Experimental and Molecular Pathology 108 (2019) 183–190

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Review

Interplay between ER stress and autophagy: A possible mechanism in multiple sclerosis pathology

Sanketh Andhavarapu[a], Fahad Mubariz[a], Muhammad Arvas[a], Christopher Bever Jr.[a,b,c], Tapas K. Makar[a,b,c,*]

[a] Department of Neurology, University of Maryland, Baltimore, MD 21201, United States of America
[b] VA Medical Center, Baltimore, MD 21201, United States of America
[c] VA Multiple Sclerosis Center of Excellence East, Baltimore, MD 21201, United States of America

A B S T R A C T

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system that results in demyelination, neurodegeneration, and axonal loss. During MS pathology, autoreactive T cells specific for self-antigens migrate the blood-brain-barrier and are responsible for the axonal and neuronal damage. ER stress, a disruption in cellular homeostasis due to the accumulation of misfolded proteins, is a hallmark of MS pathology. In response to the homeostatic imbalance, ER stress activates the unfolded protein response, an intricate system of signaling pathways that aims to restore cellular balance. During the UPR, various autophagy pathways are also activated. Autophagy is a diverse network of regulatory catabolic processes which direct the clearance of damaged and unnecessary organelles and proteins while recycling necessary cellular components. In respect to its role in the health of the immune system, autophagy is critical to the survival and proliferation of T cells. This review consolidates current knowledge and recent literature about ER stress, UPR, and autophagy in MS and implicate their crosstalk as a characteristic feature of MS, potentially aiding in the development of novel therapeutic strategies for MS research.

1. Introduction

Multiple Sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system that results in demyelination, neurodegeneration, and axonal loss. Clinically, MS exists in 4 forms: 1) Relapse-remittance 2) Secondary-progressive 3) primary-progressive 4) progressive-relapsing and it is the most common cause of nontraumatic disability in young adults (Noseworthy et al., 2000). Relapse-remittance is the most common form of MS, accounting for 85% of all cases. All forms of the disease are progressive, but only RRMS is characterized by periods of relapse and remission throughout the entirety of the disease (Loma and Heyman, 2011). MS symptomology includes changes in vision, tremors, muscle weakness, sensory changes, fatigue, and cognitive deficits (Loma and Heyman, 2011). As with most disorders, the incidence of MS increases with age, reaching a peak between 20 and 40 years and an average of 30 years (Inglese, 2006). The causes of MS are largely unknown, but there are known environmental and genetic factors that play a pivotal role in the development of MS. A potential environmental risk factor is latitude, as the exposure to sunlight in relation to vitamin D levels has been inversely correlated with MS risk (Kamm et al., 2014). Such environmental risk factors may also contribute to some genetic factors. For example, low vitamin D levels results in reduced expression of the HLA *DRB1*15* gene, a MHC class II allele that is a significant MS genetic risk factor as it is responsible for and central tolerance (Miljkovic and Spasojevic, 2013). Although MS is not considered a hereditary disease, the influence of genetics on the susceptibility of MS is highlighted by its familial recurrence of about 20%. Variations in the human leukocyte antigen (HLA) region, found on the sixth chromosome short arm, have also been discovered to increase susceptibility to MS (Gourraud et al., 2012) (Lin et al., 2012).

Two main neuropathological results have characterized the current understanding of the pathophysiological mechanisms of MS. Autoreactive T cells activated outside the CNS cross the blood-brain barrier and are reactivated by local antigen-presenting cells. Secretion of proinflammatory cytokines stimulates microglial and astrocyte cells, recruits additional inflammatory cells, and induces antibody production by plasma cells (Glass et al., 2010). The cortex is also affected in early stages of the disease. This is observed with the presence of cortical inflammation and demyelination, cortical neurodegeneration, including neuronal, neurotic and oligodendroglia injury, and finally cortical atrophy (Lassmann et al., 2007). The immune system is triggered to upregulate cytokine and antibody activity upon upregulated inflammatory processes due to demyelination, leading to further damage of the blood brain barrier. This eventually activates more

* Corresponding author at: Department of Neurology, University of Maryland, 655 West Baltimore Street, BRB-12043, Baltimore, MD 21201, United States of America.
E-mail address: tmakar@som.umaryland.edu (T.K. Makar).

https://doi.org/10.1016/j.yexmp.2019.04.016
Received 31 March 2019; Received in revised form 18 April 2019; Accepted 27 April 2019
Available online 30 April 2019
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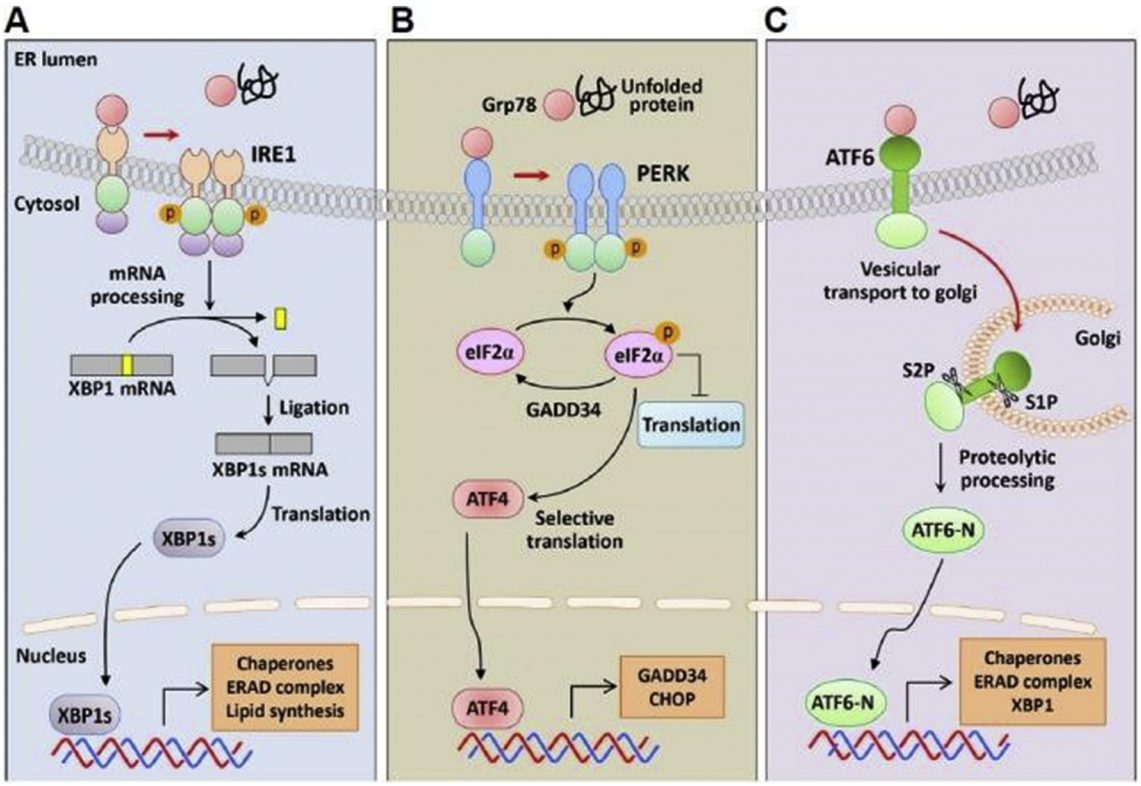

Fig. 1. Signaling pathways of the UPR. ER-resident proteins IRE1, PERK, and ATF6 sense ER stress and deliver distinct signals from the ER to the cytosol. Under normal conditions, Grp78 binds to the ER luminal domains of sensor proteins and inhibits their activation. However, Grp78 dissociates from the sensors in response to ER stress and binds to unfolded proteins, leading to activation of the sensors. (A) IRE1 pathway; IRE1 has Ser/Thr kinase and RNase domain in the cytoplasmic region, and ER stress induces IRE1 oligomerization and autophosphorylation of the kinase domain. The RNase domain of activated IRE1 performs unconventional splicing and cleaves 26 intronic nucleotides from XBP1 mRNA in mammalian cells. This splicing induces a translational frame-shift, and the truncated XBP1 mRNA encodes XBP1s, which contains a new carboxyl terminus. As a transcription factor, XBP1s activates UPR-related genes including ER chaperones, ERAD components, and lipid-biosynthetic enzymes. (B) PERK pathway; PERK is a protein Ser/Thr kinase that undergoes oligomerization and autophosphorylation of the kinase domain under conditions of ER stress. Activated PERK phosphorylates eIF2α at serine 51, resulting in general inhibition of protein translation. However, phosphorylated eIF2α selectively increases the translation of ATF4, which upregulates CHOP and GADD34 mRNA. As a negative feedback mechanism, GADD34 promotes dephosphorylation of eIF2α to restore protein synthesis following elimination of ER stress. However, failure to alleviate ER stress leads to CHOP-mediated apoptosis. (C) ATF6 pathway; ATF6 has a bZIP domain in the cytosol and translocate from the ER to the Golgi apparatus under ER stress. ATF6 is then cleaved by the proteases S1P and S2P to produce the amino terminus of ATF6 (ATF6-N), which then migrates to the nucleus and upregulates target genes encoding ER chaperones, ERAD components, and XBP1. RNase, endoribonuclease; XBP1, X-box binding protein 1; eIF2α, α-subunit of eukaryotic translation initiation factor 2; ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; GADD34, growth arrest and DNA damage-inducible protein 34; S1P, site-1 protease. Figure and caption adapted from So (2018).

destructive bodies such as cytokines, macrophages, and inflicting proteins (Compston and Coles, 2002). Inflammatory processes negatively influence the efficiency of information transfer in the central nervous system through upregulation of cytokines and antibodies through three main mechanisms: halting the production of neurotransmitters by the directed attack neuron, destructing the myelin sheath, and causing axonal damage (Compston and Coles, 2002). The cytokines are important in the initiation of many immune refluxes. Interleukin-21 represents one of the major immune factors, inducing many immune refluxes to increase autoimmunity by upregulating and functionally improving of helper T-17 and follicular helper T (THF) cells, initiating NK cells, strengthening B-cell differentiation, secreting antibodies, and attenuating regulatory T (T-reg) cells (Ghalamfarsa et al., 2016).

No cure for MS currently exists, but many drugs have shown promise, and more are entering clinical trials that will soon be available in the market. Interferons and glatiramer acetate are currently the most effective treatments in slowing MS disease progression, as they are safe for an extended period of therapy and significantly decrease MS relapses (Lassmann et al., 2007; Tsang and Macdonell, 2011). One already established mechanism of action for both these forms of treatment include the shift from the proinflammatory Th1 response to the anti-inflammatory Th2 response by interfering with antigen presentation (Martin et al., 2016). With the cytokine signaling pathways being so intricately involved with cellular homeostasis, specifically with context to the endoplasmic reticulum and autophagy systems, future treatments must persist in mediating this shift. Studies have proven that both treatments are equally effective in both children and adults. Natalizumab has shown to be far more efficient than the interferons and glatiramer acetate in decreasing MS relapses. However, due to its side effects, it is only used after treatment with interferons and glatiramer acetate have failed. Similarly, mitoxantrone is only employed after failure of the treatments above to potentially avoid side effects. Other new and upcoming treatments include dimethyl fumarate, fingolimod, and teriflunomide (Tsang and Macdonell, 2011). All such treatment options have been considered effective in slowing down the progression of MS as a multitude of studies demonstrate their ability to reduce relapse rates, promote remyelination, and ameliorate neuroinflammation.

In both animal models and humans of the relapse type of MS, and autoimmune disorders in general, combinations of chimerism and allogeneic donors have worked well but have also been found to the auto reactivity of CD4+ T cells in the spleen and lymph nodes. Intravenous methylprednisolone pulse therapy is also another option as it effectively ameliorates MS symptoms during the first, second, and third clinical

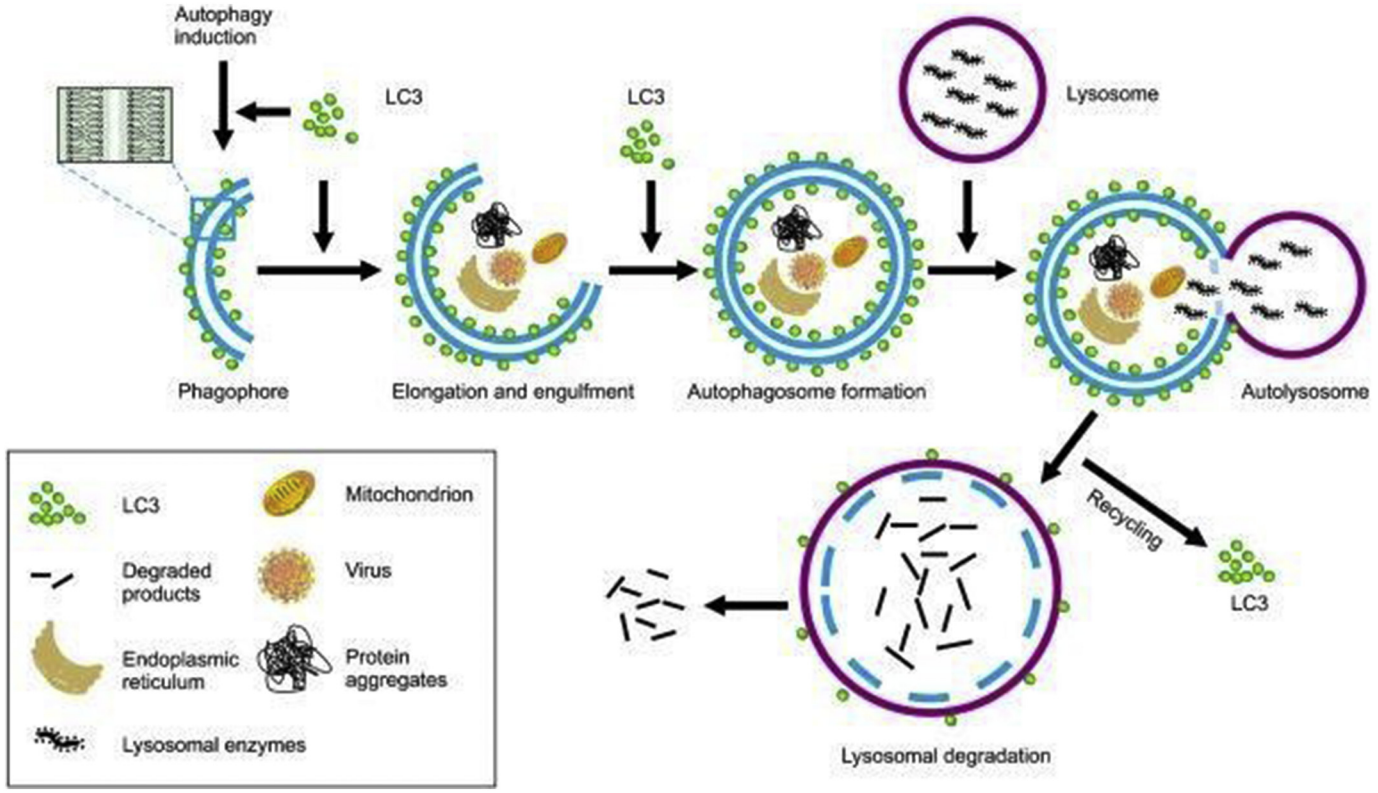

Fig. 2. Schematic diagram of autophagic progression. Autophagy induction signal leads to form a sequestering membrane called phagophore. Following a sequence of ubiquitination-like reactions, LC3 conjugates to the sequestering membrane and controls the elongation of phagophore. As the phagophore expands, cytoplasmic constituents, including organelles such as mitochondria and endoplasmic reticulum, aggregated proteins and foreign organisms (bacteria and virus) are enwrapped. At the end of elongation, sequestering membrane closes and results in the formation of a double-membrane vesicle, autophagosome. Once the autophagosome is formed, it is delivered to fuse with lysosome to form autolysosome for degradation. Lysosomal hydrolases degrade the cargo together with the inner membrane of autophagosome, and LC3 from the outer membrane as well as the autophagy-derived nutrients are recycled. This autophagic process can act as a mechanism to keep homeostatic balance and support cell survival. However, it can also cause cell death directly or indirectly. Figure and caption adapted from Jing and Lim (2012).

courses, bur an insignificant impact of the fourth and fifth clinical course of treatments for MS (Tsang and Macdonell, 2011). By decreasing pain hypersensitivity and reducing neuroinflammation, myelin-derived altered peptide ligands treatments have been highly effective in improving neuropathic pain, visual disturbances, and MS-associated optic neuritis, all of which are present is a great majority of MS cases. Orally, laquinimod, ozanimod, and the PEGylated form of interferon-β-1a are also rising treatment options (Saidha et al., 2012). Monoclonal antibodies are another relatively new form of treatments. Some examples of these include ocrelizumab, rituximab and ofatumumab. However, these treatments' side effects also primarily include adaptable infection. At higher doses, the intravenous injection of methylprednisolone speeds the treatment for visual neuritis treatment in MS patients, potentially very beneficial as visual neuritis in MS patient also leads way to various other symptoms (Saidha et al., 2012).

1.1. Endoplasmic reticulum stress and the unfolded protein response

Endoplasmic Reticulum (ER) stress is defined as any chronic perturbations to the homeostasis of the ER and is primarily characterized by the accumulation of aberrant proteins, which influences the ER's protein folding capacity due to an inability of the ER protein folding capacity to keep up with cellular demand (Gardner et al., 2013). Although ER stress originates with the intent of self-preservation, it can ultimately lead to cell death if the stress is unmanageable and persists in cell. If an apoptotic event is necessary, there have been several mechanisms that have been proposed to link ER stress to cell death. These mechanisms include the activation of proteases, kinases, transcription factors and even Bcl-2 proteins (Sano and Reed, 2013). In response to ER stress, the unfolded protein response (UPR), an evolutionarily conserved process that aims to restore homeostasis and prevent aggregation, is activated. The UPR is primarily regulated by three prominent ER-transmembrane proteins: the pancreatic endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). These three proteins work in conjunction with each other to stop improper translation and ameliorate protein-folding machinery. However, failed attempts to achieve homeostasis by the UPR after a prolonged period of time results in a shift towards apoptotic cell death (Sano and Reed, 2013; Xu et al., 2005).

On a molecular level, the release of ER chaperone Grp78 due to the buildup of aberrant proteins activates the UPR. The PERK protein is composed of a regulatory luminal domain, a cytosolic kinase domain, and an ER transmembrane domain. PERK is activated when the regulatory luminal domain senses ER stress and follows through via oligomerization and auto-phosphorylation (Harding et al., 2002). By phosphorylating eIF2α, PERK stops the translation of mRNA and translates ATF4, which, in turn, activates the apoptotic gene CHOP. CHOP is interestingly recognized as beneficial during conditions of less stress while detrimental in conditions of severe stress (Li et al., 2015). CHOP, in turn, brings about a regulatory subunit of the phosphatase complex growth arrest and DNA damage 34 (GADD34) (Marciniak et al., 2004). GADD34 also specifically targets the dephosphorization of p-eIF2 through protein phosphatase 1 (PP1), ultimately resulting in a negative feedback loop that contributes to protein synthesis (Novoa et al., 2001). The activation of PERK allows cells to adapt to conditions of ER stress by the downregulation of global protein biosynthesis and upregulation of cytoprotective genes, but can, in the contrary, also potentially lead to cell apoptosis (Tabas and Ron, 2011). One regulator

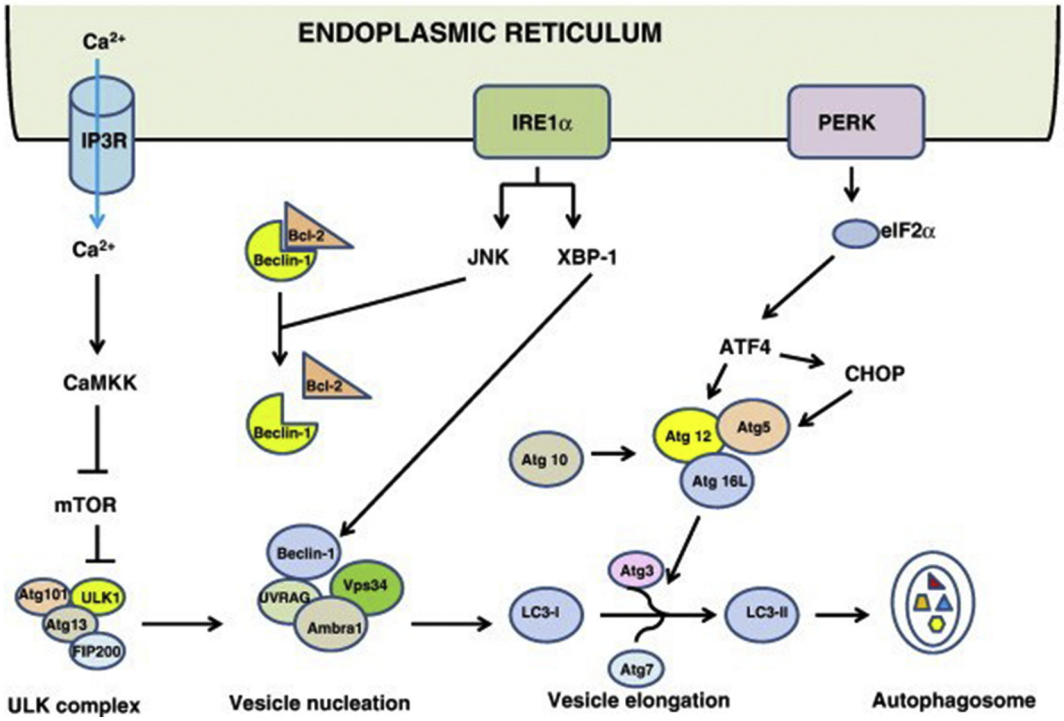

Fig. 3. Cross-talk of ER stress and autophagy. ER stress can induce autophagy, through at least two UPR pathways, PERK-elF2α and IRE1α. Activatedα can recruit TRAF2 and ASK1, which subsequently activates JNK. JNK-mediated phosphorylation of Bcl-2releases Beclin-1 from its inhibitory interaction with Bcl-2. Free Beclin-1 associates with other members of the ULK1 complex to promote vesicle nucleation. In parallel, spliced XBP-1 can also trigger transcriptional up-regulation of Beclin-1 expression. The elongation process involves two ubiquitin-like conjugation systems that promote the assembly of Atg5-Atg12-Atg16L complex and the LC3 processing (cleavage and lipidation). Activated PERK can induce autophagy through ATF4-driven transcriptional regulation of Atg12 whereas ATF4-mediated CHOP activation can also transcriptionally induce Atg5. Ca^{2+} release from the ER lumen through the IP_3R can activate CaMKK and subsequently relieves mTOR inhibition on the ULK1 complex. Figure and caption adapted from Sano and Reed (2013).

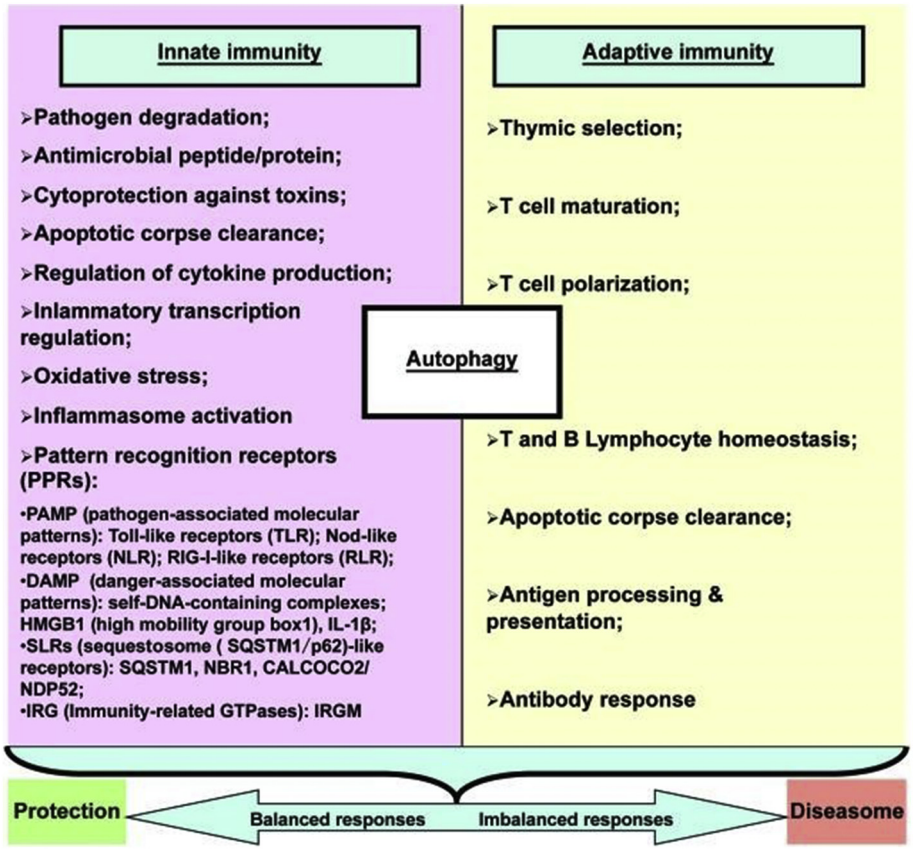

Fig. 4. The multifaceted roles of autophagy in immunity. The many currently recognized roles of autophagy in innate and adaptive immunity have been steadily increasing in complexity. Normal autophagy function contributes to balanced immunity responses, resulting in protection against disease. Imbalanced autophagy results in maladaptive responses and more severe disease. Here "diseasome" stresses more on convergence of different diseases as "every road leads to Rome" in which autophagy may be a common pathway. Figure and caption adapted from Zhou and Zhang (2012).

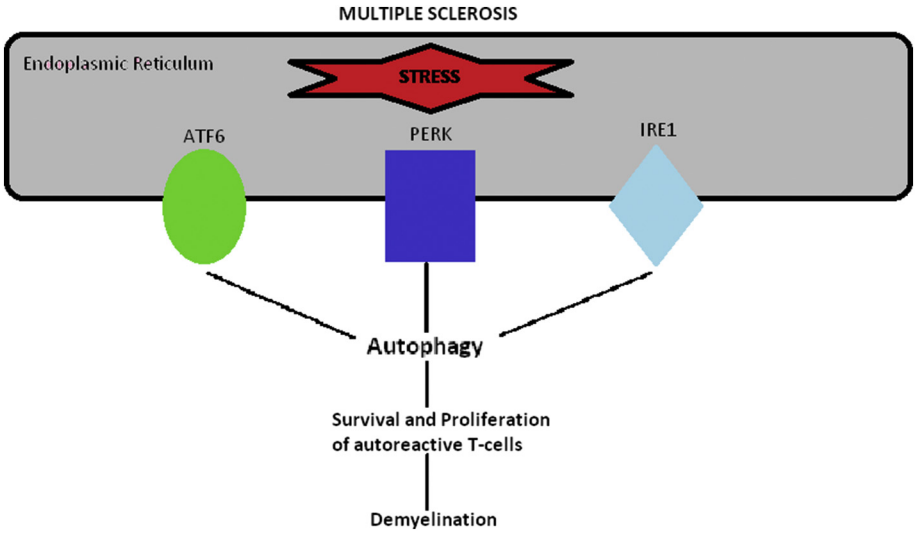

Fig. 5. A summary of the proposed interplay between ER stress and autophagy in context to multiple sclerosis pathology. ER stress is a prominent characteristic during multiple sclerosis, resulting in the activation of the UPR's three branches ATF6, PERK, and IRE1. This in turn upregulates autophagic signaling pathways directly or indirectly. This chronic activation of autophagic systems then contributes to the survival and proliferation of autoreactive T cells due to the adaptive immunity functions of autophagy. This increased infiltration of immune cells contributes to the demyelination that characterizes MS.

of the mechanisms mediating the splicing and activation of the transcription factor XBP-1 is the IRE1 RNAse domain. These mechanisms then lead to ERAD by increasingly expressing chaperones. Additionally, the IRE1 RNAse domain has a significant role in promoting mRNA decay to degrade mRNA. The third branch ATF6 is cleaved by Site-1 protease (S1P) and Site-2 protease (S2P) in the Golgi, resulting in an active transcription factor that regulates CHOP, grp78, and ERAD components (Senft and Ronai, 2015).

1.2. ER stress and UPR in multiple sclerosis

ER stress has been reported to be prominent in several neurodegenerative and neuroinflammatory disorders. In parallel, the UPR also plays a critical role in inflammatory diseases through the elevation of inflammatory mediator cells such as cytokines, reactive oxygen species, and reactive nitrogen species. In MS, ER stress is a hallmark, and the UPR has been observed in various different cell types in multiple sclerosis and EAE lesions (Stone and Lin, 2015). It is important for eIF2a phosphorylation to be regulated due to its important role in maintaining the health of myelinated neurons in the central nervous system. However, in MS, ER stress affects the timing of phosphorylation of eIF2a, resulting in an exacerbation of disease pathology (Roussel et al., 2013). One study conducted by Cunnea et al. demonstrated how ER stress molecules are present in elevated levels in post-mortem MS lesioned tissue using real-time PCR. While there exist four clinical forms of MS, ER stress plays the same role in all four forms on the molecular level during times of which the disease is clinically progressing in the chronic stage (Cunnea et al., 2011). During MS, oligodendrocytes, astrocytes, T cells, and microglia express the ER stress-associated C/EBP homologous protein, immunoglobulin heavy chain-binding protein, and X-box-binding protein 1 in increased numbers (Mhaille et al., 2008). Furthermore, studies have displayed that UPR-responsive genes ATF4 and hsp70 are upregulated in the mRNA, while CHOP and BiP are upregulated in the oligodendrocytes, astrocytes, T cells, and microglia in MS lesions, specifically in the mRNA (Stone and Lin, 2015). Yohannes et al. demonstrated that ER stress is also strongly associated with the upregulation of Rab32, a prominent mitochondrion associated membrane (MAM) regulatory protein. Rab32 was shown to be a marker for chronic and active inflammation (Haile et al., 2017). IFN-y has also been well documented as a critical role player in the development of MS and EAE; through several studies, it has been proven that IFN-y induces inflammatory mediators and activates macrophages. These proinflammatory mechanisms have shown to advance demyelination and oligodendrocyte death in MS (Brahmachari and Pahan, 2008). Altogether, ER stress and the UPR contribute greatly to MS pathology. Therefore, targeting ER stress and UPR mediators should remain a focus of research during the search for novel and promising therapeutic strategies.

1.3. Autophagy in relation to ER stress and the UPR

Autophagy is a fundamental cellular mechanism that plays a vital role in the maintenance of cellular homeostasis. The term describes a diverse network of regulatory catabolic processes which direct the clearance of damaged and unnecessary organelles and proteins while recycling necessary cellular components It is different than apoptosis in that apoptosis is a type 1 cell death mediated by caspases and responsive to cellular stress while autophagy causes cell death directly or indirectly in response to endoplasmic reticulum stress and altered metabolism (Kesidou et al., 2013). The three different basic pathways of autophagy include macroautophagy, chaperone-mediated autophagy, and microautophagy. When properly activated, all three maintain intracellular balance, especially in conditions of internal and external stress such as nutritional deprivation, oxidative stress, and accumulation of misfolded proteins (Moloudizargari et al., 2017; Kesidou et al., 2013). Macroautophagy, hereinafter 'autophagy,' can be divided into four main stages: nucleation, expansion, maturation, and degradation/recycling (Kesidou et al., 2013). In the nucleation phase, autophagosomes, double membraned vesicles containing cellular material, are assembled. The autophagosomes are elongated through the undergoing of two ubiquitin-based systems, in which autophagy-related genes (Atg) undergo a series of signaling and communication, making up the expansion phase (Levine and Kroemer, 2008; Liang and Le, 2015). Specifically, autophagy is initiated by the inhibition of mechanistic target of rapamycin complex I (mTORC1), a down-regulator of autophagy (Moloudizargari et al., 2017; Rashid et al., 2015). In the maturation and degradation/recycling phases, the autophagosomes fuse with neighboring lysosomes in the cytosol and degrade any cargo, often selectively to those deteriorating the state of the cell. This cargo can include damaged organelles, protein aggregates, foreign pathogens, or aberrant lysosomes mitochondria (Yang et al., 2015; Smith and Wilkinson, 2017).

Yeast and mammalian models have shown that autophagic activity is very prominent in the unfolded protein response during ER stress. 2 pathways of selective autophagy in the ER that are specifically activated during ER stress to directly modulate the ER function. These pathways include ER-associated degradation (ERAD) and ER-phagy, or selective autophagy in the ER. ERAD can either be Type I or Type 2; while ERAD I autophagy aims to clear just soluble misfolded proteins, ERAD II targets both soluble and insoluble proteins (Smith and

Wilkinson, 2017). ER-phagy works in conjunction with ERAD to fully activate the autophagic response. FAM134B, SEC62, CCPG1, and RTN3 are four identified ER-phagy receptors that facilitate the remodeling and trimming of the ER by directly targeting specific regions of the ER. SEC62 and CCPG1 specifically bridge portions of the ER and autophagosomes in a highly specialized manner; however, only CCPG1 requires contact with FIP200-interacting region for activation. Nevertheless, all four of these receptors can perhaps be correlated directly with the UPR, as PERK, ATF6, and IRE1a all express the ability to upregulate the transcription of ATGs (Smith and Wilkinson, 2017). Overall, the mechanisms of ER stress open several pathways for increased autophagic activity.

The unfolded protein response contributes to autophagy directly and indirectly through its three arms. The activation of PERK during UPR directly phosphorylates EIF2a and NRF2 and translates transcription factor ATF4, which then translates CHOP, SESN2, and DDIT3, all of which contributes to the induction of autophagy by inhibiting mTORC1, either directly or indirectly (Rashid et al., 2015). Due to its wide upregulation of autophagy genes, the induction of PERK is considered the most involved and dominant of the three UPR branches. Knockout of the PERK axes in the UPR displayed numerous pathological and physiological deficits along with increased overall ER stress and compromised autophagy. This correlation is further supported by the knockout of the ATF6 and EIF1 axes of the UPR as in vitro models again exhibit compromised autophagic function (Rashid et al., 2015). These results signify the role of the UPR in regulating autophagy, especially during ER stress.

The PERK sensor protein primarily depletes ATP during the ER stress response, resulting in increased AMPK signaling. The upregulation of AMPK signaling is also directed by ATF6 and IRE1a. Through CHOP translated directly and indirectly by ATF4 and PERK respectively, as well as RPS6KA3 through IREa, ULK1 is activated while mTOR is inhibited, again inducing autophagy. ATF6, although less involved, still greatly influences the activation of autophagy during ER stress by directly upregulating HSPS5 during the ER stress response, which in turn downregulates AKT1 and consequently lessens mTOR activity. All 3 branches work to increase autophagy by also targeting BECN1 and Atgs; PERK and ATF6 act traditionally, while IRE1a activates p-MAPK8 and XBP1s to indirectly upregulate autophagy. In neurodegeneration, however, the IRE1a pathway often acts through the downregulation of the FOXO intracellular regulator by upregulation of p-MAPK8s and XBP1s. The intricate model of communications amongst various components of the UPR highlights the induction of autophagy as central to counterbalancing the ER stress response (Rashid et al., 2015; Smith and Wilkinson, 2017).

1.4. Autophagy in the immune system

The immune system is largely dependent on cellular autophagy to fulfill some of its primary functions: intracellular pathogen destruction, antigen presentation, lymphocyte development, and inflammatory regulation (Yang et al., 2015). In innate immunity, autophagy is significant in clearing the by-products of apoptosis as well as activating inflammasomes. Furthermore, it provides cytoprotection against toxins and regulates pattern recognition receptors such as those detecting pathogen-associated molecular patterns (PAMP), danger-associated molecular patterns (DAMP), and sequestosome/p62-like receptors (SLRs), and immunity-related GTPases (IRG). Adaptive immunity measures include thymic selection, T cell maturation, and T cell polarization, and other actions towards the maintenance of homeostasis of T and B lymphocytes. When these functions are properly carried out, disease is unlikely. However, altered autophagic function will increase susceptibility to disease (Zhou and Zhang, 2012).

Autophagy is vital to the maintenance of homeostasis for the proliferation of T cells. T cell activation signals exist in in two main systems: the major histocompatibility complex (MHC) I and MHC II. Autophagy enhances both the MHC I and MHC II to mediate antigen presentation and sustain autoreactive T cells (Liang and Le, 2015; Yang et al., 2015). Furthermore, it has been shown that Atg5 and Atg7 deficient T cells are unable to properly proliferate or control mitochondrial load resulting in a disturbed homeostasis and increased cell death (Yang et al., 2015). During disease, however, the immune response may ameliorate or progress the pathogenesis, depending on the population of T cells (Liang and Le, 2015). B cells are also regulated through autophagy, as it has been shown that Atg5 has aided in the development and survival. However, reduced or excess autophagy can contribute to autoimmune disease (Qian et al., 2017).

Autophagy also regulates proinflammatory signaling through the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). In general, blocking autophagy activity upregulates the proinflammatory response (Liang and Le, 2015). Through NLRP3-inflammasome activation, reduced autophagy upregulates the production of Interleukin-1 alpha (IL-1a)and Interleukin-1 beta (IL-1B), both proinflammatory cytokines (Alirezaei et al., 2011). Autophagy works to elevate the levels of Th2 cytokines/chemokines such as IL-6 and TNF-a to promote the immune response, while decreasing Th2 cytokines/chemokines such as IL-1b to control inflammation. Perhaps to maintain intracellular balance via a compensatory mechanism, a reverse relationship is observed in the effects of cytokines on autophagy. In parallel, the proinflammatory response may induce autophagy or the anti-inflammatory Th2 response may ablate autophagy for increased cell survival, depending on the context (Levine and Kroemer, 2008; Plaza-Zabala et al., 2017).

1.5. Autophagy in multiple sclerosis

Due to its prominent role in the immune system, disturbances in autophagic activity can potentially contribute to the development of diseases, specifically autoimmune diseases. Previous studies have shown that during the chronic stage of multiple sclerosis, elevated levels of Atg5 are present in the T cells of MS lesions and peripheral blood (Liang and Le, 2015; Plaza-Zabala et al., 2017). Blood samples of MS patients also show elevated levels of Atg genes such as Atg-9A and Atg16L2. Even in EAE, upregulated Atg5 expression was observed. Atg7 in dendritic cells has also been shown to contribute to T cell activation, as their Atg7 deficient dendritic cells have exhibited lessen presentation of T cells in an EAE model (Plaza-Zabala et al., 2017). This suggests that excess autophagy contributes to the pathogenesis of MS, since MS demyelination is characterized by the prolonged survival of autoreactive T cells and autophagy promotes these mechanisms.

As with many disorders and diseases of the brain, mitochondrial dysfunction is a large component of the pathology of MS. During MS, glutamate is upregulated through inflammatory molecules such as macrophages and microglia. This in turn induces neurotoxicity by elevating intracellular Ca^{2+} ions, which then can cause mitochondrial damage (Alirezaei et al., 2011). One study has shown that in the EAE model, mitochondrial dysfunction paralleled neurological deficits. For example, a decrease in mitochondrial respiratory chain complex I was observed on the first day of neurological signs and axonal depolarization was associated with neurological deficits. Furthermore, this study discovered that mitochondrial dysfunction was associated with infiltrating immune cells such as CD3+ t cells and that at the onset of neurological deficits, mitochondrial trafficking is significantly reduced (Sadeghian et al., 2016). Specifically, the metabolite N-acetyasparate (NAA), a monitor of neuronal integrity produced by neuronal mitochondria, is present in lower levels in correlation with relapses and neurological decline of MS patients. This correlation is also known to be prominent in acute inflammatory lesions, and chronic fatal white matter lesions, further supporting that mitochondrial dysfunction is characteristic of MS pathology (Su et al., 2013). Connections between signaling pathways of autophagy and mitochondrial function can be traced to certain proteins involved in mitophagy and nonselective

autophagy. Autophagy works to restore the altered functions of mitochondria that result from ER stress through these processes (Levine and Kroemer, 2008). During times of excess autophagy, GTpase Drp1 is phosphorylated by protein kinase A (PKA) that elongates mitochondria and thus maintains ATP levels (Okamoto and Kondo-Okamoto, 2012). Autophagy works to restore the altered functions of mitochondria that result from ER stress, as described above, in a process called mitophagy (Levine and Kroemer, 2008). However, after extended periods of stress and accumulation of damaged mitochondria, autophagic mechanisms may be disturbed, potentially leading to neuronal damage and contributing to the neurodegeneration that defines MS (Alirezaei et al., 2011).

It is widely understood that disruptions in autophagy are notable characteristics of neurodegenerative and neuroinflammatory disorders. Several studies have shown that autophagy is both beneficial and detrimental in theses pathologies, demonstrating that its contributions to the disease are specific to different cell types (Liang and Le, 2015). Glial cells are becoming of greater interest in relation to autophagy and neuroinflammation. Microglial autophagy can be induced through the proinflammatory molecules produced by the microglia, and in response, mTOR inhibitors work to suppress this autophagy to reduce neuroinflammation. In contrast, reduced autophagy in astrocytes has resulted in astrocytic cell death through the strengthened production of neurotoxic factors, suggesting the importance of autophagy in the survival of astrocytes during MS. Specific to the pathogenesis of MS, autophagy is also strongly related to demyelination and remyelination. Studies in Long-Evans shaker rats have exhibited the favorable effects of increased autophagy on restoring myelin. However, in the cerebrospinal fluid, autophagy is inhibited through nerve growth factors, suggesting the deleterious effects of autophagy (Liang and Le, 2015). From this, it is difficult to come to a conclusion about the relationship between autophagy and the progression of MS.

1.6. Conclusion and future perspectives

MS is an autoimmune disorder in which the body's own T-cells attack the myelin sheath in the central nervous system. During MS, ER stress is a prominent occurrence due to disruptions in cellular homeostasis. To counteract the detrimental consequences of ER stress, the UPR is activated and works to restore cellular balance; one such mechanism this is possible is by triggering programmed cell death through autophagy. Like ER stress, mitochondrial dysfunction is another prominent feature of MS and also initiates autophagic pathways. Therefore, autophagy is upregulated in MS lesions and is supported by past studies that it potentially contributes to demyelination, neuronal damage, and neuroinflammation.

In our recent work, we have explored the dysregulation of various proteins related to ER stress, autophagy, and mitochondrial homeostasis in the EAE model. Our results thus far support the proposed mechanisms described throughout this review. We found that in the EAE model, both PERK and CHOP are upregulated, indicating the perturbed homeostasis of the ER defined as ER stress. Moreover, transmission EM pictures demonstrated that in EAE mice, the vesiculated endoplasmic reticulum was irregularly arranged and disrupted. Interestingly, treatment with glatiramer acetate, which reversed these disruptive effects in the EAE model, was associated with improvements in clinical score (unpublished data). Additionally, we found that in both the white matter and gray matter sections of the spinal cord in the EAE mouse model, there was an upregulation of autophagic/apoptotic proteins BAX, BCL2, and CC3, indicating the role of excess cell death in MS pathology. Furthermore, various mitochondrial and metabolic regulator proteins such as SIRT1, NAMPT, PGC1-a, TFAM, MtTF-1 were also significantly dysregulated in the EAE model. Interestingly, treatment with 7,8 dihydroxyflavone, another drug that significantly improved clinical score in the EAE mice, also reversed these dysregulations in apoptotic/autophagic and mitochondrial/metabolic proteins (unpublished data).

In health, autophagy is critical to the survival and proliferation of T cells. However, in MS, this role may actually be contributing to disease progression because the autophagic signaling system is chronically activated. This is further supported by the increased presence of T cells in MS plaques and spinal fluid, as well as an upregulated number of T cells attacking the myelin sheath. In Fig. 5, we summarize the proposed mechanisms of the interplay between ER stress and autophagy in contributing to the demyelination that characterizes MS. It is important for the reasons mentioned in this review that future research in drug treatments for MS focus on how ER stress and autophagy are interconnected in MS pathology and how this directly/indirectly affects immune cell infiltration. While further research must be performed to confirm these mechanisms as pertinent to the pathogenesis of MS, targeting ER stress and autophagic mechanisms in MS is an emerging field with promising therapeutic value. (See Figs. 1–4.)

Acknowledgements

Funding for this submission is being supported by the Center for Veterans Affairs at University of Maryland, Baltimore.

Author's contribution

All authors listed have significantly contributed to the review and agreed jointly to approve it for submission for publication.

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