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What do we know about the mechanism of action of disease-modifying treatments in MS?

Multiple sclerosis (MS) is a progressive, autoimmune disease affecting the central nervous system. However, although a proinflammatory response has been implicated in the aetiology of MS, the exact cause of the disease remains unclear.

Current therapies for MS include interferon (IFN) β and glatiramer acetate (GA). These therapies have both produced positive results in patients with MS, and appear to suppress the proinflammatory response. Nevertheless, despite ongoing research, the mechanisms of action for IFN β and GA are not yet fully understood. Through actions at five or more different points along the MS disease pathway, IFN β is thought to decrease T-cell activation, migration and reactivation, while GA is thought to have two primary mechanisms of action: acting as a 'myelin decoy', thus preventing myelin binding of proinflammatory molecules; and causing a population shift of T-cells from the proinflammatory T-helper (Th)1 cells towards the anti-inflammatory Th2 cells.

The three papers that follow explore what is known about the mechanisms of action for IFN β and GA, and touch on areas in which further research is needed.

Potential targets for disease-modifying treatments in MS

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Abstract Multiple sclerosis (MS), a chronic inflammatory disorder of the central nervous system (CNS), results in damage to axons and their surrounding myelin sheath. The exact cause of inflammation remains unclear, but an autoimmune response directed against CNS antigens is suspected. MS can affect the brain, optic nerve and spinal cord, thus causing many neurological symptoms. These can include limb numbness or weakness, sensory or motor changes, ataxia, blurry vision, painful eye movements, bladder and bowel dysfunction, decreased memory, fatigue and effective disorders. This article will include a concise overview of the pathogenesis of MS in order to set the stage for subsequent discussion of the mechanisms of action of disease-modifying treatments, and whether these should influence our treatment choices. Although the exact pathogenesis of MS is not fully understood, current knowledge has already led to the development of effective treatments, namely interferon (IFN) β and glatiramer acetate, both of which have been shown to reduce relapse rates, while IFN β-1a also reduces confirmed disability progression. Further increases in our understanding of the pathogenesis of MS are likely to assist in the identification of new targets for disease-modifying therapies and in the optimisation of current treatments.

Key words multiple sclerosis · disease-modifying treatments · pathogenesis

The immunopathogenesis of MS

Over recent years, there have been considerable advances in our knowledge of the pathogenesis of multiple sclerosis (MS), which in turn have pointed to new molecular targets for the development of future therapies. For example, while the cardinal pathological features of MS include white matter plaques in the central nervous system (CNS), particularly in the optic nerve, brainstem, spinal cord and periventricular regions [1], it is now apparent that changes in grey matter also play a role in the pathology of this disease [2, 3]. Similarly, although at the

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cellular level demyelination is one of the most widely recognised hallmarks of MS, astrogliosis [4, 5] and (as recently re-discovered) axonal degeneration [6–9] make important contributions to the spectrum of pathological changes that we encounter in MS.

An additional level of complexity has also now been added to the equation as a result of studies that clearly indicate the existence of considerable heterogeneity between the MS pathology of individual patients [10, 11]. This has led to a proposed categorisation of four fundamentally different types of lesion that differ from one another in terms of myelin protein loss, plaque size and distribution, patterns of oligodendrocyte destruction, and evidence of complement activation (Fig. 1) [11]. Overall, two of the patterns resemble those of T-cell-mediated or T-cell- plus antibody-mediated autoimmune encephalomyelitis, whereas the other two more closely reflect primary damage to, or intrinsic metabolic disturbances of, oligodendrocytes, resulting in primary progressive disease. Further studies will be required to confirm the widespread existence of these very distinctive patterns of pathology. However, if confirmed, such heterogeneity is likely to have considerable implications, both for the optimal use of current therapies and the selection of potential targets for new therapies.

Even without taking into account the existence of inter-patient heterogeneities, the network of factors that contribute to the pathology and pathogenesis of MS is far from straightforward. Inflammation is thought to be one of the key factors in MS disease activity and, although its exact role remains to be defined, it appears to be a major contributor to the formation of acute lesions [12]. There is also considerable evidence that the degree of inflammation within such lesions shows a strong correlation with the extent of axonal loss [7, 13]. Indeed, all the crucial elements of inflammatory interactions can be identified in active MS lesions. These include T-cells (predominantly cytotoxic cluster of differentiation [CD]8 T-cells), activated microglia, macrophages (the key executers of the immunoinflammatory response) and plasma cells (that release antibodies that may bind to CNS antigens, damaging the structure) [14].

Evidence, assembled over several decades, suggests that the systemic immune repertoire of some individuals may be influenced by genetic factors that increase their risk of developing MS at a later stage [15–18]. However, there is also what could (for the sake of simplicity) be described as an 'intrinsic immune system within the brain' and, in order to understand the evolution of MS, we need to be able to picture interactions between the systemic immune system and the local immune circuitry within the CNS [19] (the two are not completely separated, however, as the immune system is separated from the CNS by the blood-brain barrier [BBB]).

Currently, it is hypothesised that during early childhood, events (which have not yet been deciphered) result in a 'skewing' of the immune response and the generation of autoreactive T-cells. Although potentially capable of recognising CNS antigens, these T-cells remain dormant for years, as they cannot leave the systemic immune compartment. It is only if, or when, an external trigger comes into play (often in early adulthood) that these cells are rendered active, allowing them to migrate to and penetrate the BBB. Having entered the CNS, these autoreactive T-cells interact with antigen-presenting cells such as microglia and, if the correct antigenic epitope is present in the context of other molecules, undergo local clonal proliferation (Fig. 2) [20]. The same T-cells will also recruit macrophages and instruct B-cells to synthesise and deliver antibodies that can bind to epitopes on the myelin sheath, resulting in an enhanced local immunoinflammatory response.

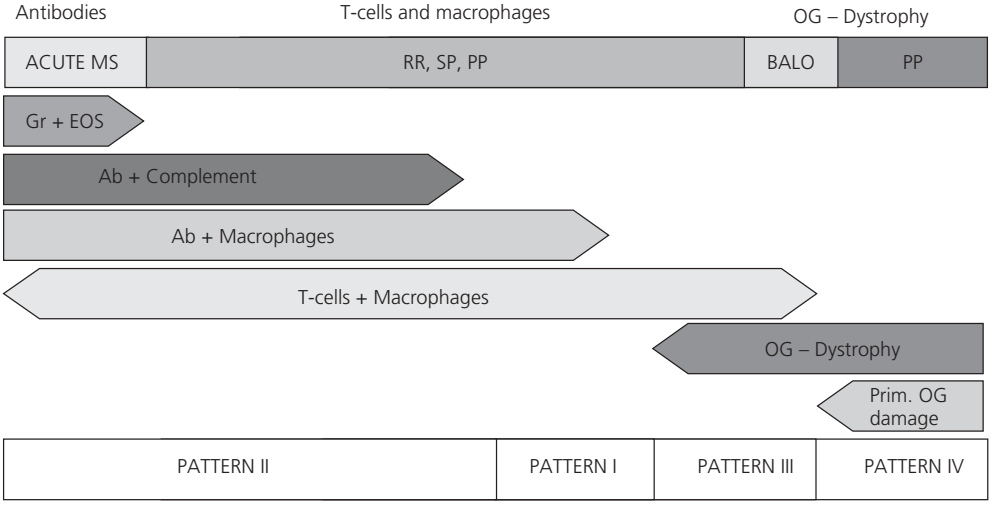

Fig. 1 Features of different patterns of active multiple sclerosis (MS) lesions in relation to clinical disease course [11]. *Ab* antibody; *BALO* Balo's concentric sclerosis; *EOS* eosinophil; *Gr* granulocyte; *OG* oligodendrocyte; *PP* primary progressive; *RR* relapsing-remitting; *SP* secondary progressive

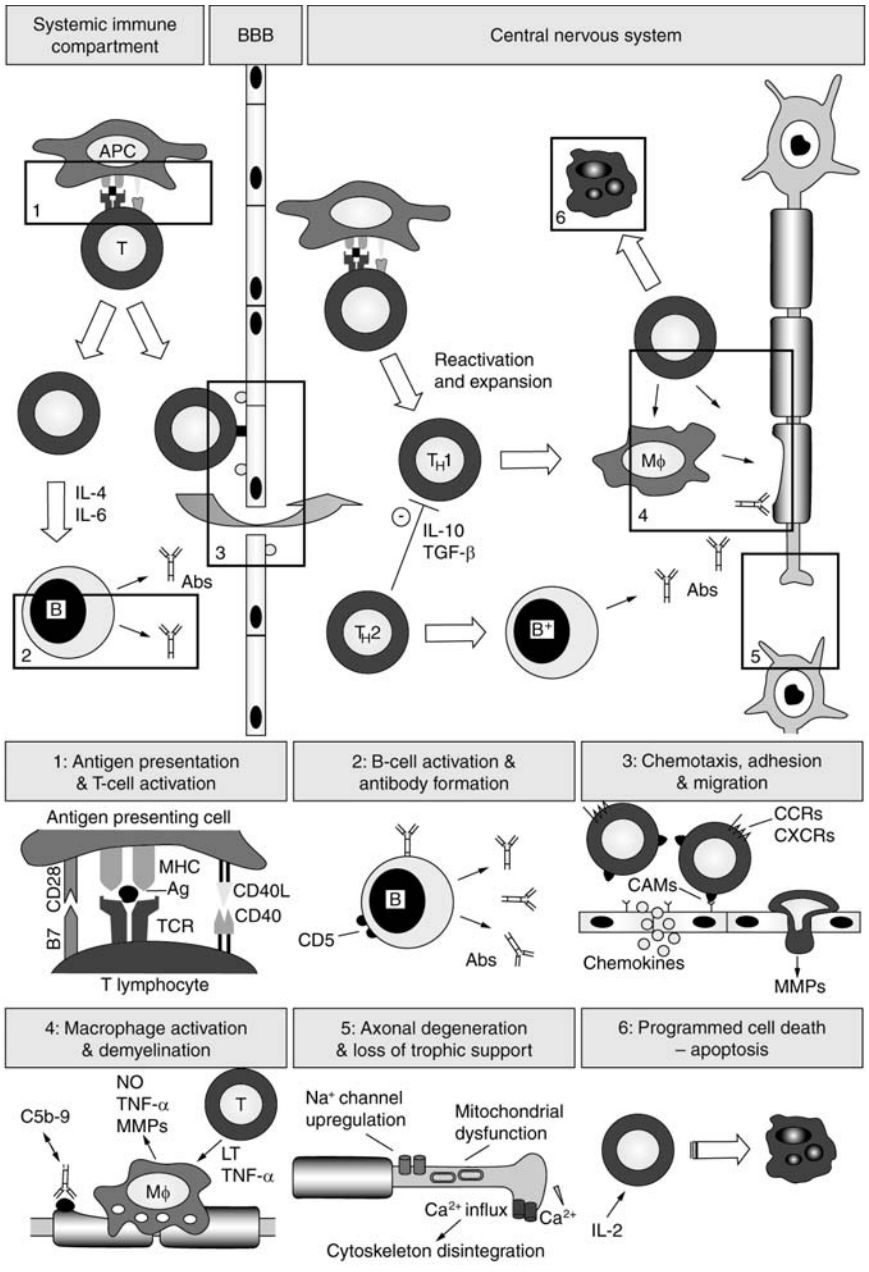

Fig. 2 Synoptic view of the immune response in the pathogenesis of multiple sclerosis. Autoreactive T-cells recognise with their T-cell receptor (TCR) a specific autoantigen presented by major histocompatibility complex (MHC) class II molecules and the simultaneous delivery of co-stimulatory signals (cluster of differentiation [CD]28, B7, CD40, CD40L) on the cell surface of antigen-presenting cells (APCs), such as macrophages, in the systemic immune compartment (Panel 1). Activated T-lymphocytes can cross the blood-brain barrier (BBB) in order to enter the central nervous system (CNS). The mechanisms of transendothelial migration are mediated by the complex interplay of cell adhesion molecules (CAMs), chemokines and their receptors (CCRs, CXCRs) and matrix metalloproteases (MMPs; Panel 3). Within the CNS, T-cells activate microglia cells/macrophages (Mϕ) to enhance phagocytic activity, production of cytokines, such as tumour necrosis factor (TNF)-α, leukotriene (LT) and the release of toxic mediators, such as nitric oxide (NO), propagating demyelination and axonal loss. Antibodies (Abs) crossing the BBB or locally produced by B-cells or mast cells (B^+) contribute to this process. Autoantibodies activate the complement cascade resulting in the formation of the membrane-attack complex (C5b-9) and subsequent lysis of the target structure (Panels 2 and 4). The upregulation of Na^+ and Ca^{2+} channels on the axon as well as mitochondrial dysfunction and loss of trophic support contribute to axonal disintegration and degeneration (Panel 5). The inflammatory response is regulated by anti-inflammatory cytokines, such as interleukin (IL)-10 or transforming growth factor (TGF)-β, as well as IL-2 inducing programmed cell death (apoptosis) in immunoreactive T-lymphocytes (Panel 6). From Wiendl H, Kieseier BC (2003) Disease-modifying therapies in multiple sclerosis: an update on recent and ongoing trials and future strategies. Expert Opin Investig Drugs 12:689–712 [20], and published with permission. *Ag* antigen; T_H T-helper cell

Migration across the BBB

The migration of autoreactive immune system effector cells, from the systemic immune system into the CNS, is crucial to the formation of inflammatory MS lesions. Upon exposure to chemokine signals, T-cells circulating in the bloodstream can attach to the endothelium of the BBB, via a process that requires reciprocal interactions with adhesion molecules expressed on both the T-cells and the endothelium. Then, by producing a cocktail of enzymes, including matrix metalloproteases (MMPs), the T-cells degrade the extracellular matrix of the BBB and enter the CNS. The processes of adhesion, penetration and migration across the BBB thus involve a number of families of molecules (including adhesion molecules, chemokines and MMPs), each of which is thought to play a fundamental role in the disruption of the BBB [21], and are therefore important targets for therapeutic intervention.

Chemokines

Chemokines form a chemoattractant gradient that is perceived by T-cells and assists them in their invasion of the CNS. They also increase binding of T-cell adhesion molecules to their reciprocal counterparts on endothelial cells (see the following section). Studies have shown that the levels of several chemokines are elevated in patients who are experiencing MS relapses. These include chemokines, such as interferon γ-inducible protein-10, which is expressed by brain microvascular endothelial cells only after inflammatory stimuli [22], while its receptor CXCR3 is expressed at enhanced levels in peripheral blood mononuclear cells of patients during MS relapses, and is found on lymphocytic cells within almost all perivascular inflammatory infiltrates in active MS lesions [23].

Adhesion molecules

The attachment of T-cells to the endothelial cells of the BBB requires the reciprocal interaction of complementary adhesion molecules expressed on the cell surface of both cell types. Three pairs of adhesion molecules that are important from the perspective of MS pathology are: E-selectin and sialyl Lewis (SLe); vascular cell adhesion molecule (VCAM)-1 and very late antigen (VLA)-4; and intracellular adhesion molecule (ICAM)-1 and lymphocyte function-associated antigen (LFA)-1 [24, 25]. However, the exact role of these adhesion molecules is not yet clear. Antibodies against LFA-1 and ICAM-1 have been reported to suppress experimental allergic encephalomyelitis (EAE) [26, 27], and studies have indicated that ICAM-1 levels are elevated just prior to the first clinical signs of the disease [28]. However, not all investigations of anti-LFA-1/ICAM-1 therapy have produced favourable results [29–31].

Matrix metalloproteases

MMPs produced by activated leukocytes assist in the breakdown of the BBB and immune cell invasion into the brain parenchyma [32]. Studies of EAE models and patients with MS indicate that some MMPs, such as gelatinase B (MMP-9) and matrilysin (MMP-7), are present at increased levels in the blood, cerebrospinal fluid and brain during periods of inflammatory disease activity [33–35]. Correlations between increased MMP production, BBB breakdown and the presence of gadolinium-positive magnetic resonance imaging lesions have also been reported [36] and, more recently, elevated levels of MMP-9 and MMP-7 have been detected in lesions and normal-appearing white matter of patients with MS [37]. As yet, the mechanism underlying the upregulation of these MMPs during the MS disease process has not been fully elucidated. However, it has been suggested that imbalances between MMP levels and those of the tissue-specific inhibitors of metalloproteases may lead to persistent proteolytic activity [37].

Thus, each of the three families of molecules listed above plays a crucial role in the process by which T-cells that have the potential to attack the brain and spinal cord move from the bloodstream to the CNS where, upon re-activation, they set in motion a cascade of inflammatory events.

Cellular mediators of the MS disease process

T-cells, B-cells and macrophages

The concept that MS is primarily a T-cell-, in particular a CD4+ T-cell, driven autoimmune disease has dominated our view of MS for a long time. Nevertheless, it is clear that T-cells can also influence or instruct B-cells to produce autoreactive antibodies, thus bringing into play the humoral part of the immune system. The proinflammatory cytokines secreted by re-activated T-cells within the CNS stimulate the activation of additional T-cells, B-cells and macrophages [38].

The inflammatory response seen in active MS plaques involves both T-cells and macrophages, but is mediated primarily by macrophages, which are abundant at the edges of these plaques and release an ensemble of molecules (including tumour necrosis factor [TNF]-α, oxygen radicals and nitric oxide) that cause tissue damage, including damage to the myelin sheath [39, 40]. The activated B-cells, in contrast, differentiate to form autoantibody-secreting plasma cells. Upon binding to antigens, these antibodies activate the complement cascade, initiating the formation of a complex, which, in very simple terms, punches holes in the myelin membrane and eventually results in myelin degradation [38].

The list of potential B-cell autoantigens is almost as long as that for T-cells. However, recently there has been particular interest in the role of myelin oligodendrocyte glycoprotein (MOG), at least in part because of its strategic extracellular location on the myelin membrane, which makes it readily accessible to antibodies [41, 42]. Furthermore, although additional studies are required, it has been reported that the presence of anti-MOG antibodies during an initial clinical event in patients with clinically isolated syndromes may be of predictive value in identifying those patients who will go on to develop clinically definite MS [43].

Cellular targets of the MS disease process

Oligodendrocytes

Oligodendrocytes are the producers of myelin in the CNS, and these cells are also targets of the immunoinflammatory response, which causes damage and eventually apoptosis of oligodendrocytes. Mechanisms that are thought to be involved in this process include the TNF-α/factor-activating exoenzyme S (Fas)-mediated death cascade, which leads to caspase activation and ultimately oligodendrocyte demise [44–46].

Axons

The importance of axonal damage as a factor in MS disease progression has been rediscovered and highlighted extensively over the past few years [6–9]. We now know that axonal damage dictates to a large extent the development of neuropathological deficit and may therefore be key to the cumulative progression of disability over time in patients with MS [47, 48].

A number of hypotheses have been proposed as to why axonal death occurs during the MS disease process [48–52]. Some of these relate to the fact that demyelinating axons appear to be particularly vulnerable to axonal degeneration, while others suggest a disturbed interaction between axons and glia resulting in the loss of trophic support. Increasing our understanding of the mechanism of such axonal damage may allow us to devise approaches that can be used alongside anti-immunoinflammatory strategies to preserve the structural and functional integrity of the CNS. Strategies aimed at the replenishment of trophic support may have an important role to play in future MS therapies. However, even greater benefits are likely to be achieved if we can prevent such damage from occurring in the first place.

At present, there is a hypothesis that suggests that in the case of axonal damage it may not be an overwhelming CD4+ T-helper (Th)1-cell-driven response, but rather a CD8+ cytotoxic T-cell response that makes the major contribution to the pathological process. While cell culture experiments have shown that CD8+ T-cells can cut axons, with an almost scissor-like motion [53], studies of brain tissue have also implicated perforin-containing T-cells in axonal dissection [54]. The final common effector pathway may involve calcium flooding (as is reported to occur in other neurodegenerative diseases), followed by calpain activation and subsequent disintegration of the cytoskeleton.

These, however, are just some of the mechanisms that are thought to underlie the axonal pathology that over time significantly contribute to atrophy, the summation of the destructive pathological processes occurring as the MS disease process evolves.

Inflammation: a role in destruction and in healing?

Anti-inflammatory properties are a key feature of many of the disease-modifying therapies currently in use or undergoing trials for use in the treatment of MS. However, although it is clear that inflammation can have extremely destructive effects, there is also evidence to suggest that inflammatory reactions may also have beneficial effects that contribute to protective or repair responses [55]. This concept (often referred to as the 'Janus face of inflammation' [56]), although not new, has been the subject of renewed focus over the past few years, and a factor that should be carefully considered when designing new therapeutic strategies. Complete abrogation of all inflammatory responses at a given time point in the disease process may not always provide the most effective route towards maximal treatment efficacy, as it may compromise the protective aspects of this basic biological phenomenon.

Conclusions

While it is true that our rapidly developing knowledge of MS immunopathogenesis presents us with an increasingly complex picture of this disease process, it also provides us with greater opportunities to try to combat disease progression and prevent the development of long-term disability. It is now clear that even from the earliest stages of the disease process, axonal damage is a prominent feature of MS and may be the pathological correlate of permanent neurological impairment. There are, however, many molecular interactions that must occur in order for autoreactive T-cells to cross the BBB, enter the CNS and then trigger the cascade of destructive events that eventually lead to axonal damage. As discussed in the following articles, many of the steps along these pathways are the targets of currently available MS disease-modifying treatments, while others may suggest targets for new or combination therapies.

Immune modulators in MS: current knowledge and questions

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Abstract Although insights into the therapeutic mode of action of multiple sclerosis (MS) therapies are

no substitute for the results of well-designed pivotal clinical trials, such an understanding does play an important role in the rational development of new and improved treatments. Elucidating the mechanisms of action of MS treatments could also help to optimise the use of current therapies, and may provide an added degree of comfort to patients and their care teams. However, it is not always easy to reconcile the rapidly growing, and at times inconsistent, information emerging from 'mechanism of action' studies. One challenge relates to how readily one can translate laboratory studies of mechanism of action to the actual ways a given medication may mediate its disease-relevant effects *in vivo*. While studying drug effects in animal models (such as the commonly used model of MS, experimental autoimmune encephalomyelitis) has become an important part of the process of drug development and approval, it is well appreciated that observations from these models do not predict effects (or toxicities) in patients. More and more studies are emerging on effects of drugs on human cells *in vitro*, though it must be kept in mind that significant and reproducible *in vitro* findings may not always be relevant to the *in vivo* situation. Therefore, the challenge is to somehow distinguish between results of 'mechanism of action' studies that are 'plausible' based on animal and/or *in vitro* studies, and those that are drawn from direct observations of patients subjected to a given therapy. Even the latter observations are not foolproof, however, since a real effect of a therapy could be measured in patients, which may or may not be relevant to the drug's impact on the disease itself. Here, we will consider a simplified model of MS immune pathogenesis to review the proposed therapeutic mechanisms of action of the four approved therapies on the inflammatory component of the illness.

Key words multiple sclerosis · mechanisms of action · immune modulators

Introduction

The aetiology of multiple sclerosis (MS) remains elusive, though most believe that the condition manifests in individuals with a polygenetic susceptibility combined with one or more environmental exposure/s during a critical period (probably early adolescence) [57–61]. The role of activated T-helper (Th)1 cells directed against self-antigens in the central nervous system (CNS), such as myelin epitopes, has been the subject of intense study [58–60, 62–65]. Until recently, the most direct data implicating these cells have come from animal models of MS, such as experimental autoimmune encephalomyelitis (EAE) [66]. In this model, injecting animals with myelin components such as myelin basic protein (MBP) results in activation of circulating Th1 MBP-reactive T-cells and in a subsequent ascending paralytic illness sharing many pathological features with MS. Notably, removing these Th1 MBP-reactive T-cells from an affected animal and injecting into the circulation of a healthy naïve animal can transfer the same CNS paralytic illness (adoptive transfer). In contrast, injecting the recipient animal first with Th2 MBP-reactive cells can protect the animal from EAE. These observations have suggested that, in MS, Th1 responses directed against myelin components may be proinflammatory and cause damage, whereas Th2 responses may be anti-inflammatory and protective.

It has been known for over a decade that all individuals – including those without MS – have T-cells circulating in their blood that have the potential to react against MBP and other CNS antigens [63, 64], yet most people do not get MS. This suggests that the mere presence of these 'self-reactive' T-cells is not enough to cause the illness. It is now known that in patients with MS, MBP-reactive T-cells are in a higher state of activation than the comparable cells in individuals without MS [67, 68]. It is also suspected that in patients with MS, the MBP-reactive cells are more likely on average to produce proinflammatory (Th1), rather than anti-inflammatory, molecules. The hypothesis is therefore that these MBP-reactive T-cells in the circulation of patients with MS encounter their antigen (MBP) or a very similar antigen (a molecular mimic of MBP) and become activated in the periphery. If the context of activation preferentially promotes Th1 differentiation, the resultant Th1 MBP-reactive T-cells can then migrate to the brain where they will recognise MBP and react by releasing Th1 mediators that contribute to the disease process.

It must be stressed that the relationship between Th1 and Th2 responses (loosely designated as 'proinflammatory' and 'anti-inflammatory', respectively) and tissue damage or protection in MS remains only partially understood, as recent studies have suggested that Th1 responses may not always be detrimental [69]. Indeed, cells of the immune system are not all 'bad' or all 'good' [70–72]. The challenge is to define which immune responses are pathogenic, and when, so that the most effective therapies can be developed. Nonetheless, a paradigm that continues to be pursued in trials of experimental therapeutics of MS is that treatments that can prevent CNS-reactive Th1 cells from entering their target, and/or treatments that can shift the immune response from a Th1 to a Th2 profile are considered beneficial [59, 61, 73–78].

The process of infiltration of myelin-reactive T-cells into the CNS depends on a series of well-coordinated interactions between several families of molecules [32, 79–82]. It is thought that, in MS, CNS-reactive T-cells in the periphery become activated by either fragments of the CNS itself (e. g. myelin), or by some antigen that closely resembles components of the CNS (molecular

mimic) and preferentially differentiates into Th1 cells (Step 1). These activated Th1 cells express the required adhesion molecules and chemokine receptors on their surface that allow them to efficiently interact with the blood-brain barrier (BBB) endothelial cells by the processes of adhesion (Step 2) and chemoattraction (Step 3). With the additional help of enzymes that degrade the BBB, such as matrix proteases, the activated Th1 CNS-reactive T-cells are then able to break into the central compartment (Step 4). There, these T-cells encounter the CNS antigen presented by resident antigen-presenting cells (APCs; microglia) or invading monocytes/macrophages, and become re-activated to produce the Th1 proinflammatory responses that contribute to tissue damage (Step 5). This model can be used to identify the probable sites of action of the currently approved immune-modulating therapies in MS [62, 83–88]. The interferon (IFN) β medications (IFN β-1b/Betaseron®, Schering; subcutaneous IFN β-1a/Rebif®, Serono; intramuscular IFN β-1a/Avonex®, Biogen Idec) are believed to share the same general mechanisms of action in MS and will therefore be discussed here as one family (IFN βs), while glatiramer acetate (GA; Copaxone®, Teva Pharmaceuticals) has a different mode of action [89].

Mechanisms of action of IFN β and GA in MS

IFN βs (Betaseron®, Rebif®, Avonex®)

Binding of IFN β to its specific receptor results in the expression of multiple IFN β response-genes, the majority of which are likely not relevant to the therapeutic potential of this family of agents in MS [90]. Drug effects that probably do contribute to the efficacy of IFN βs in MS include the ability of this class of medications to suppress T-cell responses that may relate, in part, to the property of IFN βs to decrease the expression of some co-stimulatory molecules that are otherwise able to promote T-cell activation [91]. In some cases, IFN βs may also preferentially suppress Th1-type responses, while promoting Th2 responses that could also be of benefit in MS [90]. Perhaps the most important effects of IFN βs with regards to MS are at the levels of adhesion and migration of activated immune cells at the level of the BBB. IFN βs are known to suppress the upregulation of adhesion molecules on activated T-cells, and are effective in suppressing both the transcription and translation of matrix metalloproteases, the enzymes that break down the BBB [32, 80, 82, 89, 90]. Together, IFN βs are thought to exert part of their beneficial effect by limiting the process of transmigration of activated proinflammatory Th1 cells across the BBB.

GA (Copaxone®)

Unlike IFN βs that mediate their actions by binding to specific receptors, leading to transcription of IFN β inducible genes, GA is viewed as an antigen-based therapy. Several potential mechanisms of action have been described over the years, some of which may reflect *in vitro* phenomena that are not necessarily relevant to the *in vivo* state.

GA was originally designed as a co-polymer containing a random mix of four amino acids (G, L, A, T) in proportions that resemble the content of MBP [92]. When found to inhibit MBP-induced EAE, the assumption was that GA was somehow competing with the MBP [92, 93]. It was subsequently demonstrated *in vitro* that GA can efficiently bind major histocompatibility complex (MHC) molecules and compete with MHC binding of MBP, as well as limit the T-cell receptor (TCR) engagement of MBP-reactive T-cells [94, 95]. It is not clear whether the *in vitro* conditions used in such experiments are reproduced *in vivo*. Moreover, it is now appreciated that the GA co-polymer is in fact many antigens in one, and that its effects *in vivo* are not restricted to MBP responses [96].

The aspect of the GA mechanism of action that is thought to be most relevant to its benefit in MS inflammation relates to the reproducible finding that GA injections trigger a broad T-cell response (consistent with GA being many antigens), which in most patients results in a shift in the population of GA-reactive T-cells towards a Th2 response profile [97–106]. This peripheral 'immune deviation' generates activated Th2 cells that can be identified within a month of therapy and would be expected to migrate efficiently (like other activated immune cells) into the CNS. Because they may react to multiple antigens (including, but not limited to, MBP), and given their demonstrated TCR-degeneracy, some of these GA-reactive T-cells would be expected to become re-activated within the CNS, where production of Th2 factors would counter proinflammatory Th1 responses of pathogenic CNS-reactive cells, in the process termed 'bystander suppression'. Though the exact mechanism/s by which GA therapy induces a Th2 shift is not established [89, 107, 108], recent findings extend our understanding of the mechanism of action of GA, and demonstrate that *in vivo* therapy modulates APCs in patients with MS [97, 109]. This work identifies a novel 'positive-feedback' loop between T-cells:APC, which could serve to promote further Th2 responses both in the periphery and within the CNS of patients.

Possibly of further relevance to the mechanism of action of GA are recent studies demonstrating that activated T-cells produce nerve growth factors such as brain-derived neurotrophic factor (BDNF) [110], and that BDNF-containing immune cells are found within active MS lesions [111], at sites where the BDNF recep-

tors are expressed on neural cells [112]. In this context, GA-reactive T-cells from the circulation of patients with MS have also been shown to secrete BDNF [113], raising the possibility that following migration across the BBB, these cells may contribute to both anti-inflammation and neuroprotection upon re-activation within the CNS. While direct demonstration of these effects in the CNS of patients is not feasible, studies in EAE have demonstrated that labelled GA-reactive T-cells induced by GA therapy accumulate within the CNS of the animals and produce *in situ* anti-inflammatory cytokines and BDNF [114]. Thus, in contrast to the IFN βs, GA seems to have little effect directly at the level of the BBB. Instead, peripheral induction of anti-inflammatory APC and T-cells that can migrate efficiently into the CNS would contribute to an anti-inflammatory (and possibly neuroprotective) environment within the CNS.

Conclusions

Conceptualising the MS process as a series of steps that can also be regarded as potential targets of therapy should help us discuss with our patients current as well as new treatments that will be introduced in upcoming clinical trials.

Many laboratories and clinical research activities around the world have contributed to our growing understanding of the processes leading to nervous system injury in MS. In addition to recognising the role of T-cells, there are clearly other immune responses that participate, as well as an important involvement of BBB and brain cells in these processes [72, 81]. The best approaches to treatment are likely to be those that alone, or in combination, restore a normal balance of immune responses and at the same time protect and support the functional regeneration of the nervous system [59, 61, 74–78]. While important questions remain, ongoing research efforts will undoubtedly lead to the development of new therapies that are both more effective and, importantly, easier for patients to tolerate.

Disease-modifying treatments: mode of action

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Abstract Multiple sclerosis (MS) is a putative autoimmune disorder. Various models of recognition of self and non-self have improved our understanding of autoimmunity. From simple models of direct recognition of foreign antigens by B-cells, we have moved to more complicated models that require the coordinated action of T-cells, B-cells and antigen-presenting cells (APCs). From the simple self/non-self model to the infectious non-self model and, finally, to the danger model, APCs are central in the immune process of self/non-self recognition. Genetic and environmental factors including infections are implicated in the pathogenesis of MS, although the nature of antigen stimulus remains unknown. However, our current understanding of the pathological process of the disease has led to new therapeutic approaches. Interferon (IFN) β and glatiramer acetate (GA) have well-established clinical benefits in terms of relapse rates, and IFN β has also been shown to decrease disease progression. The proposed mechanisms of action of these disease-modifying therapies reflect our understanding of the complex immune mechanisms implicated in MS. IFN β is used in the treatment of MS because of its general anti-inflammatory properties. It acts via specific receptors and by inducing a cascade of signalling. It interferes with T-cell activation, counteracts the proinflammatory effects of IFN γ, exhibits effects on co-stimulatory molecules, interacts with adhesion molecules and trafficking of T-cells into the central nervous system (CNS), downregulates specific metalloproteinases, facilitates the apoptotic process of T-cells, has an antiviral effect and probably shifts the balance of cytokine production towards the anti-inflammatory cytokines, T-helper (Th)2. The proposed mechanism of action of GA is a cross-reaction with myelin basic protein and T-cell receptors, thus closely mimicking the alleged antigen stimulus in MS. The generation of Th2 cells could act both in the periphery and the CNS, producing anti-inflammatory cytokines resulting in bystander suppression and a generalised shift in cytokine production from Th1 to Th2. However, the evidence for this is derived primarily from experimental animal models and small *in vitro* studies on T-cell lines. In conclusion, although the well-documented clinical efficacy of IFN β treatment in MS is based on its specific mechanisms of action on the immune system, that of GA treatment is poorly understood.

Key words multiple sclerosis · mechanism of action

Introduction

Multiple sclerosis (MS) is a putative autoimmune condition, characterised by central nervous system (CNS) inflammation, demyelination and axonal degeneration. Although the exact cause of MS remains unknown, our knowledge of MS pathology has increased rapidly in re-

cent years, and continues to do so. This, in turn, has greatly assisted studies of the mechanisms of action of the disease-modifying treatments (DMTs) currently used in the treatment of MS, and the identification of potential targets for new DMTs. However, such studies have also raised questions, as in some cases it has been difficult to reconcile the proposed mechanisms of action with the available clinical data. This has often led to re-appraisal of the mechanism of action, furthering our knowledge of the disease process, while emphasising the importance of a careful assessment of the data, particularly if derived from *in vitro* studies or animal models. Interferon (IFN) β and glatiramer acetate (GA) are the two most frequently used drugs in the treatment of relapsing-remitting MS. It is proposed that both achieve their effects by blocking the proinflammatory response. Nevertheless, the ways in which they are thought to act, and the extent of the data supporting these mechanisms of action, differ considerably.

What do we know about the mechanism of action of IFN β?

IFN β is a type I IFN that is produced by fibroblasts and has general antiviral, antiproliferative and immunomodulatory properties. At present, two forms of IFN β are licensed for use in the treatment of MS. IFN β-1a is produced in mammalian cells, has an amino acid sequence identical to that of natural IFN and is glycosylated, whereas IFN β-1b is produced in bacterial cells, has a slightly different amino acid sequence and is not glycosylated. However, although the structural differences do appear to impact on the level of biological activity exhibited by these two molecules (the *in vitro* antiviral activity of IFN β-1a being higher than that of IFN β-1b) and the level of clinical efficacy, there is no evidence that they differ from one another in terms of their mechanism of action.

IFN β acts through specific receptors (Fig. 3), triggering a signalling cascade, the effects of which appear to act at several different levels within the pathways that are involved in the pathological processes of MS. Events that are thought to be influenced by IFN β include the activation of immune cells, adhesion and transmigration of autoreactive T-cells across the blood-brain barrier (BBB), cytokine secretion, antigen presentation and macrophage function. It is also possible that IFN β may have neurotrophic or neuroprotective actions within the CNS, although data to support this remain inconclusive.

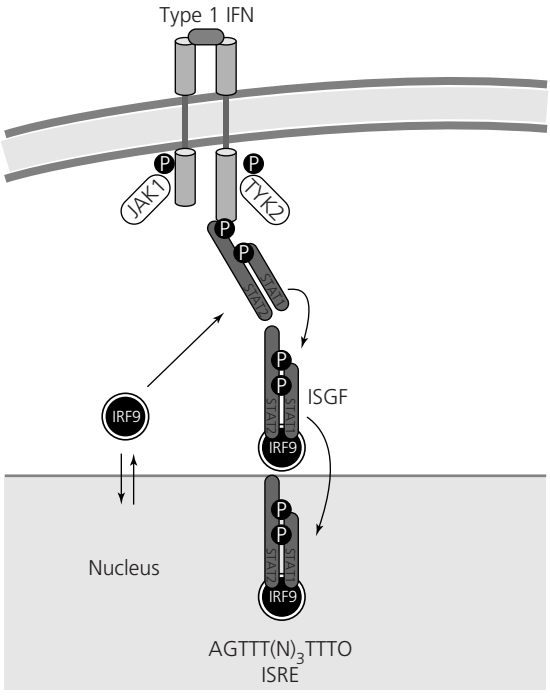

Fig. 3 Interferon (IFN) β acts through specific receptors, triggering a signalling cascade, the effects of which appear to act at several different levels within the pathways that are involved in the pathological processes of multiple sclerosis. *IRF* IFN regulatory factor; *ISGF* IFN-stimulated gene factor; *ISRE* IFN-stimulated response element; *JAK* Janus kinase; *STAT* signal transducer and activator of transcription; *TYK* tyrosine kinase

Effects of IFN β on T-cell activation and transmigration

One of the earliest actions of IFN β is the inhibition of T-cell activation. *In vitro* studies comparing the responses of peripheral blood monocytes (PBMC) from patients who have MS with those of healthy controls have shown that treatment with IFN β can significantly decrease T-cell activation and the production of the proinflammatory cytokine IFN γ [115]. Downregulation of adhesion molecules has also been reported to occur as a result of IFN β treatment. For example, studies by Gelati et al. have shown reduced expression of adhesion molecules by PBMCs and cluster of differentiation (CD)45+ cells in patients who have received 1 year of IFN β treatment [116], while studies on very late antigen (VLA)-4, the vascular cell adhesion molecule (VCAM)-1 ligand suggest that its expression on lymphocytes is decreased soon after initiation of IFN β treatment [117]. Thus, it appears that IFN β can begin to impact on the pathological processes of MS, even before the autoreactive cells make contact with the BBB.

There is also considerable evidence that IFN β inhibits transmigration across the BBB. Experiments examining the *in vitro* effect of IFN β on the ability of

PBMCs or T-cells to migrate through fibronectin have shown that IFN β decreases interleukin (IL)-2-induced secretion of the matrix-degrading enzymes matrix metalloproteinase (MMP)-2 and MMP-9, reducing migration in a dose-dependent manner [118, 119]. Similar results were reported by Lou et al., who found that IFN β dose-dependently inhibits the migration of activated leukocyte migration through a tumour necrosis factor (TNF) and IFN γ pre-stimulated human brain microvascular endothelial cell monolayer [120].

Effects of IFN β on cytokine levels

Cytokines secreted by immune system cells play an important role in mediating the inflammatory process, and can be broadly divided into two categories on the basis of whether their effects are predominantly pro- or anti-inflammatory.

IFN β decreases both the accumulation of the proinflammatory cytokine IL-2 and (as mentioned previously in relation to MMP-9) the expression of IL-2 receptors [115]. It also antagonises many of the effects of IFN γ, an IFN that has predominantly proinflammatory actions and has been implicated in several disease processes that are linked with chronic immune activation. In patients with MS, IFN γ has been found to increase the rate of exacerbations and there is some evidence to suggest that it is involved in the pathogenesis of MS lesions.

Results from early studies of animals with experimental autoimmune encephalomyelitis led to the proposal that the IL-12/IL-10 balance was a key factor in the regulation of inflammation and the pathogenesis of MS: IL-12 appeared to be critical to the proinflammatory response, while IL-10 acted as an inhibitor of this response. Furthermore, IFN β was shown to cause reciprocal changes in IL-12 and IL-10 production *in vitro*, decreasing IL-12 and increasing IL-10. However, many of these experiments were conducted using p40-deficient mice, which are now known to lack both IL-12 and IL-23 [121]. Moreover, although IL-12 is required for the development of the Th1 phenotype, it appears that it is IL-23 (a heterodimer comprising one IL-12 subunit [p40] and one IL-23-specific subunit [p19]) that is critical for the inflammatory activity and CNS-macrophage activation [122–124].

Experiments examining the production of IL-12 and IL-10 by PBMCs and myelin basic protein (MBP)-specific T-cells from patients with MS [125–127] have revealed that prior to the initiation of therapy these patients have higher levels of inducible IL-12 expression than are found in healthy controls [127], and following treatment with IFN β there is a dose-dependent decrease in IL-12 [126, 127], with a corresponding increase in IL-10 [125, 127]. To date, though, the effects of IFN β on IL-23 expression have not been fully investigated.

Effects of IFN β on antigen presentation

Antigen-presenting cells (APCs), such as macrophages and B-cells, express major histocompatibility complex (MHC) class II molecules, which, along with co-stimulatory molecules, are essential for antigen presentation and subsequent T-cell activation. IFN β decreases antigen presentation and T-cell activation by: (i) reducing the expression of co-stimulatory molecules; and (ii) reducing the expression of MHC class II molecules on APCs (Fig. 4) [89].

Studies by Genç et al. have indicated that following IFN β treatment there is reduced lymphocytic expression of the co-stimulatory molecule CD80(B7-1), which is involved in the induction of the T-helper (Th)1 proinflammatory response, while there is increased monocytic expression of CD86 (B7-2), which is involved in the induction of the Th2 anti-inflammatory response [91]. Furthermore, the essential role of co-stimulatory factors in T-cell activation and the consequent production of cytokines by these cells can be clearly illustrated through experiments that show that the levels of cytokines such as IL-10 (an anti-inflammatory Th2 cell cytokine) increase when PBMCs are treated with IFN β, but there is no such effect if the experiment is conducted with purified CD4+ or CD8+ cells.

There is also evidence that the expression of MHC class I molecules is enhanced under the influence of IFN

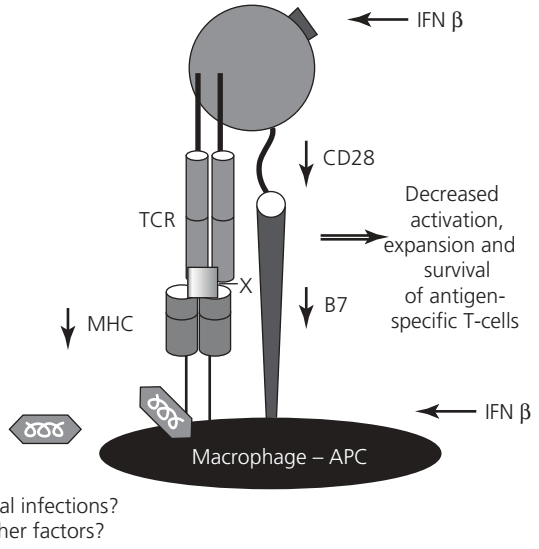

Fig. 4 Proposed mechanism of action of interferon (IFN) β on antigen presentation. IFN β acts on its receptor on T-cells and antigen-presenting cells (APCs), decreasing the expression of molecules needed for antigen presentation. Together with a further activity of IFN β on T-cell expansion and survival, this leads to the decreased generation of antigen-specific T-cells. X represents an antigen that sits on the major histocompatibility complex (MHC) groove. From Yong VW (2002) Differential mechanisms of action of interferon-beta and glatiramer acetate in MS. Neurology 59:802–808 [89], and published with permission. *CD* cluster of differentiation; *TCR* T-cell receptor

β, whereas IFN γ enhances the expression of MHC class II molecules (expressed by APCs). Co-exposure to both IFN β and IFN γ produces an IFN β concentration-dependent downregulation of MHC class II molecules [128]. Thus, it appears that IFN β can counteract some of the T-cell-activating effects of IFN γ.

Effects of IFN β on macrophage and microglia

Macrophages and microglia are thought to play an important role in the MS disease process. Although microglia can be either neuroprotective or neurodestructive, studies have indicated that in MS, activated microglia secrete proteolytic enzymes, free radicals and cytokines that contribute directly to damage of myelin, axons and the BBB, inducing the formation of a proinflammatory loop that promotes the activation of other immune cells. Studies in animal models have shown that IFN β inhibits the proliferation of microglial cells and IFN γ-induced elevation of MHC II expression, but increases expression of microglial Fc receptors. Notably, however, in the presence of IFN γ, IFN β-induced Fc receptor expression is reduced and, as IFN γ is present at increased levels during MS activity, it seems likely that the net effect of IFN β treatment in MS would be a reduction in proinflammatory microglial activity [129].

IFN β: the neuroprotection question

As is true for all currently available MS DMTs, evidence of neuroprotective effects of IFN β is inconclusive. To date, a study of embryonic mouse neuronal cells has indicated that cell survival in the presence of IFN α/β is greater than in controls [130]. There has also been a spectroscopic study, which concluded that IFN β increases the ratio of N-acetylaspartate to creatine in patients [131]. However, this conclusion is somewhat controversial, as although the results may reflect metabolic recovery they could also reflect an increase in metabolic rate.

Current knowledge of the mechanism of action of GA

GA, a synthetic polymer consisting of a mixture of four amino acids, was designed to act as a myelin 'mimic' or 'decoy' [132]. It has been proposed that GA competes with and cross-reacts with MBP, thus blocking the MHC II binding site, inducing the production of GA-specific T-cells (which are alleged to have an anti-inflammatory Th2 bias) and inhibiting the proliferation of myelin-specific T-cells or other myelin-APCs (Fig. 5). There are also claims that GA treatment may have neuroprotective effects, which are mediated by GA-induced increases in the release of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) [133].

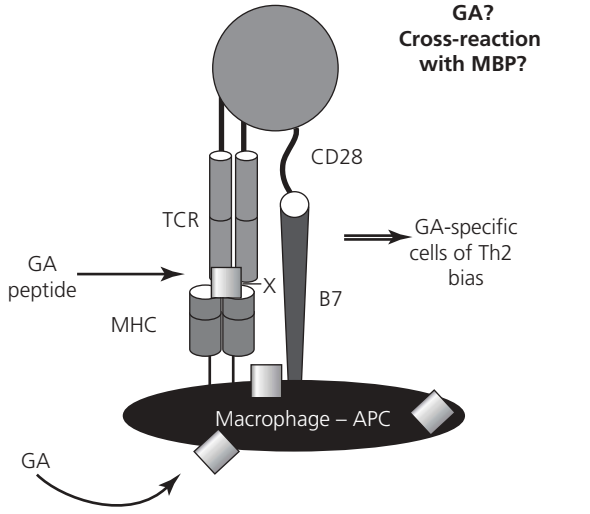

Fig. 5 Proposed mechanism of action of glatiramer acetate (GA) on antigen presentation. The high affinity of GA for the major histocompatibility complex (MHC) groove or the uptake of GA by an antigen-presenting cell (APC) leads to the presentation of GA as an antigen and the generation of GA-specific cells that are T-helper (Th)2 biased. X represents an antigen that sits on the MHC groove. From Yong VW (2002) Differential mechanisms of action of interferon-beta and glatiramer acetate in MS. Neurology 59:802–808 [89], and published with permission. *CD* cluster of differentiation; *MBP* myelin basic protein; *TCR* T-cell receptor

However, the vast majority of studies of the mechanism of action of GA have been based on *in vitro* studies of GA-reactive T-cell lines taken from patients with MS (treated and untreated) and healthy controls. This in itself raises some problems, as although there is no doubt that GA-reactive T-cell lines do exist in these patients (even before the initiation of treatment), it is very clear that there is a decrease in the number of these cells as GA treatment progresses [134]. Questions have also been raised with regard to the MBP cross-reactivity of GA, and (as pointed out in the accompanying article by Dr Bar-Or) it is now apparent that GA actually represents a multitude of antigens, the actions of which include the triggering of a more widespread T-cell response. However, whether or to what extent such widespread responses are beneficial remains unclear and, thus, despite the numerous studies that have already been conducted, it seems that there are still more questions than answers with regard to the mechanism of action of GA.

Cross-reactivity between GA and MBP

Although there have been reports of cross-reactivity between GA and MBP (and vice versa), studies examining the T-cell proliferative effects induced by such interac-

tions have failed to support this concept. Indeed, in an analysis of a panel of 721 GA-reactive T-cell lines (160 from untreated patients, 300 from patients treated with GA, 90 from healthy controls and 171 from patients treated with GA before and after therapy), there was no detectable cross-reactivity between GA and MBP at the level of cell proliferation (Fig. 6), and only 10 % of human GA-reactive T-cells secreted cytokines in response to MBP [101]. This has led to the suggestion that chronic subcutaneous administration of GA induces GA-reactive Th2 cells, which after crossing the BBB are re-activated by myelin-APCs, causing a proportion of the Th2 cells to secrete anti-inflammatory cytokines that may suppress inflammatory activity in other cells (a phenomenon known as bystander suppression).

Effects of GA on the Th1/Th2 ratio

To date, a Th1 to Th2 cytokine shift has been shown only in GA-reactive T-cell lines and not in whole PBMCs from patients. Furthermore, the suggestion that GA-reactive T-cells travel from the blood to the brain, where they are re-activated by autoantigens, becomes subject to dispute in the absence of conclusive evidence of cross-reactivity between GA and autoantigens, as does the ability of GA to induce bystander suppression and protection.

Even if we accept the evidence from GA-reactive T-cell lines, the data in favour of a Th1/Th2 shift remain far from conclusive. In the recent study conducted by Neuhaus et al., cytokine profiles of GA-reactive T-cell lines from six patients were examined before and after treatment with GA. Of the six patients studied, no T-cell shift was seen in four patients, a partial shift was seen in one patient and a transient shift was seen in another patient. Furthermore, when 111 GA-reactive T-cell lines were tested for secretion of IL-4 (an anti-inflammatory Th2 cytokine), nine (8.1 %) were positive; whereas when 53 lines were tested for secretion of IFN γ (a proinflammatory Th1 cytokine) following stimulation with MBP, eight (15.1 %) were positive [101].

Indeed, in a study by Duda et al., 590 T-cell lines generated from seven patients treated with GA over a period of 12 months failed to show a significant increase in the average levels of the anti-inflammatory cytokine IL-5 (their chosen indicator of Th2 bias). Subsequent recruitment of another three patients, whose IL-13 (rather than IL-5) levels were then monitored, did reveal an increase in the levels of this anti-inflammatory cytokine. However, there was also an increase in the production of the proinflammatory cytokine IFN γ [100]. These results are similar to those of Gran et al., who found that of 18 GA-reactive T-cell lines (taken from a single patient treated with GA), three cross-reacted with MBP, only one produced IL-5 and the same line also produced IFN γ [95]. The apparent secretion of both pro- and anti-inflammatory cytokines in response to GA is confusing with regard to the proposed mechanism of action of GA, and does little to support its ability to induce a T-cell shift.

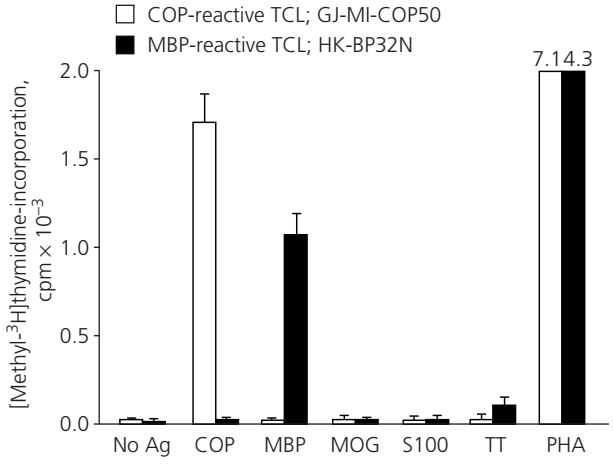

Fig. 6 Proliferative response of a representative copolymer 1 (COP)-reactive and a myelin basic protein (MBP)-reactive T-cell line (TCL). TCLs were stimulated with COP, MBP, various control antigens (Ags: human myelin-oligodendrocyte glycoprotein [MOG], S100β, and tetanus toxin [TT]), or the T-cell mitogen phytohaemagglutinin (PHA). There was no detectable cross-reaction between COP and MBP at the level of proliferation. From Neuhaus O, et al. (2000) Multiple sclerosis: comparison of copolymer-1-reactive T cell lines from treated and untreated subjects reveals cytokine shift from T helper 1 to T helper 2 cells. Proc Natl Acad Sci USA 97:7452–7457 [101], and published with permission. © 2000 National Academy of Sciences, USA

Effects of GA on monocytes

Data relating to the effects of GA on human monocytes are limited. There are indications that in the presence of GA (20 mcg/mL) there is reduced expression of the human leukocyte antigens DR and DQ. However, although GA also causes a decrease in levels of TNF-α, there is an increase in IL-1β (at GA concentrations of 5, 10 and 20 mcg/mL) [135], a proinflammatory cytokine that has been implicated in the promotion of oligodendrocyte death [136]. Nevertheless, a recent study by Kim et al. has indicated that GA therapy in patients with MS induces type 2 monocytes [97].

Is GA neuroprotective?

Recent experiments by Ziemssen et al., showing that GA-reactive T-cells produce BDNF, have led to the proposal that GA may have neurotrophic and/or neuroprotective effects in MS [113]. These findings have generated considerable interest, which is understandable, as neuroprotection is one of the key goals in the treatment of MS. In considering these data, however, it is important to re-

alise that neuroprotective factors such as this are by no means specific to GA-reactive monocytes. Indeed, earlier studies by Kipnis et al. indicate that both GA- and MBP-reactive cells secrete a variety of neurotrophic factors in response to optic nerve injury in rat, while studies of human cells revealed that such factors are secreted by all activated human T-cells, B-cells and monocytes, both *in vitro* and in inflammatory brain lesions [111, 137].

Conclusions

While much has been learnt about the mechanisms of action of DMTs in MS, there are still many gaps in our knowledge. Current data strongly suggest that IFN β has a multi-level immunomodulatory mode of action. Indeed, it appears that IFN β impacts on T-cell activity prior to transmigration across the BBB, in addition to inhibiting the transmigration process and many of the subsequent stages along the MS disease pathways. In contrast, there is considerably less certainty surrounding the mechanism of action of GA. The originally proposed mechanism of cross-reactivity with MBP is now widely questioned, and it appears that any effects of GA may be exerted at a more general level. This would certainly be consistent with the results from pivotal clinical trials of these drugs, in which high-dose, high-frequency IFN β treatment was found to show superior and more consistent efficacy than GA in reducing relapse frequency and slowing disability progression. Nevertheless, it is on the basis of this clinical efficacy, as opposed to mechanism of action, that treatment choices should be made. Although a drug's mechanism of action is likely to impact on its clinical efficacy, mechanism of action studies that are not supported by clinical data are not a reliable basis on which to make treatment decisions.

What do we know about the mechanism of action of disease-modifying treatments in MS? An overview of current clinical opinion

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Despite the advances made in multiple sclerosis (MS)-related research over recent years, much still remains unknown. The promise held out by effective therapies such as interferon (IFN) β and glatiramer acetate (GA) has led to a desire to better understand the mechanism of action of these agents, thus enabling more targeted therapy. A better understanding of the mechanisms of action of both IFN β and GA may lead to improvements in treatment optimisation, determination of appropriate therapy for individual patients and future drug development.

At present, though, our knowledge of mechanisms of action does not always allow clear correlations to be drawn between these mechanisms and the clinical outcomes produced by disease-modifying treatments (DMTs). For GA, in particular, clinical data are far from conclusive in their support of the mechanisms of action identified *in vitro*. It is to be hoped that, as research continues, we will gain a greater insight into the mechanisms of action of MS therapies. Nevertheless, a drug's mechanism of action, even when well defined, does not provide a solid basis on which to make treatment decisions. Mechanisms of action that are effective in the treatment of MS will always be reflected by clinical efficacy data, and it is on the basis of these clinical data that treatment choices should be made.

Such conclusions were strongly supported by current clinical opinion, as shown by the results of keypad voting following presentations based on the information contained within the previous three manuscripts. For example, when asked about the basis on which treatment decisions should be made when selecting a DMT, 94% of participants responded that it should be based on long-term clinical evidence, while only 4% thought that it should be based on mechanism of action, and 2% thought that magnetic resonance imaging should be the determining factor. Similarly, only 5% of participants felt that there was 'direct' or 'convincing' evidence for neuroprotection by any of the currently available DMTs. However, it is interesting to note that 64% of participants thought that the highest priority for basic research in MS should be remyelination, neuroprotection and regeneration, while 23% thought that research should focus on treatment response markers. A breakdown of the responses to these and other pivotal questions can be found in the overall conclusions by Professor Bates and the results of the interactive keypad voting (see pages 83 to 87 and 88 to 89 in this supplement).

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