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Review article

Inflammation, demyelination, neurodegeneration and neuroprotection in the pathogenesis of multiple sclerosis

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Abstract

Although axonal loss has been observed in demyelinated multiple sclerosis (MS) lesions, there has been a major focus on understanding mechanisms of demyelination. However, identification of markers for axonal damage and development of new imaging techniques has enabled detection of subtle changes in axonal pathology and revived interest in the neurodegenerative component of MS. Axonal loss is generally accepted as the main determinant of permanent clinical disability. However, the role of axonal loss early in disease or during relapsing–remitting disease is still under investigation, as are the interactions and interdependency between inflammation, demyelination, neurodegeneration and neuroprotection in the pathogenesis of MS.
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Keywords: Multiple sclerosis; CNS demyelinating autoimmune disease; Inflammation; Neuron degeneration; Experimental autoimmune encephalomyelitis

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1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS). It is the most common demyelinating disease in

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young adults. Although Charcot noted axonal loss in demyelinated MS lesions over a century ago, the majority of MS research has focused on the process of demyelination with significantly less attention paid to the neurodegenerative component (Bjartmar and Trapp, 2001; Charcot, 1868). There is little doubt that axonal loss is the main determinant of permanent clinical disability. However, the role of axonal loss early in the disease course or during relapsing–remitting (RR) disease is still unclear, as are the interactions and interdependency of inflammation, demyelination and neurodegeneration.

2. Inflammation in MS and its animal models

The CNS has long been considered to be an immunoprivileged site with few if any lymphocytes present in the absence of active or ongoing infection. However, accumulating evidence has demonstrated that a small number of T cells traffic through the CNS surveying for infection or injury and that T cells activated in the periphery can penetrate the blood–brain barrier (BBB) and enter the CNS (Hickey et al., 1991; Wekerle et al., 1987).

Autoreactive T and B cells are normal constituents of the immune system. It has been demonstrated that some of these autoreactive cells can be stimulated with myelin components in healthy individuals, but do not appear to be pathogenic unless tolerance is broken and cells activated (Diaz-Villoslada et al., 1999). Induction of autoimmune responses against myelin components in the CNS is hypothesized to occur through mechanisms such as molecular mimicry, bystander activation and epitope spreading (reviewed in Vanderlugt and Miller, 2002; von Herrath et al., 2003). Once activated, myelin-specific T cells can cross the BBB where they proliferate and secrete pro-inflammatory cytokines which in turn stimulate microglia, macrophages and astrocytes, and recruit B cells, ultimately resulting in damage to myelin, oligodendrocytes and axons (reviewed in Zamvil and Steinman, 2003).

Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS that can be induced using CNS homogenate, myelin proteins or their encephalitogenic peptides in adjuvant (reviewed in Tsunoda and Fujinami, 1996). Myelin-specific $CD4^+$ T cells are considered to be the initiators of disease in both MS and EAE, but clonal expansion of $CD8^+$ T cells has been detected in both MS and EAE lesions (Babbé et al., 2000; Jacobsen et al., 2002). In addition, adoptive transfer of myelin-specific $CD8^+$ T cells can induce an EAE-like disease in recipient animals (Huseby et al., 2001; Sun et al., 2001). Myelin oligodendrocyte glycoprotein (MOG)-induced EAE is characterized by many of the same pathophysiological processes as in MS, including encephalitogenic T cell and demyelinating antibody responses with axonal damage that is quantitatively and qualitatively similar to that seen in MS (Iglesias et al., 2001; Linington et al., 1993; Storch et al., 1998; Tsunoda et al., 2000).

The relative success of immunoregulatory drugs in RR–MS provides support that the immune system plays a role in demyelination and axonal loss. However, although there appears to be a threshold of damage above which immunoregulatory drugs are ineffective which would explain why these agents are not effective for primary progressive (PP) disease (reviewed in Ziemssen, 2005).

3. Neurodegeneration in MS and its animal models

Neurodegeneration, axonal and/or neural damage, has been recognized as a component of MS for more than a century (Charcot, 1868). However, the axon has primarily been considered an innocent bystander in the disease process occurring secondary to inflammation and demyelination rather than as a specific target for immune attack. Early studies employed silver impregnation of sections and electron microscopy to detect axonal degeneration in MS (Suzuki et al., 1969). Recently the use of immunostaining for markers of axonal damage, such as amyloid precursor protein (APP) and nonphosphorylated neurofilament (NF), as well as the development of new imaging techniques, such as magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI), have enabled earlier detection of more subtle changes in axonal pathology (Ferguson et al., 1997; Matthews et al., 1998; Simon, 1999; Trapp et al., 1998). In MS axonal loss could occur through the toxicity of effectors such as glutamate, direct attack by autoreactive antibodies or cytotoxic T cells or secondary to demyelination due to exposure of naked axons (Owens, 2003).

Accumulating evidence suggests that axonal loss occurs early in the course of the disease in MS, EAE and Theiler's murine encephalomyelitis virus (TMEV) infection, but because of compensatory mechanisms within the CNS, it remains clinically silent until a threshold level of axonal loss (15–30% in mice) is achieved and the compensatory resources exhausted (Confavreux et al., 2000; Wujek et al., 2002). Axonal injury was demonstrated to herald or trigger demyelination in TMEV-induced demyelinating disease (Tsunoda et al., 2003). In a rat model of MOG–EAE, axonal loss occurred early in the disease course, and correlated with the number of relapses in animals with RR disease and with permanent disability in animals with progressive or long-term RR disease (Papadopoulos et al., 2006). Studies examining early axonal loss in rats or mice with EAE have reported either no correlation with inflammation or axonal damage concurrent with the appearance of small numbers of parenchymal T cells (Espejo et al., 2005; Hobom et al., 2004; Wang et al., 2005).

4. Inflammation and neurodegeneration in MS and its animal models

Several possibilities exist for the relationship between inflammation and neurodegeneration: (1) that inflammation

induces neurodegeneration; (2) that neurodegeneration causes inflammation; (3) other factors contribute to the development of inflammation and/or neurodegeneration; (4) inflammation and neurodegeneration participate in a cycle or a cascade in which they augment one another; and (5) that inflammation can protect against neurodegeneration. In the context of MS and its animal models these hypotheses are not necessarily mutually exclusive.

4.1. Does inflammation alone induce neurodegeneration?

$CD4^+$ myelin-specific T cells are widely accepted as the initiators of EAE. However, $CD4^+$ T cells are not the dominant T cells found in MS lesions and they have been shown to have either pathogenic or neuroprotective functions depending on the cytokines and neurotrophins they produce or induce as well as the stage of the disease course. Defining the role of the major effector cytokines produced by $CD4^+$ T helper (Th)1 cells, interferon (IFN)-γ and tumor necrosis factor (TNF)-α, is complex in that these cytokines exert different effects in EAE and MS. IFN-γ is protective in rodent models of EAE. However, one controversial clinical trial reported administration of IFN-γ exacerbated disease in MS patients (Panitch et al., 1987). Similarly, overexpression of TNF-α induces demyelination and neurodegeneration and neutralization of TNF-α is protective in EAE, yet neutralization of TNF-α exacerbated disease in MS patients (reviewed in Lassmann and Ransohoff, 2004).

A predominance of $CD8^+$ T cells was detected in active MS lesions by Babbé et al. (2000) and analysis of T cells in the lesions suggested that expansion of the $CD8^+$ T cell repertoire was more antigen driven than the $CD4^+$ T cell repertoire. Oligoclonally expanded $CD8^+$ T cells with a memory phenotype have also been detected in cerebrospinal fluid (CSF) of MS patients (Jacobsen et al., 2002). In addition, $CD8^+$ T cells have been observed to be in direct contact with demyelinated axons in MS lesions, with their vacuoles containing granzyme B oriented toward the axon (Neumann et al., 2002). In the TMEV demyelinating disease model for MS, disease course, demyelination and axonal loss are dependent on virus-specific cytotoxic $CD8^+$ T cells (Rivera-Quiñones et al., 1998).

Antibodies against neuronal components such as tubulin and neurofilament have been detected in some patients with MS (Silber and Sharief, 1999). In addition, antibodies produced from clonally expanded B cells from the CSF of a patient with MS and a patient with clinically isolated syndrome (CIS) suggestive of MS were found to react with axons in acute MS lesions. These antibodies were found to react with axons in a variety of patterns in lesions from patients that had clinically definite MS for an average of 15 years. This suggests that an as of yet unidentified axonal antigen is capable of driving the clonal expansion of axon reactive B cells in the CNS of MS patients and that axonal degeneration is ongoing even in patients with chronic disease (Zhang et al., 2005).

4.2. Role of CNS cells in neurodegeneration and inflammation

Microglia are considered the resident macrophages of the CNS, and share similarities with cells of the monocyte lineage (Ling and Wong, 1993). Microglia become activated in response to changes in the CNS microenvironment, especially those that interfere with neuronal function (reviewed in Kreutzberg, 1996). Upon activation, microglia can proliferate, upregulate major histocompatibility complex (MHC) molecules and secrete cytokines, chemokines, nitric oxide and reactive oxygen species. Activated microglia can become phagocytic, but it is uncertain whether they can function as antigen presenting cells (APCs) *in vivo* (reviewed in Piehl and Lidman, 2001).

Astrocytes produce extracellular matrix molecules that are components of the supporting framework in the CNS. In addition, they maintain ion homeostasis by producing neurotrophic factors and clearing diffusing neurotransmitters (Bezzi and Volterra, 2001; Haydon, 2001). Astrocytes can be activated by inflammatory stimuli to proliferate and migrate toward sites of injury. The astrocytes can then form a tight glial scar around the site of injury to insulate against further damage. Although activated astrocytes express MHC class II molecules *in vitro*, they are not able to function as effective APCs for $CD4^+$ T cells due to the lack of expression of necessary costimulatory molecules (reviewed in Aloisi et al., 2000; Piehl and Lidman, 2001). A role for astrocytes in regulating immune responses in the CNS has been suggested based on their production of transforming growth factor (TGF)-β and their ability to induce apoptosis of T cells (Constam et al., 1992; Gold et al., 1996; Matsumoto et al., 1993). Astrocytes have also been demonstrated to induce a regulatory phenotype in T cells *in vitro*. These regulatory T cells suppressed both mitogen-stimulated proliferation of lymphocytes and CNS-antigen-stimulated proliferation of autoreactive lymphocytes *in vitro*. Intravenous administration of astrocyte-induced regulatory T cells led to a slight delay in disease onset, and a significant decrease in inflammation and disease severity in rats with spinal cord homogenate-induced EAE (Trajkovic et al., 2004).

Neurons are often considered to be passive bystanders in the inflammatory response. However, neurons can regulate the expression of MHC class I and II by surrounding glia in response to electrical activity in the neurons. Neurons can also produce cytokines such as IFN-γ and stimulate apoptosis of T cells (Flügel et al., 2000; Neumann et al., 1997; Olsson et al., 1989, 1994). The role of MHC class I expression by neurons in inflammatory neurodegeneration is highly controversial and not well substantiated. However, expression of MHC class I could target neurons for killing by cytotoxic $CD8^+$ T cells. MHC class I knockout mice chronically infected with TMEV had less axonal loss and preserved neurological function following extensive demyelination (Rivera-Quiñones et al., 1998).

Upregulation of B7.1 and TGF-β1 by neurons has been associated with recovery from EAE in mice (Issazadeh et al.,

1998). Neurons have been demonstrated to stimulate proliferation of activated T cells, but not naïve T cells, *in vitro*. This proliferation was dependent on cell-to-cell contact through interactions between B7 molecules on neurons and CD28 on T cells, and was associated with an increase in T cell receptor (TCR) signaling independent of MHC class II expression. Production of TGF-β1 by neurons stimulated the production of additional TGF-β1 by T cells. Direct neuron-to-T cell contact in the context of high levels of TGF-β1 was shown to stimulate differentiation of disease-causing encephalitogenic T cells into regulatory T cells expressing TGF-β, CD25 and FoxP3 *in vitro*. Green fluorescent protein labeled autoreactive $CD4^+$ T cells transferred into recipient mice also differentiated into regulatory T cells inside the CNS. Injection of both *in vitro*- and *in vivo*-derived regulatory T cells into naïve mice suppressed the induction of EAE by adoptive transfer, suggesting that neurons can modulate the function of autoreactive T cells (Fujinami, 2006; Liu et al., 2006).

4.3. Do other factors cause inflammation and/or neurodegeneration?

Zinc metallothioneins are nonenzymatic proteins that have been found to exert both anti-inflammatory and neuroprotective activity. Zinc metallothioneins have been suggested to have a role in preventing demyelination and neurodegeneration as well as decreasing inflammation during EAE and MS. Increased expression of metallothioneins I and II has been detected in microglia, macrophages and astrocytes in both MS and EAE (Espejo et al., 2001; Lock et al., 2002; Penkowa and Hidalgo, 2000). Treatment of rats with zinc metallothionein II prior to or during EAE significantly decreased the amount of demyelination and axonal loss compared to controls (Penkowa and Hidalgo, 2003).

Phospho-Akt, Bcl-2 and Bax expression was demonstrated to correlate with neuronal cell death in the early stages of MOG-induced EAE. Akt is a member of a family of serine–threonine kinases that promotes survival by negatively regulating apoptosis signaling (Kim et al., 2001). The neuroprotective Akt pathway was down-regulated, and the ratio of anti-apoptotic Bcl-2 to pro-apoptotic Bax favored the pro-apoptotic side prior to and for the first 7 days after the onset of clinical signs. The ratio of Bcl-2 to Bax then shifted toward the anti-apoptotic side, which correlated with apoptosis of retinal ganglion cells (RGCs) in the early stages of MOG-induced EAE in rats. No inflammatory cell infiltration, antibody deposition or demyelination was detected prior to clinical manifestation of EAE. Therefore, the authors concluded that death of RGCs occurs at least in part independently of inflammation (Hobom et al., 2004). The involvement of Bcl-2 was also demonstrated through the induction of MOG–EAE in transgenic mice over-expressing Bcl-2 which resulted in a less severe disease course and reduced axonal damage (Offen et al., 2000).

Influx of extracellular calcium through voltage gated ion channels was shown to be involved in demyelination and neurodegeneration in an adoptive transfer model of EAE. Administration of bepridil and nitrendipine, calcium channel inhibitors that target L-type channels, simultaneously or subsequent to transfer of myelin basic protein (MBP)-specific T cells reduced inflammation and axonal loss, delayed the onset of disease and resulted in decreased neurological disability. However, administration of these calcium channel inhibitors after the onset of inflammation had no effect on disease severity, suggesting that treatment with calcium channel inhibitors is only efficacious very early in the course of the disease (Brand-Schieber and Werner, 2004).

Sodium channels have been implicated in activation of and phagocytosis by macrophages and microglia, inflammation and neurodegeneration. Expression of the sodium channel Na_v16 is upregulated on activated microglia and macrophages in EAE and MS. Microglia from knock-out mice deficient in Na_v16 had attenuated phagocytic function (Craner et al., 2005). Persistent activation of sodium channels has been demonstrated to trigger axonal injury (Craner et al., 2004). In addition, treatment of mice and rats with sodium channel inhibitors reduced inflammation and prevented axonal degeneration in EAE (Bechtold et al., 2004; Craner et al., 2005; Lo et al., 2003).

4.4. Do inflammation and neurodegeneration cause and/or augment one another?

It is typically thought that demyelination precedes axonal loss; however, evidence from our group and others suggests that in some instances axonal loss precedes demyelination (Tsunoda et al., 2003; Tsunoda and Fujinami, 2002). We have proposed a model for the relationship between inflammation, demyelination and neurodegeneration with regard to axonal loss in which damage can be initiated either from the inside–out or the outside–in. In the inside–out model, the axon is injured by viral infection, direct attack by autoreactive T or B cells, and/or glutamate toxicity, etc., which can lead to the spread of axonal damage through Wallerian degeneration or disruption of the cross-talk between oligodendrocytes and axons. Microglia become activated, and the damage can spread to oligodendrocytes either through disruption of cross-talk, viral spread or induction of apoptosis potentially resulting in demyelination. Damaged myelin, oligodendrocytes and axons are then phagocytosed and both viral and neural antigens can be presented to T and B cells triggering an autoimmune response against myelin, axons or oligodendrocytes inducing demyelination which can lead to secondary axonal damage. Thus axonal damage has come full circle and the cycle can begin again. Alternatively, in the outside–in model demyelination occurs first and leads to secondary axonal injury, which can in turn cause demyelination. Therefore a "vicious cycle" of axonal injury and demyelination is proposed which can be triggered by

Table 1
The relationship between inflammation and neurodegeneration in MS

Hypotheses	Experimental examples
(1) Inflammation causes neurodegeneration	Adoptive transfer EAE
(2) Neurodegeneration causes inflammation	TMEV
(3) Inflammation and neurodegeneration participate in a cycle where they augment one another	TMEV $MOG_{35–55}$-induced EAE
(4) Inflammation protects against neurodegeneration	Adoptive transfer or overexpression of MBP-specific T cells in nerve crush injury; natural antibodies in EAE and TMEV

MS – multiple sclerosis; EAE – experimental autoimmune encephalomyelitis; TMEV – Theiler's murine encephalomyelitis virus; MOG – myelin oligodendrocyte glycoprotein; MBP – myelin basic protein.

either axonal injury or demyelination depending on the circumstance (Tsunoda and Fujinami, 2002).

4.5. Can inflammation protect against neurodegeneration?

The term "protective autoimmunity" was originally coined to describe the protective effect of MBP-specific T cells on injured RGCs in a rat optical nerve crush model (Moalem et al., 1999). It was also found that adoptive transfer of the same MBP-specific T cells into rats with spinal cord contusion injuries improved the recovery of hind limb motor activity (Hauben et al., 2000). In addition, overexpression of an MBP-specific T cell receptor in mice subjected to optical nerve crush was found to be neuroprotective (Yoles et al., 2001). T cells specific for MBP have been detected in rats with spinal cord injuries and transfer of these cells into naïve rats induced monophasic EAE (Popovich et al., 1997, 1998). Therefore, the autoimmune response to MBP as well as other myelin antigens is complex and caution should be used in designing therapeutic treatments which induce autoimmunity.

Protective autoimmunity induced by antibodies has also been reported. Passive transfer of anti-spinal cord homogenate serum, anti-MBP serum, MBP-specific antibodies and an oligodendrocyte reactive natural antibody have been reported to promote remyelination in the TMEV model; however, the effect on neurodegeneration was not investigated (Miller et al., 1996; Miller and Rodriguez, 1995; Rodriguez et al., 1987, 1996; Rodriguez and Lennon, 1990). Polyreactive natural antibodies purified from intravenous immunoglobulins (IVIg) have been demonstrated to decrease the inflammatory response as well as disease severity in rats with MBP-induced EAE (Bruley-Rosset et al., 2003). Interestingly, MS patients have been found to have higher levels of natural autoantibodies in their CSF compared to both healthy controls and patients with other neurological diseases (Matsiota et al., 1988). We have produced two $MOG_{92–106}$ reactive natural antibodies from an A.SW mouse with progressive-EAE that also recognize gangliosides GM1, GM3 and GD1b by enzyme-linked immunosorbent assay (ELISA), which suggests that antibodies against myelin may be capable of exerting a more direct effect in neuroprotection or neurodegeneration (Peterson et al., in press). The cause of neurodegeneration is largely unknown. One candidate molecule that could cause neurodegeneration is antibody. In a subtype of MS, Devic's neuromyelitis optica, the pathology is often described as neurodegenerative rather than inflammatory, and antibody plays a critical role (Lucchinetti et al., 2002). Therefore, although particular myelin antibodies may have a beneficial role in neuroprotection, it is generally accepted that myelin antibodies are involved in demyelination and disease pathogenesis in MS. This should be considered in designing therapies involving antibodies specific for myelin antigens.

5. Animal model for investigating the relationship between inflammation and neurodegeneration

Several hypotheses exist to explain the relationship between inflammation and neurodegeneration in MS. One theory is that the pathogenesis of MS occurs in two distinct phases, an initial inflammatory autoimmune phase with a RR disease course followed by a progressive neurodegenerative phase in which axonal loss and permanent neurological disability occur (Steinman, 2001). Another hypothesis is that the different forms of MS represent different types of pathology with RR disease classified as an inflammatory demyelinating disease and PP disease as a neurodegenerative demyelinating disease.

We have developed an experimental animal model in which RR– and PP–EAE can be induced in two strains of mice using a single encephalitogenic peptide from MOG. SJL/J mice sensitized with $MOG_{92–106}$ developed a RR disease course with perivascular cuffing and small demyelinated areas around the cuffs in the brain. Large subpial and perivenular demyelinating lesions accompanied by meningitis, but not cuffing, were detected in the spinal cord of SJL/J mice with RR–EAE. The infiltrates were predominantly mononuclear cells; however, a few neutrophils were also detected in the lesions. In contrast, A.SW mice sensitized with $MOG_{92–106}$ developed a PP disease course with large plaque-like demyelinating lesions in both the brain and spinal cord accompanied by mild meningitis and very few lymphocytic infiltrates or perivascular cuffs. Large numbers of neutrophils and macrophages and immunoglobulin deposition were detected in the areas of demyelination (Tsunoda et al., 2000). Axonal degeneration was detected in both mouse strains during the chronic stage of disease (unpublished data). This model could prove useful in investigating the hypotheses explaining the relationship between inflammation and neurodegeneration in EAE and MS, since different patterns of inflammation, demyelination and axonal loss can be induced using a single encephalitogenic peptide.

6. Conclusions and future directions

Several hypotheses exist to explain the relationship between inflammation and neurodegeneration in MS. Examples exist in which inflammation causes neurodegeneration, neurodegeneration causes inflammation, inflammation and neurodegeneration appear to occur independently of one another and in which inflammation protects against neurodegeneration (Table 1). Further studies need to focus on the relevance of these hypotheses to MS in general, or with respect to different disease courses in MS, as well as whether the hypotheses are mutually exclusive or interdependent.

Differences in whether pathogenesis is initiated by axonal loss or demyelination and in the ratios of inflammation, demyelination, remyelination and neurodegeneration in individual patients could explain the different disease courses observed. Future development of treatments for MS should focus on strategies for ending/breaking the cycle of demyelination and neurodegeneration, and it is likely that successful treatments will include a combination of agents that prevent further demyelination and axonal loss.

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