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Oligodendrocyte Metabolic Stress in Neurodegeneration

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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 shut-down 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: IRE1 α (inositol requiring protein 1 α), 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 IRE1 α 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. IRE1 α 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 IRE1 α as a monomer and prevents its activation. However, misfolded protein accumulation in the ER lumen sequesters BiP from IRE1 α and allows this receptor to homodimerize leading to transautophosphorylation and activation of its cytoplasmic endonuclease P domain [19].

A major downstream target of the IRE1 α 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 IRE1 α 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 IRE1 α signaling. ATF6 interacts with BiP, similar to IRE1 α . 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 IRE1 α 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 α subunit of eukaryotic initiation factor 2 (eIF2 α). eIF2 α 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-eIF2 α 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 eIF2 α 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 eIF2 α 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 eIF2 α 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 de-differentiation 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 eIF2 α 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 clinical

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 autoimmune 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), eIF2 α (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).

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