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The pathology of multiple sclerosis is the result of focal inflammatory demyelination with axonal damage

Abstract Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system manifested morphologically by inflammation, demyelination, axonal loss and gliosis. The inflammatory lesions are characterized by massive infiltration by a heterogeneous population of cellular and soluble mediators of the immune system, including T cells, B cells, macrophages and microglia, as well as a broad range of cytokines, chemokines, antibodies, complement and other toxic substances. The appearance of such lesions is associated with clinical relapses. Recent detailed immunopathological studies of early, acute lesions revealed profound heterogeneity in the patterns of demyelination and the factors of the immune system involved. During remission, resolution of inflammation is the main factor which leads to clinical improvement of patients. However, the immune system can play a beneficial role at this stage, promoting remyelination perhaps by production of growth factors such as BDNF. In contrast, the progressive irreversible neurological deficit in multiple sclerosis is associated with neurodegenerative processes resulting in axonal and neuronal loss. The mechanisms behind damage to axons in multiple sclerosis lesions are poorly understood. However, the close proximity of areas with prominent axonal loss and areas containing inflammatory infiltrates (*e.g.*, T cells, macrophages) suggest that axonal damage is closely associated with inflammation. Different soluble or cellular mediators of the immune response have been shown to damage axons in experimental systems, and these may be responsible for neurodegeneration in human disease.

Key words multiple sclerosis · neurodegeneration · inflammation · pathophysiology · histopathology

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Introduction

Inflammatory and neurodegenerative diseases of the nervous system differ in several important ways, including their clinical manifestation, clinical course, expression of biochemical and imaging markers of disease activity, neuropathological characteristics and treatment responses. In these respects, multiple sclerosis presents many of the hallmarks of an inflammatory disease. Its natural history is characterized, at least in the earlier stages, by relapses which are believed to be the clinical manifestation of inflammatory reactions due to episodic autoimmune attacks. On the other hand, the gradual increase in disability seen in primary and secondary progressive forms of the disease is more typical of what would be expected for the clinical course of a degenerative disease.

From a therapeutic standpoint, immunomodulatory and immunosuppressant drugs can partially ameliorate disease outcome in relapsing-remitting disease, reducing the frequency of relapses and perhaps attenuating the rate of accumulation of disability. Again, this is compatible with an inflammatory disease process. For the moment, no neuroprotective drug has been shown to be of benefit in multiple sclerosis, although this may not be surprising given the extreme paucity of drugs that have demonstrated unequivocal neuroprotective effects in classical neurodegenerative diseases.

Studies of surrogate markers of disease activity in

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multiple sclerosis reveal the presence of oligoclonal bands in cerebrospinal fluid, again consistent with activation of the immune system. There are no validated surrogate biochemical markers for neurodegeneration in multiple sclerosis. Some preliminary studies have suggested that the presence of tau protein in the CSF may be a candidate marker for axonal damage. Magnetic resonance (MR) images of brains of patients with multiple sclerosis are characterized by the presence of focal gadolinium-enhancing lesions, indicative of localized disruption of the blood-brain barrier. This would be coherent with local targeted attack of the nervous system by certain immune cells. On the other hand, other MR metrics provide evidence for a more diffuse process of neuronal damage, more compatible with a neurodegenerative process. An example is the accelerated rate of cerebral atrophy observed in certain longitudinal studies.

The focal nature of tissue damage has been recognized in neuropathological studies since the early nineteenth century. Indeed, the presence of multiple discrete sclerotic plaques in white matter of the brain and spinal cord inspired the name of multiple sclerosis for the disease. The presence of axonal damage in these plaques has also been noted from the first decades of the twentieth century, when appropriate methods became available to identify axons in histological samples by the use of silver impregnation methods.

Focal lesions such as these are characteristic of many other neuromuscular autoimmune diseases, including vasculitis, polymyositis and chronic inflammatory demyelinating polyneuropathy. On the other hand, myelin loss in non-autoimmune diseases of the nervous system, either idiopathic neurodegenerative diseases or genetic or infectious diseases, is characterized by diffuse white matter lesions. Examples include Binswanger's disease, leucodystrophies, HIV-leucoencephalopathy or progressive multifocal leucoecephalopathy. One exception from this pattern is central pontine myelinolysis, a demyelinating condition often associated with chronic alcoholism and disturbances of sodium balance, in which well-delineated areas of focal white matter loss can be observed in the pons.

Demyelination as a central feature of multiple sclerosis

The characteristic pathological feature of the sclerotic MS plaque is demyelination. Both the myelin sheath and the oligodendrocyte itself are destroyed within lesions, following attack by cells of the immune system that react with myelin-related epitopes, such as myelin basic protein. Immune attack involves both cellular immunity, with T cells directed at myelin and oligodendrocytes and inciting phagocytosis by macrophages, and humoral immunity, with the secretion of anti-myelin antibodies from B cells and subsequent fixation of complement and opsonization of the myelin sheath and the oligodendrocyte by macrophages.

Multiple, well delineated, focal lesions where demyelination has taken place can be observed in the white matter of brains from multiple sclerosis patients, and these contain a high density of immunohistochemical markers for various immune cells, including different populations of T cells, B cells, macrophages and microglia (Fig. 1). In addition, cytokines and chemokines released from these immune cells can be detected in these lesions. These immune cells are thought to have infiltrated the sclerotic plaque during the inflammatory episode that leads to demyelination.

Demyelinated plaques can also be observed in the cortical and subcortical grey matter. These cortical lesions are hard to identify by conventional MRI. Pathological examination using conventional myelin stains such as luxol fast blue (LFB) does not allow identification of these cortical lesions, and immunohistochemical staining is necessary to delineate these areas. An important difference between plaques in white matter and in cortex, albeit a relative one, is the extent of infiltration of the plaques by immune cells. It was thought for many years that inflammatory markers were absent from cortical plaques, but our own recent data (unpublished) suggest that T cell infiltrates are present in early biopsy-derived cortical lesions. Several reasons may account for the difference in the extent of inflammatory infiltration between white and grey matter lesions. The amount of myelin to be removed is much smaller in the cortex than in the white matter and therefore possibly less inflammation is required to induce demyelination in the grey matter. In the marmoset model of experimental autoimmune encephalomyelitis (EAE), there is clear evidence for the appearance of lesions in cortical grey matter early on in the disease process, with three out of four an-

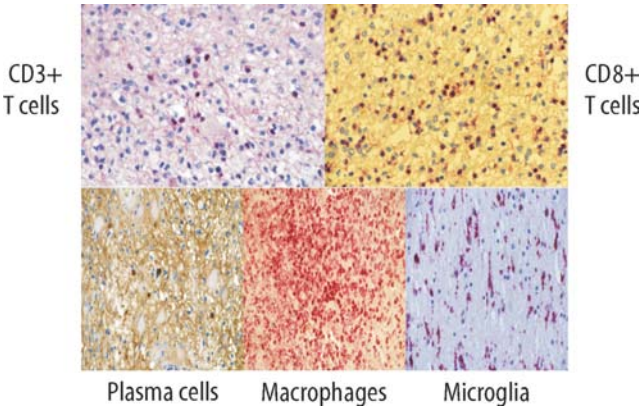

Fig. 1 Immunohistochemical markers for different populations of immune cells in a demyelinated lesion in the white matter from a patient with multiple sclerosis

imals presenting signs of cortical demyelination following inoculation. These lesions are infiltrated with CD3 + T lymphocytes, although to a lesser extent than are lesions in periventricular white matter (Fig. 2).

Mechanisms of demyelination

There are many possible biochemical and cellular mechanisms whereby activated immune cells may destroy myelin and oligodendrocytes (Table 1). Direct binding of T cells to myelin epitopes can lead to activation of macrophages and a subsequent attack of the myelin sheath leading to its phagocytosis. Similarly, release of cytotoxic cytokines or soluble toxic mediators such as nitric oxide from T cells or microglia/macrophages can lead to destruction of myelin and myelin-producing cells. Antibodies directed against myelin epitopes released from infiltrating B cells in the inflammatory lesion can bind to myelin, initiating fixation of complement, binding of macrophages, opsonization and phagocytosis of myelin and oligodendrocytes. In addition, release of cytotoxic mediators from immune or glial cells can create further tissue damage. Such materials include free radicals, which can cause oxidative stress, and glutamic acid, which can cause excitotoxicity. Moreover, initial nonfatal damage to oligodendrocytes may initiate activation of an apoptotic cascade, perhaps by activation of death ligands or receptors such as Fas, FasL or Trail, that will result in delayed oligodendrocyte death. These apoptotic proteins can be activated by tumor necrosis factor, released from pro-inflammatory T cells in multiple sclerosis lesions, and have been demonstrated to promote oligodendrocyte cell death *in vitro* [5, 13]. Blockade of these proteins has been demonstrated to protect against experimental autoimmune encephalitis in animals [1, 20]. Finally, injury to the myelin sheath may render oligodendrocytes vulnerable to environmental toxins or viruses.

On the basis of patterns of staining for different immunological and cytological markers of cell injury and death, we have recently suggested that four different patterns of oligodendrocyte cell death can be distinguished in early, acute MS plaques recovered from multiple sclerosis patients (Table 2) [12]. These are T cell-mediated autoimmune demyelination, B cell (antibody)-mediated autoimmune demyelination, distal oligodendrogliopathy and apoptosis, and primary oligodendrocyte degeneration. Type II (B cell-mediated) pathology was the most frequently seen, in 53 % of cases. These four different processes may occur to a different extent in different phases of disease; they are homogenous within a given patient but heterogeneous in different patient groups. In summary, these data suggest that the factors involved in the underlying autoimmune attack in multiple sclerosis are heterogeneous.

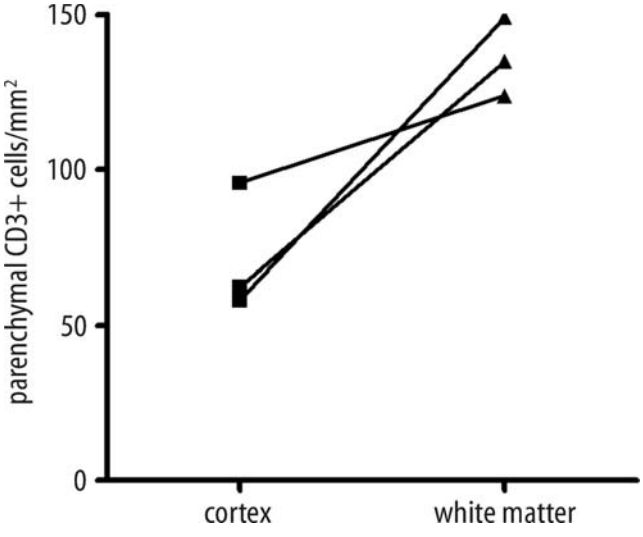

Fig. 2 Presence of CD3 + T lymphocytes in lesions from the cortex and white matter from marmosets with experimental autoimmune encephalomyelitis

Table 1 Mechanisms of demyelination in multiple sclerosis

Primary immune mechanisms	Secondary mechanisms
T cells	Excitotoxicity
Cytokines (e.g., TNF)	Free radicals
Nitric oxide	Death ligands and receptors (e.g., Fas, FasL, Trail)
Antibodies	Toxins
Complement	Viruses

Remyelination

Following acute inflammatory episodes, the resolution of the inflammatory process is probably an important factor contributing to the neurological recovery often observed following clinical relapses. However, in addition, remyelination can occur to a significant extent in lesion areas, and this probably also contributes to functional recovery. It has been estimated that up to 40 % of sclerotic plaques show signs of remyelination [2], and

Table 2 Patterns of oligodendrocyte damage. From [12]

Pattern	Phenotype	Mechanism
I	Autoimmune demyelination	T cell and macrophage mediated
II	Autoimmune demyelination	Antibody and complement mediated
III	Oligodendrocyte dystrophy	Distal oligodendrogliopathy and apoptosis. Indirect?
IV	Oligodendrocyte dystrophy	Primary oligodendrocyte degeneration. Indirect?

these correspond to the shadow plaques observed in histopathology studies. However, remyelination is generally incomplete and is characterized by thinner myelin sheaths and shorter internodal lengths than in the original myelin. For this reason, the original conduction properties of the corresponding axons may not be entirely restored.

Paradoxically, it appears that this remyelination is, at least in part, also due to the activity of immune cells infiltrating the lesion. As well as pro-inflammatory T cells and macrophages, the immune cell population also contains cells recognizing myelin epitopes that have an anti-inflammatory activity, notably T cells with a Th2 phenotype. These cells can release anti-inflammatory cytokines such as interleukin 4 and neurotrophic factors such as BDNF [3, 7]. As well as attenuating the activity of phagocytic cells such as macrophages, these agents can also actively promote remyelination. Recent histopathological studies have produced clear evidence for the production of BDNF by immune cells within multiple sclerosis lesions [27].

Axonal damage

In addition to damage to myelin and oligodendrocytes, axonal loss and injury are also characteristic features of sclerotic plaques. Axonal loss is determined by counting processes in histological samples. Injury can be detected by the accumulation of amyloid precursor protein (APP) in swellings where axonal transport has been interrupted or the axon physically sectioned. APP accumulation is a transient phenomenon and staining for this protein can thus be taken as an index of recent axonal damage. In end-state multiple sclerosis, up to 60 % of the axons present in sclerotic plaques may have disappeared [11, 16]. This proportion of axonal loss in clinically silent 'burnt-out' plaques appears to be independent of the extent of remyelination and the number of residual oligodendrocytes [16]. Axonal loss is also observed in normally appearing white matter adjacent to 'burnt-out' lesions in the cervical cord [11]. However, axonal damage is an early event in lesion formation [4, 6, 9, 28]. In fact, APP accumulation in axons is most prominent in early disease and is correlated with the extent of infiltration of T cells and macrophages into the lesions (Fig. 3) [10]. The extent of acute axonal damage is more pronounced in lesions in which active demyelination is proceeding, compared to inactive demyelinated lesions, consistent with a causal relationship between inflammation and axonal injury (Fig. 4) [10]. However, neuroaxonal damage can also be demonstrated in cortical plaques where infiltration of immune cells is less pronounced [21].

Mechanisms of neuroaxonal damage

As with loss of myelin, neuroaxonal damage is likely to be a multifactorial process. A number of cellular and humoral mediators of the immune response have been shown to be capable of damaging axons, including T cells, macrophages, antibodies, nitric oxide, glutamate and matrix metalloproteases. Immunocytochemical

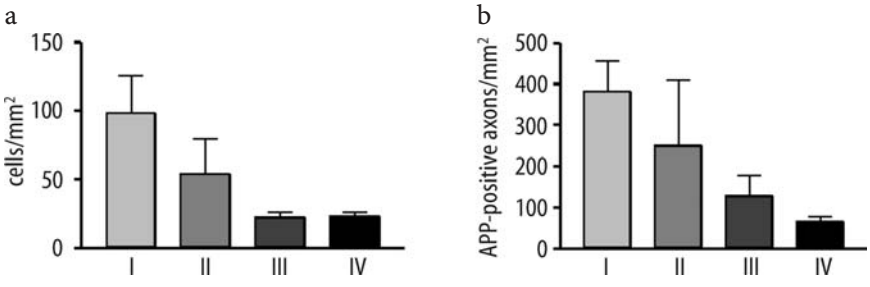

Fig. 3 Presence of macrophages (**a**) and amyloid precursor protein (APP) staining axons (**b**) in lesions from multiple sclerosis patients as a function of disease duration. I less than one year; II one to five years; III five to ten years; IV over ten years. Data are taken from [10], with permission

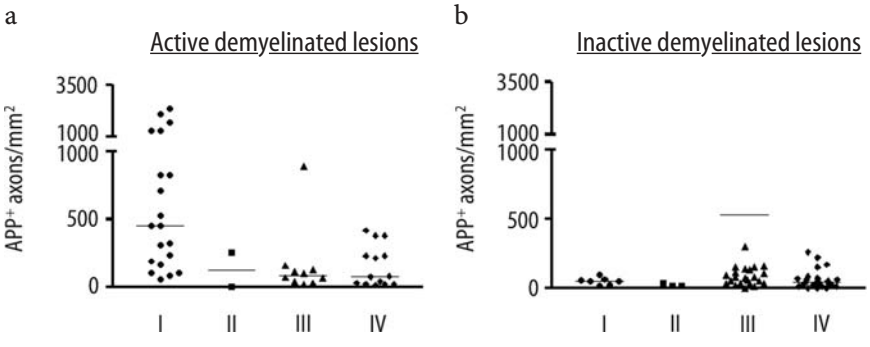

Fig. 4 Presence of amyloid precursor protein (APP) staining axons in lesions from multiple sclerosis patients as a function of disease duration in active demyelinating (**a**) and inactive demyelinated lesions (**b**). I less than one year; II one to five years; III five to ten years; IV over ten years. Data are taken from [10], with permission

studies have demonstrated T cells and macrophages in close apposition to damaged axons in multiple sclerosis lesions (Fig. 5) [18]. Moreover, when neurons and T cells are cultivated together *in vitro*, fixation of cytotoxic CD8 + T cells to axon processes followed by transection of the axon has been observed [18]. Binding of T lymphocytes to neurites may be facilitated by an increase in major histocompatibility complex proteins in neurons and their processes under the influence of interferon-γ from T cells infiltrating the lesion [19]. These observations would be consistent with the hypothesis that release of toxic soluble mediators from these immune cells may be instrumental in damaging the axons.

Among the candidates released from CD8 + T cells is perforin [23]. This cytokine has been implicated in T lymphocyte-mediated cytolysis in a variety of systems [29] and its secretion is up-regulated in active multiple sclerosis lesions [25]. Alternatively, activation of an apoptotic Fas-mediated cell death program following binding of cytotoxic T lymphocytes has been suggested [15]. In the Theiler's virus encephalopathy model of multiple sclerosis, suppression of perforin gene expression protects mice against neuronal loss and neurological impairment, whereas demyelination is unaffected [17].

Activated macrophages release nitric oxide and glutamic acid. Nitric oxide has been demonstrated to cause reversible conduction block in demyelinated axons [24]. Exposure to concentrations of nitric oxide likely to be generated by macrophages in multiple sclerosis lesions leads to Wallerian degeneration of electrically active axons and to damage to axonal membranes leading to rupture [26]. Formation of myelin ovoids and axon rupture was observed in more than 95 % of fibers in rat dorsal roots exposed to nitric oxide.

A role for glutamic acid-mediated excitotoxicity is also suggested by the observation that treatment of mice presenting an experimental autoimmune encephalitis with the excitatory amino acid receptor antagonist NBQX results in considerable and significant rescue of axons [22]. Axonal sparing was accompanied by increased oligodendrocyte survival and an improvement of the clinical state. However, markers of inflammatory activity were not affected by treatment with NBQX.

Finally, antibodies against axonal epitopes released from B cells may also be involved. In the rat experimental autoimmune encephalitis model, it has been demonstrated that depletion of the C6 complement protein prevented axonal damage as well as demyelination, in spite of normal levels of T cell infiltration into the nervous system [14].

Conclusions

The plaques that are characteristic of multiple sclerosis correspond to areas of focal demyelination that are infiltrated by a heterogeneous population of cellular and soluble mediators of the immune system including T cells, B cells, macrophages and microglia, as well as a broad range of cytokines, chemokines, antibodies, complement and other toxic substances. Demyelination is the principal feature of these lesions and is induced by or associated with mediators of the immune system present within the lesions, which cause acute damage to myelin, oligodendrocytes and axons. In multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis, massive infiltration of the brain or spinal cord with the these components of the immune system and subsequent local tissue damage have been demonstrated to be responsible for clinical relapses. Re-

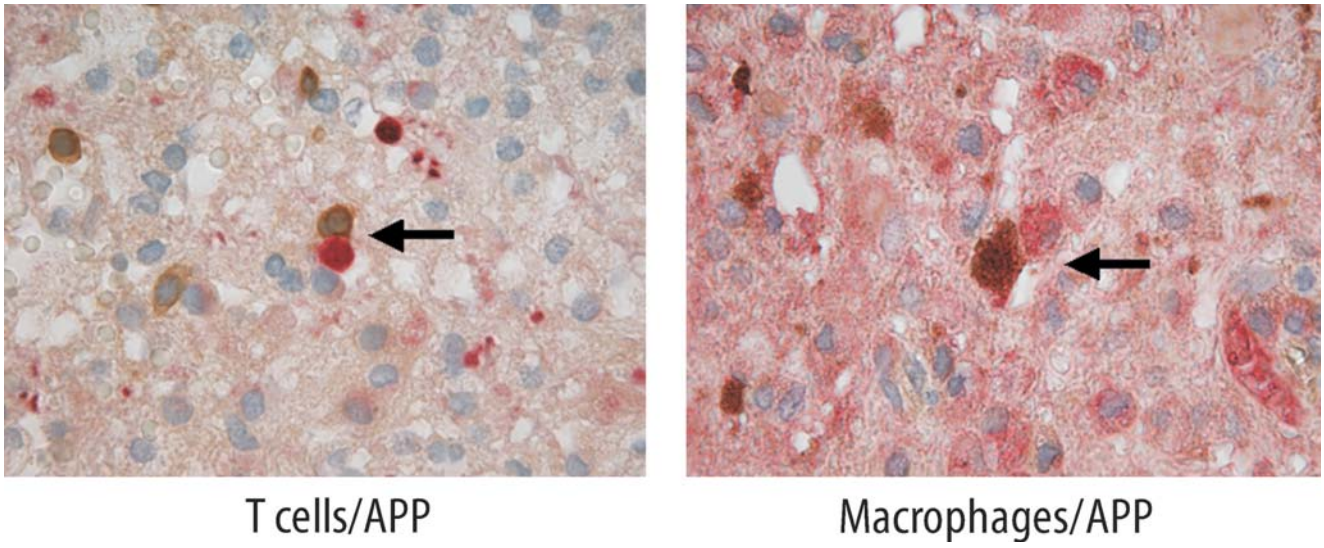

Fig. 5 Presence of T cells (**a**) and macrophages (**b**) in association with amyloid precursor protein (APP) staining axons in lesions from multiple sclerosis patients

cent detailed immunopathological studies of early, acute lesions have revealed marked heterogeneity in the patterns of demyelination and the factors of the immune system involved, suggesting that different immune mechanisms may predominate in different patients or in different stages of the disease process. Areas of active inflammation are also characterized by significant loss of axons traversing the lesions of which inflammation appears to be the major cause. The inflammatory response entails production of soluble mediators that are potentially capable of damaging axons, to which the loss of the myelin sheath renders them vulnerable. This appears to be a plausible mechanism to account for the neurodegenerative changes that are observed in multiple sclerosis and that are thought to underlie progression of disability. Therapeutic modulation of the immune response, for example by stimulation of neurotrophic factor production from T cells, may be of benefit in protecting axons and thus slowing accrual of disability [8].

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