We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Oligodendrocyte Metabolic Stress in Neurodegeneration

Daniel Radecki and Alexander Gow

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54461

1. Introduction

Neurodegeneration can be viewed in general terms as a common endpoint for a large and diverse group of nervous system diseases that arise in patients with disparate clinical symptoms. As such, neurodegeneration is a convergent pathology wherein clinical signs are largely dependent on the location and identity of the degenerating cells. For example, in patients for whom substantia nigra neurons are degenerating, the accompanying symptoms reflect Parkinson disease (PD). Many of the symptoms are unique to PD, thereby enabling diagnosis, and would rarely be confused with those of patients suffering from amyotrophic lateral sclerosis (ALS), for whom ventral horn motor neurons in the spinal cord are lost. In the same vein, disease in patients with Alzheimer disease (AD) or multiple sclerosis (MS) stem from the loss of distinct cell populations which confer unique phenotypes.

Despite these disease specific phenotypes, recent evidence indicates that their underlying pathophysiology, and that of many others, involves activation of a signaling pathway known as the unfolded protein response (UPR). This suggests the exciting possibility of a shared disease mechanism and, potentially, a common treatment strategy such as the use of a single class of drugs.

Research efforts from many laboratories have begun to elucidate the importance of the UPR to disease etiology. For example, causative mutations in familial forms of PD and AD are found in genes that encode components of protein aggregation and degradation pathways such as the ubiquitin-proteasome pathway, which strongly suggests that sporadic forms of these diseases also arise from perturbed protein folding or degradation [1-3]. In addition, the etiology of MS, which was once understood to be entirely caused by autoimmune attacks on the central nervous system (CNS), is becoming increasingly unclear because of new evidence pointing to an underlying degenerative pathology in oligodendrocytes that in-

volves UPR induction and secondary activation of the immune system. Finally, the etiology of oligodendrocyte metabolic diseases including at least two of the leukodystrophies, vanishing white matter disease (VWM) and Pelizaeus-Merzbacher disease (PMD), is known to involve UPR activation.

In this review, we begin with a general definition of normal versus disease states in terms of cell homeostasis and its relation to UPR signaling, metabolic stress and neurodegeneration. Next, we examine essential aspects of the UPR signaling cascade, as well as emerging concepts about UPR activation and function, and conclude with an examination of MS as a primary UPR disease rather than its typical consideration as a primary autoimmune disease.

2. Metabolic stress and the concept of homeostasis

An increasing awareness of the pathophysiology of neurodegeneration has led to the realization that metabolic stress is a major contributor to disease etiology. This novel view can be conceptualized as follows. Cells under normal metabolic conditions are described as maintaining homeostasis. Metabolic stress is viewed as a loss of homeostasis, defined as any pathological process that impedes cell function.

Intracellular signaling pathways have evolved to detect and counteract many forms of metabolic stress. These pathways modify cell activity and impart significant protection under pathological conditions, thereby maintaining homeostasis. However, when metabolic stress disrupts homeostasis, cells become vulnerable to apoptosis leading to brain atrophy and disease. These concepts have been principally developed to account for the pathophysiology and disease severity that we observe in animal models of PMD [4-7]. However, they are also relevant to other oligodendrocyte diseases as well as major neurological diseases like AD, PD and ALS.

3. Misfolded proteins trigger UPR signaling

Two of the most important homeostatic features of normal cell function are the consistent and efficient translation of proteins and the post-translational folding and processing of those proteins into their stable higher-ordered conformations. However, not all protein molecules achieve native conformations after translation even in normal cells, and particularly in genetic diseases when missense or nonsense mutations in coding exons of genes confer distinct misfolded conformations on the translated products [8, 9].

In cases of transmembrane or secreted proteins that are synthesized on the ER (endoplasmic reticulum) in eukaryotes, misfolded or abnormal folding intermediates are prevented from being transported beyond this compartment by the quality control machinery of the cell. These nascent polypeptide chains are either removed from the ER and degraded via the ubiquitin-proteasome system or are shunted into the lysosome by autophagy [10, 11]. How-

ever, if the synthesis of these polypeptides surpasses the rate of degradation, they accumulate in the ER, causing metabolic stress and induction of the UPR [12].

The importance of UPR signaling to cell homeostasis and survival is highlighted by the degree of conservation of this pathway in prokaryotes and in eukaryotes from yeast to mammals. Significant increases in signaling complexity in higher eukaryotes also indicates the growing importance of this pathway in multicellular organisms through evolution [13]. In most cases for eukaryotes, activation of the UPR by misfolded proteins causes rapid shutdown of global protein translation, expansion of intracellular membrane-bound compartments, induction of molecular chaperone expression and increased degradation of misfolded proteins.

In the event that such comprehensive changes in cell structure, reprogramming and metabolism are ineffective at curbing UPR signaling and reestablishing homeostasis, cells will inevitably undergo apoptosis in a manner that limits damage to neighboring cells and the survival of the organism [14]. Surprisingly, the nature of the trigger that induces apoptosis appears to be divergent in different cell types, and several hypotheses have been developed to explain the bulk of published studies as detailed below.

3.1. UPR signaling in mammals

In general terms, the UPR signaling cascade maintains cell homeostasis, metabolism and cell survival [13, 15, 16]. In higher eukaryotes, the UPR can be divided into three pathways named for the proteins that initiate signaling: IRE1α (inositol requiring protein 1α), ATF6 (activating transcription factor 6) and PERK (pancreatic endoplasmic reticulum kinase). Together, these pathways increase expression of molecular chaperone proteins, protein degradation and decrease global translation to alleviate misfolding and restore homeostasis. [12, 17].

3.1.1. The IRE1 pathway

The IRE1α receptor is a transmembrane protein that is localized to the ER and detects the accumulation of misfolded proteins or, in more general terms, serves as a sensor of changes in secretory pathway protein flux. IRE1α was the first component of the UPR cascade to be identified in any eukaryote and is the only UPR sensor present in yeast [14, 18]. The ER luminal domain is topographically similar to the Major Histocompatibility Class (MHC) proteins of the immune system and appears to bind to the molecular chaperone protein, BiP, which maintains IRE1α as a monomer and prevents its activation. However, misfolded protein accumulation in the ER lumen sequesters BiP from IRE1α and allows this receptor to homodimerize leading to transautophosphorylation and activation of its cytoplasmic endoribonuclease P domain [19].

A major downstream target of the IRE1α nuclease domain is an mRNA that encodes the bzip transcription factor, X-box Binding Protein 1 (XBP1) [20]. Processing of this mRNA removes a short internal 26 base intron and completes the major open reading frame that encodes functional XBP1. The major target genes of XBP1 include molecular chaperones which

are ER-resident proteins that bind to unfolded or misfolded polypeptides [14]. Accordingly, the IRE1α pathway detects changes in protein flux and acts to increase the folding capacity of the ER, ultimately completing a negative feedback loop on the UPR.

3.1.2. The ATF6 pathway

A second UPR pathway is initiated through activation of the membrane-tethered ATF6 protein and converges with IRE1α signaling. ATF6 interacts with BiP, similar to IRE1α. Misfolded proteins displace BiP from the ATF6 luminal domain and enable the protein to traffic from the ER to the Golgi apparatus where it is cleaved by the site 1 and site 2 proteases (S1P and S2P, respectively). The resulting cytosolic N-terminal fragment of ATF6 is the functional bzip transcription factor that heterodimerizes with XBP1 and induces expression of molecular chaperone genes including BiP, glucose-regulated protein 94 (GRP94) and other genes encoding protein folding pathway proteins [15, 19]. ATF6 also upregulates proteins associated with the ER Associated Degradation (ERAD) pathway, which is a checkpoint in the ER that ubiquitinates proteins and shuttles them into the cytoplasm for proteasome-mediated degradation [21]. Thus, ATF6 helps to upregulate chaperones to relieve mild protein misfolding, but can also activate degradation of proteins that are severely misfolded and cannot be rescued by chaperones.

3.1.3. The PERK pathway

A third UPR pathway is regulated by an ER-resident receptor known as PERK. The luminal domain of PERK functions analogously to that of IRE1α in binding BiP, and is also activated by dimerization and transautophosphorylation. The cytoplasmic domain of PERK is a protein kinase, a major target of which is the alpha subunit of eukaryotic initiation factor 2 (eIF2α). eIF2α is a critical component in ribosome assembly and can be inactivated by phosphorylation, which leads to the shut down of global protein synthesis [17].

Despite global translation arrest, a small number of proteins that are critical to the UPR signaling are actively translated, including the bzip transcription factors, activating transcription factor 4 (ATF4), ATF3 and the CCAAT-enhancer-binding protein homologous protein (CHOP) as well as the regulatory subunit of protein phosphatase 1 (PP1), known as growth-arrest and DNA damage protein 34 (GADD34) [17, 22]. The GADD34-PP1 complex is targeted to the ER membrane to dephosphorylate p-eIF2α and reinitiate protein translation. Thus, the PERK pathway temporarily halts protein synthesis to suppress additional accumulation of misfolded proteins in the ER. The pathway subsequently reactivates protein synthesis by opposing the phosphorylation activity of PERK. Thus, because of the time that is necessary to complete each of the steps downstream of eIF2α phosphorylation, the PERK pathway can be considered to be a time-delay circuit that forms a negative feedback loop to regulate UPR signaling.

3.2. Adaptive and maladaptive facets of UPR signaling

A common theme among the three branches of the UPR cascade is the similar activation of the ER-resident receptors by changes in protein flux leading to transcriptional or trans-

lational changes that reduce the accumulation of misfolded polypeptides and ultimately negatively feedback to switch off the UPR. Collectively, these activities comprise the adaptive arm of the UPR cascade, which adjusts cell metabolism to maintain homeostasis and promote cell survival. However, the UPR cascade also appears to include a maladaptive arm, the major function of which is to trigger apoptosis in the event that cells fail to maintain homeostasis.

Although the maladaptive arm of the UPR is widely known and discussed in published studies, the molecular mechanisms underlying its activation are poorly understood. Many studies identify CHOP or a decoy kinase known as Tribbles3 (Trib3) as major components of the maladaptive trigger for apoptosis [23, 24]; however, this view reveals a significant conundrum. Thus, if PERK signaling requires CHOP expression to complete the negative feedback loop that dephosphorylates eIF2α as part of the adaptive response, why would CHOP expression trigger apoptosis as part of the maladaptive response?

There are three principal hypotheses that address this issue. The first proposes that CHOP is a molecular rheostat that drives distinct downstream pathways as a function of expression level [25, 26]. The second suggests that the IRE1 and PERK pathways act in concert to effect cell survival but drive apoptosis when the activities of these pathways are unbalanced [20]. The third hypothesizes that apoptosis is triggered stochastically at a restriction point in the PERK pathway, which is more-or-less coincident with the reinitiation of protein translation upon eIF2α dephosphorylation [17, 27].

3.2.1. CHOP as a rheostat

Studies in human embryonic kidney 293 (HEK293) cells utilizing genetic and chemical induction of the UPR have led to the hypothesis of graded activation, mediated by CHOP, with apoptosis resulting from the highest levels of expression [25, 26]. Transient ER stress requires a UPR; however, the response itself would be modulated so that mild stress generates tapered transient CHOP induction, and severe prolonged stress causes sustained CHOP expression. Indeed, a modulated CHOP response has been observed during molecular and mechanical stress in vitro that activates PERK in the ER, with sustained PERK activation causing sustained CHOP expression and increased apoptosis. In contrast, oligodendrocytes undergoing severe metabolic stress and widespread apoptosis do not express CHOP, suggesting that its induction is transient even during severe stress [4, 6, 28].

3.2.2. Balanced IRE1 and PERK signaling

From their in vitro manipulation of the IRE1 and PERK pathways in HEK293 cells, Walter and colleagues [29] identified disparate roles for each pathway that could account for divergent UPR phenotypes in animal models of disease. The results showed that activating the PERK pathway alone decreased cell proliferation in vitro and triggered a morphological dedifferentiation characterized by a loss of cell processes. In contrast, unilateral IRE1 activation increased cell numbers. Because activation of the PERK and IRE1 pathways stem from the

accumulation of misfolded proteins, it is likely that the relative activation levels of these pathways generates a balance between proliferation and differentiation that determines the fate of the cells.

3.2.3. Stochastic apoptosis

The third hypothesis stems from the results of several in vivo studies involving CNS and PNS myelin mutant mice [17]. In contrast to in vitro studies in many cell types where CHOP expression drives apoptosis and CHOP loss-of-function promotes cell survival, ablation of the *Chop* gene in oligodendrocytes renders them much more susceptible to apoptosis under UPR conditions [6, 30]. Thus, CHOP promotes cell survival. In addition, induction of CHOP in Schwann cells does not induce cell death but, rather, causes dedifferentiation of these cells to promote their survival [27]. A similar mechanism is also observed in osteoblasts [31]. Together, these and other studies [29, 32] indicate that PERK signaling protects myelinating cells from apoptosis. If so, how do these cells undergo apoptosis?

One possibility is that myelinating cells become vulnerable to apoptosis at a restriction point in the PERK pathway as protein translation is restarted. At this restriction, the PP1-GADD34 complex dephosphorylates eIF2α and demand for ATP, GTP, NADH and other high-energy intermediates would dramatically increase. Sub-threshold levels of these critical molecules, perhaps also exacerbated by dissipation of the mitochondrial membrane potential, would occur stochastically in individual cells during translation-suppression and cause a loss of homeostasis leading to cell death. Under mild metabolic stress conditions, most cells would maintain supra-threshold levels of critical molecules and survive beyond the restriction point. Some of these cells would undergo apoptosis during subsequent UPR-induction cycles. Ultimately, the stronger the stress, the greater the number of UPR cycles, and the higher the likelihood that cells would undergo apoptosis.

4. Oligodendrocyte metabolic stress and neurodegenerative disease

Oligodendrocytes play a critical role in the CNS by myelinating axons to ensure efficient saltatory conduction and reliable communication between neurons over long distances as well as to promote neuronal survival [33]. The surface area of myelin membrane that is synthesized by each oligodendrocyte within a few days during development exceeds that of the cell body by several hundred fold, which makes oligodendrocytes one of the most metabolically active cell types [34]. Thus, it is not surprising that these cells are vulnerable to metabolic stress and undergo apoptosis associated with protein misfolding [4, 33, 35]. Genetic diseases that disrupt oligodendrocyte metabolism are associated with UPR signaling and are well characterized at the molecular level. It is also becoming increasingly clear that other diseases of oligodendrocytes, such as MS, involve this signaling pathway.

4.1. Leukodystrophy and metabolic stress as a model of neurodegenerative disease etiology

The leukodystrophies are a group of diseases characterized by a systemic absence of white matter in the CNS resulting in sensorimotor deficits, ataxia, hypotonia and eventual decline in cognitive function [36]. Although leukodystrophies affect all white matter tracts to varying extents, they differ in their primary causes. For example, in the case of PMD the absence of white matter stems from mutations in the gene encoding the most abundant myelin protein, proteolipid protein-1 (PLP1) [37-39], while VWM disease is caused by mutations in genes that encode subunits of the eIF2 complex [40]. In many cases, metabolic stress is severe enough that the disease develops in childhood and dramatically affects the life span of the patient [6, 38, 39, 41, 42]. The common mechanism between these leukodystrophies is the failure to manage and remove misfolded proteins, some of which rapidly activate the UPR leading to metabolic stress and apoptosis [38, 39]. Importantly, metabolic stress in oligodendrocytes also leads to secondary neuron loss [43], which demonstrates the potentially severe consequences of this disease mechanism beyond the primary cell type involved.

4.1.1. Pelizaeus-Merzbacher disease

Arguably, PMD is one of the most extensively characterized neurodegenerative UPR disease in terms of molecular and cellular etiology. In virtually all patients, disease stems from genetic lesions in the X-linked *Plp1* gene [44]. The gene products are polytopic membrane proteins that constitute approximately 50% of the total protein in the CNS myelin sheath and the developmental expression levels of this gene are among the most abundantly expressed in mammals.

Mutations in the *Plp1* gene arises from three types of genetic lesions: duplications, deletions and missense/nonsense mutations. These lesions confer disease symptoms with a wide range of clinical severity that are mild in the case of deletions, severe in the case of duplications and mild or severe for coding region mutations. In general, mild phenotypes are associated with reduced oligodendrocyte function but relatively little cell death while severe forms cause widespread apoptosis and a virtual absence of white matter [45, 46].

4.1.1.1. Gene duplications and deletions in PMD

Mild forms of disease caused by deletion of the entire *Plp1* gene or nonsense mutations in exon 1 are characterized by clinical presentation in middle age patients, often in the form of cognitive decline [43, 44] and a length-dependent dying back neuropathology. Although the absence of *Plp1* expression in patients does not significantly reduce oligodendrocyte function and the amount of myelin formed during development, the absence of this protein reduces the long-term stability of long myelinated tracts such as the corticospinal tract, which degenerate in later life. Importantly, the stability of the CNS specifically requires the PLP1 protein, and cannot be conferred by the alternatively-spliced PLP1 isoform, called DM-20, which lacks a 35 amino acid segment in the cytoplasmic domain of the protein [47].

In contrast, *Plp1* duplications cause severe phenotypes perinatally or within the first year of life. Children and adolescents with duplications exhibit severe cognitive decline in conjunction with physical disabilities including loss of motor function and coordination [6, 48]. Because of extremely high PLP1 expression levels during normal development, duplications may effectively overwhelm the secretory pathway in oligodendrocytes and disrupt cell function or survival. Whether this disruption involves defective cholesterol trafficking [49] or immune activation [50] is currently unclear.

4.1.1.2. Plp1 mutations in PMD

Approximately 30% of PMD patients harbor mutations in the *Plp1* coding region that cause missense or nonsense changes in the protein primary structure. These changes arise throughout the coding region and cause a spectrum of disease severities in patients [43, 44]. Although there does not appear to be a correlation between the location of a mutation and disease severity, most mutations in the transmembrane domains cause severe disease. This is a general feature of membrane domain mutations in many secretory pathway proteins. Accordingly, the underlying cell biology of coding region mutations is proposed to stem from a failure of protein folding and trafficking through the secretory pathway, leading to metabolic stress and activation of the UPR [6, 12, 43, 44, 51]. Two missense mutations in PMD patients have also been identified in mice. Although similarities of disease symptoms and pathology conferred by each mutation might be anticipated because the PLP1 primary structure is identical in rodents and humans, the robustness of these findings provides a strong basis for using the animal models to model PMD [4, 52-55].

4.1.2. Animal models of PMD

A common goal in the analysis and development of therapeutic strategies to treat many neurodegenerative diseases is the generation of animal models, particularly in rodents which are amenable to genetic manipulation. Naturally-occurring animal models of PMD have been described in multiple species including dog, rabbit, rat and mouse [56-59], and engineered mutations have been generated in rats and mice [47, 52, 58, 60, 61].

The *jimpy* mouse, which exhibits a severe behavioral phenotype, is the original *Plp1* allele identified and has been characterized in greatest detail [57]. More recently, the *rumpshaker* (*rsh*) and *myelin synthesis-deficient* (*msd*) alleles have become popular not only because they exhibit mild and severe phenotypes, respectively, but also because the specific single amino acid changes harbored by these strains are also found in humans [54, 62].

4.1.2.1. Mild disease in rsh mice

This allele was originally described by Griffiths and colleagues and harbors an isoleucine to threonine mutation at codon 187 (I187T) in the second extracellular domain of PLP1 [54, 63]. These mice are fertile and exhibit a normal life span with behavioral changes becoming evident between 13 – 19 days after birth (P13 – 19), depending on the background strain of the colony. The total myelin content of the brain is reduced to 40–50%. PLP1 is virtually absent

from *rsh* myelin sheaths, but DM-20 is present at normal levels[54]. This selective trafficking defect is consistent with the protein misfolding hypothesis [4, 5, 7, 51]. Oligodendrocyte metabolic stress leading to apoptosis is observed to occur at a moderate level in this strain [6, 28] despite early claims to the contrary [64].

4.1.2.2. Severe disease in msd mice

The *msd* mutation was originally described by Baumann and colleagues [65] and is characterized by an alanine to valine substitution at codon 243 (A243V) in PLP1 [66]. Mice harboring this mutation exhibit severe symptoms, with behavioral changes evident by P13 and a short life span of 3 - 4 weeks. The amount of myelin in the *msd* CNS is severely reduced to approximately 5% of normal and the phenotype is very similar to that of *jimpy* mice on the same genetic background [62, 67]. Oligodendrocyte apoptosis is widespread in these mice and, similar to *rsh* mice, involves metabolic stress and activation of the UPR [4, 6, 12]. PMD patients with the corresponding mutation have a severe form of the disease with systemic demyelination and widespread oligodendrocyte death.

Importantly, some patients with severe forms of PMD such as those corresponding to the *msd* mutation also show signs of neuron loss as a consequence of profound hypomyelination. These observations establish the principle that the survival of each of the major neural cell types is interdependent; thus, a primary insult in oligodendrocytes in the form of metabolic stress has secondary consequences for neurons [4, 6, 37]. Furthermore, symptoms in PMD can include autoimmune disease [68], which has major significance for the classification of MS as a neurodegenerative disease and suggests that the etiology may arise, at least in some instances, from primary metabolic stress in oligodendrocytes leading to secondary immune activation.

4.2. Is MS a neurodegenerative metabolic stress disease of oligodendrocytes?

Multiple sclerosis is the most common neurological disease in young adults worldwide and is typically described as an autoimmune attack on CNS white matter tracts resulting in focal lesions and degeneration of myelin throughout the CNS [69]. There are three major forms of this disease, relapse remitting MS (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS). RRMS is the most prevalent form and is characterized by patients for which lesions develop spontaneously and cause transient loss of neurological function (also known as a relapse) followed by essentially full recovery (known as remission). Disease in RRMS patients eventually transitions from these transient symptoms to SPMS, when patients do not fully recover neurological function after relapses and sensorimotor deficits become more continuous and progressive. PPMS defines the third category, which is clinically similar to SPMS but without a preceding RRMS phase. Thus, patients experience rapid severe degeneration [69-71].

Results from recent long-term clinical trials in RRMS patients that were medicated with any of several new immune suppressant therapies demonstrate that dramatic reductions in the number of new demyelinating lesions is accompanied by only modest amelioration of clini-

cal symptoms [72-75]. Moreover, patients continue to experience disease progression. These data indicate that the number of autoimmune attacks on the CNS is not strongly correlated with increasing disease severity and that there may be additional unknown mechanisms involved in the pathogenesis. If so, then immune attacks may actually be secondary to an underlying primary etiology.

Clues about the nature of such an unknown etiology in MS are scarce, but may be found in the clinical literature. For example, a few case reports detailing the misdiagnosis of PMD as childhood MS indicate that the symptoms of these two diseases overlap significantly. Indeed, the responsiveness of one of these patients to steroids suggests that PMD symptoms can be exacerbated by immune system activation at some level and perhaps similar to MS. Together, these reports provide tantalizing, if anecdotal, evidence that metabolic stress in oligodendrocytes could be one form of a primary etiology that secondarily activates the immune system [76-78].

4.2.1. Neurodegeneration in MS

The immune demyelinating lesion in white matter is an important component of MS pathology that has been studied extensively [76, 79-81]. However, a plethora of the clinical symptoms, particularly those affecting the daily activities of patients and significantly reducing their quality of life, stem from axonal transection and loss of neurons in gray matter regions [82]. The significance of this degenerative feature is that emergent immune suppressive therapies might not be expected to have a major impact in halting symptom progression [81, 83, 84]. Cognitive decline, memory loss, partial paralysis, and optic neuritis are caused by the loss of neurons in different brain regions that are spared from direct immune attacks but still contribute to disease, especially for the more severe SPMS and PPMS forms [78, 85].

Gray matter cortical atrophy may constitute the majority of the total tissue atrophy observed in MS patients, especially those with SPMS and PPMS [86-88]. Although this pathological feature has been known for decades, one of the most important advances contributing to our understanding and acceptance of neuron loss as a major, if not the principal, symptom of MS is the increasing sensitivity for detecting gray matter lesions using clinical diagnostic MRIs. Thus, with renewed interest and appreciation for this issue, there is an urgent need to understand the underlying pathogenesis. In this regard, the development of novel animal models will lead to new hypotheses and the development of novel therapeutic strategies.

4.2.2. Current and future MS models

Because of the characterization of MS as a primary autoimmune disease, a large proportion of animal model studies, particularly in mice, have focused on developing and characterizing immune models such as experimental autoimmune encephalomyelitis (EAE) [79, 81, 89, 90]. These models rely on priming the peripheral immune system with injected peptides from various myelin proteins to stimulate the immune system to attack and demyelinate white matter tracts. Damage is largely confined to spinal cord and is characterized by immune cell profiles of $CD4^+$, $CD8^+$ T-cells and $CD68^+$ macrophages as well as

proinflammatory cytokine release [79, 91-93]. However, these models have significant shortcomings in modeling MS pathology. For example, the neurological phenotypes in affected mice largely stems from tissue edema rather than demyelination. Although some models generate immune mediated demyelination, symptoms are monophasic rather than multiphasic and relapsing-remitting, in contrast to the most common form of MS [94]. Finally, in the absence of gray matter lesions and subsequent neuronal degeneration, these models fail to recapitulate the most debilitating features of MS that contribute to the declining quality of life for patients [77].

To overcome such shortcomings, we have developed a novel genetic mouse model of MS pathology that is based on primary metabolic stress in oligodendrocytes [95]. The etiology of disease in these mice has been characterized in mechanistic detail [4-7, 51] and we are currently determining if we can recapitulate the degenerative white and gray matter lesions that arise in MS patients without specifically provoking the immune system to attack the CNS. Furthermore, we are determining if our primary insult in oligodendrocytes can secondarily induce a relapsing-remitting or progressive autoimmune phenotype in the mice that would account for the pathophysiology observed in MS patients in terms of metabolic stress rather than primary autoimmune activation.

5. Identifying metabolic stress for the diagnosis of neurodegenerative diseases

For many neurodegenerative diseases, progress toward finding treatments and cures is painstakingly slow. This is in part limited by current capabilities for real-time imaging of the CNS as well as by ethical constraints that protect the health of patients and often exclude invasive procedures such as biopsies. These limitations largely confine research studies to post-mortem tissue, or generating in vitro and in vivo animal models, to develop treatments for disease. In many cases, these approaches have proved only partially effective for the study of neurodegenerative diseases [79, 94, 96, 97].

Recently, several imaging technologies have advanced significantly and become sufficiently widespread in hospitals for routine application to neurodegenerative diseases like AD and MS, including magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and positron emission tomography (PET) [98-100].

5.1. Magnetic resonance imaging

Magnetic resonance techniques are widely used in clinical diagnostics of many diseases since their development approximately 40 years ago [86, 101]. Nevertheless, there are significant drawbacks for their use in neurodegenerative diseases, particularly with respect to early disease detection [69, 102]. MRI is the most common technique used, and is particularly important for identifying white matter pathology such as hypomyelination or demyelinating lesions, as well as gray matter degeneration, because it can easily detect differences in

tissue structure or composition between normal and diseased regions. Applications to expand the utility of this technique beyond the structural realm include injectable biomarkers to detect subclinical disease or to follow the evolution of lesions in real time, but these are currently nonexistent except for animal studies [103].

The imaging of metabolic changes in structurally normal regions of the CNS can be achieved using MRS [100, 104], but this technology is currently limited to a few major neurochemicals at low resolution. MRS can be used to detect neuron cell loss by monitoring levels of the neurochemical, N-acetyl aspartate (NAA), which is specific to this cell type [99]. However, the time, expense and difficulty of scanning more than one region of the CNS at a time severely limits the use of MRS for early disease detection when clinicians are uncertain about the specific location of lesions.

5.2. Positron emission tomography

Positron emission tomography involves the incorporation of radioactive molecules into metabolites that are selectively taken up by defined cell populations so that their location and metabolic activity can be analyzed [105]. This technique has the potential to generate detailed information about the molecular basis of neurodegeneration because the metabolism of affected cells changes dramatically as they lose homeostasis. PET has been used successfully in diseases such as AD and PD where degeneration of specific neuronal populations can be monitored *in vivo* even before patients experience significant symptoms [106]. However, a significant drawback with this technique is its low resolution, which renders the technique very limited for small animal model studies.

6. Treatments for metabolic stress in neurodegenerative diseases

Increasing awareness and more sophisticated technologies have enabled earlier detection of neurodegenerative processes. However, the development of treatment strategies often has been hampered, in large measure because of the enormous plasticity of the CNS which enables neuronal circuits to compensate for ongoing damage and cell loss. Thus, these diseases only become clinically apparent at advanced stages when damage is widespread and irreparable.

The treatment of neurodegenerative diseases is also hampered by the fact that a number of these diseases stem from toxic gain-of-function, rather than loss-of-function, phenotypes. For example, deletion of the *Plp1* gene is the most mild form of PMD; thus, the loss of the protein in myelin does not confer a strong phenotype. However, mutations that cause PLP1 to misfold are toxic to oligodendrocytes because of the extremely rapid accumulation of the intermediates in the ER, which overwhelm the capacity of the UPR to eliminate them through the ubiquitin-proteasome system [107]. Therapeutic strategies to insert a wild type *Plp1* allele into these patients would fail unless the toxic protein from the mutant allele were also eliminated.

The lessons learned from PMD and other neurodegenerative diseases could be relevant to MS and may help to explain why this disease is recalcitrant to treatments that only target the immunological aspects of the pathophysiology. Thus, considering MS as a gain-of-function disease with an underlying condition of unknown etiology that is exacerbated by auto-immune activation may shed new light on the pathophysiology and lead to novel therapeutic strategies to ameliorate the symptoms [70, 108, 109].

7. Conclusion

Although neurodegenerative diseases have typically been defined as a disparate group of diseases involving neurons, there is clear evidence in the clinical and basic science literature against such a narrow viewpoint. For example, diseases of the white matter such as PMD and MS arise from primary insults to oligodendrocytes and cause neuron loss in gray matter and lead to behavioral changes and memory loss. This reflects a broader consideration that all major cell types of the CNS are interdependent and degenerative changes in one of these will lead to loss of at least some of the other cell types.

In similar vein, the fundamental belief by immunologists that MS is a primary autoimmune disease is no longer tenable. Clear evidence from large clinical trials demonstrates that the elimination of adaptive immune cells from the CNS by various forms of immune suppression does not halt the progression of disease at early or late stages. The simplest interpretation of these data is that there is an underlying etiology that is poorly understood and must be recognized. In light of overlapping symptoms between PMD and MS, it is plausible that metabolic stress could play a primary role in oligodendrocyte degeneration with secondary activation of immune cells. Indeed, several studies have demonstrated induction of the UPR in MS tissue.

Nomenclature

UPR (unfolded protein response), IRE (inositol requiring protein), PERK (pancreatic endoplasmic reticulum kinase), ATF (activating transcription factor), XBP (X-box binding protein), CHOP (CCAAT/-enhancer binding protein homologous protein), PLP1 (proteolipid protein-1), GADD34 (growth arrest and DNA damage protein 34), BiP (chaperone protein), eIF2α (eukaryotic initiation factor 2 α), PP1 (protein phosphatase 1), GRP94 (glucose-regulated protein 94), S1P (site 1 protease), S2P (site 2 protease), ERAD (endoplasmic reticulum associated degradation), ataxia (lack of voluntary muscle coordination), hypotonia (low muscle tone), RRMS (relapse-remitting Multiple Sclerosis), SPMS (secondary progressive Multiple Sclerosis), PPMS (primary progressive Multiple Sclerosis), CD4,8,68 (cluster of differentiation, immune cell specific glycoproteins), *rumpshaker* mutation (*rsh*), *myelin-synthesis deficient* mutation (*msd*), MRI (magnetic resonance imaging), MRS (magnetic resonance spectroscopy), PET (positron emission tomography).

Acknowledgements

Author details

Daniel Radecki[1] and Alexander Gow[1,2,3*]

*Address all correspondence to: agow@med.wayne.edu

1 Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI, USA

2 Carman and Ann Adams Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI, USA

3 Department of Neurology, Wayne State University School of Medicine, Detroit, MI, USA

References

[1] Coppede F. Genetics and Epigenetics of Parkinson's Disease. The Scientific World Journal 2012;2012: 12.

[2] Chen W-T, Hong C-J, Lin Y-T, Chang W-H, Huang H-T, Liao J-Y, Chang Y-J, Hsieh Y-F, Cheng C-Y, Liu H-C, Chen Y-R, Cheng IH. Amyloid-Beta (Aβ) D7H Mutation Increases Oligomeric Aβ42 and Alters Properties of Aβ-Zinc/Copper Assemblies. PLoS ONE 2012;7(4): e35807.

[3] Inayathullah M, Teplow DB. Structural dynamics of the ΔE22 (Osaka) familial Alzheimer's disease-linked amyloid β-protein. Amyloid 2011;18(3): 98-107.

[4] Gow A, Southwood CM, Lazzarini RA. Disrupted Proteolipid Protein Trafficking Results in Oligodendrocyte Apoptosis in an Animal Model of Pelizaeus-Merzbacher Disease. The Journal of Cell Biology 1998;140(4): 925-934.

[5] Southwood C, Gow A. Molecular pathways of oligodendrocyte apoptosis revealed by mutations in the proteolipid protein gene. Microscopy Research and Technique 2001;52(6): 700-708.

[6] Southwood CM, Garbern J, Jiang W, Gow A. The unfolded protein response modulates disease severity in Pelizaeus-Merzbacher disease. Neuron 2002;36(4): 585-96.

[7] Gow A, Lazzarini R. A cellular mechanism governing the severity of Pelizaeus −Merzbacher disease. Nature Genetics 1996;13: 422-428.

[8] Doyle KM, Kennedy D, Gorman AM, Gupta S, Healy SJM, Samali A. Unfolded proteins and endoplasmic reticulum stress in neurodegenerative disorders. Journal of Cellular and Molecular Medicine 2011;15(10): 2025-2039.

[9] Tosic M, Matthey B, Gow A, Lazzarini RA, Matthieu JM. Intracellular transport of the DM-20 bearing shaking pup (shp) mutation and its possible phenotypic consequences. Journal of Neuroscience Research 1997;50(5): 844-852.

[10] Scheper W, Nijholt DAT, Hoozemans JJM. The unfolded protein response and proteostasis in Alzheimer disease: Preferential activation of autophagy by endoplasmic reticulum stress. Autophagy 2011;7(8): 910-911.

[11] Yu Z, Wang AM, Adachi H, Katsuno M, Sobue G, Yue Z, Robins DM, Lieberman AP. Macroautophagy Is Regulated by the UPR–Mediator CHOP and Accentuates the Phenotype of SBMA Mice. PLoS Genet 2011;7(10): e1002321.

[12] Gow A, Sharma R. The unfolded protein response in protein aggregating diseases. NeuroMolecular Medicine 2003;4(1): 73-94.

[13] Mori K. Signalling Pathways in the Unfolded Protein Response: Development from Yeast to Mammals. Journal of Biochemistry 2009;146(6): 743-750.

[14] Zhang K, Kaufman RJ. The unfolded protein response. Neurology 2006;66(1 suppl 1): S102-S109.

[15] Walter P, Ron D. The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. Science 2011;334(6059): 1081-1086.

[16] Ye C, Verchot J. Role of unfolded protein response in plant virus infection. Plant Signaling & Behavior 2011;6(8): 1212-1215.

[17] Gow A, Wrabetz L. CHOP and the endoplasmic reticulum stress response in myelinating glia. Current Opinion in Neurobiology 2009;19(5): 505-510.

[18] Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol 2012;13(2): 89-102.

[19] Cao S, Kaufman R. Unfolded protein response. Current Biology 2012;22(16): R622-R626.

[20] Gardner BM, Walter P. Unfolded Proteins Are Ire1-Activating Ligands That Directly Induce the Unfolded Protein Response. Science 2011;333(6051): 1891-1894.

[21] Egawa N, Yamamoto K, Inoue H, Hikawa R, Nishi K, Mori K, Takahashi R. The Endoplasmic Reticulum Stress Sensor, ATF6α, Protects against Neurotoxin-induced Dopaminergic Neuronal Death. Journal of Biological Chemistry 2011;286(10): 7947-7957.

[22] Teske BF, Wek SA, Bunpo P, Cundiff JK, McClintick JN, Anthony TG, Wek RC. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Molecular Biology of the Cell 2011;22(22): 4390-4405.

[23] Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H. TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. EMBO J 2005;24(6): 1243-1255.

[24] Zou C-G, Cao X-Z, Zhao Y-S, Gao S-Y, Li S-D, Liu X-Y, Zhang Y, Zhang K-Q. The Molecular Mechanism of Endoplasmic Reticulum Stress-Induced Apoptosis in PC-12 Neuronal Cells: The Protective Effect of Insulin-Like Growth Factor I. Endocrinology 2009;150(1): 277-285.

[25] Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, Harding H, Novoa I, Varia M, Raleigh J, Scheuner D, Kaufman RJ, Bell J, Ron D, Wouters BG, Koumenis C. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. EMBO J 2005;24(19): 3470-3481.

[26] Mak BC, Wang Q, Laschinger C, Lee W, Ron D, Harding HP, Kaufman RJ, Scheuner D, Austin RC, McCulloch CA. Novel Function of PERK as a Mediator of Force-induced Apoptosis. Journal of Biological Chemistry 2008;283(34): 23462-23472.

[27] Saporta MAC, Shy BR, Patzko A, Bai Y, Pennuto M, Ferri C, Tinelli E, Saveri P, Kirschner D, Crowther M, Southwood C, Wu X, Gow A, Feltri ML, Wrabetz L, Shy ME. MpzR98C arrests Schwann cell development in a mouse model of early-onset Charcot–Marie–Tooth disease type 1B. Brain 2012;135(7): 2032-2047.

[28] Sharma R, Gow A. Minimal role for caspase 12 in the unfolded protein response in oligodendrocytes in vivo. Journal of Neurochemistry 2007;101(4): 889-897.

[29] Lin JH, Li H, Zhang Y, Ron D, Walter P. Divergent Effects of PERK and IRE1 Signaling on Cell Viability. PLoS ONE 2009;4(1): e4170.

[30] Lin W, Kemper A, Dupree JL, Harding HP, Ron D, Popko B. Interferon-γ inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. Brain 2006;129(5): 1306-1318.

[31] Shirakawa K, Maeda S, Gotoh T, Hayashi M, Shinomiya K, Ehata S, Nishimura R, Mori M, Onozaki K, Hayashi H, Uematsu S, Akira S, Ogata E, Miyazono K, Imamura T. CCAAT/Enhancer-Binding Protein Homologous Protein (CHOP) Regulates Osteoblast Differentiation. Molecular and Cellular Biology 2006;26(16): 6105-6116.

[32] Lin W, Kunkler PE, Harding HP, Ron D, Kraig RP, Popko B. Enhanced Integrated Stress Response Promotes Myelinating Oligodendrocyte Survival in Response to Interferon-γ. The American Journal of Pathology 2008;173(5): 1508-1517.

[33] Bradl M, Lassmann H. Oligodendrocytes: biology and pathology. Acta Neuropathologica 2010;119(1): 37-53.

[34] Pfeiffer SE, Warrington AE, Bansal R. The oligodendrocyte and its many cellular processes. Trends in Cell Biology 1993;3(6): 191-197.

[35] D'Antonio M, Feltri ML, Wrabetz L. Myelin under stress. Journal of Neuroscience Research 2009;87(15): 3241-3249.

[36] Perlman SJ, Mar S, Leukodystrophies Neurodegenerative Diseases, editor, Editor Editors. 2012, Springer US. p. 154-171.

[37] Mar S, Noetzel M. Axonal Damage in Leukodystrophies. Pediatric Neurology 2010;42(4): 239-242.

[38] Sakai N. Pathogenesis of leukodystrophy for Krabbe disease: Molecular mechanism and clinical treatment. Brain and Development 2009;31(7): 485-487.

[39] Sawaishi Y. Review of Alexander disease: Beyond the classical concept of leukodystrophy. Brain and Development 2009;31(7): 493-498.

[40] van der Voorn J, van Kollenberg B, Bertrand G, Van Haren K, Scheper G, Powers J, van der Knaap M. The unfolded protein response in vanishing white matter disease. Journal of Neuropathology and Experimental Neurology 2005;69(5): 770-775.

[41] Castaneda J, Lim M, Cooper J, Pearce D. Immune system irregularities in lysosomal storage disorders. Acta Neuropathologica 2008;115(2): 159-174.

[42] Powers J, Liu Y, Moser A, Moser H. The inflammatory myelinopathy of adreno-leukodystrophy: cells, effector molecules, and pathogenic implications. Journal of Neuropathology and Experimental Neurology 1992;51(6): 630-643.

[43] Garbern J. Pelizaeus-Merzbacher disease: Genetic and cellular pathogenesis. Cellular and Molecular Life Sciences 2007;64(1): 50-65.

[44] Garbern J, Cambi F, Shy M, Kamholz J. THe molecular pathogenesis of pelizaeus-merzbacher disease. Archives of Neurology 1999;56(10): 1210-1214.

[45] Boison D, Stoffel W. Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. Proceedings of the National Academy of Sciences 1994;91(24): 11709-11713.

[46] Griffiths I, Klugmann M, Thomson T, Anderson C, Vouyiouklis D, Nave K-A. Current concepts of PLP and its role in the nervous system. Microscopy Research and Technique 1998;41(5): 344-358.

[47] Stecca B, Southwood CM, Gragerov A, Kelley KA, Friedrich VL, Gow A. The Evolution of Lipophilin Genes from Invertebrates to Tetrapods: DM-20 Cannot Replace Proteolipid Protein in CNS Myelin. The Journal of Neuroscience 2000;20(11): 4002-4010.

[48] Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, Aruga J, Okano H, Mikoshiba K. Glial cell degeneration and hypomyelination caused by over-expression of myelin proteolipid protein gene. Neuron 1994;13(2): 427-442.

[49] Saher G, Rudolphi F, Corthals K, Ruhwedel T, Schmidt K-F, Lowel S, Dibaj P, Barrette B, Mobius W, Nave K-A. Therapy of Pelizaeus-Merzbacher disease in mice by feeding a cholesterol-enriched diet. Nature Medicine 2012;18(7): 1130-1135.

[50] Tatar CL, Appikatla S, Bessert D, Paintlia AS, Singh I, Skoff RP. Increased Plp1 gene expression leads to massive microglial cell activation and inflammation throughout the brain. ASN Neuro 2010;2(4).

[51] Gow A, Friedrich VLJ, R.A. L. Many Naturally Ocurring Mutations of Myelin Proteolipid Protein Impair Its Intracellular Transport. Journal of Neuroscience Research 1994;37(5): 574-583.

[52] Duncan ID, Hammang JP, Trapp BD. Abnormal compact myelin in the myelin-deficient rat: absence of proteolipid protein correlates with a defect in the intraperiod line. Proceedings of the National Academy of Sciences 1987;84(17): 6287-6291.

[53] Vela JM, González B, Castellano B. Understanding glial abnormalities associated with myelin deficiency in the jimpy mutant mouse. Brain Research Reviews 1998;26(1): 29-42.

[54] Griffiths I, Scott I, McCulloch M, Barri J, McPhilemy K, Cattanach BM. Rumpshaker mouse: a new X-linked mutation affecting myelination: evidence for a defect in PLP expression. Journal of Neurocytology 1990;19(2): 273-283.

[55] Nave K, Bloom F, Milner R. A single nucleotide difference in the gene for myelin proteolipid protein defines the jimpy mutation in mouse. Journal of Neurochemistry 1987;49(6): 1873-1877.

[56] Nadon NL, Duncan ID, Hudson LD. A point mutation in the proteolipid protein gene of the 'shaking pup' interrupts oligodendrocyte development. Development 1990;110(2): 529-537.

[57] Hudson LD, Berndt JA, Puckett C, Kozak CA, Lazzarini RA. Aberrant splicing of proteolipid protein mRNA in the dysmyelinating jimpy mutant mouse. Proceedings of the National Academy of Sciences 1987;84(5): 1454-1458.

[58] Boison D, Stoffel W. Myelin-deficient rat: a point mutation in exon III (A----C, Thr75----Pro) of the myelin proteolipid protein causes dysmyelination and oligodendrocyte death. The European Molecular Biology Organization 1989;8(11): 3295-3302.

[59] Domanska-Janik K, J. S, A. T. Immunohistochemical study of myelin-specific proteins in pt rabbits. Folia Neuropathology 1997;35(1): 1-7.

[60] Kagawa T, Nakao J, Yamada M, Shimizu K, Hayakawa T, Mikoshiba K, Ikenaka K. Fate of Jimpy-Type Oligodendrocytes in Jimpy Heterozygote. Journal of Neurochemistry 1994;62(5): 1887-1893.

[61] Schneider AM, Griffiths IR, Readhead C, Nave KA. Dominant-negative action of the jimpy mutation in mice complemented with an autosomal transgene for myelin proteolipid protein. Proceedings of the National Academy of Sciences 1995;92(10): 4447-4451.

[62] Gardinier MV, Macklin MB. Myelin proteolipid protein gene expression in jimpy and jimpy(msd) mice. Journal of Neurochemistry 1988;51(2): 360-369.

[63] Jung M, Sommer I, Schachner M, Nave K-A. Monoclonal Antibody O10 Defines a Conformationally Sensitive Cell-Surface Epitope of Proteolipid Protein (PLP): Evidence that PLP Misfolding Underlies Dysmyelination in Mutant Mice. The Journal of Neuroscience 1996;16(24): 7920-7929.

[64] Schneider A, Montague P, Griffiths I, Fanarraga M, Kennedy P, Brophy P, Nave K-A. Uncoupling of hypomyelination and glial cell death by a mutation in the proteolipid protein gene. Nature 1992;358(6389): 758-761.

[65] Baumann NA, Jacque CM, Pollet SA, Harpin ML. Fatty Acid and Lipid Composition of the Brain of a Myelin Deficient Mutant, the "Quaking" Mouse. European Journal of Biochemistry 1968;4(3): 340-344.

[66] Gencic S, Hudson L. Conservative amino acid substitution in the myelin proteolipid protein of jimpymsd mice. The Journal of Neuroscience 1990;10(1): 117-124.

[67] Wolf MK, Kardon GB, Adcock LH, Billings-Gagliardi S. Hypomyelinated mutant mice. V. Relationship between jp and jpmsd re-examined on identical genetic backgrounds. Brain Res 1983;271(1): 121-129.

[68] Kassmann CM, Nave KA. Oligodendroglial impact on axonal function and survival - a hypothesis. Current Opinion in Neurology 2008;21(3): 235-241.

[69] Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Annals of Neurology 2011;69(2): 292-302.

[70] Comabella M, Vandenbroeck K. Pharmacogenomics and Multiple Sclerosis: Moving Toward Individualized Medicine. Current Neurology and Neuroscience Reports 2011;11(5): 484-491.

[71] Hartung H-P, Lehmann HC, Kieseier BC, Hughes RAC. Novel treatment for immune neuropathies on the horizon. Journal of the Peripheral Nervous System 2011;16(2): 75-83.

[72] Kappos L, Radue E-W, O'Connor P, Polman C, Hohlfeld R, Calabresi P, Selmaj K, Agoropoulou C, Leyk M, Zhang-Auberson L, Burtin P. A Placebo-Controlled Trial of Oral Fingolimod in Relapsing Multiple Sclerosis. New England Journal of Medicine 2010;362(5): 387-401.

[73] Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW. A Randomized, Placebo-Controlled Trial of Natalizumab for Relapsing Multiple Sclerosis. New England Journal of Medicine 2006;354(9): 899-910.

[74] Comi G, Jeffery D, Kappos L, Montalban X, Boyko A, Rocca MA, Filippi M. Placebo-Controlled Trial of Oral Laquinimod for Multiple Sclerosis. New England Journal of Medicine 2012;366(11): 1000-1009.

[75] Kappos L, Li D, Calabresi PA, O'Connor P, Bar-Or A, Barkhof F, Yin M, Leppert D, Glanzman R, Tinbergen J, Hauser SL. Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. The Lancet; 378(9805): 1779-1787.

[76] Fairless R, Williams SK, Hoffmann DB, Stojic A, Hochmeister S, Schmitz F, Storch MK, Diem R. Preclinical Retinal Neurodegeneration in a Model of Multiple Sclerosis. The Journal of Neuroscience 2012;32(16): 5585-5597.

[77] Lassmann H, van Horssen J. The molecular basis of neurodegeneration in multiple sclerosis. FEBS Letters 2011;585(23): 3715-3723.

[78] Petratos S, Azari M, Ozturk E, Roula O, Papadopoulos R, Bernard C. Novel Therapeutic Targets for Axonal Degeneration in Multiple Sclerosis. Journal of Neuropathology and Experimental Neurology 2010;69(4): 323-334.

[79] Gaupp S, Pitt D, Kuziel WA, Cannella B, Raine CS. Experimental Autoimmune Encephalomyelitis (EAE) in CCR2−/− Mice: Susceptibility in Multiple Strains. The American Journal of Pathology 2003;162(1): 139-150.

[80] Bradl M, Linington C. Animal models of demyelination. Brain Pathology 1996;6(3): 303-311.

[81] Becher B, Segal B. TH17 cytokines in autoimmune neuro-inflammation. Current Opinion in Immunology 2011;23(6): 707-712.

[82] Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected Neurites, Apoptotic Neurons, and Reduced Inflammation in Cortical Multiple Sclerosis Lesions. Annals of Neurology 2001;50(3): 389-400.

[83] Pucci E, Giuliani G, Solari A, Simi S, Minozzi S, Di Pietrantonj C, Galea I Natalizumab for relapsing remitting multiple sclerosis. Cochrane Database of Systematic Reviews, 2011. CD007621 DOI: 10.1002/14651858.CD007621.pub2.

[84] Whetten-Goldstein K, Sloan FA, Goldstein LB, Kulas ED. A comprehensive assessment of the cost of multiple sclerosis in the United States. Multiple Sclerosis 1998;4(5): 419-425.

[85] Zhang J, Kramer EG, Asp L, Dutta DJ, Navrazhina K, Pham T, Mariani JN, Argaw AT, Melendez-Vasquez CV, John GR. Promoting myelin repair and return of function in multiple sclerosis. FEBS Letters 2011;585(23): 3813-3820.

[86] Bluestein KT, Pitt D, Knopp MV, Schmalbrock P. T1 and proton density at 7 T in patients with multiple sclerosis: an initial study. Magnetic Resonance Imaging 2012;30(1): 19-25.

[87] Poloni G, Minagar A, Haacke EM, Zivadinov R. Recent developments in imaging of multiple sclerosis. Neurologist 2011;17(4): 185-204.

[88] Mainero C, Benner T, Radding A, van der Kouwe A, Jensen R, Rosen B, Kinkel R. In vivo imaging of cortical pathology in multiple sclerosis using ultra-high field MRI. Neurology 2009;73: 941-948.

[89] Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FMV. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nature Neuroscience 2011;14(9): 1142-1149.

[90] Chen F, Shaw MK, Li J, Lisak RP, Tse HY. Adoptive transfer of myelin basic protein-induced experimental autoimmune encephalomyelitis between SJL and B10.S mice: Correlation of priming milieus with susceptibility and resistance phenotypes. Journal of Neuroimmunology 2006;173(1–2): 146-154.

[91] Morgan L, Shah B, Rivers LE, Barden L, Groom AJ, Chung R, Higazi D, Desmond H, Smith T, Staddon JM. Inflammation and dephosphorylation of the tight junction protein occludin in an experimental model of multiple sclerosis. Neuroscience 2007;147(3): 664-73.

[92] Shaw MK, Kim C, Ho K-L, Lisak RP, Tse HY. A combination of adoptive transfer and antigenic challenge induces consistent murine experimental autoimmune encephalomyelitis in C57BL/6 mice and other reputed resistant strains. Journal of Neuroimmunology 1992;39(1–2): 139-149.

[93] Tischner D, Weishaupt A, Brandt Jvd, Müller N, Beyersdorf N, Ip CW, Toyka KV, Hünig T, Gold R, Kerkau T, Reichardt HM. Polyclonal expansion of regulatory T cells interferes with effector cell migration in a model of multiple sclerosis. Brain 2006;129(10): 2635-2647.

[94] Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain 2006;129(8): 1953-1971.

[95] Gow A, Chapter nine - Using Temporal Genetic Switches to Synchronize the Unfold-ed Protein Response in Cell Populations In Vivo, in Methods in Enzymology. 2011, Academic Press. p. 143-161.

[96] Abraira V, Alvarez-Cermeño JC, Arroyo R, Cámara C, Casanova B, Cubillo S, de An-drés C, Espejo C, Fernández O, Ferrer J, Figueredo MA, García-Merino A, García-Sánchez MI, García-Trujillo JA, Gómez M, González-Oria C, Gosis A, Izquierdo G, Jímenez J, López-Trascasa M, Montalbán X, Moreno MJ, Muñoz D, Nuñez V, Muriel A, Navarro J, Olascoaga J, Oreja-Guevara C, Prada A, Ramil E, Ramo-Tello C, Rodrí-guez C, Rodríguez E, Rodríguez-Frías F, Rodríguez-Antigüedad A, Rodríguez-Moli-na JJ, Ruiz E, Saiz A, Sarasola E, Simó M, Yagüe J, Villar LM. Utility of oligoclonal IgG band detection for MS diagnosis in daily clinical practice. Journal of Immunolog-ical Methods 2011;371(1–2): 170-173.

[97] Denic A, Macura S, Mishra P, Gamez J, Rodriguez M, Pirko I. MRI in Rodent Models of Brain Disorders. Neurotherapeutics 2011;8(1): 3-18.

[98] Patel NH, Vyas NS, Puri BK, Nijran KS, Al-Nahhas A. Positron Emission Tomogra-phy in Schizophrenia: A New Perspective. Journal of Nuclear Medicine 2010;51(4): 511-520.

[99] Rigotti DJ, Inglese M, Gonen O. Whole-Brain N-Acetylaspartate as a Surrogate Mark-er of Neuronal Damage in Diffuse Neurologic Disorders. American Journal of Neu-roradiology 2007;28(10): 1843-1849.

[100] Tartaglia MC, Arnold DL. The role of MRS and fMRI in multiple sclerosis. Advanced Neurology 2006;98: 185-202.

[101] Lewellen T, Williams D, Olson D. Image quality with nuclear magnetic resonance imaging systems. American Journal of Roentgenology 1981;137(5): 1092-1093.

[102] Geurts JJG, Barkhof F. Grey matter pathology in multiple sclerosis. The Lancet Neu-rology 2008;7(9): 841-851.

[103] Tang KS, Shapiro EM. Enhanced magnetic cell labeling efficiency using –NH2 coated MPIOs. Magnetic Resonance in Medicine 2011;65(6): 1564-1569.

[104] De Stefano N, Filippi M, Miller D, Pouwels PJ, Rovira A, Gass A, Enzinger C, Mat-thews PM, Arnold DL. Guidelines for using proton MR spectroscopy in multicenter clinical MS studies. Neurology 2007;69(20): 1942-52.

[105] Kumar A, Asano E, Chugani HT. α-[11C]-methyl-L-tryptophan PET for tracer localiz-ation of epileptogenic brain regions: clinical studies. Biomarkers in Medicine 2011;5(5): 577-584.

[106] Kadir A, Nordberg A. Target-Specific PET Probes for Neurodegenerative Disorders Related to Dementia. Journal of Nuclear Medicine 2010;51(9): 1418-1430.

[107] Fonseca SG, Gromada J, Urano F. Endoplasmic reticulum stress and pancreatic β-cell death. Trends in Endocrinology and Metabolism 2011;22(7): 266-274.

[108] Gensicke H, Leppert D, Yaldizli Ö, Lindberg RLP, Mehling M, Kappos L, Kuhle J. Monoclonal Antibodies and Recombinant Immunoglobulins for the Treatment of Multiple Sclerosis. CNS Drugs 2012;26(1): 11-37.

[109] He D, Zhou H, Han W, Zhang S Rituximab for relapsing-remitting multiple sclerosis. Cochrane Database of Systematic Reviews, 2011. DOI: 10.1002/14651858.CD009130.pub2.