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Inflammatory demyelination is not central to the pathogenesis of multiple sclerosis

Abstract Multiple sclerosis is a disease of the central nervous system that destroys myelin, oligodendrocytes, neurons and axons. Historically considered to be caused by an autoimmune process mainly affecting myelin and oligodendrocytes in the white matter, recent data provide evidence that a generalized, diffuse neurodegenerative process plays an important role in the pathogenesis of MS. There is a high density of axonal transections in active demyelinating lesions, but also persistent low-level axonal damage in inactive plaques and diffuse axonal and neuronal loss throughout the nervous system. Initial axonal injury appears to be closely related to inflammation, but is not restricted to the lesions themselves. Damage may be propagated throughout the nervous system by anterograde Wallerian, retrograde or transynaptic degeneration. Cumulative tissue loss in the grey and white matter, especially of axons, is important and probably the principal determinant of accumulation of irreversible neurological disability and of conversion to a progressive disease course.

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

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Introduction

Multiple sclerosis has a very diverse clinical presentation and course. However, two underlying disease processes can be identified, whose interplay generates the different clinical phenotypes observed: acute relapses and progression of disability. Neurological symptoms appear abruptly during acute relapses and then remit completely or partially. Progression of disability, in contrast, is a gradual and irreversible process. In individual patients, the relative contribution of acute relapses as well as disability progression varies considerably and changes with time. The most typical course is characterized by initial presentation as a series of acute relapses whose frequency diminishes with time in parallel to a gradual accumulation of irreversible disability (Fig. 1). The underlying structural damage corresponding to the two disease processes that can be identified on magnetic resonance imaging (MRI) is also different. Acute relapses are associated with recent lesion activity visible on T1 images as gadolinium enhancement, whereas disability progression is associated with brain atrophy. The fact that the processes of acute relapse and disability accumulation are dissociated in time, between patients, and in their MRI correlates suggests that the underlying pathophysiology of the two may be different. Although there is sound evidence that acute relapses are associated with

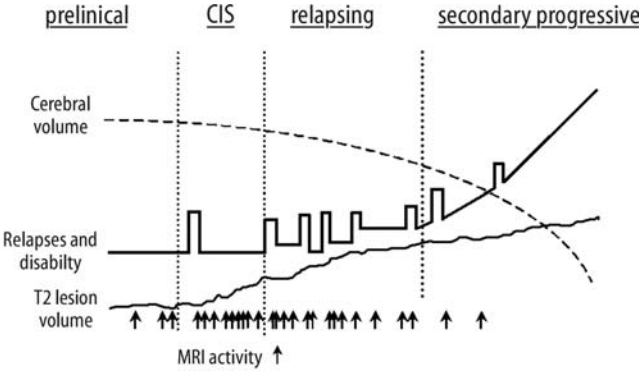

Fig. 1 Typical clinical course of multiple sclerosis and its pathological correlates observable with magnetic resonance imaging

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invasion of the nervous system by autoimmune T cells and focal inflammatory activity, it is not clear whether a primary inflammatory mechanism underlies disability progression, where irreversible neurodegeneration has also been suggested to play a role. This review presents the arguments for an independent neurodegenerative process in the pathophysiology of multiple sclerosis.

Axonal damage in the MS plaque

Axonal damage was noted in the initial description of the pathology of multiple sclerosis by Charcot, and visualized in the early years of the twentieth century by Doinikow in Poland following the development of specific histological stains. In spite of these observations, multiple sclerosis was considered historically as a demyelinating disease in which axonal damage plays a limited role, if any. However, recent studies have challenged this position. The development of immunostaining for amyloid precursor protein (APP) as a marker for recent acute axonal damage was a critical advance which allowed the demonstration of an active process of axonal injury in acute lesions [8]. This study suggested that axonal injury is an integral and early pathological feature of multiple sclerosis lesions, potentially associated with inflammation.

A subsequent study [21] showed that axonal transection was an ubiquitous, specific and extensive feature of demyelinating lesions (Fig. 2). The density of transected axons was over 10,000 per mm^3 in the center of active inflammatory lesions. In chronic inactive lesions, ongoing axonal damage was less intense, but still considerably higher than the level of axonal damage observed in white matter from neurologically healthy controls.

Although acute axonal damage is lower in inactive than in active lesions, it can still be measured in inactive lesions from patients with chronic (> 10 years) relapsing-remitting disease [13]. Since axonal transection is irreversible, low-level axonal injury persisting for many years may lead to considerable loss of axons in these lesions, in spite of the absence of continued acute inflammatory activity. In addition, the extent of acute axonal injury is greater in lesions isolated from patients with secondary progressive multiple sclerosis compared to those from patients with relapsing-remitting disease [13]. This may thus provide a plausible mechanism for the irreversible accumulation of disability in the absence of inflammatory activity seen in progressive forms of the disease.

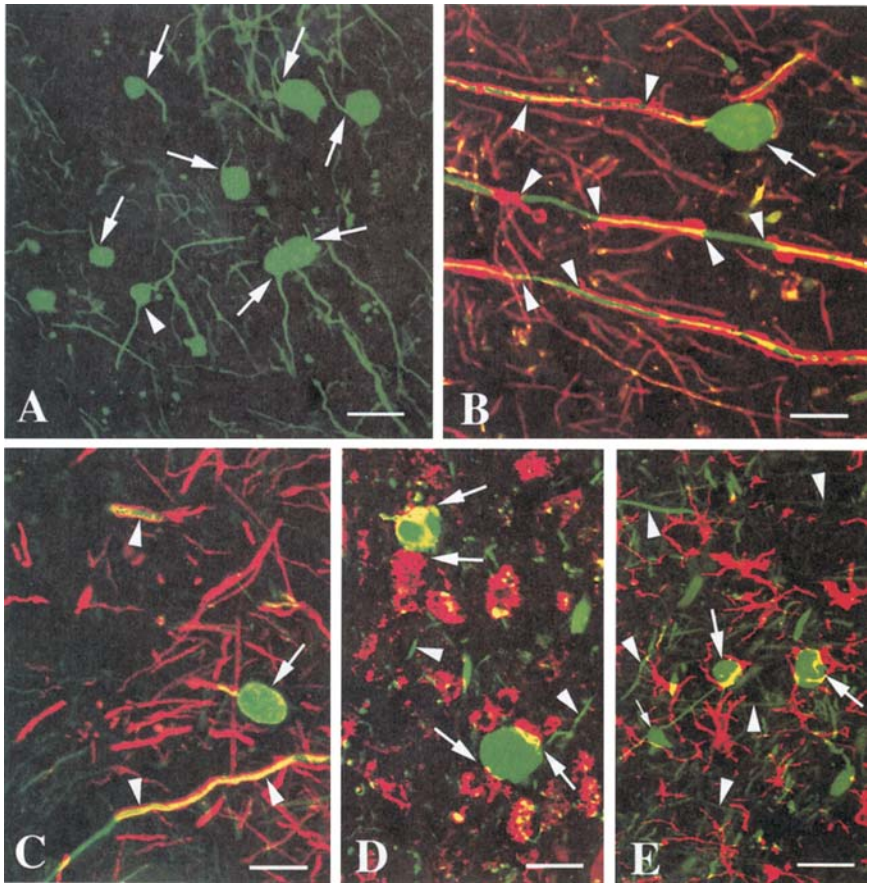

Fig. 2 Axonal injury in the centre of multiple scerosis lesions visualised by confocal microscopy. **A** Terminal axonal ovoids with single axonal connections staining for dephosphorylated neurofilaments (green stain). **B** Three large axons staining for dephosphorylated neurofilaments (green) undergoing active demyelination (arrowheads). One axon ends in a large terminal ovoid. Myelin is stained red in this image. In the centre of a associated with demyelination (red). Panel **C** Axons (green) terminating at the ends of normal-appearing myelin internodes (arrow), and many axons that express nonphosphorylated neurofilaments surrounded by normal-appearing myelin (arrowheads). In Panels **D** and **E**, macrophages (red in Panel **D**) and microglia (red in Panel **E**) surrounded and engulf terminal axonal swellings (large arrows) but have no consistent association with normal-appearing axons (arrowheads) or swellings in nontransected axons (Panel **E**, small arrow). The scale bar in Panel A represents 64 µm and in Panels **B**, **C**, **D**, and **E** 45 µm. Reproduced from [21] with permission

Axonal degeneration in normally appearing white matter

A study of the spinal cord and brainstem of a patient with early relapsing-remitting disease revealed extensive axonal degeneration in normally myelinated spinal cord, with up to 22 % of the axons lost in the cervical spinal cord [2]. Numerous myelin sheaths without axonal content were observed as well as signs of initial myelin degradation in intact myelinated axons by activated macrophages (Fig. 3). As these findings were restricted to descending fibers, with a normal axonal complement in the ascending fibers, it was suggested that axonal loss in normally appearing white matter represents anterograde Wallerian degeneration of axons transected in lesions elsewhere in the nervous system. In addition, retrograde axonal degeneration results in apoptotic death of the neuronal cell body, whereas loss of trophic factor support from degenerated axonal terminals may lead to transynaptic secondary neuronal loss. By such mechanisms, diffuse axonal and neuronal loss can develop well beyond the focal inflammatory lesions that characterize white matter pathology in multiple sclerosis.

The idea that tissue damage can develop outside inflammatory foci is also supported by a recent study of a population of seven patients with relapsing-remitting multiple sclerosis who died during or shortly after the onset of a relapse [1]. In these subjects, foci of extensive oligodendrocyte apoptosis and microglial activation were observed in white matter which contained only few or no lymphocytes or myelin phagocytes. Although several mechanisms of oligodendrocyte death have been described, including apoptosis [17], this is the first description of apoptotic cell death in the absence of infiltration by immune cells that has been described in early disease.

Axonal damage in non-inflammatory demyelination

These studies showing continued acute axonal injury in the absence of inflammation suggest that the two processes are independent of each other. This would be consistent with observations in various animal models of non-inflammatory demyelination in which axonal

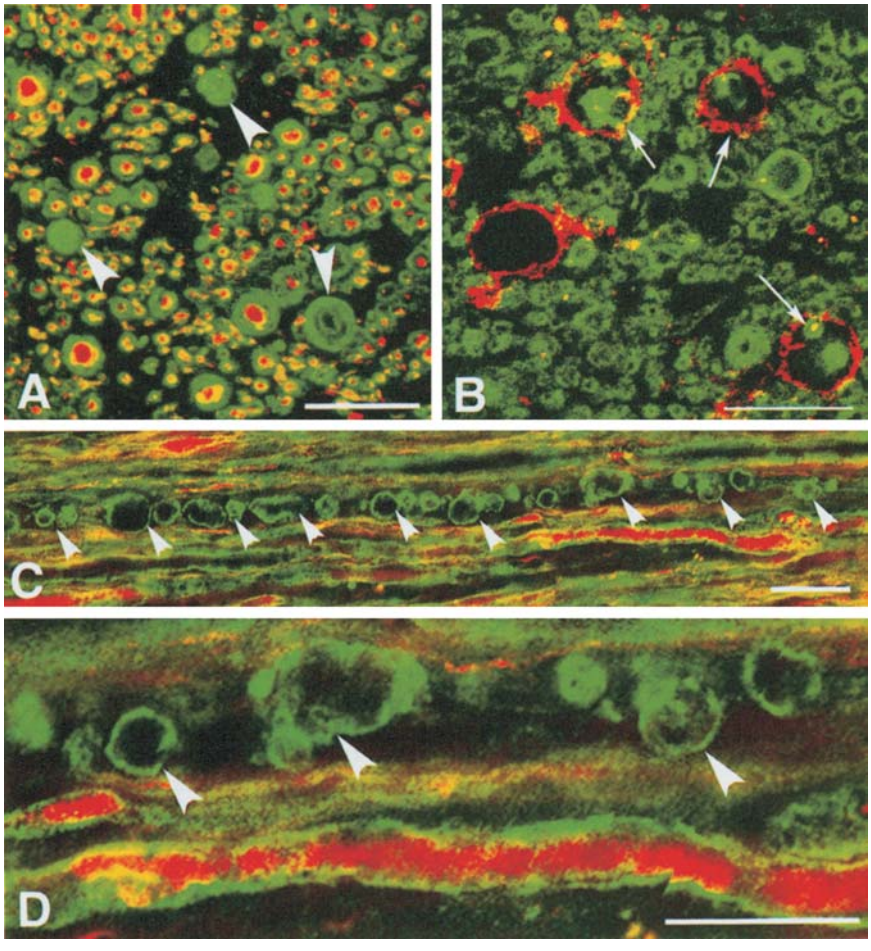

Fig. 3 Confocal images of the ventral cervical column stained for myelin (green), neurofilaments (**A**, **C** and **D**; red) or the activated microglia/macrophage marker major histocompatibility complex (MHC) class II (B, red). In cross-sections **A**, numerous myelin profiles lacking axons (arrowheads) were detected among intact myelinated axons. Some appeared as "empty" myelin circumferences (lower right profile), while others were composed of collapsed myelin membranes. Signs of primary demyelination were not detected. Activated class II cells (**B** red; arrows) often contained phagocytosed myelin (green). Overt stripping of myelin from internodes was not observed in this region. In longitudinal sections **C**, fibers undergoing degeneration appeared as rows of myelin ovoids (green; arrows) between intact myelinated axons. At higher magnification **D**, the ovoids contained disrupted myelin membranes and lacked axonal staining (red). All images represent projections of three to five contiguous confocal slices. Scale bars 25 µm. Reproduced from [2] with permission

damage has been observed. For example, in mice in which the expression of the myelin proteolipid PLP has been suppressed, the integrity of the myelin sheath is disrupted. In these mice, extensive axonal swelling and degeneration is observed [9]. Similar pathology has been observed in mice with an additional suppression of myelin-associated glycoprotein [22]. It has also been demonstrated in the *shiverer* mouse that correct myelin formation is important for the integrity of the axonal cytoskeleton [5]. One interpretation of these studies would be that axonal loss is a consequence of demyelination, which could explain the extensive neurodegeneration seen in lesions in human multiple sclerosis. However, axonal loss is also extensive in the spinal cord and corpus callosum of mice deficient in glial 2′,3′-cyclic nucleotide phosphodiesterase (CNP-1) [16]. Oligodendrocytes from these mice normally generate myelin but their metabolic function is disturbed. This might suggest that it is disturbed trophic support from oligodendrocytes rather than demyelination *per se* that is responsible for axonal degeneration.

Cortical involvement in multiple sclerosis

Although demyelinating lesions in white matter represent the most striking pathology in multiple sclerosis, the presence of lesions in the grey matter in general and in the cortex in particular has been apparent for a long time. A seminal histopathological study by Brownell and Hughes [6] found that 26 % of all lesions in the cerebral hemispheres were located outside the white matter, 5 % being localized within the cortex and a further 17 % straddling the leucocortical boundary. A later study by Lumsden [18] found cortical lesions to be present in 56 out of 60 cases examined (93 %). More recently, Kidd et al. [12] compared the sensitivity of MRI using gadolinium enhancement and histopathology to detect cortical lesions. Although it was possible to detect cortical lesions on MRI, this technique considerably underestimated the number of lesions identified histopathologically. Intracortical lesions accounted for 24 % of lesions observed in this study. Microscopically, these cortical lesions are characterized by demyelination as well as by the presence of numerous transected neurites and apoptotic neurons [20]. However, the extent of infiltration of these lesions, particularly those located entirely within the cortex, by lymphocytes and macrophages is considerably lower than that observed in white matter lesions [3, 20]. These inflammatory cells are completely absent from chronic inactive lesions, which nevertheless continue to show all the stigmata of neuronal loss.

The lesion load in the cortex varies with the clinical course and phenotype of the disease and, in some patients, can be significantly higher than the lesion load in white matter, affecting up to 26.5 % (mean value) of the cortical volume [4]. Cortical lesions are relatively sparse in the early stages of multiple sclerosis but build up progressively over the course of the disease. In chronic relapsing-remitting disease, even if this does not develop into a secondary progressive form, the cortex can contribute a significant proportion of the total lesion load (Fig. 4).

Cortical lesion load is particularly important in progressive forms of multiple sclerosis. In a series of 53 autopsy samples from patients with different forms of multiple sclerosis, we found that although cortical pathology was evident in most subjects except for those with acute disease of Marburg's type of MS, the proportion of cortical involvement was at least five times greater ($p = 0.0025$) in primary or secondary progressive forms of the disease compared to the relapsing-remitting form (Table 1) [15]. A similar difference was observed in the cerebellar cortex ($p = 0.015$). On the other hand, the extent of white matter involvement was essentially similar (21 % to 28 %) in all clinical forms of multiple sclerosis. The extent of cortical involvement may thus be a more pertinent correlate of permanent neurological disability than white matter pathology.

Independence of inflammation and neurodegeneration following autologous bone marrow transplantation

The potential of autologous hematopoietic cell transplantation has been of considerable interest in recent years following demonstration that this procedure could protect against experimental autoimmune encephalitis in rodents [11]. Pilot studies have revealed that such a treatment is beneficial in reducing lesion activity and neurological disability in patients with severe progressive disease [7, 19], although the procedure is not without risk. We have recently had the opportunity to evaluate the brains of four patients who had received autologous bone marrow transplants in North America and subsequently died.

Autopsy samples from these patients revealed that in all cases there was an almost complete absence of inflammatory markers in the brain, notably of T cells. On the other hand, there was significant staining for amyloid precursor protein (APP) inclusions, a marker of acute axonal damage (Fig. 5). This suggested that even though inflammation had been abolished, neurodegeneration was still proceeding in the brains of these patients, and thus that neurodegeneration was not a direct consequence, at least in the short-term, of inflammatory damage to the nervous system. These data are consistent with the observation from MRI studies that the rate of brain atrophy is high in patients who have received hematopoietic stem cell grafts in spite of an apparent

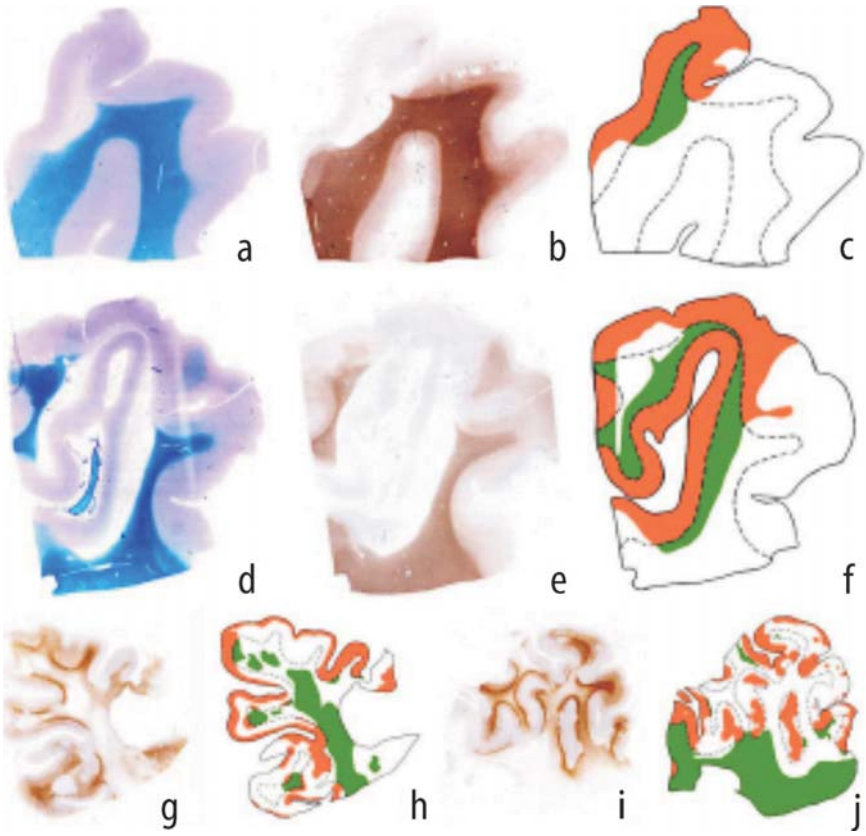

Fig. 4 Cortical and white matter lesions in multiple sclerosis. The histological sections are stained with anti-MOG antibody-peroxidase (**a**, **d**) and counterstained with hemoxylin (**b**, **e**, **g**, **i**). The camera lucida drawings (**c**, **f**, **h**, **j**) represent the same tissue sections with white matter lesions indicated in green and cortical lesions in orange. **a–f** lesion volume in a 41 year old male patient with chronic progressive multiple sclerosis of 13 years disease; **g–j** lesion volume in a 35 year old female patient with chronic inactive multiple sclerosis of 10 years disease duration (**g–j**). Taken from [14] with permission

Table 1 Cortical lesions in patients with different clinical forms of multiple sclerosis. Data are presented as the number of cases with cortical involvement, the proportion of the cortex with lesions calculated for the cases only and for the total sample and the proportion of white matter with lesions calculated for the total sample. Data are taken from [15]

	Number of cases	Cortical lesions % (cases)	Cortical lesions % (total)	White matter % (total)
Acute	1/11	3.93	0.18*	22.13
RRMS	5/6	5.22	3.75*	20.93
PPMS	9/10	18.42	26.51*	23.56
SPMS	17/20	21.85	19.58*	27.91

complete cessation of inflammatory lesion activity [10]. A possible explanation for these discordant effects on inflammation and neurodegeneration would be that suppression of the normal T cell patrolling of the nervous system may interrupt correct regulation of microglial function, allowing microglia to release toxic substances indiscriminately which can in turn injure neurons.

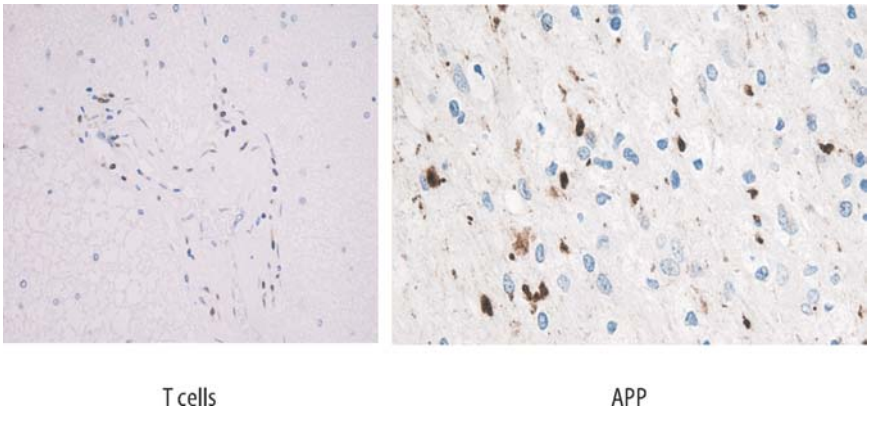

Fig. 5 Immunostaining for T cells (left) and APP (right) in brain tissue removed at autopsy from a recipient of an autologous bone marrow transplantation

Conclusions

Axonal and neuronal loss are the central pathological features of multiple sclerosis. Axonal damage is spread diffusely throughout the nervous system and may develop independently of inflammation. The mechanism of neurodegeneration may involve changes in trophic interactions between oligodendrocytes, microglia and neurons. Such trophic disturbances may represent the primary pathophysiological anomaly in multiple sclerosis, triggering both the inflammatory response and neurodegeneration. Of particular importance for the clinical presentation of the disease is neuronal loss in the cerebral cortex. Cortical pathology may develop autonomously from white matter damage and, unlike white matter lesions, may be relatively independent of inflammation. The extent of cortical pathology is particularly important in progressive forms of multiple sclerosis and may be relevant to the pathogenesis of irreversible neurological and cognitive disability in multiple sclerosis. The extension of cortical damage may be the pathological substrate of disease progression in progressive forms of the disease. For this reason, treatment strategies aimed at preventing neurodegeneration are essential to develop, in order to attenuate progression of disability and perhaps to prevent the transformation of the clinical phenotype from relapsing-remitting to a secondary progressive disease.

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