Multiple sclerosis as a neurodegenerative disease: pathology, mechanisms and therapeutic implications
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Introduction
Pathologically, demyelination with relative axonal preservation is the main and distinguishing feature of multiple sclerosis (MS) lesions. However, in recent years, evidence has accumulated from the field of neuroimaging that the extent of clinical disability does not so much correlate with the total burden of hyperintense lesions on T2-weighted MRI, but rather with the extent of total axonal damage and loss [1]. Of importance also, a reduction of N-acetylaspartate (NAA), an amino acid found at high levels in axons and neurons that is considered a marker of axonal integrity, is observed very early in the disease [2]. Furthermore, it has been proposed that the transition into the progressive disease phase is due to a critical loss of axon density, as reflected by magnetic resonance spectroscopy (MRS), MRI, and pathological studies [3–5]. Especially, brain atrophy as visualized by MRI correlates well with clinical parameters in MS, as well as experimental models [6–8].

Purpose of review
Multiple sclerosis (MS) treatments targeting the inflammatory nature of the disease have become increasingly effective in recent years. However, our efforts at targeting the progressive disease phase have so far been largely unsuccessful. This has led to the hypothesis that disease mechanisms independent of an adaptive immune response contribute to disease progression and closely resemble neurodegeneration.

Recent findings
Nonfocal, diffuse changes in the MS brain, especially axonal loss and mitochondrial dysfunction, prove better correlates of disability than total lesion load and have been associated with disease progression. Molecular changes in nondemyelinated MS tissue also suggest that alterations in the MS brain are widespread and consist of pro-inflammatory as well as anti-inflammatory responses. However, local lymphocytic inflammation and microglial activation are salient features of the chronic disease, and T-cell-mediated inflammation contributes to tissue damage. In addition, neuroaxonal cytoskeletal alterations have been associated with disease progression.

Summary
Our knowledge of the molecular mechanisms leading to neuroaxonal damage and demise in MS is steadily increasing. Experimental therapies targeting neuroaxonal ionic imbalances and energy metabolism in part show promising results. A better understanding of the molecular mechanisms underlying chronic progression will substantially aid the development of new treatment strategies.

Keywords
cytoskeletal alterations, mitochondrial damage, multiple sclerosis, neurodegeneration, T-cell infiltration

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favor a central nervous system (CNS) autonomous neurodegenerative process, but rather consider inflammation central to its pathogenesis [9,10].

Although the mechanisms leading to axonal damage and especially the most likely divergent mediators and mechanisms precipitating acute vs. chronic axonal damage and loss are not yet fully understood, during the last years, it has become increasingly clear that axonal loss and dysfunction, respectively, occur very early in the disease course [2]. However, strikingly, recent data from pathological studies underline the fact that T lymphocytes are still present in substantial numbers in advanced lesion stages and are associated with parenchymal tissue loss [4,11,12,13]. Mechanistically, inflammatory damage to mitochondria is increasingly considered a key feature of axonal damage in acute as well as chronic lesions [14,15]. Cytoskeletal changes in the neuroaxonal as well as glial compartments may reflect a disturbed balance of kinase and phosphatase activity and be amenable to therapeutic modification [4,16]. Therapeutically, evidence is accumulating that early and vigorous anti-inflammatory treatments reduce the extent of ensuing neuroaxonal damage [17]. At the same time, recent molecular studies support and direct the development of new experimental therapies targeting neuroaxonal metabolism [18,19].

The present overview addresses recent insights gained into the process(es) of neurodegeneration in inflammatory demyelination with regard to pathological findings and new mechanisms identified, comments on selected experimental therapies, and aims to deduce therapeutic consequences for MS patients.

The concept of neurodegeneration in multiple sclerosis
The term ‘neurodegeneration’ has now come to be widely accepted in MS research, although with a slightly different implication than in the classical neurodegenerative diseases. Pathological, imaging, serological, and genetic studies as well as the response to anti-inflammatory treatments strongly support the concept that MS is a primary inflammatory disease [20]. However, clinically, features reminiscent of neurodegenerative diseases, that is, a steady decline in function without much response to current treatments, and little if any inflammatory activity on MRI, dominate the progressive disease stage [21]. Epidemiological data indicate that the rate of disability progression is very similar in different patients who have reached a certain threshold of disability, suggesting a common underlying mechanism of progressive CNS dysfunction, the most likely pathological substrates being a steady accumulation of neuroaxonal dysfunction or insidious neuroaxonal demise [22–24]. Although brain atrophy as measured by MRI and reduction of the predominantly neuroaxonal amino acid NAA by MRS correlate with increasing disability, it has so far proven difficult to identify the exact pathological substrate(s) of the progressive disease phase.

Key points
- It is recognized more and more that the brain and spinal cord are globally and not only focally affected in multiple sclerosis (MS).
- Lymphocytic infiltrates, indicators of an adaptive immune response, are still present in advanced stages of the disease.
- It is still not clear whether an autonomous neurodegenerative process contributes to nervous system degeneration in MS.
- New insights into the mechanisms of neuroaxonal damage are currently leading to more specific therapies.

Neuroaxonal dysfunction and demise in multiple sclerosis: recent data from pathology
A wealth of pathological studies has examined the impact of the immune and nervous system on damage and loss of neural structures in MS.

Autonomous neurodegeneration or a role for innate and adaptive immune responses?
The presence of extensive demyelinated cortical lesions, the loss of axons and neurons as well as diffuse, non-lesion-related lymphocytic infiltration have been related to disability progression in MS [25]. Recent pathological studies further emphasize the role of an adaptive immune response for the extent of CNS parenchymal damage. Maggiozzi et al. [12] recently described a gradient of neuronal cell loss depending on the severity of meningeal inflammation. Furthermore, a relationship between the severity of meningeal inflammation and spinal axonal loss has been established [13]. Similarly, ongoing adaptive immune cell infiltration in chronic lesions was recently observed in a number of studies and has been correlated with the density of amyloid precursor protein (APP) accumulations in axons, indicating acute axonal damage [4,11]. Experimentally, even nonneuronal antigen-specific T-helper 17 cells are capable of inducing reversible neuronal damage [26]. As to the question of the role of activated microglia for neuroaxonal dysfunction and demise, Howell et al. [27] found disrupted nodes of Ranvier in brain diseases with enhanced microglia activation, exemplified by MS and Parkinson’s disease. Importantly, genetic studies so far strongly support the concept that polymorphisms in inflammatory genes contribute to the pathogenesis of the disease [28]. Molecular studies of normal-appearing and periplaque white matter indicate a subtle inflammatory response also in
nonlesioned brain tissue; however, in addition, they point to the fact that anti-inflammatory pathways are upregulated, which may indicate the activation of endogenous protective mechanisms [29–31].

The neuroaxonal cytoskeleton in multiple sclerosis

It is well established that axonal damage occurs early during lesion formation and is easily visualized by demonstration of disturbed axonal transport, for example, by staining for APP in tissue sections [32]. However, although some APP-positive axonal spheroids are still present in long-standing MS patients and correlate with the extent of T-cell infiltration [4,11], their exceedingly low numbers could indicate that the mechanisms leading to axonal dysfunction and demise in long-standing disease might be different from those observed in acute inflammatory damage. Being a key feature of classical neurodegenerative diseases, cytoskeletal abnormalities in neurons, axons, and glial cells have recently received some attention. It is long known that the phosphorylation status of neurofilament proteins is an indicator of neuroaxonal damage [33]. With regard to MS, phosphorylated neurofilament species appear in the neuronal cell body in neurons undergoing a chromatolytic reaction after inflammatory axonal transection [34]. Demyelinated axons often display immunoreactivity using the Sternberger monoclonals incorporated 32 (SMI32) antibody, indicating increasingly nonphosphorylated neurofilament proteins [4]. Apparently, with increasing disease duration, motor neurons in the MS spinal cord progressively lose their (normal) SMI32 immunoreactivity, an indicator of increasingly disturbed neurofilament metabolism not yet fully understood [4]. Experimentally, hyperphosphorylated tau protein has been detected in the brainstem of rats in an early stage of experimental autoimmune encephalomyelitis (EAE) [35]. Similarly, in MS, hyperphosphorylated and even insoluble tau aggregates have been reported [36,37]. A recent immunohistochemical study points toward hyperphosphorylated tau in astrocytes [16]. However, further experimental work is required to elucidate whether the cytoskeletal abnormalities so far reported form part of a self-sustaining neurodegenerative process.

Mechanistic insights into neuroaxonal damage in multiple sclerosis

Our knowledge on the diverse mechanisms of neuroaxonal damage in inflammatory demyelination has substantially increased in recent years, thus paving the way for the development of new therapeutic interventions.

Axonal loss as a sequel of focal demyelinated lesions

In acute MS lesions, an abundance of APP-positive axonal spheroids indicates a disturbance of axonal transport in a substantial number of axons. The question as to what extent these changes are reversible is currently a matter of debate. Data from experimental brain trauma suggest that at least after mild injury, part of the axons accumulating APP will resume function [38]. However, the appearance of very large spheroids in acute lesions may indicate that axonal transections leading to consecutive anterograde (Wallerian) and retrograde degeneration are not a rare phenomenon in newly forming MS lesions. This is in line with the presence of numerous axonal profiles immunoreactive for neuropeptide Y–Y1 receptor, a proposed marker for Wallerian degeneration [39], in the periplaque white matter of MS biopsy tissue [40]. Mechanistically, reactive oxygen species and nitric oxide presumably play a key role in inducing this early, acute axonal damage [41]. In a model of optic nerve transection, inhibitors of autophagy can block early axonal degeneration and, thus, imply a central role for autophagic processes in early axonal degeneration [42].

Mitochondrial dysfunction in inflammatory demyelination

Acute inflammation leads to increased local levels of reactive oxygen species and nitric oxide, all capable – in addition to their immediate damaging effects, especially on cell membranes – of mitochondrial damage [15]. Axonal transport is highly energy demanding and, as such, axons are extremely sensitive to fluctuations in energy supply. Evidence for mitochondrial alterations in acute and chronic MS lesions, especially in neurons and axons, has accumulated in the last few years [43]. Specifically, a reduced activity of respiratory chain complex IV (cytochrome C oxidase) has been found in demyelinated axons in active as well as chronic active lesions and was inversely related to macrophage/microglia density [44,45]. Interestingly, mitochondrial mass and complex IV activity were increased in demyelinated axons without apparent structural damage in chronic inactive lesion areas, probably indicating adaptive changes [45]. Most recently, microdissection of single cortical neurons from brains of secondary progressive MS patients has revealed respiratory-deficient neurons with deletions in the mitochondrial genome that the authors hypothesized were secondary to inflammation [46]. Extremely destructive demyelinating lesions with lymphocytic infiltrates have been observed in a patient with Leber’s hereditary optic neuropathy, providing further evidence that mitochondrial energy metabolism plays a role in the survival of the neuroaxonal unit under an inflammatory attack [47]. As a further mechanism, disturbances of mitochondrial transport may cause insufficient energy supply. Inflammatory mediators can initiate a disruption of axonal transport via a translocation of the histone deacetylase 1 (HDAC1) from the nucleus to the cytosol and ensuing complex formation with kinesin motor proteins [48].
Recently, in-vivo techniques to assess mitochondrial function were established. In addition to being a marker of neuroaxonal integrity, the above-mentioned amino acid NAA reflects mitochondrial function and can be assessed using spectroscopic techniques [49]. A faster recovery of NAA in acute MS lesions has been correlated with faster recovery of the patients [50]. In another study, reduced mitochondrial function was associated with greater clinical disability, independent of structural damage to axons [49*].

Alterations of connectivity in inflammatory demyelination

In addition to a blunt loss of neurons and axons, more subtle changes in connectivity have recently been reported. Microarray data of human nondemyelinated MS cortex revealed a reduction of components for γ-amino-butyric acid (GABA)ergic transmission accompanied by an immunohistochemical reduction of interneurons [43]. Electrophysiological studies on the striatum of animals with EAE now reproduce and extend these results showing a link of altered transmission to chronic exposure to pro-inflammatory cytokines [51]. Along the same lines, an enhanced activity of the Na⁺/Ca²⁺ exchanger has been reported in experimental CNS inflammation, which leads to enhanced glutamatergic transmission, favoring neuroaxonal demise [52]. Morphologically, reductions of synaptophysin immunoreactivity have been reported in several studies of MS gray matter [53,54] and also in experimental models [55,56]. Functional MRI data indicating plastic changes in patients very early in the disease as well as the well established reduction of NAA very early on are in line with the notion that widespread changes in neuronal connectivity occur already quite early in disease and may be only in part correlated with the lesion load [57,58].

Therapeutic efforts targeting the neuroaxonal unit

At present, patients with MS are treated with immunosuppressive or immunomodulatory therapies. However, these approaches in general only work in patients with active inflammatory disease, as visualized by gadolinium–DTPA (gadopentetate dimeglumine)-enhancing lesions. In contrast, patients with primary or secondary progressive MS are much less likely to respond to anti-inflammatory and immunomodulation, a fact that has significantly endorsed the concept of an – at least in part autonomous – neurodegenerative component in MS. Several therapeutic approaches targeting the neuroaxonal unit have been assessed so far in MS; however, none of these has yet reached the level of clinical applicability. Among these, modulators of ion channel permeability, such Na⁺ channel blockers, inhibitors of the Na⁺/Ca²⁺ exchanger or blockers of the acid-sensing ion channel 1, hold promise [18,59,60]. Recently, in addition, strategies to improve mitochondrial functioning and, thus, axonal energy metabolism were found to be beneficial in experimental models of MS [61]. Furthermore, repair strategies similar to those tested in traumatic spinal cord injury seem promising in inflammatory demyelination [62].

Still, however, the concept of indirect axonoprotection by means of combating inflammation remains highly valuable. Recent clinical studies clearly point to the fact that early and vigorous anti-inflammatory treatment improves the outcome of MS patients [17,63*]. However, there certainly remains the urgent need for potent drugs targeting the progressive disease phase. Of note, the presence of T-lymphocytic infiltrates correlates with damage to and loss of neuroaxonal structures, even in advanced stages of disease [11,13*]. It remains enigmatic why current anti-inflammatory therapies do not seem to efficiently target these cells. The concept of inflammation barely accessible behind a closed blood–brain barrier has been established and may at least in part explain the little benefit observed with anti-inflammatory treatments in late-stage patients [64].

Apart from T-lymphocytic infiltrates, activated microglia cells are prominent in MS and might serve as a target of anti-inflammatory and indirectly neuroprotective therapies [27,65,66]. Experimental evidence indicates that a further indirect mechanism of axonoprotection is remyelination, and extending the concept of neurodegeneration, oligodendrocyte demise is clearly a feature of MS [67]. A novel concept that has evolved in recent years predominantly from molecular studies of the nonlesional, so-called normal-appearing white and gray matter is the idea that periplate brain tissue might engage in anti-inflammatory and neuroprotective strategies that possibly help to counteract inflammatory tissue damage and maybe even restrict plaque extension [30,31]. A better understanding of endogenous neuroprotective strategies might help to identify new therapeutic targets and devise novel treatment strategies [19].

Conclusion

Although the complex interplay between inflammation and neuroaxonal damage and demise, especially during the different phases of a disease lasting for many decades, is not yet fully understood, recent advances in the field support a combined use of both anti-inflammatory and neuroprotective therapeutic approaches in MS. With regard to drugs directly targeting neuronal processes and cell bodies, it seems that we have by now finally overcome the therapeutically frustrating decade and are entering an era in which closer insights into the molecular pathways of axon degeneration offer intriguing new possibilities of therapeutic modification.
Demyelinating diseases

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 302–303).


13 The influence of meningeal lymphoctic infiltration for cortical neuronal damage is highlighted by this pathological study, which suggests a gradient of neuronal loss in the cortex caused by a putative cytotoxic factor diffusing from the meningeal compartment.


15 The importance of an adaptive immune response in the meninges for axonal loss in the spinal cord is underlined by this study.


30 Okenberg JR, Baranzini SE. Multiple sclerosis genetics: is the glass half full, or half empty? Nat Rev Neurol 2010; 6:429–437.


48 Campbell GR, Zabreva I, Reeve AK, et al. Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis. Ann Neurol 2010 [Epub ahead of print]. This study finds deletions of mitochondrial DNA in cortical neurons, irrespective of lesions, and, thus, provides a novel mechanism for neuronal dysfunction in progressive MS.


The nuclear export of HDAC1 is presented as a novel mechanism that impairs axonal transport of mitochondria and, thus, may initiate axonal energy failure and damage in several pathological conditions, including cuprizone-induced demyelination.


This is an elegant study that demonstrates the feasibility of an in-vivo assessment of mitochondrial function in human brain tissue by MRS.


64 Lassmann H. Pathophysiology of inflammation and tissue injury in multiple sclerosis: what are the targets for therapy? J Neurol Sci 2010. [Epub ahead of print]

