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Modulating processes within the central nervous system is central to therapeutic control of multiple sclerosis

Abstract Historically considered to be an autoimmune demyelinating disease, multiple sclerosis is now recognized to be characterized by significant axonal and neuronal pathology. Addressing this neurodegenerative component of the disease is an important treatment objective, since axonal injury is believed to underlie the accumulation of disability and disease progression. The precise relationship between the inflammatory and neurodegenerative components in multiple sclerosis remains poorly elucidated, although neurodegeneration appears to be at least partially independent from neuroinflammation. The mechanisms underlying axonal injury appear complex and are likely to be multifactorial. Specific treatment strategies need to be developed that act within the central nervous system to prevent neurodegeneration and need to be provided from the earliest stages of disease. It is likely that immunomodulatory treatments acting purely in the periphery will provide only indirect and not direct neuroprotection. A promising approach is to enhance neuroprotective autoimmunity inside the brain, believed to be mediated, at least in part, by the release of neurotrophic factors within the nervous system from infiltrating immune cells. Such a beneficial process would be inhibited by a non-selective immunosuppressive strategy. In summary, treatments of multiple sclerosis should take into account the heterogeneous pathophysiology of the disease. The pathogenic process in the central nervous system itself should be the major focus in multiple sclerosis therapy in order to protect against demyelination and axonal loss and to promote remyelination and regeneration directly in the target tissue, independently of peripheral immune status. In conclusion, selective treatment strategies aimed at preventing axonal injury within the central nervous system are required to complement existing, peripherally acting treatments targeting the immune system.

Key words multiple sclerosis · neurodegeneration · neuroprotection · inflammation · treatment

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Introduction

Multiple sclerosis is a complex autoimmune disease involving disturbances to the peripheral immune system, although the detailed pathogenic cascade remains unknown. Many different immune cells are believed to be involved in the disease process, including myelin-reactive CD4 + T cells which carry the immune response to the nervous system, CD25 + regulatory T cells, which can control autoreactive CD4 + cells, myelin-reactive B cells, CD8 + killer cells, macrophages and brain microglia [42]. In the central nervous system, these cells infiltrate discrete areas of tissue, where they cause damage to oligodendrocytes (e.g., demyelination) and neurons (e.g., axonal transection), resulting in the formation of a sclerotic plaque. The interactions between these different immune cell populations itself and between immune cells, neurons and glia are highly complex. However, a better understanding of these interactions in the central nervous system itself is necessary for the development of more rational treatment strategies which can modulate these interactions in a specific way and thereby prevent disease activity. Of particular interest is the poten-

JON 5007

tial to prevent the neurodegenerative changes in the nervous system that are thought to be responsible for the accumulation of permanent neurological disability.

Treatment targets in the periphery

To date, treatments for multiple sclerosis have been developed with the intention of intervening at the level of certain autoimmune responses in the periphery. This approach is hampered by limited knowledge of the pathogenic cascade in human multiple sclerosis, which compromises the development of rationally designed immune treatments. Peripherally acting drugs targeting the immune system in multiple sclerosis include immunosuppressants, such as mitoxantrone, which produce a non-specific inhibition of immune cell function, or immunomodulators such as the beta-interferons, glatiramer acetate and natalizumab which target more or less well-defined processes involved in the assumed pathogenic autoimmune response. Unfortunately, since there is no specific immunological abnormality in patients with multiple sclerosis, it is not possible to develop treatments that selectively target these processes. For this reason, a major limitation of such drugs is that the immune processes targeted are more or less non-specific, leading to unwanted effects on immune function such as immunosuppression.

Because of the limited pathogenetic data in multiple sclerosis, nearly all studies of agents targeting the disease have been performed not in the human disease but in animal models. In particular, the experimental autoimmune encephalomyelitis (EAE) model has been used widely to evaluate immunomodulatory treatments for multiple sclerosis as well as to explore the pathophysiology of the disease. This model involves the generation of an autoimmune response in the immunological periphery by immunizing animals with myelin proteins such as myelin basic protein (MBP). In this quite simple model, the disease can also be transferred from affected animals to healthy recipients by adoptive transfer of myelin-reactive T cells. The animals develop a clinical and pathological pattern which is quite different from human multiple sclerosis (spinal cord lesions in EAE) although several histopathological hallmarks of multiple sclerosis, including focal inflammatory lesions in the nervous system can be found.

As more and more scientific data from animal experiments accumulate, it is important to keep in mind that EAE is *not* human multiple sclerosis. In fact, each EAE experiment only represents a small part of the still unknown pathogenetic cascade of autoimmune demyelination. It is perhaps for this reason that divergent results have been observed for a number of treatments assessed in both the EAE model and in clinical trials in multiple sclerosis [39] (Table 1). The most striking example is perhaps that of lenercept, a recombinant TNF receptor p55 immunoglobulin fusion protein. In the EAE model, such TNF receptor fusion proteins ameliorate clinical symptoms [22, 23]. However, a double-blind, randomized placebo-controlled trial in relapsing-remitting multiple sclerosis found that treatment with lenercept was associated with a higher proportion of patients experiencing relapses, a shorter time to first relapse and more severe neurological deficits [36]. Other examples include glatiramer acetate (GA) and natalizumab which provide complete abrogation of the disease process in the EAE model in contrast to their limited clinical benefit in MS patients. For these reasons, all information obtained from the EAE model should be interpreted with caution and within the context of what is known of the overall pathophysiology of multiple sclerosis.

Table 1 Comparison between outcomes of treatment in clinical trials in multiple sclerosis (MS) and in animal models of experimental autoimmune encephalomyelitis (EAE)

Therapy	MS	EAE
IFN-γ, systemic	Worsens	Cures
Anti-TNF-α, systemic	Worsens	Cures
IL-4 transduced T cells	Not tested	Cures
TNF-α transduced T cells	Not tested	Worsens
Glatiramer acetate	Improves	Cures
Beta-interferons	Improves	Improves
Anti-α4 integrin antibodies	Improves	Cures

Developing treatments that target the immune response within the central nervous system may be more promising than peripherally acting treatments. Brain targets have the advantage over peripheral targets in that they directly address the core disease process and will have less non-selective effects on systemic immune function. Interesting potential targets include activation of microglia, antibody-mediated injury to myelin and axons and B cell interactions with oligodendrocyte precursor cells [29]. A drug that appears to down-regulate microglial activation is minocycline [37] and this agent also slows the appearance of EAE and attenuates its severity [6, 31]. Preliminary clinical data in multiple sclerosis indicate that minocycline may reduce lesion activity [26] and these findings merit confirmation in a randomized controlled trial.

The two faces of multiple sclerosis: inflammation and neurodegeneration

It is now clear that the pathophysiology of multiple sclerosis cannot be adequately explained uniquely by acute, focal inflammatory attack inside the central nervous system [21]. Our understanding also needs to take into

account the neurodegenerative processes of axonal loss, which may occur to some extent independently from inflammation, and certainly arise very early in the disease process.

These two facets of the pathology of multiple sclerosis provide an elegant model to explain the heterogeneous clinical presentation and course of the disease [12]. If it has been accepted for many years that the acute relapses observed in relapsing-remitting multiple sclerosis reflect flairs of inflammatory activity, it is now thought that permanent clinical disability is mainly determined by the extent of axonal loss. Once axonal loss has reached a certain critical threshold, irreversible neurological deficits emerge. During the course of the disease, inflammatory events become rarer, whereas neurodegeneration continues or even becomes more prominent (Fig. 1). This picture would account for the transition from a relapsing-remitting to a secondary progressive form of the disease. However, if inflammation and neurodegeneration are to some extent independent, the relative importance of the two processes in individual patients may account for the different patterns of clinical presentation seen between patients and explain the imperfect correlation between exacerbations, inflammatory activity in MRI and accumulation of disability.

The biochemical events underlying the inflammatory and neurodegenerative phases of the disease are not known in detail and may be quite different [33]. Inflammation involves activation of T and B cells in the periphery, crossing the blood brain barrier and homing to the lesion site. In the lesion, T cells are reactivated by myelin antigens, release cytokines that attract macrophages and activate microglia which start to destroy the myelin sheath. Anti-myelin antibodies bind complement, attract macrophages and stimulate opsonization of myelin [42]. Demyelination leads to reversible and to some extent irreversible impairment of function of the axon whose conduction properties are deteriorated, thus accounting for the clinical symptoms associated with relapses.

Neurodegeneration is likely to be a complex process [11, 18], especially when it takes place in inflammatory disorders like multiple sclerosis. To a significant extent, axonal loss seems to be a major consequence of demyelination and inflammation, for example by binding of CD8 + T cells to exposed axons and secretion of toxic factors. However, other mechanisms not directly related to demyelination and inflammation are also likely to be important. An example is excitotoxicity: glutamic acid can bind to excitatory amino acid receptors on the cell bodies, dendrites or axon terminals of neurons and initiate a process of necrotic cell death [34]. Theoretically, there are several possible relationships between inflam-

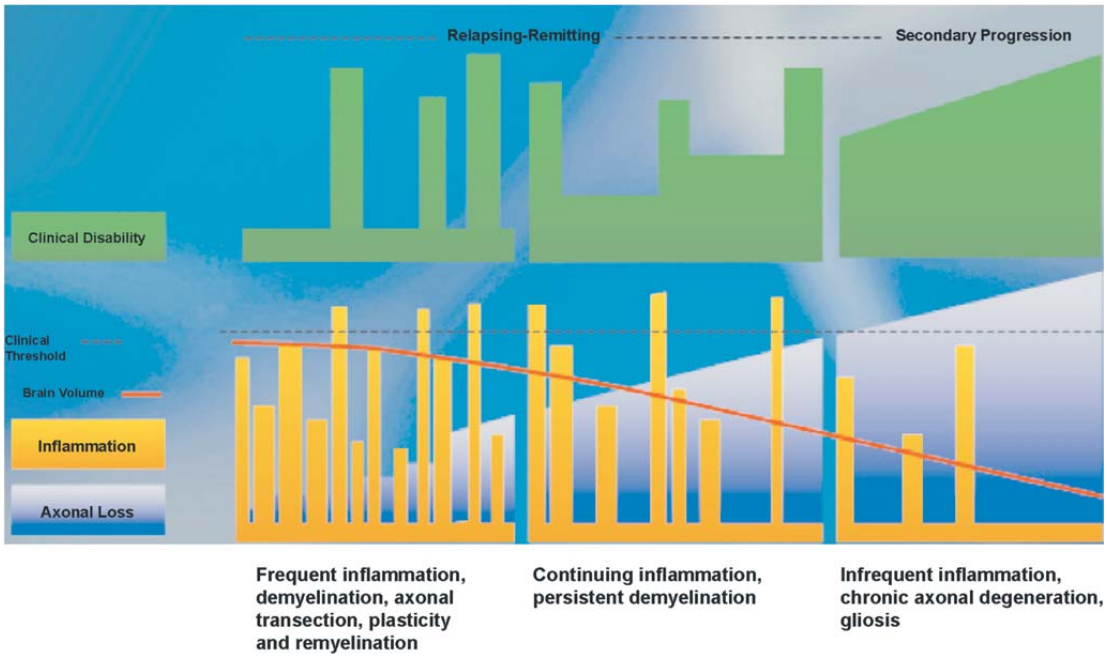

Fig. 1 Different clinical phenotypes of multiple sclerosis and the underlying pathology. Adapted from [13]

mation and neurodegeneration in multiple sclerosis (Fig. 2). Three exclusive hypotheses can be envisaged: (a) that neurodegeneration is entirely secondary to inflammation, (b) that inflammation is entirely secondary to neurodegeneration or (c) that inflammation and neurodegeneration are entirely independent. On the other hand, non-exclusive hypotheses where neurodegeneration is partially dependent and partially independent of inflammation or vice versa can also be put forward, and these seem intuitively more likely.

There are a number of clinical arguments in favor of some independence between the inflammatory and neurodegenerative processes. For example, in a clinical trial of alatuzemab (Campath-1H), a monoclonal antibody directed against CD52 which leads to T cell depletion, in secondary progressive multiple sclerosis, a gradual extinction of exacerbations and lesion activity visible on MRI was demonstrated [12]. However, disability continued to progress in about half the patients in whom progressive brain atrophy and axonal degeneration could be observed using MRI and magnetic resonance spectroscopy (MRS). The investigators concluded, first that inflammation and demyelination were responsible for relapses of multiple sclerosis and could be prevented by alatuzemab treatment and, second that continuing axonal degeneration accounted for the progressive phase of disability. Even though axonal injury may have been conditioned by prior inflammation, this process can continue despite complete suppression of inflammatory activity or it is for example the case in bone narrow transplanted MS patient.

There is also neuropathological evidence for a dissociation between inflammatory demyelination and axonal injury from a series of 42 biopsy samples obtained from patients with multiple sclerosis [3]. Acute axonal injury was visualized by amyloid precursor protein (APP) staining. There was no relationship between the expression of APP or axonal density and the extent of demyelination, with axonal injury being observed even in lesions that were successfully remyelinating. Similarly, there was no association between the extent of axonal injury and markers of acute inflammation such as TNF-α or inducible NO synthase. However, axonal injury was correlated to some extent with the extent of infiltration by CD8 + T lymphocytes and by macrophages. A dissociation between neurodegeneration and inflammatory demyelination is also observed in lesions within the cortex. These lesions are characterized by a significant degree of axonal transection and apoptosis of neuronal cell bodies. However, the extent of infiltration by T lymphocytes and macrophages and the expression of inflammatory markers is low [4, 30].

MRI studies in very early disease also suggest that inflammation and neuronal injury are not strictly related. A study of 31 subjects presenting with a clinically isolated syndrome evaluated inflammatory lesion activity with classical T2- and T1-weighted images after gadolinium enhancement and measured a surrogate marker of axonal injury, the size of the *N*-acetylaspartate peak (NAA) determined in the whole brain [14]. In these patients the mean size of the NAA peak was some 20 % lower than that observed in matched controls. No correlation was observed between the size of the NAA peak and lesion volume on either T1 or T2 images. The investigators concluded that significant axonal injury occurs early in the disease and that this is only indirectly linked to inflammatory activity.

Studies such as these suggest that treatment strategies for multiple sclerosis need to address both the inflammatory and neurodegenerative components of the disease, and that anti-inflammatory therapies may only be able to control the inflammation-related neurodegenerative process adequately [11].

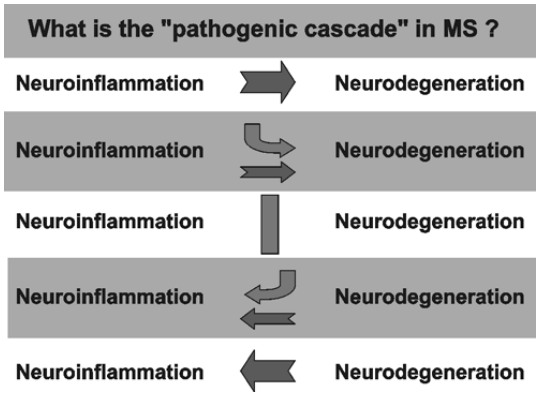

Fig. 2 Possible hypotheses for the causal relationship between inflammation and neurodegeneration in the pathogenesis of multiple sclerosis

The pathogenic process takes place in the brain

Degeneration of oligodendrocytes, neurons and axons are the key pathological features of multiple sclerosis which are responsible for the irreversible neurological handicap that accumulates in the course of disease. For this reason, rational therapies need to target these core disease processes in a more specific and a more effective way than it is possible with only peripherally acting treatments [11]. Nevertheless, the processes underlying axonal damage in multiple sclerosis are extremely complex and certainly multifactorial [16, 18], which has hampered development of neuroprotective treatments in the past. However, recent developments have identified several promising avenues of research for developing such drugs.

Treatments targeting the brain are also more appro-

priate with respect to the phase of the disease at which treatment is initiated. Although the initial trigger of disease in multiple sclerosis is likely to occur in the peripheral immune system, by the time the disease is diagnosed, it is already a localized disease in the central nervous system. The initial symptoms correspond to reactivation of autoimmune cells, their entry into the brain and the triggering of a local demyelinating event. However, at this stage, the disease process is already characterized by primary and secondary axonal and neuronal degeneration and the local environment has become to some extent inhospitable for repair and regeneration of axons and myelin. Due to the built-in redundancy of the nervous system, clinical manifestations are only apparent once a critical degree of neuronal damage has been reached. That is why the earliest stages of the disease are as a consequence clinically silent. This can be visualized by the reduced brain volume and NAA compartments observed by imaging studies performed at the first clinical presentation of disease.

Therapeutic strategies need to take into account this burden of tissue damage inside the brain that is already present when treatment decisions are first being made. Although prevention of future flairs of inflammatory attack on the nervous system are obviously required, it is also important to address existing damage and vulnerability. For example, strategies could be implemented to promote oligodendrocyte survival and repair, to prevent further axonal degeneration and neuronal dysfunction and to promote axonal and neuronal regeneration. Delivery of growth factors to the areas of tissue damage would be an interesting possibility to achieve these objectives.

The neuroprotective side of neuroinflammation

A promising avenue of research for drug development in multiple sclerosis is the concept of protective autoimmunity. Although the traditional view has been that autoimmune responses are exclusively deleterious, especially inside the brain, it now appears clear that autoimmune cells can, under certain conditions, promote neural repair. This was first demonstrated in an animal model of traumatic optic nerve injury [27] in rats. If the rats were injected with activated anti-MBP T cells, they retained three times as many retinal ganglion cells with functionally intact axons than did rats injected with activated T cells specific for other antigens. Since then, this concept has been extended to many other experimental paradigms and appears to be a universal principle for both immune and non-immune degenerative diseases of the central nervous system [32]. For example, in Parkinson's disease, activated microglia is present in the substantia nigra. On the one hand, these cells may contribute to tissue damage by, for example, the release of reactive oxygen species or pro-inflammatory cytokines, but, on the other, they may protect neurons by the release of neurotrophic factors or by removal of excitotoxic glutamic acid from the extracellular *milieu* [35]. Regulation of activated microglia in Parkinson's disease as a potential target for new neuroprotective therapies is an exciting new prospect which is receiving much interest.

A possible explanation of this neuroprotective role of T cells may be the release of neurotrophic factors from immune cells that promote neuronal repair or protect against injury [20] (Fig. 3). In particular, brain-derived neurotrophic factor (BDNF) has been shown to be produced and secreted by a variety of immunocompetent cells [2, 5, 19]. BDNF is a potent neurotrophic factor which can rescue neurons following axonal transection [17]. In autopsy material from multiple sclerosis patients, BDNF is present in T cells and macrophages infiltrating the lesions [33]. BDNF expression is higher in immune cells from active lesions compared to inactive ones, consistent with the observation *in vitro* that expression is up-regulated following activation of T cells [19]. In addition, the BDNF receptor *trk*B appears to be up-regulated in damaged neurons in the immediate vicinity of active lesions [33]. The cellular machinery is therefore in place for BDNF-mediated neuroprotective immunity in multiple sclerosis lesions.

The potential neuroprotective effects of growth factors have been evaluated extensively in the EAE model of human multiple sclerosis. These studies have concerned nerve growth factor (NGF), leukemia inhibitory factor (LIF), insulin-like growth factor-1 (IGF-1) and glial growth factor-2 (GGF-2), but not BDNF. In the majority of studies, administration of growth factors either delayed the onset of disease or reduced the severity of the neurological deficit (Table 2).

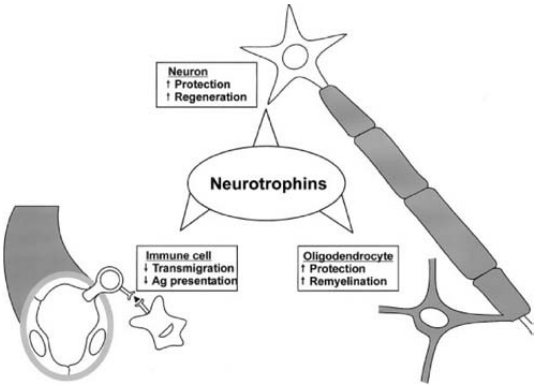

Fig. 3 Potential neuroprotective effects of neurotrophins released from immune cells in inflammatory diseases of the nervous system. Reproduced from [20] with permission

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