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Therapeutic strategies in multiple sclerosis. I. Immunotherapy

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THE ROYAL SOCIETY

Therapeutic strategies in multiple sclerosis. I. Immunotherapy

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This review first addresses several general aspects of the immunotherapy of multiple sclerosis. Next, two approved immunomodulatory treatments, interferon-β and copolymer-1 (glatiramer acetate), are reviewed in more detail. Finally, other immunosuppressive therapies and experimental strategies are briefly discussed.

Keywords: autoimmunity; T lymphocytes; immunotherapy; multiple sclerosis

1. PRINCIPLES OF IMMUNOTHERAPY

(a) *Implications of disease heterogeneity*

In multiple sclerosis (MS) as in other diseases, rational treatment depends on a thorough understanding of the disease's aetiology and pathogenesis. Research into the pathogenesis of MS and especially the rapidly growing number of different animal models are beginning to reveal a remarkable heterogeneity and complexity of the pathogenetic mechanisms of inflammatory demyelinating central nervous system (CNS) disease. In view of these developments, it seems likely that much more refined classifications can be developed in the future for the disease we today call MS. Clearly, this will increase the possibility of a more differentiated therapeutic approach.

The evidence for an immunopathogenesis of MS is strong but indirect, because much of it has been derived from autoimmune animal models (Wekerle *et al.* 1994; Lassmann and Wekerle 1998*b*) (Wekerle *et al.* 1994; Lassmann and Wekerle 1998*b*) (Wekerle *et al.* 1994; Lassmann & Wekerle 1998). Experimental support has been accumulated for a variety of distinct autoimmune mechanisms, and it is likely that different human autoimmune diseases (and different forms of MS?) are triggered by different mechanisms. In addition, different mechanisms may operate at different stages of the same disease. Obviously, the identification of the aetiology in individual cases is not just an academic problem, but of the utmost practical relevance for therapy. Furthermore, it is still unclear whether virus(es) or other infectious agents play a role. In principle, there are at least four possibilities: (i) viruses are not involved in MS; (ii) a primary early viral infection triggers a secondary autoimmune reaction, e.g. by 'molecular mimicry'; (iii) a primary autoimmune reaction is complicated by episodic reactivation of a latent virus, particularly in the CNS; or (iv) MS is caused by a persistent viral infection of the CNS. Although numerous viruses have been incriminated over the years, most of these claims have not been substantiated. This is not surprising as it is extremely unlikely that a single virus would be involved in all forms of MS. It is possible, however, that subtypes or 'variants' of MS are related to viral infection. Clearly, for appropriate treatment it is essential to identify such cases.

(b) *Cell-mediated versus antibody-mediated effector mechanisms*

Although transfer experiments have demonstrated that autoreactive T cells are critically important in the immunopathogenesis of experimental autoimmune encephalomyelitis (EAE) (and by analogy, probably also MS), it is becoming increasingly clear that B cells and their products, antibodies, are equally important, especially for demyelination (figure 1). The lesions of classic myelin basic protein (MBP)-induced EAE in Lewis rats, are produced by the transfer of purified MBP-specific T cells alone, are mainly inflammatory, not demyelinating. If, however, a monoclonal antibody (mAb) specific for myelin oligodendrocyte glycoprotein (MOG) is co-injected with the T cells, large demyelinating lesions develop (Linington *et al.*, 1988; Genain *et al.*, 1995). That the transfer of T cells is necessary, but by no means sufficient for demyelination, has been observed not only with MBP-specific T cells, but also with T cells specific for other CNS autoantigens, such as MOG and S-100 β. Demyelinating lesions also develop in these models after co-injection of anti-MOG antibody (Linington *et al.* 1993; Kojima *et al.* 1994). These observations support the concept that T cells specific for various CNS autoantigens initiate inflammation and disturb the blood–brain barrier, whereas autoantibodies against surface antigens of myelin or oligodendrocytes are required to produce demyelination.

Direct support for a pathogenetic role of MOG-specific antibodies comes from a study that demonstrated the

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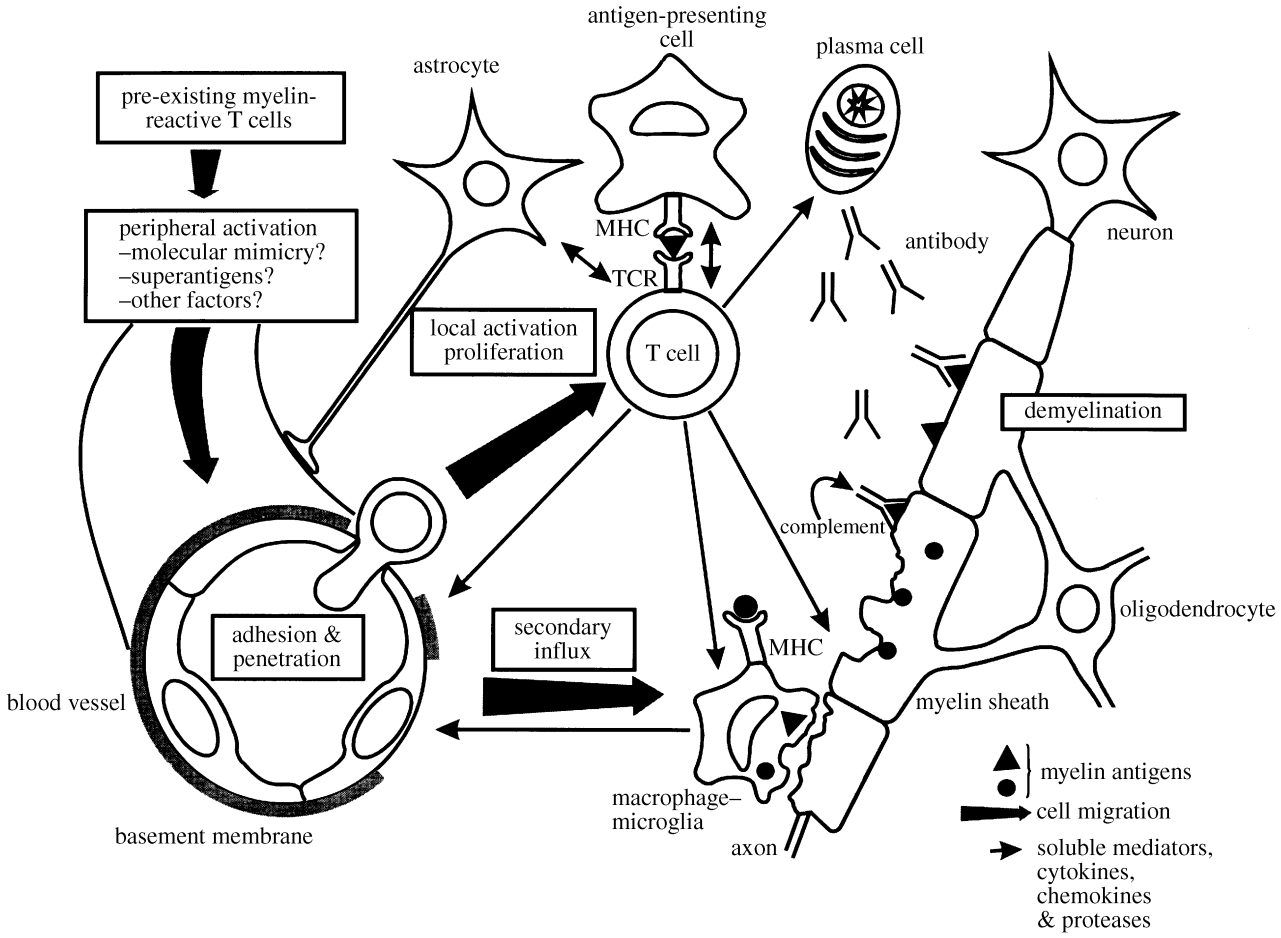

Figure 1. Schematic view of some crucial steps in MS pathogenesis (reprinted from Hohlfeld 1997, by permission). Pre-existing autoreactive T cells are thought to be activated outside the CNS. The activated T cells traverse the blood–brain barrier and are locally re-activated when they recognize their antigen on the surface of APCs. The activated T cells secrete cytokines that stimulate microglia and astrocytes, recruit additional inflammatory cells and induce antibody production by plasma cells. Anti-myelin antibodies and activated macrophages–microglia are thought to cooperate in demyelination.

association of these antibodies with myelin debris in MS lesions (Genain *et al.* 1999). Assays using the recombinant extracellular domain of MOG allow the detection of anti-MOG autoantibodies in serum by Western blotting and enzyme-linked immunosorbent assays. Several studies demonstrated that anti-MOG antibodies are more frequent in MS patients than in normal controls (Karni *et al.* 1999; (Reindl *et al.* 1999; Lindert *et al.* 1999). However, these antibodies are clearly not specific for MS as they are also frequently detected in patients with other (inflammatory) neurological diseases. It is presently unknown whether assaying anti-MOG antibodies can help in treatment decisions.

Obviously, the relative role of the different effector mechanisms (T cells, B cells and/or antibodies, others) and autoantigens (MBP, MOG, encephalitogenic proteolipid protein (PLP), S-100 β and others) is a critical factor for immunotherapy. Extensive efforts have recently been made to tilt pathogenic T-cell responses from TH1-type to TH2-type. The rationale behind this strategy is that TH1 T cells, which produce proinflammatory cytokines such as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α), often act as disease-mediating inflammatory cells, whereas TH2 T cells, which produce interleukin-4 (IL-4) and interleukin-10 (IL-10) and provide help for antibody-producing B cells, are often beneficial and can ameliorate autoimmune disease. Both TH1 and TH2 cells seem to play a pathogenic role in MS. This role may vary in different forms and cases. Thus, tilting the T-cell response may have unforeseen deleterious rather than the expected beneficial consequences (Genain *et al.* 1996).

An unresolved problem is how to determine the relative importance of the different autoantigens and effector mechanisms in an individual patient. Whether assays of anti-MOG antibodies are helpful for identifying subgroups of patients, such as responders or non-responders to different therapies, needs to be established in the future. Histological studies provide evidence for pathogenetic heterogeneity (Lassmann *et al.* 1998; Lassmann, this issue). For example, Lucchinetti *et al.* (1996) have presented a new classification scheme for lesional activity based on the composition of myelin degradation products in macrophages. These criteria allow one to distinguish between different patterns of demyelination, including demyelination with relative preservation of oligodendrocytes, myelin destruction with concomitant, complete destruction of oligodendrocytes, and primary destruction of oligodendrocytes with secondary demyelination (Lucchinetti *et al.* 1996). It is noteworthy that the different immunopathological variants of MS can be reproduced in different variants of MOG-induced EAE (Storch *et al.* 1998). The different lesional morphologies may in part

reflect different pathogenetic mechanisms, which require a differentiated immunotherapy (Lassmann *et al.* 1998).

(c) *Phase-specific immunotherapy*

Common sense and clinical judgement suggest that the sooner immunotherapy is initiated, the better the chances are to prevent deficits. Arguments against very early treatment include the high cost of long-term therapy and adverse reactions, which could diminish or abolish therapeutic effectiveness at a later stage when therapy is urgently needed (e.g. development of neutralizing antibodies to IFN-β). At the other end of the clinical spectrum, patients with severe impairment have an increased risk for various adverse reactions to immunotherapy. Furthermore, immunotherapy cannot be expected to reverse a pre-existing, stable chronic deficit. Therefore, patients with advanced disease are bad candidates for immunotherapy, and aggressive immunosuppressive treatments are usually contraindicated.

Ideally, the intensity of immunosuppressive or immunomodulatory treatment should be adjusted to disease activity. How can disease activity be measured? One of the first lessons learned from magnetic resonance imaging (MRI) studies was that clinical activity is a very poor indicator of disease activity. It is probably unrealistic to assume that the currently available MRI techniques alone can provide reliable and feasible indicators of disease activity for therapeutic monitoring. However, the various imaging techniques have an enormous potential, and it is conceivable that in the future they can be developed into practical and reliable tools for assessing disease activity (McDonald 1998). As far as laboratory markers of disease activity are concerned, the consensus is that none of the proposed 'activity markers' (e.g. T-cell subsets, myelin degradation products, cytokine levels and expression) has yet proven suitable for routine monitoring of disease activity. There are many reasons for this, mostly related to limitations of the feasibility, reliability, sensitivity and specificity of the various assays. In spite of the unsolved problems, it is likely that more dependable and feasible laboratory tests of disease activity will soon be developed.

There are presently no reliable criteria for predicting the individual course and severity of MS. Clearly, such criteria are crucial for deciding whether and when to initiate immunomodulatory treatment. There is no need to treat benign MS, but MS cases with poor prognosis should be treated early. There is some evidence that imaging studies may help assess the prognosis in individual cases, but a precise prediction is not possible (Kappos *et al.* 1999). It is also unclear whether the clinical course provides helpful criteria to define subtypes of MS for differentiated therapy. The published clinical trials have differentiated (more or less rigorously) between relapsing–remitting and progressive MS, excluding primary progressive MS. More results are needed to decide whether such a distinction is meaningful.

(d) *Combination therapy*

It seems logical to consider combining different immunotherapies that work via different mechanisms. Striking examples for the effectiveness of such therapy are provided by the combination of antiviral therapies in HIV infection and the combination of the immunosuppressive regimes routinely used in transplantation. Obviously, the difficulty is to select the right agents to combine, as well as the right type of MS. An important potential problem is that the different immune mechanisms targeted by different agents may be interdependent, so that one agent depends on the intactness of mechanisms inhibited by another agent. Clinical trials need to look carefully for such adverse interactions, which are difficult to predict. Examples of possible combinations (which, incidentally, present a major challenge for trial design) include IFN-β plus copolymer-1 (COP-1), or IFN-β plus azathioprine.

(e) *Neuroprotective side-effect of inflammation: relevance for immunosuppressive therapy?*

It has been proposed that inflammatory cells, including macrophages and T cells, are not necessarily always harmful but under certain conditions are even protective. For example, the intraperitoneal injection of MBP-specific T cells protected optic nerve axons and retinal ganglion cells after experimental crush injury of the optic nerve (Moalem *et al.* 1999). The transferred T cells were shown to home to the crush lesion. The exact mechanism of T-cell mediated neuroprotection is still unknown. However, we have recently demonstrated that activated human immune cells, including MBP-specific T cells, unexpectedly express brain-derived neurotrophic factor (BDNF) *in vitro* and in inflammatory brain lesions of MS (Kerschensteiner *et al.* 1999). We therefore propose that the functional neuroprotective effect of T cells seen in the optic nerve crush experiments (Moalem *et al.* 1999) is related to the focal production of neurotrophic factor(s) by autoimmune T cells.

Until recently, neurons were considered to be the major cellular source of BDNF in the nervous system (Hofer *et al.* 1990; Lewin and Barde 1996). Indeed, BDNF has potent effects on neuronal survival and plasticity during development and after injury. Considering that inflammation is a universal tissue reaction crucial for defence and repair, we asked whether the immune cells accumulating in traumatic, degenerative, ischaemic, infectious and autoimmune lesions of the nervous system might provide an external source of BDNF. We found that indeed activated human CD4+ T cells, CD8+ T cells, B cells and monocytes, as well as myelin antigen-specific T-cell lines, produce BDNF. The immune cell-derived BDNF supports neuronal survival *in vitro*. Furthermore, BDNF is expressed in inflammatory infiltrates in the brain of patients with acute disseminated encephalitis and MS (figure 2). The expression of BDNF by immune cells provides a striking example for the close functional integration between the immune and nervous systems (Steinman 1993; Neumann and Wekerle 1998).

Obviously, the novel concept of 'protective autoimmunity' has far-reaching implications. Inflammatory reactions are very common not only in infectious and autoimmune diseases but also in ischaemic, degenerative, traumatic and metabolic lesions of the nervous system and muscle (e.g. stroke, Duchenne muscular dystrophy, adrenoleucodystrophy). It now appears that these lesions represent an attempt (often futile) of the immune system to protect the nervous system. An important implication for the therapy of MS is that non-selective immunosuppressive

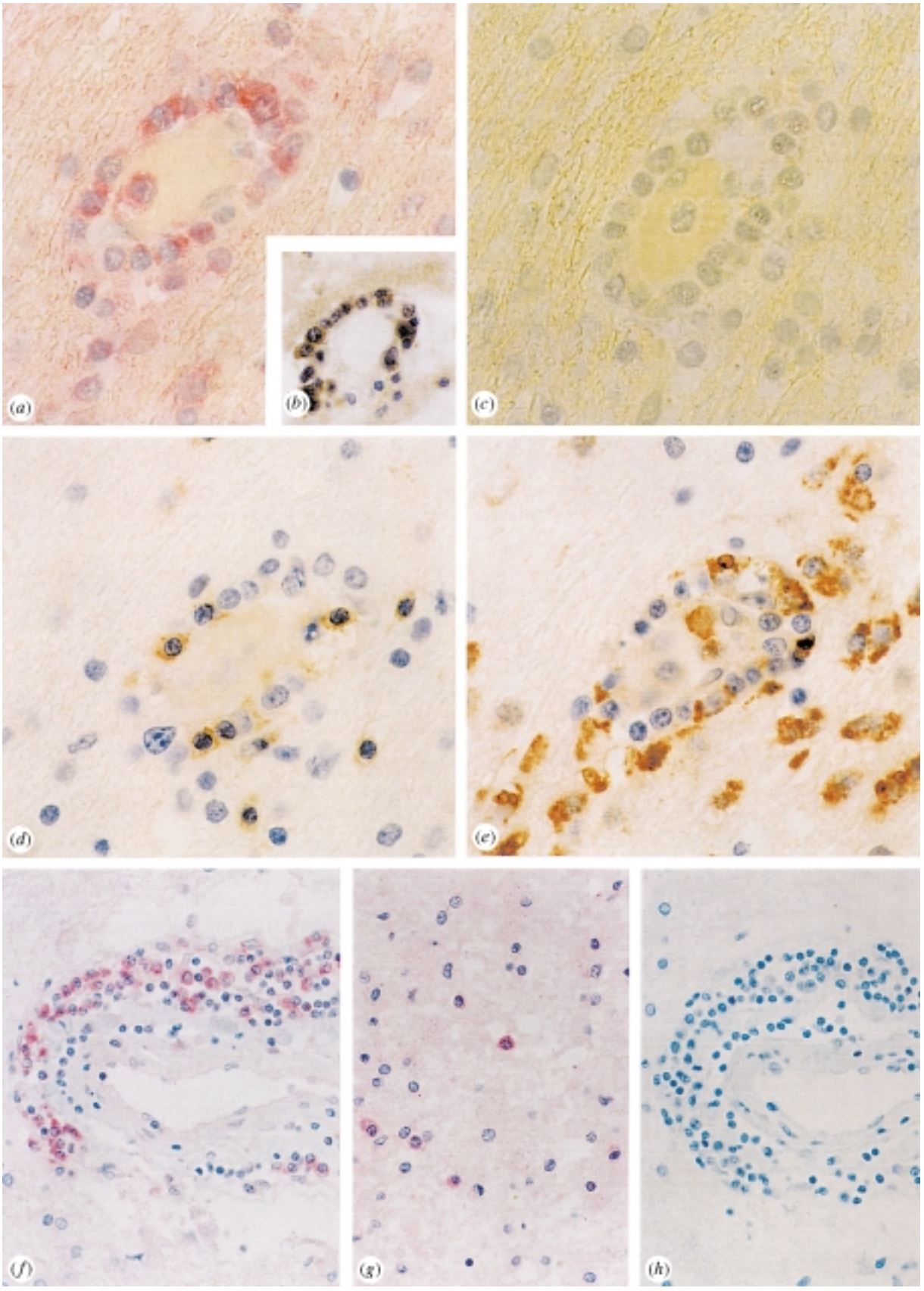

Figure 2. Expression of BDNF in inflammatory cells in acute disseminated (post-infectious) leucoencephalitis and MS (reprinted from Kerschensteiner *et al*. 1999, by permission). (*a*, *c*, *d*, *e*) Serial sections of a perivascular inflammatory infiltrate in post-infectious leucoencephalitis (×1000); (*a*) immunolabelling of BDNF (mAb); (*c*) negative control without primary antibody; (*d*) CD_3-labelled T cells; (*e*) CD68+ macrophages. (*b*) Inflammatory cells in a cryostat section from the same patient stained with a polyclonal anti-BDNF antiserum (×500). (*f*–*h*) BDNF immunoreactivity in MS (×450); (*f*) in inflammatory cells forming a perivascular infiltrate, and (*g*) in inflammatory cells invading the plaque area; (*h*) negative control.

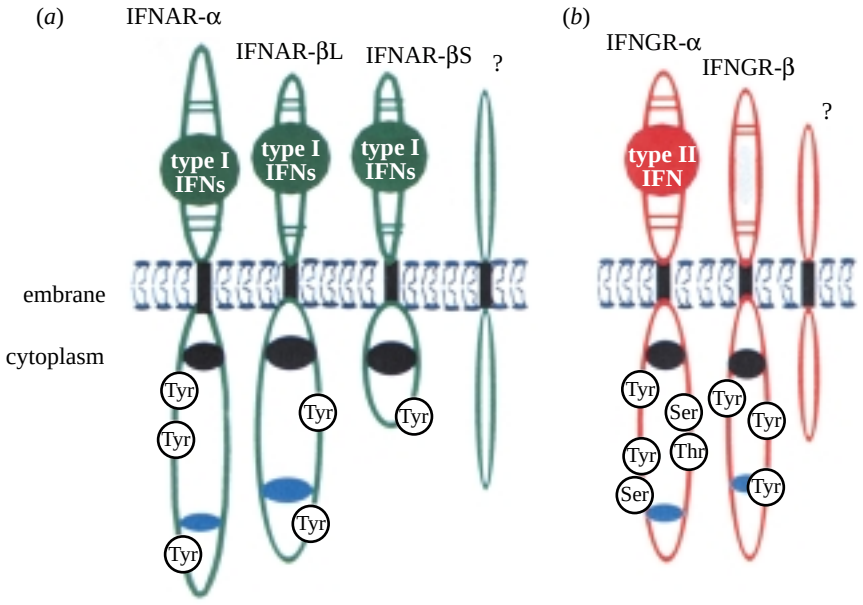

Figure 3. Schematic drawing of the receptor for IFN-α and IFN-β ((*a*) IFNAR, green) and IFN-γ ((*b*) IFNGR, red). Each receptor is composed of multiple chains. The cytoplasmic domains of the receptor chains contain tyrosine (Tyr) and serine (Ser) phosphorylation sites. Courtesy of M. Müller, Vienna.

treatments are likely to eliminate the neuroprotective autoimmune cells along with the autoaggressive offenders. This may be one of the reasons why treatment with non-selective immunosuppressive agents often fails to have a convincing clinical benefit.

2. INTERFERONS

(a) *Hypothetical mechanisms and clinical effects*

IFNs were initially tried in MS for their antiviral effect (reviewed in Jacobs & Johnson 1994). A pilot trial of systemic recombinant IFN-γ resulted in a sharp increase of exacerbations (Panitch *et al.* 1987). In contrast, IFN-β turned out to have a beneficial effect, which has meanwhile been corroborated in several large controlled trials (Sibley & IFNB Multiple Sclerosis Study Group 1993; IFNB Multiple Sclerosis Study Group 1995; Jacobs *et al.* 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998).

IFN-γ is produced by activated T cells and natural killer cells. A potent activator of macrophages and monocytes, it induces an array of inflammatory mediators in these cells. One of its many important functions is to increase the expression of class I and class II major histocompatibility complex (MHC) molecules on a variety of cell types, including both professional (obligatory) and facultative antigen-presenting cells (APCs). The increase in MHC expression facilitates antigen presentation, thereby augmenting and accelerating immune responses. This can plausibly explain why treatment of MS patients with IFN-γ was unsuccessful (Panitch *et al.* 1987).

Different trials of IFN-β showed a 30% reduction of the exacerbation rate (Sibley & IFNB Multiple Sclerosis Study Group 1993; IFNB Multiple Sclerosis Study Group 1995; Jacobs *et al.* 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998), a significant reduction of MRI activity (Paty *et al.* 1993; IFNB Multiple Sclerosis Study Group 1995; Stone *et al.* 1995; Jacobs *et al.* 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998), and/or a delay in time until sustained clinical progression (Jacobs *et al.* 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998). IFN-treated MS patients also exhibit reduced cerebrospinal fluid (CSF) pleocytosis (Rudick *et al.* 1999*b*). Recombinant IFN-α also seems to reduce exacerbation frequency and MRI activity (Durelli *et al.* 1994; Jacobs *et al.* 1996; Myhr *et al.* 1999).

The mechanisms of the effects of IFN-β are unknown. Antiviral and antiproliferative actions might contribute, although immunomodulatory effects such as reduced transcription of MHC class II molecules are considered more important. Consistent with this notion, type I IFNs are also effective in EAE.

IFNs have antiproliferative and various immunomodulatory effects (Arnason & Reder 1994; Weinstock-Guttman *et al.* 1995; Yong *et al.* 1998; Hall *et al.* 1997). IFN-α is part of a multigene family, whereas IFN-β and IFN-γ are encoded by single genes. Because IFN-α and IFN-β share components of the same receptor, they are referred to as type I IFNs. IFN-γ uses a separate receptor and is referred to as type II IFN. The receptor for type I IFNs is composed of at least three chains (IFNAR-α, IFNR-βL, IFNAR-βS; figure 3, left). The receptor for IFN-γ is composed of the IFNGR-α and IFNGR-β chain, plus at least one additional chain (figure 3, right). Although the surface receptors are different, several components of the intracellular signalling pathways are shared between type I and type II IFNs. Thus, the signalling pathways of the different IFNs partially overlap (Darnell *et al.* 1994; Briscoe *et al.* 1996). Additional IFNs have been discovered, but they have not been as well characterized.

Type I IFNs—the IFN-α family and IFN-β—are produced by almost all mammalian cells upon stimulation. One (but not the only) inducer of IFN synthesis is double-stranded RNA, which is part of the infectious cycle of most viruses, but is not found in mammalian cells. IFNs trigger the synthesis of many host-cell proteins that contribute to the inhibition of viral replication, and are believed to mediate most of the biological effects of the IFNs. Like IFN-γ, type I IFNs increase expression of

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MHC class I proteins and thereby enhance the ability of virus-infected cells to present viral peptides to CD8+ T cells. In contrast to IFN-γ, however, IFN-α and IFN-β do not induce but suppress the synthesis of MHC class II proteins. This effect may be important for the immunomodulatory activity of type I IFNs (Weinstock-Guttman *et al.* 1995; Yong *et al.* 1998; Hall *et al.* 1997). The exact mechanism of IFN-β-mediated MHC class II inhibition is not completely understood. It appears that IFN-β reduces the activity of the class II transactivator CIITA, a factor necessary for IFN-γ-induced MHC class II transcription (Lu *et al.* 1995).

Apart from the inhibition of MHC class II expression, numerous other immunomodulatory effects of type I IFNs have been described. For example, IFN-β has been shown to upregulate IL-10 expression and secretion by T cells and monocytes (Rudick *et al.* 1996, 1998*a*; Rep *et al.* 1996; Weber *et al.* 1999), indicating that part of the clinical effects of IFN-β are in fact mediated by IL-10. Both IFN-β-1a and IFN-β-1b induce the production of IL-10 in MBP-specific CD4+ T-cell lines, but inhibit proliferation and production of lymphotoxin (Weber *et al.* 1999).

Furthermore, IFN-β inhibits T-cell migration across basement membrane *in vitro*, presumably by decreasing the secretion of matrix-degrading enzymes (Leppert *et al.* 1996; Stüve *et al.* 1996). This IFN-mediated inhibitory effect on the secretion of matrix metalloproteinases, as well as other consequences of IFN cellular responses, may be pertinent for the suppression of inflammation (i.e. reduced numbers of enhancing MRI lesions and lessened CSF pleocytosis) observed in IFN-treated MS patients. Potential mechanisms include increase in soluble vascular cell adhesion molecule-1 (VCAM-1) (Calabresi *et al.* 1997b) and concomitant downregulation of the corresponding partner adhesion molecule very late antigen-4 (VLA-4) on peripheral blood lymphocytes (Calabresi *et al.* 1997*a*). Increased soluble VCAM-1 and decreased cellular VLA-4 would both tend to block leucocyte–endothelial adhesion at the blood–brain barrier. Leucocytes also require stimulation by chemoattractants, called chemokines, during extravasation, and several chemokines are increased in the CSF of patients with relapses of MS (Sörensen *et al.* 1999). It is interesting that IFNs upregulate the expression of chemokines by numerous cell types, including haematopoietic cells. Elevated levels of chemokines in the circulation would tend to reduce transvascular chemokine gradients, favouring the entry of cells into the CNS.

A myriad of other effects of IFN-β have been described. A few examples include the inhibition of T-cell proliferation (Rudick *et al.* 1999*a*; Pette *et al.* 1997), different immunomodulatory effects on microglia (e.g. Chabot *et al.* 1997; Hall *et al.* 1997), the reduction of circulating CD80+ B cells (Genc *et al.* 1997), the stimulation of nerve growth factor production by astrocytes (Boutros *et al.* 1997), the inhibition of human glial inducible nitric oxide synthase (Hall *et al.* 1997; Guthikonda *et al.* 1998), the inhibition of mitogen-induced astrocyte proliferation (Malik *et al.* 1998), the antagonistic effect on IFN-γ-induced expression of high affinity Fc receptors for IgG (Van Weyenbergh *et al.* 1998), and the inhibition of IL-12 production by human dendritic cells (McRae *et al.* 1998). The relative importance and mutual interdependence of this bewildering variety of actions are not well understood, so that at the present time the mechanisms of IFN-β treatment of MS remain unknown.

There is evidence that recombinant IFN-α also reduces exacerbation frequency (Durelli *et al.* 1994) and MRI activity (Myhr *et al.* 1999) in relapsing–remitting MS. Its efficacy as regards MRI activity was comparable with that observed in trials of IFN-β (Myhr *et al.* 1999). A low frequency of injection site reactions and the absence of neutralizing antibodies in the high-dose arm (nine million international units of IFN-α 2a subcutaneously (s.c.) three times weekly for six months) suggest that it is advantageous (Myhr *et al.* 1999). Otherwise, the profile of side-effects was similar to that of IFN-β (see §2(b)). In addition, however, there was a high incidence of moderate to severe (reversible) hair loss (40% in the high-dose group after six months) (Myhr *et al.* 1999). Whether IFN-α will ever be as important in MS treatment as IFN-β now seems doubtful. However, it should be noted that although IFN-β and IFN-α bind to the same receptor, they bind at different receptor sites. Furthermore, the induction of signalling by IFN-α requires the simultaneous binding of IFN-α to both the IFNAR-α and IFNAR-β chain (figure 4, left). In contrast, IFN-β can induce signalling by binding to both chains (figure 4, middle), or by binding to two IFNAR-β subunits of two different receptors (figure 4, right). These differential binding modes of IFN-α and IFN-β result in differential cytoplasmic signalling. These differences could translate into different clinical effects. This is one reason why it may be worthwhile to explore further the clinical effects of IFN-α in MS.

(b) *Adverse reactions*

The major short- and medium-term side-effects of IFN-β observed in MS trials include flu-like symptoms and (usually mild) laboratory abnormalities (IFNB Multiple Sclerosis Study Group 1995; Jacobs *et al.* 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998). Skin necrosis at injection sites occurred in up to 5% of patients in trials of s.c. IFN-β (IFNB Multiple Sclerosis Study Group 1995; Ebers & PRISM 1998; Kappos & European Study Group 1998).

Initially up to 75% of patients experience flu-like symptoms such as fever, myalgia, headache, fatigue and chills. The reaction begins 3–6 h after injection and usually improves within 24 h. The individual pattern and intensity of these symptoms varies greatly from patient to patient and even from injection to injection. These transient flu-like symptoms are probably related to a temporary upregulation of inflammatory cytokines such as IL-6, TNF-α and IFN-γ (Dayal *et al.* 1995; Brod *et al.* 1996). The flu-like reactions usually resolve during the first three months of treatment. Patients should be advised to take the injection before bedtime in order to 'sleep off' most of the side-effects and to take non-steroidal antiphlogistics (NSAPs) as co-medication. Oral prednisone (10 mg per day) with or without NSAPs is highly effective in reducing flu-like symptoms, if NSAPs alone are not sufficient. The efficacy of low-dose oral steroids is probably related to a decreased induction of IL-6 (Martínez-Cáceres *et al.* 1998). Alternatively, pentoxifylline (800 mg twice a day) has been shown to reduce fever,

chills and myalgia during the first six months of therapy (Weber *et al*. 1998).

Many patients experience a transient worsening of pre-existing MS symptoms, especially increased spasticity. This usually appears within the complex of flu-like symptoms, especially in the first 12 weeks of treatment, and is probably similar in nature to the functional deterioration of the neurological status of MS patients as a consequence of stress, heat, fever or inflammatory mediators. Symptoms typically appear 3–24 h after IFN injection and can last between several hours and several days. If the drug is administered in the evening, most patients report that they feel worse on the day after the injection. Co-medication with NSAPs and, sometimes, antispastic medication (e.g. baclofen) is helpful.

Neither IFN-β-1a nor IFN-β-1b has been associated with significant hepatic or renal dysfunction or bone marrow suppression. The most commonly observed laboratory abnormalities are lymphopenia, neutropenia, leucopenia and raised liver aminotransferase values. These changes were seldom serious and always reversible during clinical trials and post-marketing surveillance. Complete blood counts should be obtained each month as well as serum chemistries with liver function tests in the first three months, and quarterly thereafter. If significant laboratory changes occur, the dosage should be temporarily reduced or discontinued. Once the parameters have normalized, it is usually possible to gradually increase the dosage of IFN without any complications. Although no evidence for any drug interactions has emerged so far, the concomitant administration of IFN-β and other potentially hepato- or myelotoxic medications such as anticonvulsants, antidepressants or ticlopidine should be closely monitored.

Menstrual disorders, such as breakthrough bleeding or spotting, were reported in 28% of premenopausal women during the IFN-β-1b trial, compared with 13% of placebo-treated women. In the IFN-β-1a (Avonex™) trial, placebo- or IFN-β-1a-treated women were equally affected by menstrual disorders. Earlier studies noted a decrease in serum progesterone and oestradiol levels during IFN therapy, suggesting that IFN interacts *in vivo* with the function of both luteinizing hormone and follicle-stimulating hormone. However, a decrease in fertility was not observed.

Pre-clinical studies with both IFN-β-1b and IFN-β-1a in rhesus monkeys demonstrated that high doses of IFN-β were not teratogenic for the embryo, but they had a dose-dependent abortive effect. It is, however, not known if IFN-β is teratogenic in humans. It is generally recommended that IFN therapy should be discontinued during pregnancy. However, IFN-β therapy is not necessarily an indication for inducing an abortion of an intact pregnancy once the drug has been discontinued until delivery. It is not known whether IFN-β is secreted into breast milk. Although the protein is most likely degraded in the acidic environment of the infant's stomach, therapy should nevertheless be discontinued during nursing for safety reasons. To lower the risk of a relapse post-partum, one can consider early weaning and begin treatment with IFN-β soon after delivery.

Symptoms of depression such as passiveness, lack of interest or appetite, sleep disorders, and pessimistic hopelessness are frequent in MS patients (see McDonald & Ron, this issue). Up to 50% of patients who begin IFN-β therapy have unrealistically optimistic treatment expectations, and consequently their compliance is significantly less than that of patients with more realistic expectations. These facts should be taken into account when evaluating the significance of IFN-β in depression. The results of large, controlled, clinical trials with both IFN-β and IFN-α are controversial. Out of 372 patients in the North American study, four attempted and one committed suicide in the course of five years (Sibley & IFNB Multiple Sclerosis Study Group 1993; IFNB Multiple Sclerosis Study Group 1995). All five patients had belonged to the group receiving IFN-β-1b; there were no suicide attempts in the placebo group. These differences, however, are not statistically significant (Sibley & IFNB Multiple Sclerosis Study Group 1993). In contrast, in the European trial on IFN-β-1b treatment of secondary progressive MS, depression was more likely to occur in patients receiving placebo (Kappos & European Study Group 1998). Both IFN-β-1a trials reported no significant difference in Beck Depression Inventory scores between the two treatment arms (Jacobs *et al*. 1996; Ebers & PRISM 1998). If depression and mood disorders appear during therapy with IFN-β, they should be treated like all other depressive syndromes, i.e. with antidepressant medication and psychotherapy. The individual pros and cons of IFN-β treatment in light of the actual course of the disease must be openly and critically discussed with the patient in order to decide whether IFN-β should be continued.

The incidence and character of injection site complications depends on the route of administration. Generally, intramuscular (IM) injection causes less skin reactions, and so far no necroses have been reported. Locally painful, irritated, as well as hardened skin lesions at injection sites are found in patients treated with s.c. IFN-β-1b or IFN-β-1a. The individual extent and pattern of skin reactions vary greatly. Subtle, uninflamed sclerotic dermal plaques, erythematous plaques, ulcers and sarcoid-like granulomatous dermatitis have been described. Most events appear within the first month of treatment. Women seem to be at a greater risk of skin reactions. The mechanisms of injection site inflammation are unknown; however, they are currently thought to be local, non-specific inflammatory responses to IFN-β and to be influenced by the injection depth. This is consistent with the observation that skin reactions are more likely to occur after injections in the arms or thighs and less frequently in the abdomen or buttocks (IFNB Multiple Sclerosis Study Group 1995). Cutaneous necroses were reported in up to 4.7% of all cases for IFN-β-1b and 2.1% for IFN-β-1a (Rebif®) (IFNB Multiple Sclerosis Study Group 1995; Jacobs *et al*. 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998). Risk factors include incorrect injection techniques, insufficient needle length, cold IFN-β solution, repeated use of the same injection site, and excessive exposure of recent injection sites to sunlight or ultraviolet rays (e.g. in a solarium). The pathogenetic mechanism is not yet known, but microthromobosis and vasculitis are suspected.

In general, IFN therapy can be continued if mild to moderate skin irritations occur. Applying ice to the

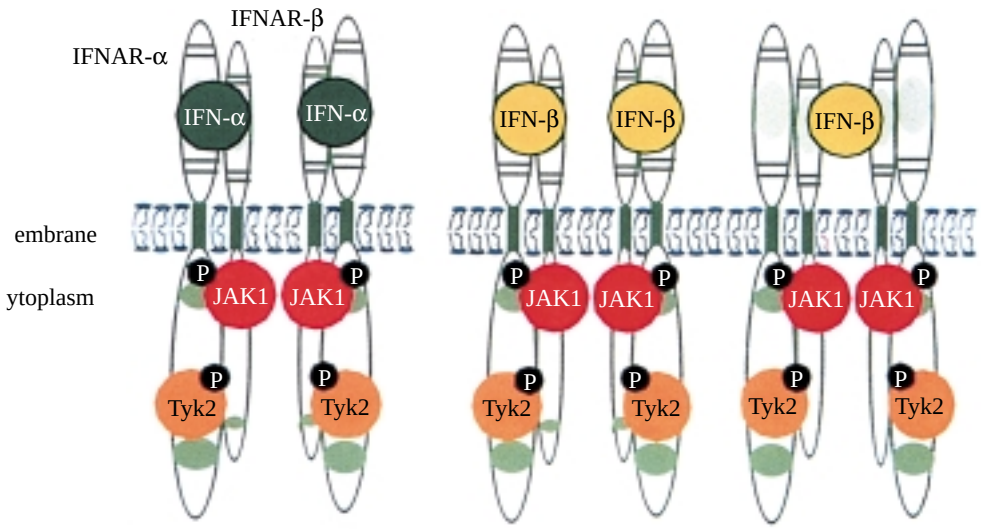

Figure 4. Differential binding of IFN-α (left, dark green) and IFN-β (right, yellow) to the α- and β-chain of the receptor for type I IFNs (IFNAR). JAK1 and Tyk2 denote the receptor-associated Janus kinases. Binding of the ligand (IFN-α or IFN-β) leads to phosphorylation of tyrosine residues on the kinases and receptor. The induction of signalling by IFN-α requires the simultaneous binding of IFN-α to both the IFNAR-α and IFNAR-β chain (left). In contrast, IFN-β can induce signalling by binding to both chains (middle), or by binding to two IFNAR-β subunits of two different receptors (right). Courtesy of M. Müller, Vienna.

injection site before and after injection can reduce injection site pain. The drug should be completely dissolved and adjusted to room temperature before injection. Ibuprofen or other NSAP drugs generally relieve pain in cases of moderate to severe injection site discomfort. The complaint of reddened, patchy injection sites can be improved by applying 1% hydrocortisone ointment locally. The frequency and extent of injection site reactions usually diminish gradually over the first six months of treatment. If stronger reactions appear (necrosis), s.c. IFN-β should be discontinued and i.m. IFN-β should be considered. If the necrosis is not infected, a sterile covering together with an antibacterial ointment is sufficient after documenting the size and severity of the lesion. Cortisone ointments are contraindicated, since they delay the slow, natural healing process and increase the likelihood of secondary infection. A diagnostic biopsy is not only unnecessary, but it can lead to additional complications, because it is often slow to heal. Superinfected necroses need surgical intervention (debridement, peroxide, etc.) and broad-spectrum antibiotics.

The development of other autoimmune diseases is a rare but documented side-effect of IFN-α therapy. Several cases of similar complications recently reported for IFN-β include myasthenia gravis, hyper- and hypothyroidism, Raynaud's phenomenon, autoimmune hepatitis, rheumatoid arthritis and lupus erythematosus. Since autoimmune complications may be serious, other immunoactive drugs should be considered for MS patients with known autoimmune thyroid dysfunction or other autoimmune diseases.

A single case of a capillary-leak syndrome associated with a pre-existing C1-esterase-inhibitor deficiency and a monoclonal gammopathy in a patient treated with eight million international units of IFN-β-1b has been reported. The patient died within 80 h of injection due to shock and multi-organ failure (Schmidt *et al.* 1999). IFN-β should not be prescribed in MS if the patient's history or blood tests suggest a concomitant disease with abnormalities of the complement system or increased B-cell activation (e.g. monoclonal gammopathy of unknown significance).

Both IFN-β-1b and IFN-β-1a may be associated with a worsening of psoriasis vulgaris. Very rarely, anaphylaxis or shock may occur.

(c) *Neutralizing antibodies*

During therapy with IFN-β, patients may develop antibodies that neutralize its biological effects. Such neutralizing antibodies have been observed in MS patients treated with IFN-β-1b and in patients treated with IFN-β-1a (IFNB Multiple Sclerosis Study Group 1995; Jacobs *et al.* 1996; IFNB Multiple Sclerosis Study Group and UBC MS/MRI Analysis Group 1996; Ebers & PRISM 1998; Kappos & European Study Group 1998; Khan & Dhib-Jalbut 1998; Yong *et al.* 1998; Rudick *et al.* 1998*b*). Neutralizing antibodies have also been detected in patients treated with natural IFN-β (Fierlbeck *et al.* 1994), suggesting that the immunogenicity of IFNs is not simply a consequence of structural differences between natural and recombinant IFNs. It is interesting in this connection that naturally occurring autoantibodies against a number of cytokines, especially IL-1α, IL-6, IL-10, IFN-α, IFN-β and leukaemia inhibitory factor have been detected in serum of normal individuals and patients with various autoimmune diseases (reviewed by Bendtzen *et al.* 1995). The functional importance of these autoantibodies is not clear, but they could be involved in the network of cytokine regulation.

The clinical significance of the neutralizing antibodies remains uncertain. It seems clear that they may attenuate or abolish the treatment effect. However, the following points should be considered. First, although the reported incidence of neutralizing antibodies varies between trials, the results of different trials are definitely not directly

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comparable because of different assays, cut-off levels and/or methods for confirmation of antibody positivity. Second, there are no reliable criteria to predict whether individual patients will develop neutralizing antibodies. Third, antibody titres may fluctuate widely in individual patients. Notably, the antibodies may completely disappear despite continued treatment. Fourth, some antibodies to IFN-β may serve as carriers, prolonging the half-life for biological activity and enhancing the bioactivity.

There is evidence that neutralizing antibodies to IFN-β-1a and IFN-β-1b are cross-reactive (Khan & Dhib-Jalbut 1998). In addition, theoretically neutralizing antibodies induced by therapy with one of the recombinant IFN-β preparations might cross-react with endogeneous IFN-β and abate its physiological effects. Fortunately, thus far there has been no evidence for such a far-reaching effect, but the possibility cannot be completely dismissed. Therefore, it will be important in the future to gather additional information on the incidence and long-term effects of neutralizing antibodies, using reliable assays. It is likely that the extent of immunogenicity of IFN-β depends not only on the route and dose of administration, but also on many other, as yet unidentified factors.

3. COP-1 (GLATIRAMER ACETATE)

(a) *Hypothetical mechanisms*

COP-1 is a synthetic basic random copolymer of L-alanine, L-glutamic acid, L-lysine and L-tyrosine in a molar residue ratio of 6.1:1.9:4.7:1.0. It was originally studied along with other basic copolymers in an attempt to simulate the activity of MBP in inducing EAE, but was then found to suppress EAE in various species including the guinea-pig, rabbit, mouse, rhesus monkey and baboon (reviewed by Arnon *et al.* 1996). Different encephalitogenic determinants of MBP are involved in the different species. Furthermore, there are some indications that the suppressive effect of COP-1 is not restricted to MBP, but extends to EAE induced in mice with PLP (Teitelbaum *et al.* 1996) or MOG (Ben-Nun *et al.* 1996) peptides. In contrast, COP-1 had no effect on experimental myasthenia gravis and experimental thyroiditis, indicating that it has some specificity for myelin-induced autoimmunity (Arnon *et al.* 1996).

It seems unlikely that a single mechanism can explain all these observations. It has been proposed that COP-1 competes with MBP and perhaps other myelin autoantigens for binding to MHC class II molecules expressed on APCs (Arnon *et al.* 1996). Indeed, COP-1 binds promiscuously to purified HLA-DR molecules (Fridkis-Hareli & Strominger 1998). This would place COP-1 in the category of MHC inhibitors. It seems that the binding to MHC class II molecules does not require internalization or processing of COP-1 by APCs. Since D-COP-1, a stereoisomeric form of COP-1, binds as efficiently to MHC class II molecules as COP-1 but does not inhibit EAE, one can speculate that MHC binding is a necessary but insufficient step that must be followed by a more specific step such as induction of regulatory cells or T-cell receptor (TCR) antagonism.

It is difficult to understand how COP-1 can compete with myelin antigens for MHC binding at the s.c. injection site. In this regard, the actions of COP-1 in MS may differ from those in EAE. In MS, COP-1 is abundantly available at s.c. injection sites. It is unlikely, however, that COP-1 peptides reach the brain. On the other hand, the repeated s.c. 'immunization' with COP-1 may induce a population of COP-1-reactive regulatory T cells. Since activated T cells can traverse the blood–brain barrier (Wekerle *et al.* 1986), these COP-1-reactive regulatory cells should be able to reach the brain parenchyma and particularly MS lesions. Here they might be confronted with myelin degradation products, including MBP peptides bound to MHC molecules expressed on the surface of perivascular microglia and secondarily recruited macrophages. It can be further postulated that upon recognition of cross-reactive MBP, the COP-1 reactive 'suppressor' T cells are activated (or modulated) to exert their downregulatory functions. The suppressive effect might extend to T-cell responses against other myelin antigens, e.g. PLP ('bystander suppression') (Aharoni *et al.* 1998).

Conversely, systemically circulating MBP-reactive T cells might be altered (e.g. downregulated, 'tolerized' or shifted in cytokine profile) after recognition of cross-reactive components of COP-1 in the periphery. Indeed, there is some evidence that COP-1 peptides can act as 'altered peptide ligands' (APLs) or 'TCR antagonists' on MBP-specific T cells (Aharoni *et al.* 1999). *In vitro*, human T cells specific for MBP epitope 82–100 were inhibited when they were cocultured with APCs pulsed with MBP and APCs pulsed with COP-1, suggesting a TCR-antagonistic effect of COP-1 (Aharoni *et al.* 1999). Note that in contrast to MHC competition and TCR antagonism, APL-like effects do not require the simultaneous exposure of T cells to both COP-1 and MBP. Therefore, APL effects could occur in the periphery at s.c. injection sites. After exposure to COP-1 in the periphery, MBP-specific T cells might be altered in their properties and respond differently when they encounter their original antigen MBP in the brain.

In addition to the effects mentioned above, there is some evidence that treatment with COP-1 induces a systemic shift from TH1 to TH2/TH3 responses: patients treated with COP-1 had elevated serum levels of IL-10 and transforming growth factor-β, and increased transcription of IL-4 in peripheral blood cells (Miller *et al.* 1998). Considering the various effects of COP-1 that have been described, it is probably fair to conclude that at the current time, the exact mechanisms of COP-1 remain largely speculative.

(b) *Clinical effects*

The therapeutic effects of COP-1 on relapsing–remitting (Bornstein *et al.* 1987; Johnson *et al.* 1995) and chronic progressive (Bornstein *et al.* 1991) MS were investigated in a number of clinical trials. A phase III multicentre, double-blind, placebo-controlled trial of patients with relapsing–remitting MS showed a 29% reduction in relapse rate (Johnson *et al.* 1995). The safety profile and clinical benefit of COP-1 were maintained during an extension study (Johnson *et al.* 1998). COP-1 also has a beneficial effect on MRI lesions.

Patients chronically injected with COP-1 develop binding antibodies. There is no evidence, however, that the COP-1-binding antibodies compromise the clinical effects of COP-1. Further studies are underway to obtain

Table 1. *Conventional immunomodulatory and immunosuppressive treatments*

(For most of the listed treatments, there are presently insufficient data to definitely judge their usefulness for MS.)

azathioprine
bone marrow and stem cell transplantation
cladribine
corticosteroids
cyclophosphamide
cyclosporin-A
15-deoxyspergualin
i.v. immunoglobulin
irradiation (total lymphoid irradiation; low-dose total-body irradiation)
Linomide[a]
metalloproteinase inhibitors
methotrexate
mitoxantrone
mycophenolate mofetil
pentoxyfilline
phosphodiesterase IV inhibitors
plasma exchange
psoralen UV-A irradiation
sirolimus (rapamycin)
sulphasalazine
tacrolimus (FK506)

[a] Definitely shown to be ineffective or unfavourable in MS, or had unacceptable toxic effects.

more detailed information on the effects of COP-1 on clinical progression and on MRI activity in different clinical types of MS. Whether combination therapy with COP-1 and IFN-β will be additive awaits further study.

COP-1 was generally well tolerated in the published trials. The most common adverse experience was an injection-site reaction. A transient systemic reaction occurred at least once in 15% of patients treated with COP-1 (Johnson *et al.* 1995). This sporadic and unpredictable reaction occurred within minutes after injection, and was characterized by flushing or chest tightness with palpitations, anxiety or dyspnoea and usually lasted between 30s and 30 min. Very rarely, a severe allergic reaction occurred, requiring the discontinuation of therapy.

4. OTHER IMMUNOSUPPRESSIVE AND IMMUNOMODULATORY AGENTS

(a) *'Conventional' immunotherapies*

Apart from IFN-β and COP-1, many other agents were previously tested or are currently being tried for the immunosuppressive or immunomodulatory treatment of MS. A comprehensive review of these agents is beyond the scope of this chapter. Recent overviews on the subject may be found in Hohlfeld (1997), Compston (1998), Noseworthy *et al.* (1999) and Weilbach & Gold (1999) (tables 1 and 2).

It is fair to state that in the trials published to date, none of the many 'conventional' immunomodulatory treatments and non-selective aggressive immunosuppressants showed sufficiently positive effects to warrant their official approval for the treatment of MS (table 1). However, most clinicians who treat MS patients have observed that some of their patients in whom other, less toxic therapies have failed, respond to more aggressive forms of immunosuppressive treatment. This has been nicely documented in a series of patients who were followed by frequent MRI scans at the US National Institutes of Health (J. Frank and H. McFarland, personal communication). An example is shown in figure 5. It is to be hoped that sometime in the

Table 2. *Biotechnological agents and experimental approaches for the immunomodulatory therapy of MS (Hohlfeld 1997; Weilbach & Gold 1999)*

(Most treatments are experimental. FDA-approved agents are printed in bold.)

immunosuppressive agents
anti-CD3 mAbs
anti-CD4 mAbs
anti-CD52 mAb (Campath-1H)
anti-IL-2 receptor α-subunit mAbs (e.g. Daclizumab, Basiliximab)
cytokines and cytokine inhibitors
IFNs
IFN-β-1a
IFN-β-1b
IFN-α
other IFNs
TNF inhibitors
TNF-receptor–IgG soluble dimeric p-55 (Lenercept)[a]
anti-TNF human/murine chimeric mAb cA2[a]
metalloproteinase inhibitors (e.g. BB-3644)
downregulatory cytokines
IL-1 inhibitors
IL-4
IL-10
IL-13
TGF-β2 (BetaKine)[a]
chemokine antagonists and receptor blockers
neurotactin antagonist
monocyte chemoattractant-1 (MCP-1) receptor antagonist
CXC-chemokine receptor 3 (CXCR3) receptor antagonist
CC-chemokine receptor 1 (1CCR1) receptor antagonist
CC-chemokine receptor 5 (CCR5) receptor antagonist
therapies directed at cell interaction molecules
adhesion molecules
humanized anti-CD11/CD18 mAb (Hu23F2G)
antagonistic peptide inhibitors of integrins
anti-VLA-4 and anti-α4 integrin mAbs or peptide inhibitors
anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) mAb
costimulatory molecules
anti-CD2 mAb
anti-leucocyte function-associated antigen-3 (LFA-3) (CD58) mAb
anti-CD154 mAb
cytotoxic T-lymphocyte antigen 4 (CTLA4-Ig)
anti-CD45 mAb
immunotherapies targeting the 'TMC'
Copolymer-1
MHC blockers
altered peptide ligands (so far mostly MBP analogues)
oral tolerance (oral bovine myelin)[a]
other strategies of tolerance induction by modification of antigen presentation
vaccination with T cells or TCR peptides
anti-TCR mAbs
agents affecting both the immune and nervous systems (Scolding, this issue) e.g. neurotrophic factors

[a] Treatments that were shown to be ineffective or unfavourable, or had unacceptable toxic effects.

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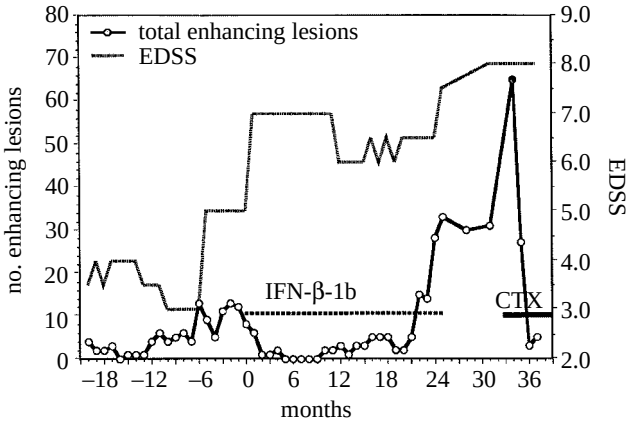

Figure 5. Course of a patient who was sequentially treated with IFN-β-1b and cyclophosphamide (CTX). The solid line indicates the number of gadolinium-enhancing lesions on brain MRI. The dotted line indicates the clinical course (expanded disability status scale). Treatment with IFN-β-1b initially showed a positive effect on MRI, but from month 20, there was a sharp increase in enhancing lesions. Subsequent treatment with CTX drastically reduced the number of enhancing lesions. Courtesy of J. Frank and H. McFarland, Neuroimmunology Branch, National Institutes of Health.

future it will be possible to identify subgroups of patients who are most likely to benefit from one or the other form of immunosuppression.

(b) *Biotechnological agents*

Advances in biotechnology have promoted the development of a new class of biotechnological product for immunotherapy and even immunological gene therapy. These biotechnological agents are used to manipulate the immune system by selectively mimicking, inhibiting, or otherwise interacting with naturally occurring polypeptides or oligonucleotides (table 2) (reviewed by Hohlfeld 1997; Weilbach & Gold 1999). Some of the new biotechnological agents hold the promise of an unprecedented selectivity of action. However, these agents also present a number of specific problems, ranging from inconvenient application (by s.c., i.m. or intravenous (i.v.) injection) to immunogenicity (stimulation of neutralizing antibodies). Thus, it is obvious that biotechnological agents are not necessarily superior to chemical immunomodulators.

Ideally, immunotherapy would not have to be applied indefinitely, but only for a limited, short time to achieve a permanent result. Such tolerance-inducing therapy has indeed been achieved in experimental situations, especially in transplantation. While these experiments have only indirect relevance for MS, they demonstrate that it is possible to achieve a state of permanent and selective immune tolerance by combining different immunological manipulations. By analogy, it may be possible to 'silence' an ongoing immune reaction permanently by combining different immunotherapies. Possible approaches include combinations of anti-leucocyte differentiation antigens, combinations of anti-costimulatory agents, and various strategies for 'immune deviation' (Hohlfeld 1997).

Selective, antigen-specific immunotherapies target the trimolecular complex (TMC) of T-cell stimulation (Hohlfeld 1989; Wraith *et al.* 1989) (table 2). In principle, each component of the TMC can be targeted. The MHC molecule could be blocked by anti-MHC antibodies or 'blocking peptides'; the antigen (or antigenic peptide) could be applied in such a way that the autoreactive T cells are inhibited rather than stimulated; and the TCR could be targeted with anti-TCR antibodies or by T-cell or TCR-peptide vaccination (table 2).

Antigen-selective immunotherapy seems very promising, but it poses special practical problems. For example, it has been shown that the T-cell response to various candidate CNS autoantigens is much more complex in humans than it is in certain inbred rodent strains. This implies that selective immunotherapy needs to be 'individualized' (tailored for individual patients). Furthermore, there is growing evidence that the autoimmune response is not static but dynamic. For example, new autoantigens may be recruited over time. Conceivably, this 'antigen spreading' might not be slowed but accelerated by certain immunotherapies (reviewed in Hohlfeld 1997).

5. CONCLUSION: PROSPECTS FOR A DIFFERENTIATED IMMUNOTHERAPY OF MULTIPLE SCLEROSIS

In order to make optimal use of the increasing number of immunotherapies, it is essential to improve our understanding of the pathogenetic and clinical heterogeneity of MS. In order to tailor the therapy for each patient, it must be possible to classify and stage the disease. We are presently at a relatively early stage of this development. Therefore, much remains to be done, although our current therapies are clearly better than those of only a few years ago.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES