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REVIEW

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Mitochondrial damage and histotoxic hypoxia: a pathway of tissue injury in inflammatory brain disease?

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Abstract The immunological mechanisms leading to tissue damage in inflammatory brain diseases are heterogeneous and complex. They may involve direct cytotoxicity of T lymphocytes, specific antibodies and activated effector cells, such as macrophages and microglia. Here we describe that in certain inflammatory brain lesions a pattern of tissue injury is present, which closely reflects that found in hypoxic conditions of the central nervous system. Certain inflammatory mediators, in particular reactive oxygen and nitrogen species, are able to mediate mitochondrial dysfunction, and we suggest that these inflammatory mediators, when excessively liberated, can result in a state of histotoxic hypoxia. This mechanism may play a major role in multiple sclerosis, not only explaining the lesions formed in a subtype of patients with acute and relapsing course, but also being involved in the formation of diffuse ''neurodegenerative'' lesions in chronic progressive forms of the disease.

Keywords Mitochondrial dysfunction · Histotoxic hypoxia · Inflammatory brain disease · Multiple sclerosis

Introduction

Chronic inflammatory diseases in the central nervous system (CNS), such as multiple sclerosis or virus-induced encephalomyelitis are generally considered to be T lymphocyte-driven disorders [38]. T cells, which are activated in the peripheral immune system, can enter the CNS. When they find their respective antigen within this compartment, they become re-activated, produce pro-inflammatory cytokines and start a focal inflammatory lesion, leading to tissue injury [23].

Immune-mediated tissue injury in inflammatory conditions of the CNS can be accomplished by various means. Cytotoxic T lymphocytes may recognize their target antigen in the context of major histocompatibility molecules and directly attack the target tissue through their cytotoxic machinery [37]. Specific antibodies directed against an antigen, which is accessible from the extracellular space, can opsonize target structures and mediate tissue destruction either by activating complement or by interaction with activated macrophages or microglia [2, 30, 37]. Besides these specific immune reactions, tissue injury can also be accomplished in a nonspecific way through effector cells, such as granulocytes or macrophages, a process called ''bystander tissue damage'' [59]. Such effector cells may become directly activated by products of infectious agents and altered tissue components, or through pro-inflammatory cytokines produced in the course of the inflammatory reaction.

The pathological correlate of such immune-mediated tissue injury is a classical inflammatory lesion of the CNS, reflected by perivascular accumulation and diffuse infiltration of the tissue by lymphocytes and macrophages, which is associated with more or less selective destruction of nervous tissue elements. However, depending upon the dominant type of tissue injury different inflammatory cells prevail within the lesions and the patterns of tissue injury may vary.

However, recent observations, mainly obtained in the course of systematic studies of multiple sclerosis pathology and pathogenesis suggest that there may be an additional mechanism of tissue injury, which bears similarities with the tissue damage seen in hypoxic conditions of the CNS [1]. As clearly illustrated in the paradigm of acute bacterial meningitis, brain inflammation can be associated with vasculitis, thrombosis and vascular occlusion. However, in many cases, such inflammation-induced hypoxia-like lesions occur in the absence of significant vascular pathology. It is, thus,

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suggested that certain inflammatory mediators, such as nitric oxide and oxygen radicals, can induce mitochondrial dysfunction [4, 7, 8, 19, 32], which results in a form of histotoxic hypoxia. The recognition of this pathway of tissue injury in brain inflammation may have major therapeutic consequences, since neuroprotective strategies developed for stroke lesions may also have beneficial effects in certain inflammatory brain diseases.

The pattern of tissue injury in acute white matter ischemia

Studies on brain ischemia have mostly concentrated on changes in the gray matter. However, white matter lesions are particularly informative, since they follow a highly characteristic pattern of tissue injury, at least during the earliest stages of tissue destruction [1]. Earliest alterations, found during the first days after onset of the disease, are reflected by the appearance of circumscribed areas of myelin pallor (Fig. 1a) with some acute ischemic changes in glia cells, which are demarcated from the surrounding normal tissue by a small zone of edematous vacuolization. Axons are still well preserved within these lesions. At later stages, myelin is removed and taken up by local macrophages. Axons disintegrate mainly in the center of the lesions, but may remain in part preserved in the periphery [60]. This finally leads to a lesion with a cystic core, surrounded by an area of primary demyelination of variable size. During this stage numerous oligodendrocytes can be seen in the areas of partial tissue preservation, which express high levels of myelin protein mRNAs and show cytoplasmic reactivity for myelin proteins [52]. These are unequivocal signs of remyelination (Fig. 1m–p). An important feature of ischemic white matter lesions is that perivascular tissue is in general well preserved, resulting in small rims of normal myelinated tissue around small and medium-sized vessels (Fig. 1d–f).

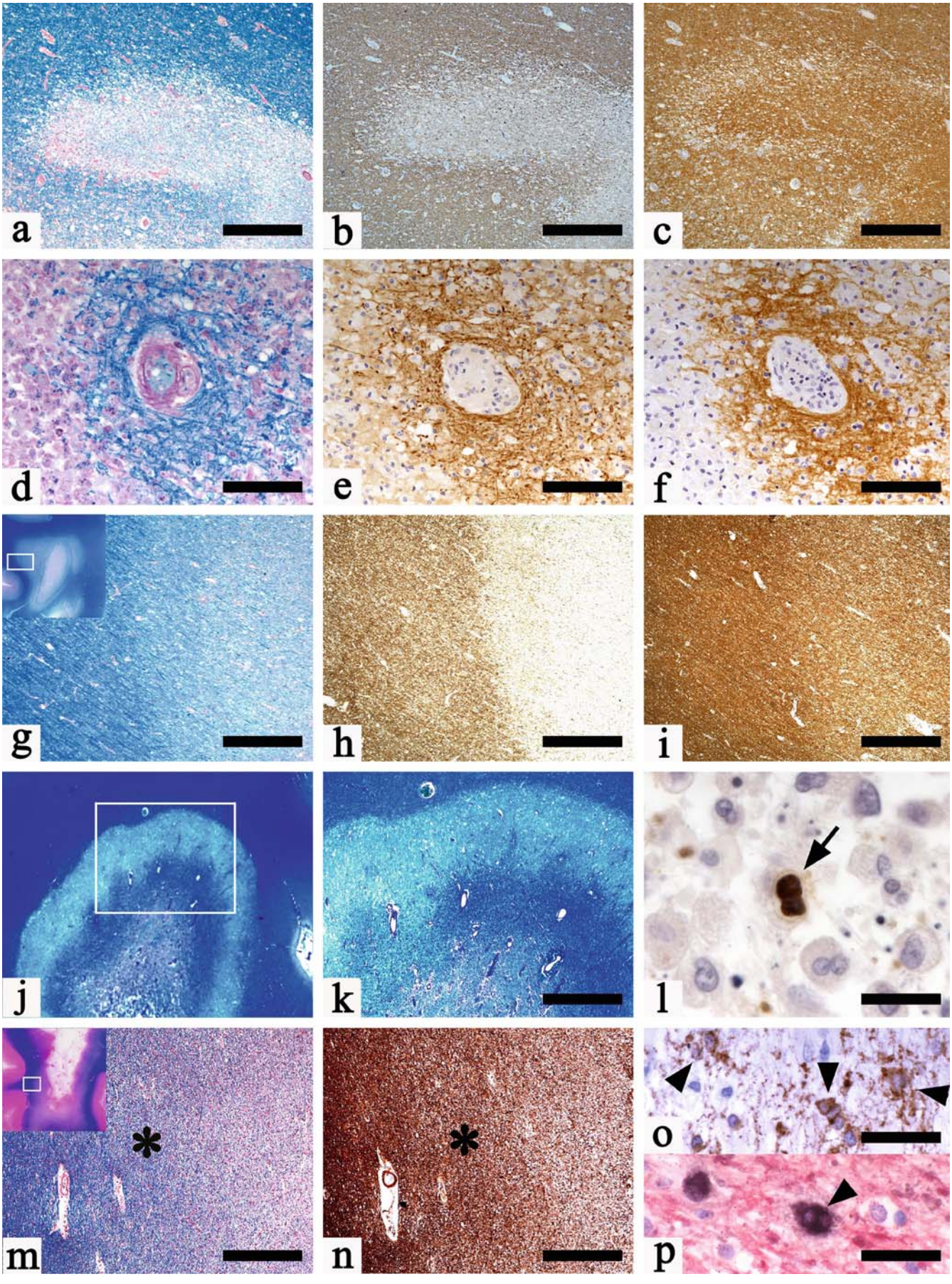
When the molecular changes in such initial white matter lesions are studied in more detail further characteristic patterns of tissue injury appear [1]. In the areas of myelin pallor, those myelin proteins that are located in the most distal processes of oligodendrocytes (as for instance myelin-associated glycoprotein and cyclic nucleotide phosphodieserase; [9, 53]) are completely lost, while proteins present in the compact myelin sheaths (myelin basic protein or proteolipid protein) and on the outer surface of myelin and oligodendrocytes (myelin oligodendrocyte glycoprotein) remain preserved (Fig. 1a–c). This differential loss of myelin proteins suggests that the process of tissue injury starts in the most distal processes of oligodendrocytes, reflecting a "dying back" injury of these cells [34]. This is further supported by confocal microscopy and electron microscopy, showing an early clumping and dissolution of the periaxonal oligodendrocyte processes [1, 58]. This distal oligodendrogliopathy in early stroke lesions is associated with nuclear condensation and to a lesser extent fragmentation of oligodendrocytes (Fig. 1c insert). Degeneration of oligodendrocytes reveals morphological changes of apoptosis, which is associated with DNA fragmentation, but not with the expression of caspase-3, and should thus be classified as apoptotic-like cell death [28].

Fig. 1 Myelin changes in stroke and inflammatory brain lesions: **a–f** 85-year-old female, acute stroke, 0.3 weeks disease duration; **g–i** 35-year-old male, acute MS, 6.0 weeks disease duration; **j–l** 55-year-old male, cytomegalovirus encephalitis, 9.0 weeks disease duration; **m–p** 70-year-old female, chronic stroke, 35 weeks disease duration. **a, g, j, k** Acute ischemic brain lesion (**a**), actively demyelinating lesion edge of an acute MS lesion (**g**) of Balo's type (**g**, *inlet*) and concentric layered cytomegalovirus brain lesion (**j**, **k**) show pallor in conventional Luxol-fast blue myelin stains. Lesions are demarcated from the surrounding white matter by an edematous rim. Immunocytochemistry for myelin proteins reveals a nearly complete loss of immunoreactivity for myelin-associated glycoprotein (**b**, **h**), whereas immunoreactivity for myelin oligodendroglial glycoprotein (**c**, **i**) remains well preserved. Note, in perivascular areas myelin sheaths remained intact (**d–f**). **l** Nuclear expression of cytomegalovirus protein in a mononuclear cell within CNS lesion (*arrow*). **m–p** Chronic stroke lesion with area of partial tissue preservation and remyelination (**m**, **n** *asterisks*) exhibit numerous axons (**n**) and oligodendrocytes, which show immunoreactivity for myelin proteins within their cytoplasm (**o** CNP; *arrowheads*) and high levels of myelin protein mRNA (**p** *arrowheads*). **m** Luxol-fast blue myelin stain; **n:** Bielschowsky's axonal silver impregnation; **o** CNP; **p** mRNA for PLP: *black*; PLP protein: *red* (*PLP* proteolipid protein). *Bars* **a–c, g–i** 500 μm; **d–f, o** 50 μm; **l, p** 20 μm; **k, m, n** 1,000 μm; **j,** *inlets* in **g** and **m** represent scans of complete slides

A subset of inflammatory brain lesions show a pattern of demyelination closely resembling that present in acute white matter ischemia

In the course of a systematic study on the pathology of actively demyelinating multiple sclerosis lesions, a profound heterogeneity of the patterns of demyelination and tissue injury was found [34]. Four different basic patterns of demyelination were identified, three of them showing variations which are expected in classical inflammatory brain lesions. The fourth, however, designated pattern III [34] revealed very unusual properties. It was originally defined by an early and complete loss of myelin-associated glycoprotein with preservation of proteins located in compact myelin [20, 21] and apoptotic like cell death in oligodendrocytes [34]. In a subsequent comparative study, it turned out that the pattern of demyelination and tissue injury in these lesions was structurally indistinguishable from that occurring in acute white matter stroke lesions (Fig. 1g–i). The only exception was that in multiple sclerosis these lesions occurred on the background of profound inflammation [1].

In multiple sclerosis such lesions are mainly present in a subset of patients with acute MS as well as in patients with chronic MS with fulminate exacerbations [34]. Moreover, lesions characterized by this specific type of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demyelination were not restricted to multiple sclerosis, but were also present in a subset of patients with various virus-induced brain diseases, such as herpes simplex virus encephalitis, cytomegalovirus encephalitis (Fig 1 j-k) and progressive multifocal leukoencephalopathy [1]. From these observations we concluded that in inflammatory brain diseases a pattern of tissue injury can occur, which closely mimics that found in acute hypoxic and/or ischemic conditions in the CNS.

Molecules associated with hypoxic preconditioning are expressed in a subset of inflammatory brain lesions

Hypoxic brain damage leads to the destruction of glia cells and neurons in the lesions. However, in case hypoxia is incomplete, a cascade of events is set in motion, which increases the resistance of the tissue to subsequent hypoxic damage and is thus instrumentally involved in limiting the expansion of structural damage [6, 44]. This process is called hypoxic preconditioning and is regularly found to take place at the border between the lesion and the surrounding normal tissue.

One master switch in the induction of hypoxic preconditioning is the expression of hypoxia-inducible factors (HIFs) α and β [5, 43, 44]. Forming heterodimers and being translocated into the nucleus, they act as transcription factors, which induce gene expression of various other molecules with neuroprotective functions. These downstream molecules are known to be involved in vasomotor control (inducible nitric oxide synthase, endothelin 1), angiogenesis (vascular endothelial growth factor), cell growth (growth factors) as well as in energy metabolism [44]. Altogether, these proteins render the tissue more resistant to further hypoxia-induced injury. In addition, sublethal damage of the CNS parenchyma at the border of a stroke lesion also induces the expression of so-called stress proteins [10, 55]. In particular, the heat shock protein (HSP) 70, a molecular chaperone, helps to re-nature damaged proteins and also confers profound protection to the tissue against subsequent injurious stimuli.

The expression of these "survival" proteins does not necessarily imply that these lesions occur on a pathogenetic background of hypoxia and/or ischemia. HIF expression can be induced or increased by the action of certain pro- or anti-inflammatory cytokines, such as interleukin-1β, tumor necrosis factor-α and transforming growth factor-β or inflammatory mediators such as nitric oxide [25, 44]. Furthermore, protein denaturation in injured tissue itself induces the expression of HSPs.

Interestingly, all these proteins are expressed at the border between some active inflammatory lesions and the adjacent normal brain parenchyma. While HSP 70 is found at the edges of all inflammatory lesions in the CNS, the expression of HIF-1α, at least at levels detected by immunocytochemistry, is restricted to lesions, which follow the above-described hypoxia-like tissue injury [1]. In a systematic study, performed in more than 80 cases of inflammatory and degenerative CNS white matter disease and in 20 controls, nuclear translocation of HIF-1α was highly significantly ($P < 0.00000001$) associated with lesions, characterized by distal oligodendrogliopathy and apoptotic-like cell death of oligodendrocytes. These cases included multiple sclerosis, various forms of virus encephalitis, stroke and metabolic encephalopathy. The close association between a hypoxia-like pattern of tissue injury and the expression of HIF-1α strongly suggests that energy failure may not only account for lesion formation in stroke, but may also play a role in a subset of patients with inflammatory brain lesions.

The expression of molecules involved in tissue preconditioning may have further metabolic consequences. In active multiple sclerosis lesions following the pattern of hypoxia-like tissue injury, but not in other multiple sclerosis lesions, a concentric layering of myelinated and demyelinated tissue can frequently be observed at the lesion margins [3]. In extreme cases this may form large multilayered concentric plaques, the hallmark of Balo's type of concentric sclerosis. The formation of concentric lesions, however, is not restricted to multiple sclerosis. They may sometimes also be seen in patients with virus infections of the white matter and, in rare instances, at the edge of progressive stroke lesions [11] or in experimental brain damage induced by cyanide intoxication [17]. Within the concentric rims of preserved myelin, HIF-1α and HSP 70 are found mainly in oligodendrocytes, but to a smaller extent also in astrocytes and macrophages (Stadelmann et al., unpublished). Thus, concentric demyelination could at least in part be explained by tissue preconditioning in an expanding active lesion.

Recent microarray data provide further support to the concept, that (hypoxic?) tissue preconditioning is a feature of multiple sclerosis pathology. In a study comparing gene expression in the "normal" white matter of patients with secondary progressive multiple sclerosis with that in normal controls, one of the most consistent difference was found in the expression of HIF-1α and its downstream genes [18].

Patterns of axonal injury in multiple sclerosis are consistent with a mechanism involving energy failure

Multiple sclerosis is an inflammatory demyelinating disease with relative preservation of axons. However, since the earliest descriptions of multiple sclerosis pathology it is well known that axonal injury and loss is a feature found in all plaques (for review see [26]). Furthermore, profound diffuse axonal injury is present in the "normal" white matter of patients with primary or secondary progressive disease [14]. The most sensitive tool nowadays to detect axonal injury in vivo in multiple sclerosis patients is the determination of *N*-acetylaspartate (NAA) levels by magnetic resonance tomography

spectroscopy. With this imaging technique profound reduction of NAA peaks is not only found within plaques, but also in normal-appearing white matter of the entire brain. It was, however, an unexpected finding in these studies that NAA reductions were sometimes reversible. For example, NAA levels in the brain were found to increase in the course of interferon-β treatment, suggesting axonal metabolic recovery [36]. Although part of this recovery of NAA can be explained by the resolution of edema within the plaques, this alone can hardly account for all of it. NAA is mainly present in neurons and their processes and found to be accumulated within mitochondria [33]. Could the marked NAA loss within lesions reflect a transient and reversible mitochondrial dysfunction during the phase of active demyelination and tissue injury ? A similarly reversible NAA loss has also been described in brain hypoxia and CNS trauma [13, 45].

Injury and loss of axons have now been shown convincingly in many studies of multiple sclerosis pathology [16, 27, 54]. Detailed analysis of axonal and neuronal loss in the optic system, however, suggested that small axons are preferentially susceptible to injury in multiple sclerosis patients [15]. It is hard to explain this differential axonal vulnerability by direct toxic action of inflammatory mediators alone. In such a situation differential vulnerability of the axolemma, possibly due to different expression levels of ion channels or excitotoxin receptors between small and large diameter axons, has to be postulated. A better, although so far unproven, explanation for this differential vulnerability may be that energy failure preferentially affects small diameter axons due to their large surface to cytoplasmic volume ratio.

Is hypoxia-like tissue injury in inflammatory brain lesions due to vascular pathology or driven by mitochondrial dysfunction?

Inflammation may result in vascular damage by several different mechanisms. Since endothelial cells constitutively express MHC class I molecules, cytotoxic T cells may recognize their target antigen directly on the luminal surface of brain vessels. This may result in apoptosis of endothelial cells, activation of the clotting cascade and microvessel thrombosis. Similarly, specific antibodies may recognize their antigen at the vessel wall and induce vascular or endothelial damage by complement activation or through macrophage or granulocyte toxins. Endothelial cells can also be activated or damaged directly by pro-inflammatory cytokines. Finally, inflammation leads to edema with focal tissue swelling. This may result in a disturbance of microcirculation, in particular in areas, in which swelling is constrained by bones or tight connective tissue layers.

Microvessel thrombosis has exceptionally been observed within multiple sclerosis lesions [56]. To prove or exclude, whether vascular mechanisms are involved in the formation of hypoxia-like tissue injury in inflammatory brain diseases, we systematically analyzed blood vessels in a large sample of inflammatory and non-inflammatory brain diseases. For this purpose we used by immunocytochemistry markers for endothelial cells (Factor VIII), leukocytes (CD3, CD8, CD20, CD68), thrombocytes (CD 42b), complement C9neo antigen and fibrin (Aboul-Enein et al., unpublished). Although we found microvascular thrombosis, characterized by endothelial damage as well as thrombocyte and fibrin attachments at the vessel wall in some cases, this was only occasionally present in patients with either very severe brain inflammation or in patients, who died due to terminal septic complications. There was no significant association of vascular pathology with hypoxia-like tissue injury. In particular, many patients revealed hypoxia-like injury in the brain lesions in the complete absence of vascular pathology. We thus concluded that vascular pathology is not the main driving force of hypoxia-like tissue injury in inflammatory brain diseases. What could then be the reason for this type of pathology in inflammatory brain diseases?

A variety of different mediators, which are produced in activated effector cells in inflammatory lesions can provoke mitochondrial dysfunction, the most important being reactive oxygen [32] or nitric oxide intermediates [4, 7, 8, 19] or their combined product, peroxynitrite. Exposing cells to nitric oxide in vitro causes mitochondrial damage, leading at high concentrations to cell death but in lower concentration to the induction of genes involved in hypoxic preconditioning. The enzyme responsible for nitric oxide production (NOS-2), is highly expressed in macrophages in multiple sclerosis [12, 31], its expression being particularly prominent in the lesions following a hypoxia-like tissue injury. Furthermore, oxidative damage of mitochondrial DNA with subsequent reduction in the activity of mitochondrial enzymes has been detected in active lesions of chronic multiple sclerosis [32]. Finally, recent micro-array studies in cortical lesions of multiple sclerosis patients with progressive disease have shown a reduction in the expression of certain mitochondrial genes in cortical lesions [35].

Both, oligodendrocytes and axons are highly vulnerable to the action of nitric oxide [46, 47]. Axonal injury induced by nitric oxide is particularly interesting. Not surprisingly, demyelinated axons are more vulnerable to the action of nitric oxide compared to myelinated axons. Interestingly, nitric oxide itself induces a functional, hence reversible conduction block in demyelinated axons [41], while axons, which were additionally exposed to high metabolic stress by repetitive stimulation, degenerate [48]. These observations suggest that irreversible axonal damage by nitric oxide results from a combined effect of direct toxicity and energy failure.

Mitochondrial damage in inflammatory brain lesions can also be triggered by heme oxygenase 1 (HO-1). HO-1 is also highly expressed in actively demyelinating MS lesions. Mitochondrial damage may be accomplished by HO-1 in concert with reactive oxygen species by pathological iron sequestration [42].

Provided the concept is correct that inflammatory mediators can induce brain damage through the induction of mitochondrial dysfunction in multiple sclerosis and other inflammatory brain diseases, it seems likely that a concomitant genetic mitochondrial defect could augment the severity of a disease, such as multiple sclerosis. Mutations in mitochondrial DNA have so far not been identified as a risk factor for multiple sclerosis susceptibility, but are associated with particularly severe disease of the optic system [24]. It is currently not clear whether this is a coincidence or reflects a pathogenetic relation.

Conclusions

As outlined above evidence has accumulated over the last few years that certain inflammatory mediators that are liberated by activated effector cells in virus-induced or putative autoimmune diseases of the CNS can disturb mitochondrial function. This may lead to a state of chronic energy failure within the tissue and subsequent histotoxic hypoxia. Such a pattern of brain damage is found mainly in a subset of MS patients with very severe exacerbations of the disease, but may also play a role in the diffuse brain injury, which is the hallmark of patients with primary and secondary progressive multiple sclerosis. Thus, the ''neurodegenerative component'' of multiple sclerosis, which is thought to drive cortical damage and progressive axonal injury in the ''normal'' white matter of multiple sclerosis patients [22, 29, 39] may largely reflect such a pattern of brain damage. In case this hypothesis holds true, neuroprotective therapeutic strategies that have been developed to cope with white matter injury in stroke may turn out to have beneficial effects in multiple sclerosis patients. In white matter ischemia axonal injury and possibly also damage of oligodendrocytes seems to be driven by ionic imbalance, resulting in intracellular sodium and later calcium accumulation, which is followed by the activation of calcium-dependent proteases [40, 50, 51, 57]. This process can be counteracted by agents, which block sodium channels, the sodium/calcium exchanger [50] and AMPA/kainate receptors [49]. It will have to be shown in the future whether such agents also exert neuroprotective effects in multiple sclerosis patients.

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