



Review

Axonal and neuronal pathology in multiple sclerosis: What have we learnt from animal models

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ABSTRACT

Axonal and neuronal injury and loss are of critical importance for permanent clinical disability in multiple sclerosis patients. Axonal injury occurs already early during the disease and accumulates with disease progression. It is not restricted to focal demyelinated lesions in the white matter, but also affects the normal appearing white matter and the grey matter. Experimental studies show that many different immunological mechanisms may lead to axonal and neuronal injury, including antigen-specific destruction by specific T-cells and auto-antibodies as well as injury induced by products of activated macrophages and microglia. They all appear to be relevant for multiple sclerosis pathogenesis in different patients and at different stages of the disease. However, in MS lesions a major mechanism of axonal and neuronal damage appears to be related to the action of reactive oxygen and nitrogen species, which may induce neuronal injury through impairment of mitochondrial function and subsequent energy failure.

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Introduction

Although multiple sclerosis (MS) has originally been defined as an inflammatory demyelinating disease, it was already noted in its earliest pathological descriptions that axons and neurons are also affected and that axonal injury and loss is a major substrate for permanent neurological deficit of the patients (for review see [Kornek and Lassmann, 1999](#)). The situation is best exemplified by a statement of [Marburg \(1906\)](#) in his study on the lesions of acute multiple sclerosis. He claimed that multiple sclerosis is a demyelinating disease

with relative preservation of axons. However, he then added that emphasis has to be laid upon the term “relative” and that a variable degree of axonal loss is present in every multiple sclerosis lesion. Thus, in the literature published between 1880 and 1930 much attention was paid to axonal pathology in MS and it became clear that axonal loss within the lesions is variable, but very severe in selected cases, that acute axonal injury is associated with inflammatory macrophage infiltration and that there are some, but rather inefficient attempts of axonal regeneration. Furthermore, by analysing clinico-pathological correlation in patients with spinal cord lesions, it became clear that permanent neurological deficit is more related to axonal loss than to demyelination ([Kornek and Lassmann, 1999](#)). However, when it was found that a disease, with similarities to MS, can be

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induced by active sensitisation of experimental animals with nervous system tissue, isolated myelin or purified myelin proteins, interest focused on demyelination and the importance of axonal pathology was ignored for many decades. Emphasis on axonal injury and loss in MS brains was reintroduced, when new technologies in magnetic resonance imaging (MRI) revealed changes, most likely consistent with profound axonal injury and loss, leading to a boom of neuropathological studies during the last two decades, which focused on this issue (Kornek and Lassmann, 1999).

Key features of axonal and neuronal pathology in multiple sclerosis

As mentioned above, axonal injury and loss is present in all demyelinated lesions in MS, but their extent is variable. In chronic established lesions axonal density is reduced in average by 60–70% (Mews et al., 1998; Bjartmar et al., 2000). Acute axonal injury, as seen by the presence of axonal spheroids and end bulbs or detected by the focal accumulation of proteins, which are moved along the axon by fast axonal transport, is mainly seen in actively demyelinating lesions. However, even in inactive plaques a low background of acute axonal injury, reflected by local accumulation of amyloid precursor protein, is present (Kornek et al., 2000). These data superficially suggest that the bulk of axonal injury occurs during lesion activity. However, the stage of lesion activity only lasts for a few days to weeks, while chronic demyelinated lesions persist for months to years. Thus, slowly progressive loss of demyelinated axons in chronic MS lesions may make a major contribution to global axonal loss in MS brains. Stable remyelinated shadow plaques in general show a very low degree of ongoing axonal injury, which suggest that remyelination protects axon integrity (Kornek et al., 2000). However, at early stages of remyelination quite pronounced ongoing axonal injury is seen, suggesting that at early stages of remyelination axons may even be more vulnerable than when they are completely demyelinated (Kuhlmann et al., 2002). This may be related to a particular pattern of Na⁺ channel expression at the widened nodes of Ranvier in early stages of remyelination (Smith, 2006).

Axonal injury, however, is not restricted to focal demyelinated lesions. It is also seen, although in lower extent, in the normal appearing white matter (Kutzelnigg et al., 2005; Frischer et al., 2009). There, ongoing axonal destruction can in part be explained by secondary Wallerian degeneration, leading to nerve fiber degeneration in tracts, traversing focal white matter lesions (Evangelou et al., 2000). This, however, cannot be the sole explanation, since the correlation between diffuse axonal injury in the normal appearing white matter with the numbers, size and destructiveness of focal lesions is poor (Kutzelnigg et al., 2005; DeLuca et al., 2006; Evangelou et al., 2005). Since inflammation in MS is not restricted to focal white matter lesions, but affects the brain of patients with progressive MS diffusely, axonal injury in the normal appearing white matter appears in part to occur independently from focal plaques (Kutzelnigg et al., 2005). All these data suggest that there are two patterns of axonal destruction in MS. One takes place within demyelinated lesions and is associated with primary demyelination and its extent at a given time point correlates with lesional activity. In addition, there is a diffuse axonal injury, which is also associated with inflammation, but is present within the whole brain and spinal cord and seems to occur also in non-demyelinated nerve fibers. Overall, there is a highly significant correlation between inflammation and the extent of axonal injury not only in focal white matter lesions but also in the normal appearing white matter (Frischer et al., 2009). Furthermore, in some patients, at the late stage of the disease, inflammation in the central nervous system of MS patients may decline to levels, seen in age matched controls. In these patients axonal injury within lesions and the normal appearing white matter, too, declines to levels seen in aged matched controls (Frischer et al., 2009). In line with this finding,

axonal injury within inactive white matter lesions also correlates well with the degree of residual inflammation and microglia activation (Kornek et al., 2000). All these data show that axonal injury in the MS brains is invariably associated with inflammation, suggesting that inflammation drives axonal damage in MS.

An important issue in understanding mechanisms of axonal injury in MS is the size selective axonal degeneration within the lesions (Evangelou et al., 2001). Small calibre axons are much more vulnerable to injury compared to thick axons. Thus, in inactive chronic MS lesions thick axons are much better preserved than thin ones and these thick axons may even show larger diameters compared to those in the normal appearing white matter (Shintaku et al., 1988). The explanation for this preferential destruction of thin axons is their high energy demand in relation to focal mitochondrial mass. As will be discussed below, mitochondrial injury with subsequent energy deficiency seems to be an important factor in the induction of axonal injury in MS lesions (Trapp and Stys, 2009).

In relation to that for axonal injury, much less information is available regarding neuronal injury and loss in MS patients (Wegner et al., 2006; Kutzelnigg et al., 2007; Vercellino et al., 2005). Several studies have addressed the question of neuronal loss in the cerebral cortex. The results are quite divergent, describing neuronal loss ranging from about 10% to no neuronal loss in comparison the respective controls, when present, being most pronounced in demyelinated cortical lesions (Wegner et al., 2006). The reason for these discrepancies may reside in the variability of MS lesions, some lesions showing profound and aggressive tissue injury, while in others demyelination is slowly developing, leaving neurons and axons largely unaffected. In more fulminate cortical lesions acute neuronal death and apoptosis has been described (Peterson et al., 2001). Neuronal loss has also been seen in lesions in the brain stem grey matter (Cifelli et al., 2002) and in the grey matter of the spinal cord (Gilmore et al., 2009). In these areas neuronal loss may be more pronounced than in the cortex.

Axonal loss in lesions of autoimmune encephalomyelitis is similar to that seen in MS lesions

Like in MS, axonal injury and loss is also pronounced in the lesions in experimental autoimmune encephalomyelitis. The extent of axonal loss, however, depends upon the species and strain of animals and the procedure of the induction of experimental autoimmune encephalomyelitis (EAE). Widespread axonal damage is seen in most EAE models, induced in mice by active sensitisation. In such lesions primary demyelination, although present, is sparse and axonal destruction with secondary demyelination is profound. In these models EAE lesions are basically induced by auto-reactive T-cells of the Th-1 or Th-17 phenotype (Aranami and Yamamura, 2008). Much more selective primary demyelination is seen in models, which are induced by a combined action of encephalitogenic T-cells and demyelinating antibodies. In these models, which are mainly induced in guinea pigs, rats and primates, T-cell mediated inflammation results in only limited tissue damage (Lassmann, 1983; Ben Nun et al., 1981; Aboul-Enein et al., 2006). When, however, antibodies against a conformational epitope of myelin oligodendrocyte glycoprotein (MOG), which is a target for demyelinating antibodies, are present, large focal lesions of primary demyelination are seen (Lington et al., 1988). Also in these lesions axonal injury is invariably present and axonal injury at different stages of lesion development is very similar to that seen in MS lesions analysed at comparable stages of their development (Kornek et al., 2000). As will be discussed below, also some of the downstream mechanisms of axonal injury, which are relevant for MS, are well reflected in the experimental models of EAE. These include expression of ion channels, calcium dependent activation of proteases, disturbance of fast axonal transport and final axonal dissolution. Thus, the conclusion is that neuroprotective

strategies aimed to ameliorate axonal damage in MS lesions can in principle be tested in respective EAE models.

This, however, mainly accounts to the study of axonal injury in focal white matter lesions, as seen in patients with acute and relapsing MS. A modelling of progressive MS in EAE is so far not well established. Although some mouse and rat models show a progressive disease course from the onset and are, thus, regarded as experimental models of progressive MS (Hampton et al., 2008), their pathology resembles MS lesions in the progressive stage only poorly. A chronic inflammatory process, which is trapped within the central nervous system and is associated with slow expansion of pre-existing focal white matter lesions or profound diffuse damage of the normal appearing white matter, which is typical for progressive MS (Kutzelnigg et al., 2005), has so far not been described convincingly in chronic EAE models. Extensive subpial cortical demyelinating lesions, a hallmark of the pathology seen in progressive MS (Bo et al., 2003; Peterson et al., 2001; Kutzelnigg et al., 2005), can be induced in some rat strains and in primates by active sensitisation with MOG (Pomeroy et al., 2005; Storch et al., 2006). However, in these animals cortical demyelination is induced by demyelinating anti-MOG antibodies, while in MS evidence for antibody mediated cortical demyelination is so far lacking and most cortical lesions in MS patients show very little leakage of immunoglobulins and no complement activation in actively demyelinating cortical areas (Brink et al., 2005).

Finally, as will be discussed below, many different mechanisms of axonal injury have been described in experimental models of brain and spinal cord inflammation and these mechanisms seem to be different, depending upon the model analysed. Which of these mechanisms of axonal injury are most relevant for MS is currently uncertain.

Experimental models reveal different mechanisms of axonal injury in inflammatory lesions of the central nervous system

Depending upon the experimental model different mechanisms of axonal injury in inflammatory lesions of the brain and spinal cord have been described. They involve mechanisms of adaptive immunity, mediated through T-lymphocytes and antibodies, as well as of innate immunity, mainly driven by activated macrophages and microglia.

Axonal and neuronal injury driven by class I MHC restricted cytotoxic T-cells

A pre-requisite for the formation of an immunological synapse between cytotoxic effector lymphocytes and their target cell is MHC restricted antigen presentation. Class I MHC molecules, required for the presentation of antigen to CD8⁺ cytotoxic T-cells, can be induced in neurons and glia cells by pro-inflammatory cytokines, such as γ -interferon (Neumann et al., 1995). A further regulatory step for the expression of MHC class I antigens is the electrical activity of neurons. Intact, electrically active neurons do not express the full machinery necessary for antigen presentation to cytotoxic T-cells, but when neurons are silenced they do (Neumann et al., 1995). In this situation, neurons can be attacked by class I restricted cytotoxic T-cells, provided they contain the cognate antigen (Medana et al., 2000). This not only applies for neuronal cell bodies, but also for their processes, such as axons (Medana et al., 2001). Thus, axonal transection can be induced by an antigen-specific cytotoxic T-cell reaction. This mechanism seems to be particularly important in the pathogenesis of virus induced inflammatory diseases of the central nervous system. As an example, disease severity and axonal destruction is ameliorated in Theiler's virus induced encephalomyelitis, when cytotoxic T-cells or MHC class I expression is blocked, in spite of similar inflammation and demyelination (Howe et al., 2007). In this model, however, blockade of axonal destruction is not necessarily

beneficial, since it facilitates virus spread and the propagation of infection through axonal transport (Tsunoda et al., 2007).

Cytotoxic, class I MHC restricted T-cells seem to play a major role in the pathogenesis of multiple sclerosis. They dominate the inflammatory infiltrates in MS at all stages of disease and lesions and they show preferential clonal expansion within the central nervous system (Babbe et al., 2000). In lesions from patients with fulminate disease close attachment of CD8⁺ T-cells to demyelinated axons is sometimes seen and these cells show a polarized arrangement of their cytotoxic granules towards the site of contact with the axons (Neumann et al., 2002). This may reflect the formation of an immunological synapse between axons and cytotoxic T-cells, similar to that seen in the above-described *in vitro* studies. However, this scenario is rare in MS and restricted to lesions with very severe inflammatory reaction.

Axonal and neuronal injury induced through class II restricted T-cells

Classical models of experimental autoimmune encephalomyelitis are driven by class II MHC restricted Th-1 or Th-17 cells (Aranami and Yamamura, 2008). Pure CD4⁺ T-cell mediated EAE in most animal species results in inflammation with very little tissue injury (Ben Nun et al., 1981). This is different in mice, where pure T-cell mediated EAE is associated with large lesions predominantly in the spinal cord, brain stem and cerebellum, which are characterized by demyelination with massive axonal injury (Kerlero de Rosbo et al., 1995). Generally such models are driven by T-cells, which react against myelin antigens, in particular against MOG. However, in a recent study it was shown that immunization of Biozzi mice with neurofilament leads to severe EAE. In comparison to MOG EAE more severe axonal and grey matter pathology was seen in animals with neurofilament induced EAE (Huzinga et al., 2008). A similar situation was recently reported in MOG T-cell receptor transgenic animals. In these animals MOG directed T-cells not only recognized MOG but also a peptide of neurofilament (Krishnamoorthy et al., 2009). Also in these animals large lesions were present with very extensive axonal injury. Superficially, these data suggest that class II restricted T-cells may induce axonal injury, when they are directed against a protein component of the axon. This interpretation, however, is unlikely, since neurons and axons do not express MHC class II molecules and, therefore, direct cytotoxicity, involving the formation of an immunological synapse, is hard to imagine. An alternative explanation is that neurofilament is much more abundant in the CNS compared to MOG and neurofilament peptides are hydrophilic. Thus, in contrast to MOG it can be expected that the extracellular concentration of neurofilament, available for antigen presentation, is much higher and thus reactivation of T-cells in the CNS will occur much more efficiently. This may lead to more severe inflammation with more tissue damage. In support of this view neurofilament protein can be easily detected in the human cerebrospinal fluid (Teunissen et al., 2005), while this has so far not been seen for MOG.

Some data suggest that CD4⁺ T-cells can induce tissue injury also in an antigen independent way. Using dual photon confocal laser microscopy on live brain tissue Nitsch et al. (2004) found that T-cells against a myelin antigen (proteolipid) as well as against an irrelevant antigen (ovalbumin) induce calcium oscillations and cell death in neurons, which they contacted in the passage through the brain extracellular space. This neuronal injury was independent from specific antigen recognition, but could be prevented by the blockade of either perforin or glutamate receptors (Nitsch et al., 2004). These data imply that just the migration of activated T-cells through the brain may induce neurodegeneration in a way, which is independent from antigen recognition. The caveat regarding this study is that it was performed in *ex vivo* brain slices co-cultured with T-cells, where the threshold of neurons for degeneration may be lower than in the intact tissue *in vivo*. Furthermore, many models of acute T-cell mediated

autoimmune encephalomyelitis are reflected by massive T-cell infiltration within the CNS without evidence for any neuronal or axonal destruction. Thus, whether such a non-specific T-cell induced neurodegeneration plays a role in brain inflammation *in vivo* is currently uncertain.

Antibody mediated axonal or neuronal destruction

It is now well established that auto-antibodies, when they reach their target in the central nervous system under inflammatory conditions, lead to selective damage of their target cells (Linnington et al., 1988). Auto-antibodies against molecules, expressed on the surface of neurons and axons, are thus potential candidates for mediating axonal or neuronal injury. Recently it was found that a subset of patients with multiple sclerosis mount an antibody response, which is directed against neurofascin, a molecule, which is expressed on axons and oligodendrocyte processes at the node of Ranvier (Mathey et al., 2007). When such antibodies against neurofascin are systemically injected in animals with brain inflammation due to T-cell mediated autoimmune encephalomyelitis, a profound augmentation of clinical disease is seen. This is associated with significantly more severe axonal injury within the lesions. Furthermore, these antibodies selectively bind to axons at the node of Ranvier and may also disturb axonal conduction by interference with the function of nodal sodium channels (Mathey et al., 2007). This is the first example, providing unequivocal evidence that auto-antibodies against axonal components can directly be involved in the induction of functional and structural axonal damage. Since neurofascins are also expressed on oligodendrocytes and the antigen is exposed and accessible during remyelination, such antibodies could also impair myelin repair. Whether neurofascin is the sole axonal or neuronal target for pathogenic antibodies in inflammatory brain lesions is currently unresolved.

Axonal and neuronal injury induced by toxic products produced by activated macrophages or microglia

In MS lesions axonal injury is closely associated with macrophages and microglia, which are seen in close contact with axons, that reveal acute axonal injury, such as the formation of axonal spheroids or a disturbance of fast axonal transport (Trapp et al., 1998; Ferguson et al., 1997). Activated macrophages and microglia produce a large array of toxic molecules, which may potentially induce axonal injury. They include proteo- and lipolytic enzymes, cytotoxic cytokines, excitotoxins and reactive oxygen or nitric oxide intermediates. For axonal injury oxygen and nitric oxide radicals seem to be of particular importance. In rats with acute T-cell mediated autoimmune encephalomyelitis profound neurological deficit is seen, despite the fact that spinal cord lesions just show inflammation with very little demyelination or axonal destruction. However, inflammation leads to profound disturbance of axonal transport in axons, adjacent to perivascular inflammatory infiltrates and the time course of acute and reversible axonal injury correlates well with the clinical severity of the disease (Aboul-Enein et al., 2006). Acute axonal injury is seen only in areas, which contain macrophages with acute axonal injury is seen only in areas, which contain macrophages with profound cytoplasmic expression of inducible nitric oxide synthase and nitrotyrosin reactivity within the tissue. These data suggest that nitric oxide radicals may induce reversible axonal dysfunction in EAE, which is associated with clinical deficit (Aboul-Enein et al., 2006). Spinal cord axons undergo reversible conduction block, when exposed to nitric oxide radical donors and demyelinated axons are more vulnerable compared to intact myelinated ones (Redford et al., 1997). Furthermore, when axons, exposed to nitric oxide, are electrically active, irreversible axonal injury and destruction ensues (Smith et al., 2001). Thus a high energy-demand increases the

susceptibility of axons for radical mediated injury. One possible explanation for this observation is, that both, oxygen and nitric oxide radicals impair mitochondrial function.

A number of recent data suggest that mitochondrial injury and subsequent energy failure is a major component in the pathogenesis of MS lesions. MS lesions in their most aggressive forms show pathological changes reminiscent of a hypoxia-like tissue injury, which is associated with up-regulation of molecules, involved in (hypoxic) preconditioning (Aboul-Enein et al., 2003; Stadelmann et al., 2005). Within such lesions, profound mitochondrial injury is seen, which most severely affects the expression of COX1, a molecule of the mitochondrial respiratory chain, which is particularly vulnerable to radical mediated damage (Mahad et al., 2008a). Similar mitochondrial changes are also seen in chronic active MS lesions, albeit in less severe manifestation (Mahad et al., 2009). In chronic established lesions mitochondrial number and function is increased in axons, suggesting a compensatory reaction of preserved axons to injury (Mahad et al., 2008b, 2009).

Mitochondrial injury in MS lesions is not restricted to axons. It is also pronounced in oligodendrocytes and may be involved in the induction of oligodendrocyte apoptosis in active lesions (Mahad et al., 2008a). It is also seen in astrocytes, but these cells appear to be more resistant to energy deficiency. Furthermore, mitochondrial injury may also play a major role in neurodegeneration in cortical lesions in MS (Dutta et al., 2006).

Consequences of mitochondrial dysfunction and energy failure for axons and neurons

Energy deficiency has fatal consequences for axons (Trapp and Stys, 2009; Mahad et al., 2008b). The axonal Na⁺ pump is dependent upon the availability of ATP. Thus, in electrically active nerve fibers Na⁺ accumulates in the axonal cytoplasm in conditions of energy deficiency. Axonal Na⁺ is then replaced by calcium due to a reverse operation of the Na⁺/Ca⁺⁺ exchanger, resulting in increasing Ca⁺⁺ concentrations. Ca⁺⁺ dependent proteases, such as calpains, are activated in such a condition and degrade structural proteins, such as neurofilaments and microtubules (Stys and Jiang, 2002). This finally invokes a disturbance of axonal transport with the consequence that certain channels, which are normally expressed in synapses, become locally inserted in the cell membrane of dystrophic axons. An example for this is the aberrant accumulation of voltage gated calcium channels at sites of injury, which may further increase Ca⁺⁺ overload within and final degeneration of the affected axonal segment (Kornek et al., 2001; Gadjanski et al., 2009). The consequences of this process are further augmented by the energy demand of demyelinated axons, which is higher than that in intact myelinated fibers. Furthermore, energy deficiency within axons and neurons increases their susceptibility to excitotoxic (glutamate mediated) injury (Abramov and Duchon, 2009; Mahad et al., 2008b). It is important to note that not all axons are affected by this process equally. Thin calibre axons, which are the main target for neurodegeneration in MS, are much more vulnerable than thick axons. The reason for this size dependent susceptibility is that thin axons have much lower numbers of mitochondria in relation to their membrane surface area and are thus more vulnerable to energy deficiency (Stys, 2005).

One possible neuroprotective strategy to ameliorate axonal injury is the blockade of Na⁺ channels, thus preventing the above-described secondary pathogenic cascade of axonal injury. Indeed, treating EAE animals with sodium channel blockers reduced clinical disease and axonal injury in the lesions (Lo et al., 2002; Bechtold et al., 2006; Black et al., 2006). However, Na⁺ channels are also present on immune cells and therefore also inflammation was reduced in the animals. Thus, it is difficult to judge whether the beneficial effect was due to neuroprotection or the anti-inflammatory effect. Furthermore, rapid

Table 1
Mechanisms of axonal and neuronal injury identified in experimental models.

Type	Mechanism	Relevance for MS
Antigen-specific T-cell cytotoxicity	Cytotoxic class I MHC restricted T-cells; Formation of an immunological synapse Destruction through cytotoxic granules or death receptor interactions	Major pathway in virus induced encephalomyelitis Few evidence in MS (only in highly inflammatory fulminate lesions)
Antigen independent T-cell cytotoxicity	Both CD4 and CD8 positive T-cells Perforin dependent	So far only established in vitro In vivo relevance uncertain
Antibody mediated axonal injury	Specific auto-antibodies against neurofascin Augment disease and axonal injury in autoimmune encephalomyelitis	Neurofascin auto-antibodies seen in a subset of multiple sclerosis patients
Macrophage mediated axonal injury	Activated macrophages and microglia produce a large battery of cytotoxic molecules Macrophage blockade effectively reduces disease and lesions in autoimmune encephalomyelitis	Axonal injury in multiple sclerosis is associated with close apposition of activated macrophages/microglia
Mitochondrial injury and energy failure	Most likely induced by oxygen and nitric oxide radicals Blockade of reactive oxygen species is beneficial in autoimmune encephalomyelitis Nitric oxide radicals induce conduction block and axonal degeneration, depending upon energy demand Energy failure potentiates glutamate excitotoxicity Energy failure induces ionic imbalance (Na ⁺ , Ca ⁺⁺) in axons	Evidence for oxidative damage in multiple sclerosis lesions Profound mitochondrial injury in acute and chronic MS lesions Preferential injury of thin axons supports energy failure as a pathogenic mechanism

cessation of treatment induced a rebound of the disease with high mortality of the animals (Black et al., 2007). Clinical studies are currently underway to test a neuroprotective effect of a Na⁺ channel blocker in multiple sclerosis patients. In light of the results described above, there are potential dangers associated with this trial. In addition, one has to keep in mind that blockade of Na⁺ channels in MS patients with demyelination and profound axonal loss may also increase clinical disease by functionally impairing conduction of nerve fibers. To circumvent these potential problems acid sensing Na⁺ channels have recently been targeted for experimental therapy. Such channels are preferentially expressed in lesions and their function depends upon a low tissue pH. Selective blockers for these channels, too, were found to be neuroprotective in EAE (Friese et al., 2007).

Mechanisms of axonal and neuronal injury in inflammatory lesions of the central nervous system are in part different

Most studies on neurodegeneration in EAE and MS have so far focused on axons. During the last years, however, a model has been developed, which allows simultaneous and accurate evaluation of both axonal and neuronal injury. This model is based on the analysis of the optic nerve and the retina in EAE animals, specifically selected for their preferential affection of the optic system (Sättler et al., 2008). The advantages of this system for such studies are the rather simple anatomical orientation of the neuronal population in the retina, which can accurately be quantified. In addition, the retina as well as the optic nerve are accessible for in vivo imaging (Boretius et al., 2008) and by tracing methods the cell bodies of axons, affected in the optic nerve, can be identified. In this model inflammatory demyelinating lesions in the optic nerve are associated with profound apoptotic degeneration of retinal ganglion cells (Meyer et al., 2001). Due to these advantages this model has extensively been used during recent years to study mechanisms of axonal and neuronal injury in EAE and to test neuroprotective strategies (for review see Diem et al., 2007). A key lesson learnt from this model is that the mechanisms of degeneration of axons and their respective neuronal cell body in part differ from each other and that different neuroprotective strategies have in part differential (or opposing) effects. An example is methylprednisolone, which ameliorates inflammatory demyelination in the optic nerve, but simultaneously increases retinal ganglion cell apoptosis (Diem et al., 2003). Interestingly, neuronal degeneration in EAE animals, treated with methylprednisolone, was prevented, when the animals were additionally treated with erythropoietin (Diem et al., 2005). A similar dissociation was seen when animals were treated with

glatiramer acetate or β -interferon (Maier et al., 2006). Both treatments had an effect on the inflammatory demyelinating lesions in the optic nerves, but only glatiramer acetate also protected retinal ganglion cells (Table 1).

All the data indicate that degeneration of axons and their cell bodies follow in part different pathogenic pathways, and that a neuroprotective treatment may have to target both in part by different therapeutic strategies.

Conclusions

Degeneration of axons and neurons is an important feature of MS pathology and appears to be the major correlate for permanent clinical deficit in the patients. Experimental models show that in the context of inflammation axons and neurons can be destroyed by a variety of different mechanisms and that these mechanisms are in part different for axons and their respective cell bodies. In a complex disease such as MS it is likely that most of these different mechanisms play some pathogenetic role in different patients or stages of disease and lesion formation. However, recent data suggest that in MS lesions a major pathway of neurodegeneration involves mitochondrial injury with subsequent energy failure, which is most likely induced by oxygen and nitric oxide radicals. First clinical trials to target these mechanisms are currently underway.

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