General introduction

Adapted from:

**Mitochondrial dysfunction: A potential link between neuroinflammation and neurodegeneration?**

**Radical changes in multiple sclerosis pathogenesis**
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Chapter 1

1. Multiple sclerosis
1.1 Demographics and disease course

Multiple sclerosis (MS) is the most common cause of neurologic disability in young adults, affecting over 2.5 million people worldwide. In North-Western Europe MS has a prevalence of ~1:1000,$^1$ and women are affected approximately three times as often as men.$^2$ Prevalence of MS in both males and females seems to have risen steadily during the 20$^{th}$ century.$^3$ MS is a global disease with lowest prevalence around the equator which increases when moving towards both poles, however many exceptions exist to this rule of thumb.$^4$ First symptoms generally appear in early adulthood (20-40 years of age), and 50% of patients will need help walking within 15 years.$^5$ Initial symptoms are often sensory disturbances, optic neuritis, diplopia and limb weakness. As disease progresses, symptoms also include fatigue, bladder dysfunction and cognitive impairment.$^1$

Depending on progression of clinical symptoms, 3 distinct MS subtypes can be distinguished. In relapsing-remitting (RR) MS, present in 80% of cases, patients suffer from clearly defined periods of neurologic deficits followed by complete recovery.$^6$ In 65% of patients suffering from RRMS, disease course gradually evolves into secondary progressive (SP) MS, characterised by progressive permanent neurological deficits.$^6,7$ 20% of all MS patients have a progressive disease course from onset, known as primary progressive (PP) MS. The age of onset of PPMS is ~40; remarkably similar to the mean age of conversion from RR to SPMS.$^7$ For all subtypes combined, median time to death from onset of disease is ~30 years.$^8$

1.2 Aetiology

Despite extensive research over the last 100 years, the cause(s) and initial triggers of MS are still largely enigmatic. We do know that both genetic and environmental factors contribute substantially to disease susceptibility. Genotype makes up ~30% of susceptibility, as shown by studies in monozygotic twins.$^9-11$ Moreover, the risk of developing disease in first-degree relatives is 20-40% higher than in the general population.$^{12-14}$ This increased risk is solely attributable to genetics, as the families of adopted children and step-siblings with MS have equal susceptibility as the general population.$^{15,16}$ In addition to susceptibility, genetics also influence disease severity and age of onset.$^{17-19}$ Thus far, only a few genes have been consistently shown to influence disease susceptibility, which together still only account for a small proportion of the aforementioned 30%. Genes with the strongest link to MS, although still modest, are located on chromosome 6 and are part of the major histocompatibility complex class II (MHC II), namely HLA-DR15 and HLA-DQ6.$^3,20$ More recently, certain alleles of interleukin-2 & interleukin-7 receptor α chains have been linked to MS susceptibility, albeit with only a weak association.$^{21,22}$ So, up to know, all the genes linked to MS are involved in immunological processes.

Over the years many environmental factors have been linked to the occurrence of MS, clear-cut proof for any of these factors, however, is lacking. One of the strongest indications for environmental triggers is the global distribution of MS, which, with many exceptions, has the lowest prevalence around the equator and increases when moving towards both poles.$^4$ Firm support for the importance of the environment in MS susceptibility comes from studies correlating risk of MS with place of residence in the earlier years of life.$^{23-25}$ These studies showed that the risk of MS decreases when people migrate from high-risk regions to lower-risk regions during childhood, and increases after migration in the
opposite direction. On these premises, several environmental factors have been proposed to attribute to or cause MS: low exposure to sunlight, vitamin D deficiency and a wide variety of bacterial and viral infections.\(^{26}\) Currently, much emphasis is put on Epstein-Barr virus (EBV) as a possible contributor to MS. Infection with EBV, one of the most widespread viruses with infection rates in adults over 90\%, in the first two decades of life seems to increase the risk of developing MS later.\(^{27,28}\) Moreover, EBV seropositivity in MS patients is 99-100\%, significantly higher than the 90-95\% found in controls.\(^{29}\) These epidemiological studies, however, are certainly no proof of a causal relation between EBV infection and MS.

### 1.3 Pathogenesis

MS is a chronic inflammatory disease of the central nervous system (CNS), characterised by focal demyelinated lesions scattered throughout brain and spinal cord. It is not a pure white matter (WM) disease as there is also significant cortical and deep grey matter involvement, especially in later stage of the disease.\(^ {30,31}\) The course of the disease is generally episodic, with frequent intervals of exacerbations followed by periods of remission. The relapsing-remitting phase is characterized by immune-mediated responses, such as widespread microglial activation and massive cellular infiltrates in the CNS. In time, patients gradually develop secondary progressive MS, which is mainly characterized by neuronal and axonal degeneration and extensive cortical demyelination.\(^ {30,32,33}\)

Histopathologically, our group distinguishes, based on the stage of lesion development, the following WM areas in MS brains.\(^ {34}\) Normal-appearing white matter (NAWM) is characterized as normal myelinated white matter with no signs of leukocyte infiltration. Within the NAWM we identified clusters of activated microglia without evident signs of demyelination, the so-called preactive lesion. However, though likely, it still has to be proven that preactive lesions actually develop into demyelinating lesions. Lesions with ongoing demyelination are termed active lesions and contain abundant activated myelin-laden microglia and macrophages throughout the lesion area. Several groups divide this category in early and late active; the former containing macrophages with myelin protein-positive inclusions and the latter macrophages containing neutral lipids.\(^ {34,35}\) In time, active lesions gradually convert into chronic active lesions, which are characterized by a hypocellular demyelinated gliotic center with a hypercellular rim containing activated macrophages and microglia. In the chronic lesion stage, when inflammation has subsided, hypertrophic astrocytes form a dense network, the so-called astroglial scar. Lesions in grey matter generally lack most of the WM lesion characteristics, e.g. there is no significant infiltration of leukocytes, no astrogliosis and microglial activation is less prominent than in most WM lesions.\(^ {36,37}\) Due to the lack of infiltration of blood-derived leukocytes in virtually all GM lesions, we discriminate GM lesions on the basis of location. Type 1 GM lesions are leukocortical lesions; all other types are purely cortical with type 2 being lesions not touching the WM/GM border and pial surface, type 3 lesions are located subpially and type 4 lesions encompass the whole width of the cortex.\(^ {33,37}\)

The trigger for disease initiation is arguably the largest enigma in MS pathogenesis. Over the years, several triggers have been proposed, including primary oligodendrocyte apoptosis and primary axonal damage/alterations.\(^ {38-40}\) However, the prevailing hypothesis is that self-tolerance is breached in the periphery, possibly virus-mediated, leading to activation of autoreactive CD4\(^ +\) T-cells directed against myelin. Subsequently, activated
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T-cells cross the brain endothelium into the perivascular compartments where they are reactivated (fig 1).\textsuperscript{41,42} Reactivated T-cells then attract and stimulate monocytes and lymphocytes to cross the local blood-brain barrier into the brain parenchyma. There, macrophages and activated microglia destroy the axon-encapsulating myelin sheaths by secreting several toxic mediators, such as matrix metalloproteinases, reactive oxygen species (ROS) and nitric oxide, and subsequently phagocytise myelin remnants. Unfortunately, myelin is not the only victim of the relentless attack by macrophages, as evidenced by massive loss of oligodendrocytes, the myelin producing cell, and axonal destruction (fig 1).\textsuperscript{43,44} Similar pathogenetic mechanisms underlie the symptoms and pathology that are present in the experimental autoimmune encephalomyelitis (EAE) model, a validated animal model of MS developed in the 1930's.\textsuperscript{45-47} In this model, disease is generally induced by injecting animals with myelin proteins together with Freud's adjuvant or adopted transfer of autoreactive T-cells. The widespread use of EAE in the last decades explains the historic focus of MS research on understanding and targeting T-cell driven neuroinflammation. As a result, current therapies are mainly immunomodulatory (IFN-\(\beta\), natalizumab), which are effective in reducing the number of relapses. Disease progression, however, remains largely unchanged by these therapies as they do not

Figure 1. Infiltrated immune cells mediate demyelination and axonal degeneration in multiple sclerosis. 1) peripherally activated T-cells (blue) infiltrate the central nervous system (CNS). 2) There, they are reactivated and produce chemokines (yellow dots), which attract circulating monocytes (brown) and other T-cells to migrate across the blood-brain barrier into the CNS. 3) In the CNS, blood-borne macrophages and resident microglia (green) are triggered by cytokines (red dots) released by T-cells to become phagocytic macrophages (pink). 4) These phagocytes then destroy and phagocytose the axon-enwrapping myelin sheaths. In this process, axons and intra-axonal structures like mitochondria are also damaged.
reduce axonal/neuronal degeneration in later stages of the disease, demonstrating the need for novel therapeutic strategies oriented towards neuroprotection. For that reason, research interest in the last decade has shifted more towards mechanisms leading to axonal degeneration, which is now considered the best correlate of disease progression in MS. Axonal degeneration is most prominent in inflammatory MS lesions, although axonal injury continues at a ‘slow-burning’ rate in chronic MS lesions, where inflammation has largely subsided. However, there is some evidence that suggests that upon complete disappearance of inflammation axonal degeneration returns to levels similar to age-matched controls. In inflammatory lesions, axonal damage is likely to be a bystander effect of myelin destruction by macrophages. In chronic MS lesions, however, intra-axonal mechanisms are most likely responsible for axonal degeneration and evidence is accumulating that mitochondrial dysfunction is an important feature of axonal degeneration in the chronic stage of the disease.

2. Mitochondria

2.1 Mitochondria, powerhouses of the cell

Mitochondria are double-membraned organelles present in all eukaryotic cells and are the sole carriers of non-nuclear DNA. Their main function is to provide the cell with adenosine triphosphate (ATP), the cellular energy currency, by oxidation of metabolic fuels. In addition, mitochondria are involved in various processes vital to the cell, such as fatty acid oxidation, apoptosis, and Ca\(^{2+}\) homeostasis. Production of ATP in mitochondria is essentially a two step process: first, energy stored in fats and carbohydrates is released and used to form the high energy bonds in nicotinamide adenine dinucleotide (NADH) and 1,5-dihydro-flavin adenine dinucleotide (FADH\(_{2}\)). These molecules, together with oxygen, are then used to produce the bulk of ATP through a process called oxidative phosphorylation (OxPhos) (fig 2). The OxPhos chain consists of 5 multi-subunit complexes located on the inner mitochondrial membrane; the first 4 complexes, collectively called the electron transfer chain (ETC), are committed to oxidizing NADH and FADH\(_{2}\), transferring electrons to molecular oxygen and pumping protons from the mitochondrial matrix to the intermembrane space, thus creating an electrochemical gradient called the mitochondrial membrane potential (MMP). The MMP is used by complex V, also known as ATP synthase, to phosphorylate ADP into the energy-rich ATP (fig 2).

2.2 Mitochondrial generation of reactive oxygen species

Besides producing ATP, mitochondria are also a constant source of reactive oxygen species (ROS): small highly reactive molecules that can cause damage to virtually all cellular macromolecules. However, ROS also function as important signalling molecules in many physiologic cellular processes. Therefore, it is important for each cell (and each mitochondrion) to tightly control ROS production and detoxification. When ROS production exceeds the antioxidant capacity oxidative stress occurs, a state implicated in a wide range of pathologies, including neurodegenerative diseases and autoimmune disorders.

ROS production in mitochondria is almost exclusively the result of electrons leaking out of the electron chain at complex I and III (fig 2). As a consequence, molecular oxygen is reduced to superoxide (\(O_2^-\)), which serves as the main precursor for other ROS. Studies in isolated mitochondria have shown that 1%-2% of the consumed molecular oxygen
is converted to superoxide. Locations of superoxide production are the mitochondrial matrix site of complex I & III and the intermembrane site of complex III. In the mitochondrial matrix superoxide is very rapidly dismutated into hydrogen peroxide (H$_2$O$_2$) by superoxide dismutases 2 (SOD2). Hydrogen peroxide itself is relatively stable and can easily pass the mitochondrial membranes. However, hydrogen peroxide also serves as the substrate for the iron- or copper-driven Fenton reaction, which generates the very toxic and harmful hydroxyl radical (OH$^\cdot$).

In addition, peroxynitrite (ONOO$^-$), the result of the reaction between nitric oxide (NO) and superoxide, and nitric oxide itself are important ROS in mitochondrial biology, as they can directly inhibit electron transfer. Nitric oxide, which can be locally produced by mitochondrial nitric oxide synthase (mtNOS), can reversibly inhibit complex IV and thus regulate ATP synthesis. Furthermore, nitric oxide and peroxynitrite can irreversibly inhibit complex I leading to a marked increase in superoxide generation.

### 2.3 Mitochondrial ROS defence

To cope with the continuous production of superoxide and its various metabolites, mitochondria are equipped with an organelle-specific pool of antioxidants. As stated previously, SOD2, an iron/manganese superoxide dismutase, resides in the mitochondrial matrix.
matrix where it catalyzes the reaction of superoxide into hydrogen peroxide. In addition to SOD2, mitochondria contain specific enzyme systems capable of detoxifying superoxide metabolites: the thioredoxin and glutathione/glutaredoxin system. These antioxidant systems are widely expressed in various cellular compartments, with specific family members within mitochondria. The mitochondrial thioredoxin system is composed of peroxiredoxin 3 (Prx3), peroxiredoxin (Prx5), thioredoxin 2 (Trx2) and thioredoxin reductase 2 (TrxR2). Prx3 is the mitochondrial-localized member of a family of antioxidant enzymes which catalyze the reduction of various peroxides, including hydrogen peroxide and peroxynitrite. It possesses a redox-active cysteine residue that can be oxidized by peroxides to cysteine sulfenic acid (-SOH). To regain antioxidant function Prx3-SOH must be reduced by Trx2, which in turn becomes oxidized. Trx2 is a mitochondrial-specific member of the thioredoxin family which are capable of reducing disulfide bonds in proteins. The end of this redox cascade is the reduction of Trx2 by TrxR2 at the expense of NADPH (fig 2). The mitochondrial glutathione/glutaredoxin system works in a similar fashion, although with different players: glutathione peroxidase 4 (Gpx4), glutaredoxin (Grx2) and glutathione.

Besides having its own ROS detoxifying apparatus, mitochondria also contain a family of proteins known for their ability to reduce ROS production, the uncoupling proteins (UCPs). In 1978, UCP1 was the first to be described and found to be specifically expressed in mitochondria in brown adipose tissue (BAT). There, UCP1 regulates proton leak from the intermembrane space into the mitochondrial matrix, and in doing so it bypasses ATP synthase and induces non-shivering thermogenesis. More recently, 4 proteins with significant sequence homology to UCP1 have been described and numbered according to order of discovery. UCP2 and UCP3 are remarkably similar, but have completely different expression patterns. UCP2 is ubiquitously expressed, including in the CNS, whereas UCP3 is only present in skeletal muscle and BAT. UCP4 and UCP5 (or brain mitochondrial carrier protein 1 (BMCP1)) are exclusively expressed in the CNS. There is heavy debate on how UCP2-5 exactly function and some claim they are not true “uncouplers” like UCP1. Nonetheless, it has been extensively shown that all UCPs reduce ROS production in mitochondria and protect cells from oxidative insults.

2.4 Transcriptional regulation of mitochondrial proteins

Mitochondria are unique organelles, not in the least because they are the sole carriers of non-nuclear DNA in mammalian cells. This circular DNA, present in each mitochondrion, encodes 13 proteins, 22 tRNAs and 2 ribosomal RNAs. The RNAs are necessary for intra-mitochondrial synthesis of the 13 proteins, which are all essential subunits of OxPhos complexes I, III, IV and V. All other proteins expressed in the mitochondrial matrix or membranes, estimated to be between 1000-1500 proteins, including 77 subunits of the OxPhos complexes, are encoded in the nucleus. Such a vast amount of proteins, of which the majority is involved in the same process, namely energy metabolism, requires responsive and coordinated transcriptional regulation.

In mammalian cells, transcription of genes coding for proteins expressed in mitochondria is under the regulation of only a handful of transcription factors and coregulators. The most important (or at least best studied) DNA-binding transcription factors are nuclear respiratory factors (NRF1 and -2), estrogen-related receptors (ERRα, -β and -γ) and peroxisome proliferator-activated receptors (PPARα, -γ and -δ). These
transcription factors can induce transcription of overlapping but specific sets of genes involved in mitochondrial and non-mitochondrial processes (fig 3). Other, less well-studied, transcription factors involved in transcription of nuclear-encoded mitochondrial genes are CRE-associated binding protein (CREB), c-MYC and YY1. Expression levels of all these transcription factors is highly cell-type specific. Transcription and replication of mitochondrial DNA is under control of nuclear-encoded proteins Tfam, TFB1M and TFB2M, which in turn are under control of both NRFs. In this way, the cell is able to simultaneously induce expression of nuclear- and mitochondrial- encoded proteins.

Activity of the NRFs, ERRs and PPARs is regulated by a family of transcriptional coregulators, the so-called PPARy–coactivator (PGC) family. The best known member of this family is PGC-1α (fig 3), which was first discovered by Spiegelman and colleagues to bind to PPARγ in response to cold in BAT. The other two family members, PGC-1β and PGC-1α-related coactivator (PRC), were discovered on the basis of sequence homology and were found to exert similar effects as PGC-1α. All three PGC-1s regulate the activity of transcription factors in a similar fashion: they bind to their DNA-bound target transcription factors and subsequently attract histone acetyltransferases and the Mediator complex to enhance transcriptional activity. The ability of PGC-1s to bind to NRFs, ERRs and PPARs is regulated by various posttranslational modifications such as (de-)phosphorylation and...
Unsurprisingly, proteins that modify PGC-1 activity, such as sirtuin1 and AMP-activated protein kinase (AMPK), are responsive to changes in metabolic demands and oxidative stress. Taken together, PGC-1s (especially PGC-1α) are activated in response to an increased cellular energy demand and enhance transcription of a vast array of mitochondrial proteins by interacting with different transcription factors (fig 3). Furthermore, PGC-1α and PGC-1β were shown to exert strong neuroprotective effects by increasing mitochondrial ROS-detoxifying enzymes.

### 2.5 Mitochondrial dynamics

Mitochondria are not generated de novo; instead mitochondria continuously fuse and divide through processes called mitochondrial fusion and fission, respectively. Moreover, severely damaged mitochondria are removed by mitochondrial autophagy, termed mitophagy. Together, these three processes guard mitochondrial function and shape the cells mitochondrial content to meet its metabolic needs. In healthy cells, fission and fusion are balanced processes with different points of equilibrium. Metabolically active cells generally have elaborate mitochondrial networks caused by extensive fusion of mitochondria, whereas fission is more prominent in quiescent cells where mitochondria are often observed as small distinct spherical organelles. Thus far, several proteins have been identified that mediate fission and fusion. Mitofusin 1 & 2 (MFN1&2) mediate fusion of the outer mitochondrial membrane and OPA1 is essential for inner mitochondrial membrane fusion, whereas fission is executed by Fis1 and DRP1.

Besides shaping mitochondrial content to different physiological demands, fission and fusion are also essential in several other biological processes. Before cell division, fission is increased to equip the daughter cells with enough functional mitochondria. In addition, evidence is accumulating that increased fission and reduced fusion of mitochondria is an essential step in apoptosis. Together, fusion and fission are essential for maintaining a healthy mitochondrial population. As stated previously, mitochondria are a constant source of ROS which renders mtDNA particularly vulnerable to ROS mediated damage. As a result, many cells contain a mixture of mitochondria containing damaged or mutant mtDNA and mitochondria with healthy mtDNA, a state termed heteroplasmy. Fusion of these healthy and damaged mitochondria, or two damaged mitochondria with mutations in different mtDNA alleles, leads to a full restoration of respiratory activity. Thus, intermixing of mitochondrial contents of different mitochondria by fusion serves as a mechanism to safeguard mitochondrial functioning. The result of this process is fully functional mitochondria harbouring damaged or dysfunctional components (e.g. mutated mtDNA). Currently, it is believed that fission of these mitochondria can lead to functional and non-functional mitochondria, of which the non-functional mitochondria are subsequently targeted for degradation by mitophagy. Interestingly, mitochondrial fission, fusion and mitophagy seem particularly important in neuronal cells, as mutations in OPA1 lead to autosomal dominant optic atrophy, mutations in MFN2 to Charcot-Marie-Tooth disease 2a and mutations in Pink1 and Parkin, which are essential proteins in mitophagy, cause hereditary early onset Parkinson’s disease.

### 3. Mitochondria in multiple sclerosis: the story so far

Interest in mitochondrial involvement in MS was ignited by the finding of an MS-like
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disease in a subset of patients with Leber’s hereditary optic neuritis, a disease caused by
mutations in mitochondrial (mt)DNA.\textsuperscript{123,124} Although large genetic screening studies did
not reveal an association between mtDNA mutations and the occurrence of MS, distinct
mtDNA haplotypes were actually shown to be significantly associated with MS, however
the unknown biological relevance of these haplotypes warrants further investigation.\textsuperscript{125,126}

Later, it was hypothesized that mitochondria might also contribute to axonal
degeneration in chronically demyelinated axons. In the chronic phase of MS, when
inflammation has abated, axonal degeneration is still ongoing albeit more subtle than
in inflammatory MS lesions.\textsuperscript{127} In acutely demyelinated axons conduction is blocked,
which can be restored in chronic demyelinated axons by upregulation and redistribution
of Na$^+$ channels along the entire demyelinated axon.\textsuperscript{128} Concurrently, Na$^+$/K$^+$ ATPase is
upregulated to restore intra-axonal Na$^+$ concentration. Hence, more ATP is needed in the
chronic demyelinated axon; which leads to the hypothesis that more mitochondria are
needed to provide the demyelinated axon with sufficient energy. Proteomics analyses of
MS lesions indeed revealed enhanced expression of various mitochondrial proteins.\textsuperscript{129}
As mitochondria are a constant source of ROS, the increase in mitochondria is likely to
increase oxidative stress in these demyelinated axons. Moreover, mitochondria in neuronal
cell bodies in non-demyelinated MS grey matter were found to have decreased activity
of complex I and III, and, as neurons partly provide their axon with mitochondria, this
might lead to impaired mitochondrial function in chronically demyelinated axons.\textsuperscript{130} As
mitochondrial ROS production and decreased activity of complex I and III strengthen each
other, this will lead to a self-perpetuating cycle where mitochondrial function becomes
progressively compromised as oxidative damage accumulates, eventually leading to
axonal degeneration. Thus, although mitochondria are initially needed to preserve
demyelinated axons, they might ultimately seal the axon’s faith.

However, it is also conceivable that mitochondria contribute to axonal degeneration in
the inflammatory stage of the disease. ROS and NO production by infiltrated macrophages
and activated microglia is believed to significantly contribute to demyelination and
axonal damage in inflammatory MS lesions.\textsuperscript{52,131} Accordingly, there is ample evidence of
oxidative damage to lipids, proteins and DNA in these inflammatory MS lesions.\textsuperscript{132,133} Not
surprisingly, most oxidative damage to DNA was found in mtDNA,\textsuperscript{134} as mtDNA is \textit{\sim}10
times more susceptible to DNA damage than nuclear DNA, due to the absence of histones
and repair mechanisms.\textsuperscript{135} The same study also described decreased activity of complex I
of the OxPhos chain in MS lesions, which is likely to be the result of ROS-induced mutations
in mtDNA-encoded complex I genes.\textsuperscript{134} Thus, ROS produced by infiltrated macrophages
and activated microglia in inflammatory MS lesions might damage mtDNA and decrease
oxidative phosphorylation, which, in analogy to several other neurodegenerative diseases,
leads to decreased ATP production and increased ROS production by the OxPhos chain
itself, thereby contributing to axonal degeneration in inflammatory MS lesions.\textsuperscript{136-138}

Mechanisms underlying axonal degeneration in EAE are believed to be similar to those
seen in MS. Consequently, several studies addressing mitochondrial dysfunction in EAE
have been performed. Thus far, microarray studies revealed altered expression of several
genes encoding mitochondrial proteins. In spinal cords of EAE animals at advanced
disease stages, several complex IV subunits were significantly upregulated.\textsuperscript{139} In contrast,
decreased expression of mitochondrial genes has been found in non-affected gray matter
of EAE brain tissue. These results are in line with previous findings, where decreased expression of genes encoding mitochondrial proteins, particularly OxPhos genes, in non-affected MS gray matter has been observed. In addition, it has been elegantly shown that mitochondrial proteins are heavily nitrated in EAE. Proteomics revealed OxPhos, glycolytic and chaperone proteins to be nitrated, indicating alterations in energy metabolism in EAE. Interestingly, increased mitochondrial protein nitration appeared as early as 3 days after sensitisation, suggesting that mitochondrial dysfunction precedes leukocyte infiltration and the occurrence of the first clinical symptoms. Based upon these results, it is tempting to speculate that experimentally evoked autoimmunity to myelin swiftly leads to mitochondrial dysfunction in axons, independently of inflammation. However, microglial activation, representing mild CNS inflammation and occurring early in EAE pathogenesis, was not assessed and therefore remains a likely reason for causing early mitochondrial dysfunction. Thereafter, Qi and co-workers assessed the effect of adenoviral injection of SOD2, a mitochondrial superoxide scavenger, on optic nerve integrity of EAE animals. They observed a striking reduction in optic nerve atrophy, demonstrating the important role of mitochondria-derived ROS in axonal degeneration in EAE. Moreover, these results allude to a causal relation between the observed mitochondrial changes and increased mitochondrial ROS production in EAE. Further evidence for mitochondrial involvement in EAE pathogenesis was obtained in mice lacking cyclophilin D, a key regulator of the mitochondrial permeability transition pore (MPTP). The induction of MPTP can lead to depolarization of mitochondria, decreased ATP production and increased ROS production, eventually leading to cell death. In cyclophilin D knock-out mice an attenuated disease course and a remarkable sparing of axons was observed compared to wild type animals, whereas the extent of inflammation was similar. Notably, neurons derived from these knockout mice were less sensitive to oxidative and nitrosative stress, indicating that their axonal mitochondria are more resistant to inflammation-derived ROS and subsequent mitochondrial dysfunction, resulting in less axonal degeneration.

Taken together, several studies have observed mitochondrial changes in MS and its animal model, EAE. However, many questions regarding the role of mitochondria in MS pathogenesis remain unanswered. In this thesis, several of these remaining questions will be addressed.

4. Aims and outline of this thesis

The realization that for successful treatment of MS not only the immune system should be targeted but also the CNS itself, led to renewed interest in mechanisms underlying neurodegeneration in MS. As discussed above, there is substantial circumstantial evidence suggesting that mitochondria mediate degeneration of axons. Moreover, a handful of studies already described mitochondrial changes in MS lesions and MS grey matter, warranting further investigation. The aim of this thesis is therefore to further explore mitochondrial changes in the CNS of MS patients as well as unravelling the underlying mechanisms, hopefully this might eventually lead to new therapeutic targets to combat neurodegeneration in MS.

In chapter 2, we quantify the mitochondrial content of astrocytes and axons in NAWM and WM MS lesions. Also, we look at the expression of several OxPhos proteins and mtHSP70, which is upregulated upon increased mitochondrial oxidative stress. Lastly, we study complex IV activity in WM MS lesions and compared it with NAWM and control WM.
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Chapter 3 will be dedicated to the expression of Parkin in MS and Alzheimer's disease CNS tissue. Parkin is an essential protein for mitophagy, and is therefore important in mitochondrial quality control. In chapter 4, we explore altered expression of mitochondrial antioxidants in MS WM and use in vitro techniques to study the consequences of these alterations. Chapter 5 describes an elaborate study on the distorted mitochondrial transcription machinery in the cortex of MS patients and its effects on mitochondrial ROS defence mechanisms. In chapter 6, we investigate the expression of proteins involved in mitochondrial fission and fusion in MS cortex. Finally, the results of the previous chapters will be discussed and put into perspective in chapter 7.

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