected in a favourable change in EDSS. The changes in lesion load on MRI are favourable.

In relapsing remitting MS methotrexate induced a decrease of the number of exacerbations but the side-effects of cytostatic drugs are not negligible.

Experimental Treatments

T cell vaccination

Subcutaneous vaccination of MS patients with irradiated autologous MBP reactive T cells leads to a decrease of MBP reactive cells in the circulation^{6, 9, 46, 66, 102, 117, 118}. In 5 out of 8 patients this correlated clinically with less exacerbations and radiologically with a smaller increase in lesion load on MRI.

A disadvantage of this therapy is the reappearance after 1 year of specific T cells against other epitopes (of MBP). These cells are then resistant to the vaccine and exacerbations reappear. Moreover, this strategy assumes the crucial role of MBP as responsible antigen in the immunopathogenesis of MS.

Antibodies

Monoclonal anti-CD4

Demyelination may result from an inflammatory process mediated by CD4⁺ T cells. Blocking of this activity was attempted by monoclonal antibodies^{6, 9, 46, 61, 86, 96, 99, 105, 110}. Large studies still need to be done.

Monoclonal anti-TNF- α

TNF- α exerts its disease-promoting influence by stimulation of several cytokines and metabolites of arachidonic acid and by induction or enhancement of the expression of adhesion molecules on endothelial cells^{6, 46}.

In a recent study¹⁰⁴ two rapidly progressing MS patients were treated with such an inhibitory monoclonal antibody. There was an increase of gadolinium enhanc-

ing lesions on the MRI and also of cytosol and IgG index in the cerebrospinal fluid. These signs of immune activation were not reflected in a clinical deterioration but the study was interrupted.

Campath-1H

This monoclonal and humanized antibody recognizes the CDw-52-antigen on lymphocytes and monocytes, resulting in a lymphocytic depletion^{21, 46}. Intravenous administration in 7 patients resulted in a reduction of disease activity on MRI.

Anti- $\alpha 4\beta 1$ integrin

Antibodies against this adhesion molecule impair the adhesion of leukocytes to endothelial cells. This prevents inflammatory cells to infiltrate perivascularly⁵⁷. In the guinea pig this treatment resulted in a reduction of clinical and pathological hallmarks of EAE. Further studies are warranted.

Rolipram

The antidepressant ($\pm$) -4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone increases the intracellular cAMP by inhibition of the type IV phosphodiesterase^{37, 46, 91}. This leads to an inhibition of the transcription of the TNF- α gene. There is also a decrease of lymphotoxin- α and INF- γ bioactivity. Rolipram protects animals against EAE. This therapy has not yet been applied in humans.

Mofetil

Mofetil⁴⁶ inhibits the *de novo* pathway of the purine synthesis. There is a suppression of T and B cell function with a consequent inhibition of antibody production.

Future Perspectives

Based on the role of cytokines and proteases in autoimmune diseases⁷⁵ and in the control of inflammatory cell infiltrations⁷⁴, it has been suggested to use this knowledge in the treatment of MS.

Suppression of inflammatory activity by using anti-inflammatory cytokines (IL-4, IL-10, TGF- β) and inhibition of pro-inflammatory cytokines (IL-1, IL-2, TNF- α , TNF- β , IFN- γ , IL-12) will remain an important goal in future research^{84, 88}.

Similarly, inhibition of metalloproteinases may become a novel target. Unspecific inhibitors of gelatinase B, such as hydroxamate³⁸, D-penicillamine and tetracyclines^{39, 71}, have shown antiinflammatory properties in animal models. The only clinical study²⁹ in MS

patients with a combination of D-penicillamine and metacycline showed, however, toxicity. There is an urgent need for more selective and efficacious metalloproteinase inhibitors. Further improvements are also expected by optimized timing and dosing of the currently available agents and by combination of partially effective drugs. Finally, promotion of remyelination by growth factors and by transplantation of myelin producing oligodendrocytes²¹ is under investigation as an alternative route to treat demyelinating diseases.

In conclusion, it is clear that the therapy of MS needs to be improved, although much progress has been made during the last years. Mainly IFN- β appears currently to give hope for many patients and their physicians. However, it must be stressed that this therapy is far from ideal, with side-effects and an influence on disease progression that still needs to be confirmed. Indeed, for MS patients it is certainly important to have less exacerbations, but their global functional state with disease progression must not be overlooked.

There exists an urgent need for less toxic and more effective (immune) therapies that can be administered early in the disease progress and without limitation in time. The complexity and interconnection of immunological processes, the heterogeneity of contributory genetic factors and the interactions with partially unknown exogenous risks make rapid scientific advances difficult in the treatment of this mutilating disease.

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Note added in proof. Meanwhile it has been established that treatment with interferon β -1b delays sustained neurological deterioration in patients with secondary progressive MS (*Lancet* 1998, **352**, 1491–1497).