Enhanced number and activity of mitochondria in multiple sclerosis lesions

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Abstract
Mitochondrial dysfunction has been implicated in multiple sclerosis (MS) lesion development and progression. Mitochondrial alterations might occur as a response to demyelination and inflammation, since demyelination leads to an increased energy demand in axons and could thereby affect the number, distribution and activity of mitochondria. In this study we aimed to elucidate the expression of mitochondrial proteins and mitochondrial enzyme activity in active demyelinating and chronic inactive MS lesions. Mitochondrial protein expression and enzyme activity in active and chronic inactive MS lesions was investigated using (immuno)histochemical and biochemical techniques. The number of mitochondria and their co-localization with axons and astrocytes within MS lesions and adjacent normal appearing white matter (NAWM) was quantitatively assessed. In both active and inactive lesions we observed an increase in mitochondrial protein expression as well as a significant increase in number of mitochondria. Mitochondrial density in axons and astrocytes was significantly enhanced in active lesions compared to adjacent NAWM, whereas a trend was observed in inactive lesions. Complex IV activity was strikingly upregulated in MS lesions compared to control white matter and, to a lesser extent, NAWM. Finally, we demonstrated increased immunoreactivity of the mitochondrial stress protein mtHSP70 in MS lesions, particularly in astrocytes and axons. Our data indicate the occurrence of severe mitochondrial alterations in MS lesions, which coincides with enhanced mitochondrial oxidative stress. Together, these findings support a mechanism whereby enhanced density of mitochondria in MS lesions might contribute to the formation of free radicals and subsequent tissue damage.

Keywords
Astrocytes; axon; mitochondria; multiple sclerosis; oxidative stress
Mitochondrial alterations in MS lesions

Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) and the most common cause of nontraumatic disability in young adults. Histopathological hallmarks of MS include inflammation, demyelination, loss of oligodendrocytes and neuroaxonal degeneration. The clinical consequence of neuroaxonal injury has been demonstrated in several studies, which report a correlation between the amount of axonal loss, measured by N-acetylaspartate proton magnetic resonance spectroscopy, and clinical disability. Additionally, post-mortem studies have reported a strong association between axonal damage and degree of inflammation in active MS lesions. Remarkably, axonal loss is also observed in non-inflammatory lesions and normal appearing white matter (NAWM), suggesting that other factors also contribute to axonal loss. Currently, it is widely accepted that during inflammation macrophages are responsible for tissue damage and axonal loss by production of nitric oxide (NO), proteolytic enzymes and reactive oxygen species (ROS). Under pathological conditions, high levels of ROS are produced by several enzymes, such as NADPH oxidase and myeloperoxidase and by dysfunctional mitochondria.

Mitochondria play a central role in ATP production, cellular homeostasis, and are involved in apoptotic and non-apoptotic cell death. ATP generation mainly occurs in the oxidative phosphorylation system (OXPHOS), which is located in the inner-mitochondrial membrane and consists of the electron transport chain (complex I-IV) and ATP synthase (complex V). Under physiological circumstances, low levels of ROS are produced as a by-product of oxidative phosphorylation, mainly at complex I and III, which are efficiently removed by mitochondrial antioxidant enzymes. Mitochondrial alterations, however, can lead to increased ROS generation, which cause damage to proteins, lipids and nucleotides. Upon exposure to ROS, cells upregulate the production of endogenous protective proteins, like antioxidant enzymes and mitochondrial heat shock protein 70 (mtHSP70).

Recently, a limited number of studies focused on the involvement of mitochondria in the pathogenesis of MS. Several studies have shown associations between mitochondrial DNA (mtDNA) polymorphisms and MS. Similarly, a number of papers described the occurrence of MS in patients suffering from Leber’s hereditary optic neuritis (LHON), which is caused by mutations in mtDNA. However, primary LHON mutations do not commonly occur in MS, and MS is not caused by mtDNA mutations of pathogenic significance.

Oxidative damage to mitochondrial DNA (mtDNA) and impaired activity of complex I has been observed in chronic active MS lesions. Mitochondrial dysfunction could occur as a response to chronic demyelination of axons. Upon demyelination upregulation and redistribution of sodium channels from the nodes of Ranvier along the entire demyelinated axon occurs in order to facilitate conduction. This requires additional ATP to maintain a functional membrane potential, which is under control of Na+/K+ ATPase. As mitochondria provide 99% of ATP in eukaryotic cells through the OXPHOS system, the amount of mitochondria or OXPHOS enzyme activity per mitochondrion needs to be upregulated to meet the enhanced energy demand in the demyelinated axon. Interestingly, increased numbers of mitochondria have been reported in non-myelinated axons of an experimental animal model, in which myelin formation is abnormal. Enhanced OXPHOS activity and mitochondrial number lead to increased production of intracellular ROS and subsequent
damage to mtDNA, mitochondrial proteins and neurofilaments\textsuperscript{12} and could further contribute to neuroaxonal degeneration.

Surprisingly, little is known about the distribution of mitochondria and mitochondrial activity in MS white matter lesions. Hence, we studied the density, distribution, cellular localization and enzyme activity of mitochondria in distinct MS lesion stages.

**Methods**

*Brain tissue*

Brain tissue from 7 non-neurological controls and 26 patients with clinically diagnosed and neuropathologically confirmed MS was obtained at rapid autopsy and immediately frozen in liquid nitrogen (in collaboration with The Netherlands Brain Bank). White matter MS tissue samples were selected under post-mortem magnetic resonance imaging as published previously.\textsuperscript{25} Relevant clinical information was retrieved from the medical records and is summarized in table 1. The age of MS patients at time of death ranged from 34 to 80 years (mean 60 years) with a mean post-mortem delay of 9 hours. The age of the non-neurological controls ranged from 24 to 84 years (mean 63 years) with a mean post mortem delay of 6 hours. All donors or next of kin provided written informed consent for brain autopsy and use of material and clinical information for research purposes.

*Lesion Classification*

MS lesions were detected and classified by immunohistochemical analysis using antibodies directed against proteolipid protein (PLP), a myelin component, and anti-major histocompatibility complex class II, clone LN3, as described previously.\textsuperscript{26} Active lesions are characterized by abundant perivascular and parenchymal leukocyte infiltration throughout the lesion area, whereas chronic inactive lesions are hypocellular and contain few infiltrating inflammatory cells throughout the lesion. White matter areas without any signs of demyelination and abundant microglial activation were classified as normal appearing white matter (NAWM). Based on the above-mentioned classification, we identified 12 active and 30 chronic inactive lesions.

*Immunohistochemistry*

For immunohistochemical stainings, 5 µm serial cryosections of 12 active lesions, 25 chronic inactive lesions and 7 control WM blocks were air-dried and fixed in acetone for 10 minutes. Next, sections were preincubated with rabbit serum (1:50 diluted in 1% bovine serum albumin in phosphate buffered saline (PBS), pH 7.4, Dako, Copenhagen, Denmark), for 10 minutes. Then, sections were incubated for 60 minutes with the appropriate primary antibody and subsequently incubated with a biotinylated rabbit-anti-mouse F(\text{ab}')\text{2} (1:500, Dako, Copenhagen, Denmark) for 30 minutes. After incubating for 60 minutes with s-ABC-HRP complex (1:200, Dako, Copenhagen, Denmark), peroxidase labeling was visualized by 3,3-diaminobenzidine (Sigma, St. Louis MO). Finally, tissue sections were counterstained with haematoxylin and mounted with DEPEX (Gurr, BDH Laboratories, Poole, UK). Between all incubation steps, sections were extensively washed in PBS (pH 7.4). All antibodies were diluted in PBS containing 1% bovine serum albumin, which also served as a negative control. Negative controls were essentially blank. Lesion and control tissue were processed simultaneously.

For double-immunohistochemical staining, sections were incubated overnight
with the mitochondrial marker anti-ERAB (endoplasmic reticulum associated binding protein) and one of the following antibodies GFAP (glial fibrillary acidic protein; astrocytes), NF (neurofilament; axons), Nogo (oligodendrocytes) and MHC class II (major histocompatibility complex class II; inflammatory cells). ERAB immunopositivity was visualized with biotinylated rabbit anti-mouse F(ab’)2, (1:500) followed by 60’ incubation with Streptavidine Alexa488 (1:400, Molecular probes, Leiden, The Netherlands). The other antibodies were fluorescently labeled with Alexa-594-labelled goat anti-rabbit (1:400, Molecular probes, Leiden, The Netherlands). Between all incubation steps, sections were extensively washed in PBS (pH 7.4). Negative controls for the fluorescent double-labelling experiments involved omission of both primary antibodies, and were essentially blank. A detailed description of the primary antibodies used in this article is provided in table 2.

Quantification of co-localization

To quantify the density of mitochondria within astrocytes and axons in different lesion stages, as well as the total density of mitochondria, we collected 6 active and 6 inactive lesions with adjacent NAWM from 12 patients. Cryosections were immunostained as described above to analyse the amount of mitochondria (ERAB-positive elements) in axons (NF-positive elements) and astrocytes (GFAP-positive elements). Images were acquired at random in lesions and adjacent NAWM using a standard confocal scanning microscope (TCS SP2, Leica Microsystems Heidelberg GmbH, Mannheim, Germany) equipped with an Ar/Kr laser (488 nm) and three HeNe lasers (543, 594, and 633 nm). Images were scanned in two channels, 488 for mitochondria and 594 for astrocytes and axons. Of each section, the maximum number of Z-images (average of 54 z-images, z-step was 0,12 µm) were taken at 630 magnification with a double zoom. The basic premises and procedures of the quantification of co-localization are described before.27,28 In brief, the software is capable of analyzing image series acquired in two- or multichannel experiments. Because the number of overlapping or touching objects in Channel A compared to Channel B can be different from the number of overlapping or touching objects when Channel B is compared to Channel A (example: six small objects in Channel A all overlap on a single, large structure in Channel B, and therefore only one object from Channel B overlaps with six in Channel A) the comparison of two channels was done in duplex by reversing the channels to be compared. Overlay of graphic output synchronized the results of the duplicate channel. The procedure involved in the software is as follows. The software first recognized 3D objects in each channel in the Z-series of images. A 3D object was defined as an aggregate of at least 20 voxels with above-threshold intensity. Thresholds for both channels were set manually. Then the program continued by analyzing which identified 3D objects in images belonging to the first channel showed (partial) voxel overlap with identified 3D objects in images belonging to the second channel. Next the software determines which of the objects from one channel did not overlap with any object from the other channel. These remaining 3D objects were then processed to determine which of them touched a 3D object present in the other channel. Output consisted of text data files and colour-coded bitmapped projection images of the recognized 3D objects. Finally, the number of co-localized objects was corrected for differences in section thickness.

Complex IV enzymehistochemistry

Complex IV enzyme histochemistry on serial sections of 11 active and 20 chronic inactive lesions was performed as described previously.29 Detection of complex IV activity
was analysed in sections, which were also used for complex IV immunostainings. In short, freshly cut 5 µm cryosections were immediately incubated for 1 hour at 37°C with substrate mix containing 10 mg cytochrome C (Sigma, St. Louis MO), 5 mg 3,3′-diaminobenzidine (Sigma, St. Louis MO), 0.5 mg catalase (Sigma, St. Louis MO) and 750 mg sucrose in 10 mL phosphate buffer (pH 7.4). Tissue sections were counterstained with haematoxylin and mounted with DEPEX.

Complex IV and citrate synthase activity assay

For biochemical measurements of complex IV and citrate synthase activity in chronic inactive lesion and NAWM, 100 mg brain tissue of both lesion and NAWM was obtained from 9 patients. 5 µm Cryosections were stained for PLP and LN3 to locate and classify lesion area and surrounding NAWM. Lesion and NAWM tissue were cut out of consecutive 10 µm cryosections. Hereafter, two additional cryosections were stained for PLP and LN3. In parallel, 100 mg of white matter tissue was collected from 7 non-neurological patients and used as control. All samples were prepared in 10 % (w/V) homogenate SEF buffer (0.25 M sucrose, 2 mM K-EDTA, 10 mM phosphate buffer pH 7.4) using a Potter-Elvehjem homogenizer. Homogenates were stored at – 80 °C. Prior to complex IV and citrate synthase measurements, samples were freeze-thawed three times, and subsequently diluted with 2 volumes of SEF buffer. Citrate synthase and complex IV activities in brain extracts were measured according to methods described before. All values were given as µmol/min/g wet weight tissue.

Statistical analysis

The Mann-Whitney U test was used to compare complex IV activity and co-localization between lesion/NAWM and control, student’s paired t-test was used to compare between NAWM and lesion.

Results

Mitochondrial protein expression in NAWM and control white matter

To study mitochondrial protein expression in MS lesions and NAWM, we performed immunohistochemistry using different antibodies directed against distinct mitochondrial proteins. In control white matter brain tissue, COX V immunostaining displayed a diffuse parenchymal staining, whereas a more punctuate pattern, typical for mitochondria, was observed with ERAB, complex I 20kDA & 30kDA and COX I. In NAWM and control tissue, astrocyte cell bodies and processes showed immunopositivity for all mitochondrial antibodies. Microglia, oligodendrocytes and endothelial cells lining the cerebral blood vessels, showed a moderate cytoplasmic staining.

Mitochondrial protein expression is enhanced in active and CIA MS lesions

In both active (Fig 1) and CIA (Fig 2) lesions we observed enhanced immunoreactivity of complex I 20kDA & 30kDA, COX I and V and ERAB compared to surrounding NAWM (Fig 1C to L, Fig 2C to L). Particularly cells with an astrocyte-like morphology showed a marked increased staining in active demyelinating and CIA lesions (arrows in Fig 1C to L & Fig 2C to H).

To identify the cellular localization of mitochondrial proteins in MS lesions, we performed co-localization studies using anti-ERAB with distinct cellular markers (GFAP,
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NF, MHC class II, and Nogo). In active lesions, a markedly increased co-localization of mitochondria with astrocytes and axons compared to adjacent NAWM was observed (Fig 3A to F). This increase was also observed in CIA lesions, albeit to a lesser extent (Fig 3G to L). Oligodendrocytes showed a small perinuclear rim of mitochondria (supplementary data), but no clear differences were observed in the amount of mitochondria comparing NAWM with lesion areas. Infiltrating leukocytes in active lesions contained multiple ERAB-positive mitochondria (supplementary data).
Figure 1. Immunohistochemical staining of active MS lesions for various mitochondrial proteins. Active lesions are characterized by demyelination (PLP, A) and contain abundant MHC class II-positive leukocytes (B). Increased expression of ERAB in active MS lesions (D) compared to adjacent NAWM (C) indicates increased mitochondrial density. In active lesions, complex I subunit 20kDA (F) and 30kDA (H) are enhanced compared to NAWM (E, G). Complex IV subunits I (J) and V (L) expression is increased in lesions compared to NAWM (I, K). Upregulation of mitochondrial proteins was particularly observed in astrocyte cell bodies and processes (arrows). Abbreviations: PLP = proteolipid protein, MHC = major histocompatibility complex, ERAB = endoplasmatic reticulum associated binding-protein, MS = multiple sclerosis, NAWM = normal appearing white matter.
Figure 2. Immunohistochemical staining of chronic inactive MS lesions for various mitochondrial proteins. Chronic inactive lesions are characterized by demyelinated areas (PLP, A) with few MHC class II positive cells (B). In chronic inactive lesions, an increase in expression of ERAB is observed (D) compared to NAWM (C). Expression of all mitochondrial proteins tested was upregulated in chronic inactive lesions (complex I subunits 20kDA (F), 30kDA (H) and complex IV subunits I (J) and V (L)) compared to adjacent NAWM (E, G, I, K respectively). Upregulation of mitochondrial proteins was particularly observed in astrocyte cell bodies and processes (arrows). Abbreviations: MS = multiple sclerosis, PLP = proteolipid protein, MHC = major histocompatibility complex, ERAB = endoplasmatic reticulum associated binding-protein, NAWM = normal appearing white matter.
Mitochondrial density is significantly increased in astrocytes and axons in MS lesions

To quantify the mitochondrial density in astrocytes and axons, we studied co-localization of ERAB-positive mitochondria with GFAP- and NF-positive structures in MS lesions and surrounding NAWM. In line with our immunohistochemical data we demonstrated an increased mitochondrial density in active and CIA lesions compared to NAWM (Fig 4A).
Figure 3. Confocal images representing an image stack of double immunofluorescence staining of mitochondria (anti-ERAB, green) and astrocytes (GFAP, red) and axons (NF, red) in active (A-F) and chronic inactive (G-L) MS lesions. In active lesions, mitochondria colocalize with astrocytes and astrocyte processes (A-C) and axons (D-F, inset). Similarly, mitochondria colocalize with astrocytes (G-I) and axons (J-L, inset) in chronic inactive lesions. Abbreviations: MS = multiple sclerosis, ERAB = endoplasmatic reticulum associated binding-protein, GFAP = glial fibrillary acidic protein, NF = neurofilament.
The number of mitochondria within axons and astrocytes was significantly enhanced in active lesions compared to adjacent NAWM, whereas a trend was observed in CIA lesions (Fig 4B). Mitochondrial density in astrocytes was also significantly enhanced in active lesions, and only slightly in CIA lesions (Fig 4C).

**Complex IV enzyme activity is upregulated in MS lesions**

Histochemical analysis of complex IV activity in NAWM (Fig 5B-C) and control (data not shown) demonstrated a weak parenchymal staining and a clear perinuclear staining in cells with an astrocytic appearance. No difference in staining intensity between NAWM and control white matter was observed. We revealed an enhanced complex IV enzyme activity in CIA lesions (Fig 5B and 5E) and active lesions (Fig 5D). Complex IV activity was predominantly increased in large hypertrophic astrocytes and axons in active as well as CIA lesions. Biochemical measurements of complex IV activity in CIA lesions were in line with the histochemical data (Fig 5F) and demonstrated a tendency of increased complex IV activity per mitochondrion in CIA lesions compared to NAWM. Interestingly, complex IV activity was significantly increased in CIA lesions compared to control white matter. Complex IV activity is depicted relative to citrate synthesis activity due to differences in mitochondrial number between control, NAWM and lesion tissue.

**MtHSP70 expression is increased in MS lesions; predominantly in astrocytes**

To examine whether the observed increase of mitochondria in lesions was associated with enhanced mitochondrial stress, we qualitatively assessed the expression of the mitochondrial heat shock protein 70 (mtHSP70), which is known to be upregulated upon
Mitochondrial alterations in MS lesions

In NAWM and control white matter, anti-mtHSP70 weakly stained mitochondria in astrocytes and no differences were observed between NAWM and control white matter (Fig 6A-B). Interestingly, mtHSP70 expression was markedly increased in MS lesions compared to control white matter.

Figure 5. Complex IV activity is increased in MS lesions compared to control white matter. Complex IV enzyme activity (B) is increased in demyelinated areas (PLP, A) compared to NAWM. Complex IV activity is low in NAMW (C) and strikingly increased in active (D) and chronic inactive (E) lesions. Complex IV activity is predominantly observed in astrocyte-like cell bodies and processes (D, E, arrows). Biochemical analysis confirmed enhanced complex IV enzyme activity in chronic inactive MS lesions compared to NAWM and control white matter (F). Abbreviations: NAWM = normal appearing white matter, MS = multiple sclerosis, PLP = proteolipid protein.
in mitochondria in both active and CIA lesions (Fig 6C-D). Confocal microscopical analysis demonstrated enhanced mtHSP70-immunoreactive mitochondria in astrocytes (Fig 6C-D, inset) and axons (Fig 6D, inset).

**Discussion**

This study provides detailed information on the distribution and activity of mitochondria in active and chronic inactive MS white matter lesions. We show a significant increase in mitochondrial protein expression in different MS lesion stages. Moreover, we demonstrate that the activity of complex IV is markedly upregulated in MS lesions compared to control white matter and surrounding NAWM, which correlates with the increase in complex IV protein expression. Enhanced numbers of mitochondria in axons and astrocytes are observed in active lesions and, to a lesser extent, in CIA lesions. Interestingly, increase in complex IV activity and mitochondrial density coincides with a marked upregulation of the mitochondrial heat shock protein 70 (mtHSP70) in MS lesions, indicative of mitochondrial oxidative stress in MS lesions.
Recently, Mahad and colleagues reported a general reduction of mitochondria and a decrease in complex IV expression in pattern III MS lesions. Type III lesions are characterized by extensive oligodendrocyte apoptosis and hypoxia-like tissue injury. In the present study, we use brain tissue from patients with established MS, in which hypoxia-like damage and oligodendrocyte apoptosis are absent or very rare. Although decreased mitochondrial activity might occur in a subset of early lesions, we evidently demonstrate enhanced density of mitochondria and complex IV activity in MS lesions in patients with established disease. Recently, Mahad and colleagues demonstrated decreased activity of complex IV in axons in the rim of chronic active lesions, whereas axons within inactive lesions displayed increased activity of complex IV. Furthermore, mitochondrial mass was found to be increased in axons in chronic inactive lesions, which is in line with our data. We observed increased complex IV activity in active lesions, whereas they found a reduction of complex IV activity in the inflammatory rim of chronic active lesions. However, we have investigated complex IV activity within the whole active lesions, not specifically in axons. Hence, it is likely that even though axonal complex IV activity is decreased, total complex IV activity is increased in inflammatory MS lesions.

We demonstrate an increased numbers of mitochondria in demyelinated axons in MS lesions. Most likely, mitochondria are recruited to demyelinated axons to meet the increased energy demand needed for proper conduction. In active inflammatory lesions, axons are subjected to elevated levels of ROS and NO, which leads to irreversible inhibition of complex I, and subsequent increased ROS production by mitochondria, AT depletion, influx of Ca through the reversed Na/Ca exchanger and eventually axonal injury. The suggested role of mitochondria in axonal loss is further supported by our finding that the increase in mitochondrial density in axons is more evident in active compared to CIA lesions. This might explain the higher occurrence of axonal damage and loss in active lesions. Similarly, the increase in the number of mitochondria in axons in CIA lesions might contribute to the ‘slow burning’ axonal loss observed in these lesions.

The increase in mitochondria and complex IV activity in astrocytes likely reflects their active involvement in various pathological processes in MS pathology. Astrocytes produce a vast array of molecules involved in MS lesion formation and persistence, such as matrix metalloproteinases, extracellular matrix proteins, chemokines and cytokines (for review, see Williams). Furthermore, reactive astrocytes express high levels of proteins involved in glutamate metabolism, suggesting an important role in sequestering and metabolizing extracellular glutamate. Taken together, astrocytes play an active role in various energy-demanding processes underlying MS pathology. As a consequence, enhanced mitochondrial activity in astrocytes, as reported in this study, might contribute to the formation of toxic free radicals.

In fact, reports demonstrated the occurrence of lipid peroxidation and nitrotyrosine residue formation within astrocytes in MS lesions indicating that astrocytes are a target of ROS-induced damage. Lipid peroxidation and tyrosine residue formation can be mediated by peroxynitrite which is formed by a spontaneous reaction between superoxide and nitric oxide. In active MS lesions, macrophages produce ROS, whereas both astrocytes and macrophages produce NO. Peroxynitrite is able to decrease ATP production through irreversible inhibition of complex I and III. Previously, a decrease in the activity of mitochondrial respiratory chain complexes I and III was reported in non-demyelinated areas of the motor cortex of MS patients, although the cellular origin was
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not reported. In chronic active WM lesions, a general reduced complex I activity and a slight increase in complex IV activity has been observed by Lu and colleagues. They postulate that enhanced complex IV activity is compensatory for decreased complex I activity, however this remains to be established. It is conceivable that the observed increase in mitochondria and complex IV activity might be a compensation for the reduced ATP production.

In contrast to complex IV, where increased immunopositivity coincides with increased activity, complex I immunoreactivity is increased and activity is decreased, as described previously. Inhibition of complex I activity by either NO or peroxynitrite might explain this apparent contradiction.

Finally, we demonstrate enhanced immunoreactivity of mtHSP70 in MS lesions, particularly in reactive astrocytes. MtHSP70 is a chaperone protein involved in mitochondrial protein import (for review, see Kaul) and is upregulated upon oxidative stress. Overexpression of mtHSP70 reduces ROS formation, improves mitochondrial function and protects astrocytes against ischemic injury in vitro. Therefore, upregulation of mtHSP70 in astrocytes in MS lesions might function as a protective mechanism to counteract oxidative damage and mitochondrial stress. Remarkably, enhanced mtHSP70 immunoreactivity is also observed in CIA lesions, indicating that astrocytes in MS lesions are subjected to oxidative stress even in the aftermath of acute inflammation.

In summary, we demonstrate an increase in mitochondrial density and activity in active MS lesions and to a lesser extent in CIA MS lesions, particularly in astrocytes and axons. In addition, we show enhanced mtHSP70 immunoreactivity in MS lesions, indicating the occurrence of mitochondrial oxidative stress. We speculate that the mitochondrial alterations, as demonstrated in this study, play an important role in various processes underlying MS pathology such as axonal injury. To date, therapies that prevent neuro-axonal loss or promote axonal regeneration are scarce. We speculate that therapies based on enhancement of endogenous antioxidant expression or supplementation of mitochondria-targeted antioxidants might be an attractive strategy to reduce tissue damage in MS.

Reference List

Mitochondrial alterations in MS lesions


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Mitochondrial alterations in MS lesions


Tables

Table I Clinical data of MS patients and non-neurological controls

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<th>Case</th>
<th>Age (years)</th>
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<th>Disease Duration (years)</th>
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<td>-</td>
<td>f</td>
<td>Unknown</td>
<td>-</td>
<td>Unknown</td>
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<tr>
<td>Control</td>
<td>91</td>
<td>-</td>
<td>f</td>
<td>Unknown</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Control</td>
<td>84</td>
<td>-</td>
<td>m</td>
<td>4:45</td>
<td>-</td>
<td>Resp. Failure</td>
</tr>
<tr>
<td>Control</td>
<td>72</td>
<td>-</td>
<td>m</td>
<td>9:45</td>
<td>-</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

SP = secondary progressive MS; PP = primary progressive MS; ND = not determined; m = male; f = female; CVA = cerebrovascular accident

Table 2 Antibody details

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Dilution</th>
<th>Antibody type</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLP</td>
<td>1:500</td>
<td>IgG2a</td>
<td>Serotec</td>
</tr>
<tr>
<td>MHC class II (LN3)</td>
<td>1:100</td>
<td>IgG2b</td>
<td>Dako</td>
</tr>
<tr>
<td>ERAB</td>
<td>1:250</td>
<td>IgG1</td>
<td>Abcam</td>
</tr>
<tr>
<td>Complex I subunit 20kDA</td>
<td>1:50</td>
<td>IgG1</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Complex I subunit 30kDA</td>
<td>1:100</td>
<td>IgG1</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Complex IV subunit-I (COX-I)</td>
<td>1:50</td>
<td>IgG2a</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Complex IV subunit-V (COX V)</td>
<td>1:50</td>
<td>IgG2a</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>GFAP</td>
<td>1:500</td>
<td>rabbit polyclonal</td>
<td>Dako</td>
</tr>
<tr>
<td>NF</td>
<td>1:100</td>
<td>rabbit polyclonal</td>
<td>Sigma</td>
</tr>
<tr>
<td>NOGO</td>
<td>1:1000</td>
<td>rabbit polyclonal</td>
<td>Gift from Dr. Yan</td>
</tr>
<tr>
<td>mtHSP70</td>
<td>1:50</td>
<td>IgG1</td>
<td>Stressgen</td>
</tr>
</tbody>
</table>

PLP, proteolipid protein; MHC, major histocompatibility complex; COX, cytochrome-c oxidase; ERAB, endoplasmatic reticulum associated binding protein; GFAP, glial fibrillary acidic protein; NF, neurofilament; mtHSP70, mitochondria associated heat shock protein 70

* Sources: Serotec, Oxford, UK; Dakocytomation,Glostrup, Denmark; Abcam, Cambridge, UK; Invitrogen, San Francisco, CA; Sigma-Aldrich, St Louis, MO; Stressgen, San Diego, CA.
Supplementary data. Immunohistochemical staining of ERAB in MS lesions. Mitochondria (anti-ERAB, green) are observed in MHC class II-positive leukocytes (red, A) and Olig2-positive oligodendrocytes (red, B) in MS lesions. Perivascular infiltrates are immunopositive for ERAB (C). Abbreviations: ERAB = endoplasmatic reticulum binding-protein, MS = multiple sclerosis, MHC = major histocompatibility complex.