The pathology of multiple sclerosis: a paradigm shift

Michael H. Barnett[a,b] and Ian Sutton[c,d]

Purpose of review

Detailed immunopathological assessment of multiple sclerosis tissue remains the research tool most likely to elucidate the major processes involved in disease pathogenesis and tissue injury. Such studies steer and provide the impetus for refining cellular/molecular investigations and developing more relevant disease models in animals.

Recent findings

Recent observations in early multiple sclerosis lesions challenge the traditional hypothesis that multiple sclerosis arises as the result of a primary autoimmune process that specifically targets myelin antigen(s). A new multiple sclerosis paradigm proposes that oligodendrocyte apoptosis is the earliest change in newly forming lesions and that tissue injury is amplified by the subsequent recruitment of a systemic immune response. Over months to years the pathology of multiple sclerosis is transformed and the changes which accompany the late phase of the disease suggest that the inflammatory response becomes progressively 'compartmentalized' and therefore largely isolated from systemic influence with time.

Summary

Recent pathological studies raise important questions regarding the aetiology of oligodendrocyte apoptosis, the mechanisms by which the accompanying inflammatory response amplifies tissue injury and the regulation of central nervous system immunity. An improved understanding of these processes is essential for advancing therapeutic interventions applicable to different stages of the disease.

Keywords

microglia, multiple sclerosis, oligodendrocyte, pathology, T lymphocyte

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[a]Brain and Mind Research Institute, University of Sydney, NSW, Australia, [b]St George Hospital, Kogarah, NSW, Australia, [c]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia and [d]St Vincent's Hospital, Darlinghurst, NSW, Australia

Correspondence to Dr Michael Barnett, Brain and Mind Research Institute, University of Sydney, 100 Mallett Street, Camperdown NSW 2050, Australia
Tel: +61 2 93510730; fax: +61 2 93510653; e-mail: mbarnett@mail.usyd.edu.au

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Abbreviations

CNS	central nervous system
CSF	cerebrospinal fluid
MS	multiple sclerosis

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Introduction

Almost 150 years have elapsed since Charcot synthesized the principal clinical and histological features of chronic multiple sclerosis (MS) [1,2]. He and others recognized MS as a venocentric focal inflammatory demyelinating disease of the central nervous system (CNS). The subsequent identification of perivascular lymphocytes and plasma cells by Dawson [3] and Brain [4] indicated the possibility of an underlying 'allergic' process, forming the basis of the modern interpretation of MS as a Th1 lymphocyte-mediated autoimmune response that is directed against myelin-derived antigen(s).

Knowledge of the neuropathology of MS, particularly in the early relapsing phase of the disease, has increased significantly in recent years and is reshaping our concepts of disease pathogenesis. A spectrum of early MS lesion pathologies is now recognized and has been interpreted by some as indicating heterogeneous disease pathogenesis. Others propose that a uniform pathogenetic mechanism underlies formation of new plaques. In this paradigm, oligodendrocyte apoptosis and ensuing molecular changes in the myelin sheath attract and activate local microglia, with subsequent recruitment of a systemically activated immune response resulting in dramatic amplification of the inflammatory process. As disease progresses, relapses become less frequent and a significant proportion of patients enter a secondary progressive phase. Neuropathological examination during this 'late' phase of the disease rarely discloses acute lesions in previously unaffected white matter, and 'activity' is usually restricted to the edge of, or superimposed upon, pre-existing lesions. This pathological metamorphosis, which is accompanied by global abnormalities including diffuse microglial activation, suggests that MS may become a 'compartmentalized' disease, largely isolated from a systemic influence, over time (Table 1). These clinical and pathological changes are paralleled by changes in the composition and distribution of the inflammatory cell populations within the CNS. In order to understand the dynamics of this inflammatory process, pathological studies have been extended to include an examination of the role of cervical lymph nodes and a greater emphasis is being placed on chronic changes that can be observed in perivascular Virchow–Robin spaces, cerebrospinal fluid (CSF) and meninges.

This review will focus on (1) evolution of the newly forming and active MS lesion during acute relapses and (2) immunopathological 'compartmentalization' in

Table 1 Characteristics of early and late-stage multiple sclerosis

	Early	Late
Disease course	Relapsing-remitting	Progressive
Magnetic resonance imaging	Gadolinium-enhancing lesions	Focal non-gadolinium-enhancing lesions; atrophy; NAWM abnormalities
Pathology	Multifocal	Multifocal and global
Inflammatory response	Systemic recruitment	Compartmentalized
Response to immunotherapy	Reduction in relapse rate	Minimal effect on progression

NAWM, normal-appearing white matter.

the chronic phase of MS, and will discuss the implications for pathogenesis and tissue injury of these two phases.

Active phagocytic multiple sclerosis lesions

Examination of MS lesions shortly after the onset of a clinical relapse presents a unique opportunity to unravel the immunopathological complexities which characterize the developed plaque. Active MS lesions, defined by the presence of myelin-laden macrophages in the presence of degenerating myelin sheaths, are most commonly described in patients with a disease duration of 18 months or less. T lymphocytes, both within perivascular cuffs and in lesser numbers infiltrating the parenchyma, are a constant feature of such lesions but are outnumbered at least 20:1 by macrophages containing myelin proteins which are visualized in sections stained histochemically with Luxol fast-blue or immunocytochemically with antibodies to myelin proteins [5–7]. Detailed analysis of the active MS lesion reveals a number of other features, chief among which is a variable loss of oligodendrocytes. While axonal transection and loss is another invariable finding, the myelin sheath appears to be the primary target of the pathophysiological process and complete lesion demyelination usually results within weeks of symptom onset. Selective myelin damage and comparisons with the animal model, experimental allergic encephalomyelitis, has led most authorities to consider the pathology of the active MS lesion to be best explained by a myelin-antigen-specific Th1-mediated response that results in macrophage-mediated destruction of the normal myelin sheath. The presence of Th1-type cytokines in the CSF of patients with active disease and CXCR3/CCR5 chemokine receptor expression in lesion-infiltrating T lymphocytes provides support for this hypothesis [8]. However, recent pathological studies that have helped to define the events preceding active demyelination in evolving MS lesions cast doubt on this conventional paradigm.

Prephagocytic lesions – the 'initial' multiple sclerosis pathology?

Conclusions drawn from pathological studies of active MS lesions are necessarily limited by the fact that tissue is sampled at a specific point in time. Rapid shifts in lesion pathology may occur over hours to days and the earliest features, which we believe are most likely to yield useful pathophysiological information, may therefore be absent in the classical actively demyelinating lesions described above.

In a recent study of autopsy-derived MS tissue [9], we identified focal prephagocytic pathology at sites which correlated anatomically with the clinical symptoms of patients who had died during or shortly after an acute relapse, and defined two principal pathologic findings: (1) circumscribed fields of oligodendrocytes exhibiting morphological features of (activated caspase-independent) apoptosis, which bear no consistent vascular relationship and (2) activated highly ramified microglia, some of which appear in close apposition to apoptotic oligodendrocytes. Such lesions are also notable for their absence of overt structural myelin pathology or significant macrophage infiltrate. Luxol fast-blue-stained sections reveal generalized myelin pallor, and more advanced prephagocytic changes include myelin vacuolation due to intramyelinic oedema in tissue completely devoid of oligodendrocytes or their remnants. While T lymphocytes are not identified within such lesions, typical lymphocytic infiltrates accompanied by active phagocytic demyelination are often present in contiguous white matter zones, and in lesions elsewhere in the neuraxis of these patients [9].

These observations suggest a novel paradigm of MS pathology, namely that oligodendrocyte apoptosis, in the absence of contact-dependent cell-mediated immunity, precedes myelin phagocytosis in the newly forming lesion. In addition, we have proposed that macrophage activity is largely scavenger-like in nature, and results from a physiological transformation of resident microglia into an amoeboid macrophage phenotype in response to the expression of phagocytic ligands on the extended plasma membranes of the apoptotic oligodendrocytes. A discussion of the potential triggers of oligodendrocyte injury in nascent MS lesions is beyond the scope of this paper, and has recently been reviewed elsewhere [10•].

Could prephagocytic MS pathology be incorporated into the traditional autoimmune theory of MS? Since we have not observed IgG deposition on either the oligodendrocyte or the myelin sheath (MH Barnett and JW Prineas, unpublished data), one hypothesis is that proinflammatory

cytokines, such as tumour necrosis factor, initiate programmed cell death in the newly forming lesion [11•]. Diffusable cytokines could derive from CD4+ Th1 lymphocytes and macrophages in nearby actively demyelinating tissue, or potentially from activated microglia, which are an invariable feature the prephagocytic lesion itself. However, it is difficult to ascribe oligodendrocyte destruction in MS to the action of proinflammatory cytokines alone, given that extensive demyelination and oligodendrocyte loss are not features of typical T lymphocyte-mediated experimental allergic encephalomyelitis [12,13], acute disseminated encephalomyelitis or paraneoplastic encephalomyelitis [14], diseases in which widespread T lymphocyte infiltration accompanied by microglial and macrophage activation is the rule. Nevertheless, convincing evidence that a Th1-mediated immune response can effect tissue damage in MS has emerged from recent clinical trials in which inhibition of $\alpha 4$ integrin-mediated T-lymphocyte trafficking into the CNS has been shown to dramatically reduce clinical relapses and the number of new gadolinium-enhancing lesions on magnetic resonance imaging [15–17].

In the absence of a defined autoantigen in MS, there are two alternative theories which reconcile prephagocytic and active lesion pathology while acknowledging the contribution of the systemically derived Th1-mediated immune response in the clinical manifestation of early, relapsing disease: (1) death of the oligodendrocyte/myelin complex unmasks an autoantigen which activates a systemic immune response that amplifies tissue injury or (2) a foreign antigen both produces oligodendrocyte apoptosis and provides the stimulus for the systemic inflammatory response. Although the healthy CNS is characterized by the absence of parenchymal dendritic cells, a recent pathological study of four acute MS cases has demonstrated that active lesions are characterized by the presence of CCR7+CD68+ myeloid dendritic cell-like cells which express major histocompatibility complex class II and co-stimulatory molecules [18]. CCR7 is a chemokine receptor essential for migration to regional lymph nodes, and CD68+ cells containing myelin degradation products can be demonstrated in the cervical lymph nodes of MS patients, both at post mortem [19] and following cytological examination of ultrasound-guided fine-needle aspirates from non-enlarged lymph nodes in life [20•]. Although it is unclear whether this myeloid dendritic cell-like CD68+CCR7+ major histocompatibility complex class II+CD86+ population is derived from resident microglia, perivascular macrophages or systemically recruited monocytes [18,21], it is apparent that antigen-presenting cells migrate from the evolving lesion to the cervical lymph nodes where they can regulate the differentiation of naïve T cells into effector and central memory T lymphocytes that are capable of entering the CNS and amplifying tissue injury.

The concept of a foreign antigen that induces both demyelination and inflammatory cell recruitment is not without clinical precedent. In the peripheral nervous system *Mycobacterium leprae* preferentially infects Schwann cells by binding of the cell-wall protein phenolic glycolipid-I to laminin-$\alpha 2$ [22] and can cause primary demyelination and delayed axonal degeneration in the absence of inflammatory cells. This effect has been demonstrated both in Schwann cell–dorsal root ganglion co-cultures and following injection of the organism into the sciatic nerve of $Rag1^{-/-}$ mice [23]. Interestingly, *M. leprae* viability is not requisite to effect demyelination, which can be reproduced using the cell-wall fraction alone [23]. In addition, a spectrum of immune responses can occur in individuals with this infection but most patients have a borderline, immunologically changeable status and often develop enhanced cell-mediated immunity-delayed type hypersensitivity (Th1) responses. These 'type 1 reversal reactions' are accompanied by severe destruction in peripheral nerves, underscoring the critical role of bystander inflammation in this condition [24].

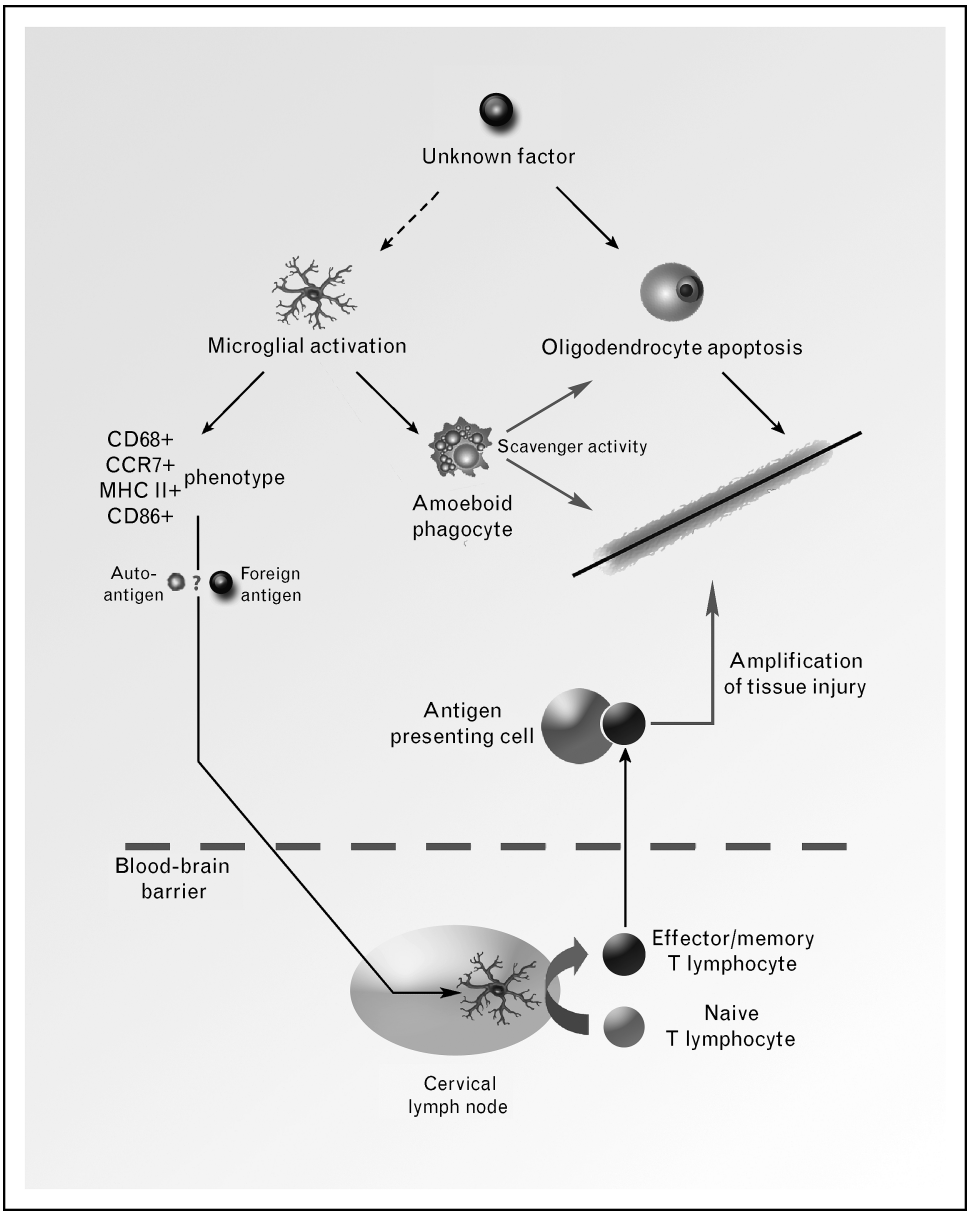
Figure 1 illustrates a paradigm for the evolution of the acute lesion based on currently available pathological information.

Pathological heterogeneity of active multiple sclerosis lesions – pathogenic heterogeneity or lesion evolution?

Lucchinetti *et al.* [25] examined a large series of actively demyelinating plaques obtained at autopsy ($n = 32$) or biopsy ($n = 51$) and reported two distinct pathological patterns in 80% of the included cases. Pattern 2 lesions were typically perivenous in location, characterized by the precipitation of activated complement and IgG on degenerating myelin sheaths and associated with the presence of remyelinating shadow plaques elsewhere in the CNS. In contrast, Pattern 3 lesions had ill-defined borders, were not centred on vessels, lacked immunoglobulin and complement deposition and 8/22 cases exhibited concentric bands of demyelinated and myelinated tissue at the lesion edge. Pattern 3 lesions showed preferential loss of myelin-associated glycoprotein immunoreactivity relative to other myelin proteins and variable oligodendrocyte apoptosis at the active plaque edge; remyelinating shadow plaques were absent in such cases. Myelin was considered to be the primary target of the inflammatory response in the Pattern 2 lesion, whereas Pattern 3 lesions were thought to reflect primary oligodendrocyte injury. Importantly, the pathology of all active plaques in a given individual was reported to conform to only one pattern, leading the authors to suggest that inter-individual pathologic heterogeneity reflected heterogeneous disease aetiology and therefore carries significant implications for treatment [26•].

Figure 1 Suggested paradigm for the temporal evolution of inflammatory changes in the newly forming multiple sclerosis lesion

(A) An as-yet-unidentified factor results in oligodendrocyte apoptosis and direct or secondary microglial activation. (B) Some microglia differentiate into macrophages that phagocytose apoptotic oligodendrocytes and degenerating myelin which express phagocytic ligands on their abluminal surface consequent to molecular changes which accompany programmed cell death. (C) A population of CCR7+ microglia differentiate into myeloid-like dendritic cells that migrate to the cervical lymph nodes and activate naïve T lymphocytes to a memory/effector phenotype. (D) These Th1 lymphocytes re-enter the central nervous system and are reactivated by antigen-presenting cells, possibly those located in Virchow–Robin spaces within the lesion. This results in amplification of the inflammatory process by recruitment of additional systemic immune populations causing further tissue injury by as-yet-undefined mechanisms. This paradigm makes no assumptions about the antigen-specificity of the T-lymphocyte response, which may be directed towards a constitutive autoantigen, an autoantigen unmasked during the apoptotic process, or epitopes derived from an unknown factor that also causes oligodendrocyte apoptosis.



Stadelmann *et al.* [27••] have recently published an important report which suggests that tissue 'preconditioning' may explain the concentric layering of damaged and preserved myelin which characterizes Balo's concentric sclerosis, a form of acute MS. Oligodendrocytes, and to a lesser extent other cell types in the outer lesion edge and the outermost rims of preserved myelin in expanding concentric lesions, showed intense expression of markers of hypoxia-like tissue damage (D-110 epitope), regulators in the induction of hypoxic preconditioning (HIF-1α) and stress proteins which afford protection against hypoxic injury (heat-shock protein 70). Mitochondrial dysfunction, perhaps induced by inflammatory mediators or nitrogen/oxygen intermediates, has been invoked as the potential basis of preconditioning. The authors equate Balo pathology with the Pattern 3 lesions described previously by the same group [25].

Lesions which conform strictly to the Pattern 3 phenotype were found in some 30% of all cases reported by Lucchinetti *et al.* [25]. In our experience, however, lesions with concentric banding but no complement activation or remyelination are much rarer and are probably confined to a small subset of patients with established Balo's concentric sclerosis. In contrast, we have

found that many patients in the early stages of relapsing and remitting MS exhibit a spectrum of pathological changes encompassing both Pattern 2 and Pattern 3 features. Complement deposition is observed on degenerating myelin sheaths and remyelinating lesions are often abundant in cases which exhibit small regions or bands of preserved/demyelinated tissue and in those with frank prephagocytic pathology [9]. Temporal evolution of the newly forming lesion, rather than discrete immunopathogenic patterns, is thus a more likely explanation of pathological heterogeneity in patients with typical relapsing and remitting disease. It is probable that the majority of, if not all, active lesions in such cases conform to the Pattern 2 phenotype described by Lucchinetti *et al.* [25], and that prephagocytic changes, which are only present transiently in the newly forming lesion, are therefore only identified in a small fraction of pathological specimens. This is supported by the findings of a recent study by Bö *et al.* [28], in which only Pattern 2 pathology could be defined in 33 actively demyelinating lesions from 22 patients drawn from a large unselected pool of MS material.

Immunopathological compartmentalization in late multiple sclerosis

Active lesions are almost never seen in previously unaffected white matter in the late phase of MS and any evidence of myelin phagocytosis is usually restricted to the edge of preexisting plaques. Prineas *et al.* [29] have also described a further type of 'arrested' activity in the periplaque white matter of patients with longstanding secondary progressive MS, in the form of microglial nodules surrounding segments of disrupted myelin which are immunoreactive for C3d, the opsonic component of activated complement. Totally demyelinated plaques, and regions of limited or aberrant remyelination, are not uncommon and extensive cortical demyelination may be present [30••,31•]. In addition to these focal white matter changes, diffuse microglial activation may be present throughout the CNS white matter in late MS, often accompanied by a significant infiltrate of perivascular CD8+ T lymphocytes and evidence of widespread axonal injury [30••]. Magnetic resonance imaging studies show parallel signal changes in otherwise normal-appearing white matter and diffuse cerebral atrophy, and document a gradual transition from the 'early' to 'late' phase of the disease [32].

Changes in immune regulation parallel the pathological changes that accompany disease progression. Pathological studies show different patterns of chemokine expression in acute and chronic lesions [33•] and while early relapsing MS is associated with recruitment of systemically derived immune cell populations, inflammatory responses in the late progressive phase appear to be locally regulated or 'compartmentalized' [30••]. Increasingly the CNS is being recognized as a unique immune environment and Ransohoff *et al.* [34] have proposed that the 'CSF is a partial functional equivalent of lymph for the CNS'. This concept is supported by Prineas' [35] description of secondary lymphoid organization within the Virchow–Robin spaces of patients with chronic MS. He observed direct contact between macrophages and lymphocytes within smooth-walled channels formed by reticular cells, and clusters of plasma cells outside these channels [35]. In addition, a more recent study demonstrated ectopic follicles containing B cells and plasma cells interacting with follicular dendritic cells in the meninges of two out of three secondary progressive MS patients [36], and CSF B-lymphocyte subset analysis is consistent with recapitulation of a germinal centre reaction inside the CNS [37].

These observations provide some explanation for lack of efficacy of immunomodulatory and immunosuppressive therapies in late MS and suggest that treatments aimed at this phase of the disease should target resident CNS inflammatory cell populations, in addition to developing strategies to promote remyelination and protect neurons.

Conclusion

There appear to be two critical neuropathological phases in MS. The first occurs in newly forming lesions over hours to days, beginning with oligodendrocyte apoptosis which is followed by transformation of microglia to a scavenger, phagocytic phenotype and subsequent activation of a systemic immune response that amplifies tissue damage. The second is a gradual transition in the overall pathology of MS, occurring over months to years and characterized by progressive immunopathological compartmentalization which renders the disease resistant to traditional immune-based therapies.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 317–320).

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