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# Hypoxia-like tissue injury as a component of multiple sclerosis lesions

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## Abstract

Recent data suggest that the mechanisms of demyelination and tissue damage in multiple sclerosis (MS) are heterogenous. In this review, evidence is discussed, which show that in a subset of multiple sclerosis patients the central nervous system (CNS) lesions show profound similarities to tissue alterations found in acute white matter stroke, thus suggesting that a hypoxia-like metabolic injury is a pathogenetic component in a subset of inflammatory brain lesions. Both, vascular pathology as well as metabolic disturbances induced by toxins of activated macrophages and microglia may be responsible for such lesions in multiple sclerosis.

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## 1. Introduction

Recent studies on the immunopathology of multiple sclerosis (MS) revealed an interindividual heterogeneity in the patterns leading to demyelination and tissue destruction [1,2]. Although all lesions are characterized by a chronic inflammatory process, dominated by T lymphocytes and macrophages, the structural and immunological features of demyelination within active lesions differ between patients. The data indicate that there is a basic mechanism of demyelination and tissue destruction, which is present in all cases and lesions and which is mediated by T lymphocytes in cooperation with activated macrophages [3]. Since Class I MHC restricted CD8+ cells dominate the T cell infiltrates in MS [4,5], tissue damage in these lesions may be accomplished by cytotoxic T cells themselves. In addition toxic products, released from macrophages and microglia cells in the course of the inflammatory process apparently are involved in myelin and axonal destruction [6–9]. This basic pattern of lesion pathology (Pattern I [2]), however, is overlaid by amplification factors, which differ between patients. Thus, in some cases a massive deposition of immunoglobulins and components of activated complement is present on degenerating myelin and oligodendrocytes, suggesting an antibody mediated demyelinating process (Pattern II [10–12]). In

other cases, profound degeneration and cell death of oligodendrocytes can be found in the periplaque white matter, adjacent to actively demyelinating plaques (Pattern IV [13]). These pathological alterations are consistent with a T-cell and macrophage-mediated demyelinating process, similar to that found in Pattern I lesions, which occurs on the background of an increased susceptibility of the target tissue for immune-mediated damage, that by itself alone is not sufficient to cause tissue damage or disease. Recent epidemiological studies highlight the genetic influence on the target tissue as a factor determining disease severity and outcome in some patients [14–19]. Finally, we identified a pattern of demyelination, which shows a characteristic disturbance of oligodendrocytes, defined as distal “dying back” oligodendroglialopathy with oligodendrocyte apoptosis (Pattern III [2]). As will be discussed below, this pattern of demyelination closely mimics the tissue alterations found in the early stages of white matter ischemia and may thus reflect hypoxic white matter damage as a pathogenetic component of the lesions.

## 2. The structural features of Pattern III lesions

The most characteristic feature of Pattern III lesions in multiple sclerosis patients is a preferential loss of those myelin proteins, which are concentrated in the most distal (periaxonal) processes of oligodendrocytes [2,20]. Thus, at early stages of demyelination myelin associated glycoprotein (MAG) and cyclic nucleotide phosphodiesterase nearly completely disappear from the lesions, while other proteins,

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which are located in the compact myelin, such as proteolipid protein (PLP) and myelin basic protein (MBP) remain well preserved. In addition, myelin oligodendrocyte glycoprotein, which is expressed on the surface of oligodendrocytes and their processes, is completely preserved or even shows increased expression in early stages of active demyelination [2]. This process is associated with apoptotic nuclear changes of oligodendrocytes, finally leading to complete destruction of these cells and the myelin sheaths. Such myelin alterations suggest that an injury in the most distal, periaxonal processes is the initiating event in demyelination, which is then followed by programmed cell death of affected oligodendrocytes. Such a mechanism has been described before as distal or dying back oligodendrogliaopathy [21].

Since this process of demyelination has not been observed in models of acute or chronic autoimmune encephalomyelitis [22], the question arises, whether it is unique for multiple sclerosis or present also in other conditions of inflammatory or noninflammatory white matter damage. Dying back oligodendrogliaopathy has first been described in a model of toxic demyelination induced by cuprizone [21]. Cuprizone is a toxin, which interferes with the cellular energy metabolism. Similar changes of myelin and oligodendrocytes have also been described in certain models of virus infection of the central nervous system (CNS) [23] as well as in some cases of human virus-induced white matter inflammation, such as progressive multifocal leucoencephalopathy [24].

We have recently studied a large spectrum of white matter diseases, both in humans and in experimental animal models [26]. As markers for this type of myelin pathology, we used the preferential loss of MAG and CNPase as well as apoptotic nuclear changes in oligodendrocytes. We were unable to find this pattern of demyelination so far in any model of acute or chronic autoimmune encephalomyelitis nor in any experimental model of virus-induced white matter pathology, such as Theiler's virus, corona virus or canine distemper virus encephalomyelitis. In the spectrum of human white matter diseases distal, dying back oligodendrogliaopathy was found, besides in a subset of MS patients, also in some cases suffering from virus-induced white matter disease, such as progressive multifocal leucoencephalopathy, (PML), herpes simplex or cytomegalovirus encephalitis. However, in each of these virus-induced diseases distal oligodendrogliaopathy was only present in a subset of cases and was not related to the overall activity of the demyelinating process or the expression of virus antigen in oligodendrocytes. Furthermore, in more chronic virus-induced diseases of the white matter, such as subacute sclerosing panencephalitis or HIV–encephalitis, similar myelin alterations were absent in spite of ongoing demyelination and/or tissue destruction. These findings led to the conclusion that a very severe and profound inflammatory process in the white matter may, in some instances, be associated with a destructive process of myelin, resembling distal, “dying back” oligodendrogliaopathy, irrespective of the primary cause of the disease.

### **3. Distal oligodendrogliaopathy is a characteristic feature of acute hypoxic/ischemic white matter damage**

The condition in which distal oligodendrogliaopathy was found most consistently was acute white matter stroke. During the first week of white matter ischemia myelin appears pale in conventional myelin stains. In these areas there is a complete loss of MAG and CNPase, while MBP and PLP are only marginally affected. MOG in contrast shows increased immunoreactivity in affected areas. Oligodendrocytes can still be detected within the lesions, but many of them show nuclear condensation and fragmentation, reminiscent of apoptosis. Axons at this stage are still preserved, although many axons show signs of acute axonal injury, such as for instance the focal accumulation of amyloid precursor protein as an indicator of disturbed axonal transport. One week after onset of clinical disease, lesions become more demarcated and are characterized by a central area of tissue necrosis. At the lesion edges, in some cases, similar myelin alterations compared to those in acute lesions can be seen. In other areas some primary demyelination with axonal preservation is found at the lesion borders, which at later stages may show signs of remyelination, characterized by the appearance of thin myelin sheaths and synthesis of myelin proteins in the oligodendrocyte cytoplasm. Thus acute ischemic white matter lesions follow a pattern of myelin destruction, which closely reflect that found in a subset of patients with multiple sclerosis or virus encephalomyelitis. We thus analyzed in a next step the expression of ischemia-associated markers in stroke and multiple sclerosis lesions at different stages of lesion development. Interestingly, we found in active lesions with distal dying back oligodendrogliaopathy a profound expression of hypoxia inducible factor (HIF 1 alpha), an antigen which is recognized as a marker for hypoxic tissue damage [25]. This was strikingly different to demyelinating lesions, following other patterns of demyelination, where expression of HIF 1 alpha was minimal or absent [26]. Thus, our data indicate that hypoxia is a pathogenetic component of inflammatory brain lesions in a subset of patients with multiple sclerosis or virus-induced white matter disease. As will be discussed below several pathogenetic mechanisms, which are instrumental in ischemic brain lesions damage oligodendrocytes and myelin more effectively than other components of the CNS. It is thus not surprising that incomplete ischemia in the penumbra of a stroke lesion may be associated with primary demyelination.

### **4. Possible pathogenetic mechanisms, responsible for hypoxic/ischemic damage in inflammatory lesions of the central nervous system**

A metabolic state, resembling hypoxia could be induced in inflammatory conditions by two principally different ways: a vascular disturbance, leading to defective microcirculation and subsequent ischemia or the local production

of toxic metabolites, which interfere with mitochondrial energy metabolism. There is evidence that both mechanisms may operate in inflammatory conditions of the central nervous system.

#### 4.1. Vascular factors

##### 4.1.1. Edema and disturbance of microcirculation

The most simple mechanism is a disturbance of microcirculation due to focal edema within inflammatory lesions. There is ample evidence for blood–brain barrier damage and edema formation in multiple sclerosis lesions [27,28]. Active disease in MS patients is best monitored by MRI, using the paramagnetic tracer gadolinium for the detection of blood–brain barrier (BBB) disturbance [29]. This massive alteration of the BBB is also reflected by leakage of serum proteins into the CNS compartment. If swelling of the tissue is impaired by stringent connective tissue or bony constrains, edema may lead to focal disturbance of microcirculation with subsequent ischemia [30]. Such a mechanism may play an important role in the pathogenesis of inflammatory lesions of the spinal cord and the optic nerves and may thus in part explain the extensive, sometimes cystic necrosis of the spinal cord gray matter in patients with transverse myelitis or Devic's neuromyelitis optica.

##### 4.1.2. Inflammatory damage of the vessel wall

Within an inflammatory process, vessel walls can be damaged by several different ways. As seen in early graft rejection or in vasculitis, an extensive inflammatory reaction at the vessel wall may lead to activation of the clotting cascade, resulting in local microvascular thrombosis and disturbance of the microcirculation. Such a reaction may either be mediated by antibodies, recognizing specific antigen within the vessel wall or—nonspecifically—through cytokines, extensively produced by macrophages, granulocytes or NK-cells during their passage through the vascular barrier. This may lead to massive endothelial activation with expression of different adhesion molecules [31], loss of thrombomodulin and activation of prothrombotic factors within the microvessels [32].

Besides, antibody or cytokine-mediated mechanisms, also a direct effect of cytotoxic T lymphocytes, may lead to endothelial damage in the inflamed CNS. In a recent experimental model, autoimmune encephalomyelitis was induced by passive transfer of cytotoxic, Class I MHC restricted T cells directed against myelin basic protein (MBP). Recipient animals developed an inflammatory disease of the central nervous system with very peculiar inflammatory lesions in the central nervous system that were characterized by perivascular tissue damage closely resembling that found in acute brain ischemia [33]. The immunological mechanism responsible for such lesions is so far undetermined. Cerebral endothelial cells constitutively express MHC Class I antigens [34] and can thus present

antigen directly to cytotoxic T cells. Since, however, MBP is not directly produced by endothelial cells, their presentation of this antigen can only occur when MBP is liberated in the lesions and reaches the luminal surface of cerebral vessels by diffusion. Alternatively, endothelial damage may also occur nonspecifically by cytokines, produced by activated cytotoxic T cells during their passage through the vessel wall.

Thrombotic occlusion of small microvessels has been observed in some multiple sclerosis lesions, in particular in cases with fulminate acute disease (Marburg's variant of MS) [35,36]. Furthermore, profound vascular alterations have been described in Balo's concentric sclerosis [37] and it has been suggested that a vasculopathy with subsequent hypoxic/ischemic tissue damage is a major pathogenic factor in the development of the concentric lesions. In other cases of acute MS, antibody and complement deposition is prominent within the walls of small microvessels. This is particularly prominent in patients with Devic's type of neuromyelitis optica, where intramural and perivascular deposition of IgM and activated complement is a typical feature of actively demyelinating lesions [38]. In addition, cytotoxic, Class I MHC restricted T lymphocytes dominate the inflammatory infiltrates in multiple sclerosis lesions and show prominent clonal expansion within the demyelinating plaques [4,5]. Attachment of activated CD8+ T lymphocytes to the luminal surface of cerebral endothelial cells is frequently encountered in active MS lesions and their profound expression of granzyme B within cytotoxic granules may suggest a direct cytotoxic attack on endothelial cells.

Thus, all these data suggest that small microvessels in multiple sclerosis lesions can be injured in the course of the inflammatory process. In line with these observation an increase in the density of small microvessels associated with signs of endothelial activation and proliferation has recently been observed in chronic multiple sclerosis lesions, suggesting reactive endothelial and vascular sprouting in the inflamed areas of the central nervous system (Ludwin, personal communication). Furthermore, in some cases perivascular depositis of haemosiderin can be found, representing footprints of chronic microvessel injury [39].

##### 4.1.3. Hypoxia-like metabolic tissue injury in multiple sclerosis lesions

An alternative explanation for the occurrence of hypoxia-like tissue alterations in inflammatory brain lesions is that effector mechanisms, responsible for tissue damage, are shared between inflammatory and ischemic conditions. One possible mechanism is the local production and liberation of excitotoxins. Excitotoxins are not only liberated by damaged neurons but also by activated macrophages and microglia [40]. Since oligodendrocytes as well as neurons and axons are highly susceptible to excitotoxic injury, such a mechanism may in part be responsible for tissue damage in an inflammatory demyelinating disease. An unbalanced

glutamate homeostasis has recently been suggested to occur in multiple sclerosis lesions, reflected by increased glutaminase expression in macrophages or microglia and reduced expression of glutamate dehydrogenase in oligodendrocytes [41]. Furthermore, blockade of excitotoxicity by specific antagonists for AMPA-receptors ameliorated clinical disease and tissue damage in autoimmune encephalomyelitis [42,43].

Another effector pathway of inflammation, which may directly impair mitochondrial energy metabolism, is the action of reactive oxygen species (ROS) and nitric oxide intermediates (RNI). In active MS lesions inducible nitric oxide synthase (i-NOS) is highly expressed mainly in macrophages and microglia [43–45] and deposition of nitrotyrosine, a footprint for peroxynitrite formation, is present [46,47]. RNIs, mainly in cooperation with ROS, may play a prominent role in the pathogenesis of oligodendrocyte injury [48] as well as in functional and structural disturbance of axons [49,50]. Besides direct effects on ion channels [51], direct lipid peroxidation and the induction of DNA damage [52], a major mechanism of the toxic action of ROS and RNI, is the impairment of mitochondrial function, leading to a state of energy failure [53,54]. This is also reflected in the expression of hypoxia associated molecules such as HIF 1 alpha within affected cells [55]. Thus the tissue damage induced by ROS and RNI apparently leads to a metabolic injury, quite similar to that present in hypoxic/ischemic conditions [25]. For these reasons a hypoxia-like tissue injury in inflammatory brain lesions not necessarily has to be due to vascular pathology, but may also reflect a metabolic impairment induced by toxic products from activated macrophages and microglia cells.

## 5. Conclusions

The pathogenetic mechanisms leading to tissue damage in multiple sclerosis are complex and may, in different patients to a different degree, involve cytotoxic T lymphocytes, toxic products of activated macrophages, specific autoantibodies, a genetic susceptibility of the target tissue and—as summarized here—hypoxia-like tissue injury. Elucidation of these different pathways of tissue damage in multiple sclerosis may lead to new paraclinical markers, which could allow a subclassification of MS patients according to the major effector pathways of tissue injury. Following this concept, we are currently investigating whether the liberation of hypoxia-related molecules into the cerebrospinal fluid may be clinically useful for this purpose.

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