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THE MECHANISMS OF MALLORY-DENK BODY FORMATION ARE SIMILAR TO THE FORMATION OF AGGRESOMES IN ALZHEIMER'S DISEASE AND OTHER NEURODEGENERATIVE DISORDERS

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Abstract

There is a possibility that the aggresomes that form in the brain in neurodegenerative diseases like Alzheimer's disease (AD) and in the liver where aggresomes like Mallory-Denk Bodies (MDB) form, share mechanisms. MDBs can be prevented by feeding mice sadenosylmethionine (SAME) or betaine. Possibly these proteins could prevent AD. We compared the literature on MDBs and AD pathogenesis, which include roles played by p62, ubiquitin UBB+1, HSPs70, 90, 104, FAT10, NEDD8, VCP/97, and the protein quality control mechanisms including the 26s proteasome, the IPOD and JUNQ and autophagosome pathways.

Keywords

neurofibrillary tangles (NFT); β amyloid (A β); protein quality control; 26S proteasome

INTRODUCTION

It would be useful to compare the mechanisms involved in the aggregation of amyloid (A β) and neurofibrillary tangles that accumulate in Alzheimer's disease (AD) with the mechanisms of Mallory-Denk body (MDB) formation in the liver. This is because MDB formation can be prevented by restoring normal protein quality control. AD and PD together affect around 50 million people worldwide (Goedert, 2015). Prevention would more likely succeed rather than treating the diseases which had already developed and where protein quality control mechanisms are defective.

What diseases cause MDB formation in the liver?

The diseases that cause MDB formation include HCV (Hu and French, 1997), alcoholic liver disease (ASH), hepatocellular carcinoma, primary biliary cirrhosis, Wilson's disease, a beta lipoproteinemia, Indian childhood cirrhosis, intestinal bypass surgery for morbid obesity, glucocorticoid therapy, Weber-Christian disease, perhexiline maleate hepatitis,

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diethylaminoethoxyhex-estrol-induced hepatitis, fatty liver in obesity (NASH), focal nodular hyperplasia, post intestinal resection, radiation pneumonitis and asbestosis (French 1981), cirrhosis due to differing etiologies, hepatic adenoma (French, 1983), 2'3'-Dideoxinosine (Hu and French, 1997), sclerosing hyaline necrosis in Bloom Syndrome (Wang et al., 1999) amiodarone, antitrypsin deficiency, von Gierke disease, porphyria cutanea tarda, congenital fibrosis, acute viral hepatitis and acute cholestasis (Zatloukal et al., 2007), HBV (Nakanuma and Ohta, 1985).

What are the proteins present in MDBs?

The following proteins co-localize in MDBs: K8, 18, 7, 19, 20, hyperphosphorylated keratins, ubiquitinated keratins, HSP70, 90 and, 25, α - β crystalline, phosphothreonine, phospho-p38 MAPK, ubiquitin, UBB+1, p62, VSP/97, NEDD8, proteasome subunits β 5, P25, Tbp7, transglutaminase and tubulin (incomplete list) (Zatloukal, 2007).

What treatments can prevent MDB formation?

Several different approaches could be used to prevent MDB formation. Mouse liver tissue culture cells derived from livers forming MDB in response to DDC feeding failed to form MDBs when incubated with agents which depolarized the liver cell microtubules i.e. colchicine. It was concluded that the formation of MDBs requires intact microtubules (Riley et al., 2003). Peripheral small aggregates formed but did not move toward the nucleus to form a MDB aggregate. Using the same tissue culture model as used for microtubules above, but using an NF κ B inhibitor, prevented MDB formation *in vitro* (Nan, 2005). The treatment reduced mRNA for Src, p105/NF- κ B, ERK, MEKK1 and JNK1/2 and decreased phosphorylated ERK 1/2. (Fig 1). Inhibition of NF- κ B prevented ERK activation, which prevented MDB formation. Using the same tissue culture model used to depolarize microtubules and NF κ B above, the inhibitor of p38 phosphorylation (SB202190) completely prevented MDB formation *in vivo* (Nan et al., 2006). When MDBs were allowed to form, phosphorylated p38 co-localized in the MDBs. Then the CK8 was phosphorylated causing the MDBs to formed. Using the same tissue culture model as used for microtubules but instead adding S-adenosylmethionine (SAME) to the media prevented MDB formation *in vitro* (Li et al., 2008).

Neuronal ERK, JNK and p38 are also involved in AD pathogenesis (Zhu et al., 2001). Both nitrogenic signaling (ERK) and cellular stress signaling (p38) were investigated in AD and found to be activated in susceptible neurons in both mild and severe cases of AD. This is similar to that seen in MDB formation. Activation of p38 and ERK were observed within neurons by IHC using antibodies to phosphorylated ERK and p38 in both the hippocampus and cortical regions in cases of AD.

Mice fed DDC and SAME showed a marked reduction in MDB formation *in vivo* (Li et al., 2008). The mechanism of the inhibition of MDB formation by SAME was achieved by preventing the inhibition of the 26S proteasome activity caused by DDC feeding. This was achieved by preventing the switch from the 26S proteasome to the immunoproteasome (Bardag-Gorce et al., 2010). DDC feeding causes the induction of the catalytic subunits of the immunoproteasome. At the same time DDC feeding decreases the expression of the

catalytic subunits of the 26S proteasome. DDC feeding also regulates FAT10, TNF α and IFN γ receptors. The interferon sequence response element (ISRE) is located on the FAT10 promoter. It responds to IFN γ to induce the immunoproteasome catalytic subunits LMP2 and 7 (Oliva et al., 2010). SAME feeding reversed these changes and restored the proteasome to normal despite DDC feeding (Bardag-Gorce 2010). Betaine feeding also inhibited MDB formation by increasing MAT1 α expression (Oliva et al., 2010b). Lastly, FAT10 KO mice fed DDC, failed to develop MDBs because, without the FAT10 promoter present, the 26s proteasomes retained the normal proteasomal chymotrypsin proteolytic activity when DDC was fed. MDBs form when the 26s proteasome is inhibited. MDBs cannot form if the 26s proteasome is not inhibited. (French et al., 2010).

Since both SAME and betaine prevent MDB formation they might also prevent AD and PD. There have been no papers reporting FAT10 involvement in aggresome formation in the human brain in AD. Only one thesis, submitted for a Ph.D. degree was found entitled “The modulation of Tau aggregation in a cell model of Alzheimer’s disease by the proteasome adaptor protein NUB1” by Richet (2012) performed at University College, London. This study focused on the presence of ubiquitin, FAT10, NEDD8 and NUB1 in the entorhinal cortex and hippocampus of AD patients and age matched controls. Ubiquitin accumulated in NFTs as well as extra-cellular structures in either dystrophic neurites, senile plaques or both only in AD patients. FAT10, in both the entorhinal cortex and hippocampus, was found to be more widespread in AD compared to the controls. It involved mainly the cytoplasm of cells.

Oxidative stress in Alzheimer’s disease (AD) compared to MDB formation

Patients with AD have an increase of oxidized nucleoside derived from RNA, 8 hydroxyguanoside (8OHG) and nitrotyrosine in their vulnerable neurons. The oxidative damage is quantitatively greatest early in the course of AD and reduces with progression of the AD. Increase in beta amyloid (A β) deposition is associated with decreased oxidative damage. Neurons with neurofibrillary tangles (NFT) showed a 40–60% decrease in relative 8OHG compared with neurons free of NFT. (Nunomura et al., 2001). In the case of MDB formation in the liver, oxidative stress is present in the *in vitro* model of Cederbaum using a hepatoblastoma cell line (HepG2 CYP2E1 transduced cells). In this model arachidonic acid and iron-NTA complex is added with ethanol to create oxidative stress. After 48 h incubation multifocal cytoplasmic cytokeratin and ubiquitin positive aggresomes formed, which resembled small MDBs. Reactive oxygen species, carbonyl proteins and 4HNE adducts were generated. 4HNE formed adducts to α 6 and RPT4 subunits of the 26s proteasome subunits. The chymotrypsin and trypsin activity of the 26S proteasome was reduced. DNA damage was increased and reactive oxygen species were increased (Bardag-Gorce et al., 2006). However, when MDBs were formed *in vitro*, after a 6 day period, and DDC was withdrawn for 1 month followed by refeeding DDC, the 4HNE and MDA adducts were formed. There was no increase in GSH and no increase in the carbonyl protein levels (Li et al., 2008). Like AD, MDB related oxygen stress seems to diminish with time.

Human liver biopsies from patients who had alcoholic hepatitis with MDB formation were immunohistochemically stained (IHC) for MDBs and 4 HNE aggresomes to see if 4HNE was located within the MDBs. Instead, 4HNE was located in separate aggresomes located in

the cytoplasm of liver cells that had MDBs. The MDBs were stained with the antibody M30 (Amidi et al., 2007). Early on, Alzheimer's neurons develop 4HNE deposits in their cytoplasm and axons (Wataya et al., 2001). Glycooxidation and lipoxidation adducts also form. They function to protect the neuron system from neurotoxic damage. 4HNE deposits from oxidative stress are present in the brains of AD and in amyloid (A β). (Siegel et al., 2007). 4HNE triggers A β aggregation, resulting in covalent crosslinking of A β peptides.

What is the role that p62 plays in AD and MDB formation?

P62/sequestome-1 is a multifunctional protein that contains several protein-interaction domains where it regulates cell signaling and protein trafficking, aggregation and degradation. p62 can bind through its UBA motif to ubiquitinate proteins and control their aggregation and degradation by way of autophagocytosis or 26S proteasome digestion as a major player in protein quality control (Fig 2). p62 has been reported to be involved in association with the intracellular inclusions in the primary and secondary tauopathies, α -synucleinopathies and other neurodegenerative brain disorders where inclusions of misfolded protein develop. In AD, p62 is associated with neurofibrillary tangles composed of hyperphosphorylated tau protein and ubiquitin (Salminen et al., 2012). The keratins in MDBs are also hyperphosphorylated and ubiquitinated (Zatloukal et al., 2007; Ohta et al., 1988). p62 appears to play a role in degradation of tau protein. The absence of p62 (p62 KO mice) leads to the development of tau pathology. p62 gene expression and protein levels are decreased in the frontal cortex of AD patients. Decrease in p62 can cause a decrease in the signal pathways of Nrf2, cyclic AMP and NF- κ B, causing increased oxidative stress and loss of neurons (Salminen et al., 2012). p62 is also involved in MDB formation. p62 in the liver functions as a scaffolding protein that binds polyubiquitin on proteins destined for digestion by the 26S proteasome. Inhibition of the expression of p62 by grip NA (gp62), *in vitro* in hepatocytes in a primary culture, markedly inhibited MDB formation (97% inhibition). When p62 was over expressed by transfecting the hepatocytes with a plasmid containing green fluorescent protein (GFP) fused p62 (p62-GFP) it induced an increase in MDB formation by 339% in DDC drug-primed hepatocytes. It is clear that p62 is involved in MDB formation (Nan et al., 2004). MDBs that form in human livers in cases of alcoholic hepatitis incorporate ubiquitin and p62 within the MDB aggresome (Bardag-Gorce et al., 2005).

The ubiquitin-proteasome system (UPS) in AD compared to MDB formation

The changes in UPS in AD consist of many factors including E3 ubiquitin ligases, ubiquitin hydrolase (Uch-LI), ubiquitin, ubiquitin-like molecules and the 26S proteasome. An extensive body of work links UPS dysfunction with AD pathogenesis and progression (Gong et al., 2016). A low Uch-LI level has been linked with A β accumulation in AD. UBB+1 (frame-shift mutation) inhibits the proteasomal function and is associated with increased neurofibrillary tangles in AD. Defective proteasome activity is detected in the early phase of AD, along with synaptic dysfunction. The late stage of AD is associated with the accumulation and aggregation of ubiquitin proteins, which precede tangle formation (Garcia Gil et al., 2001; Upadhyya and Hedge, 2007; Oddo, 2008; Bedford et al., 2009). When the proteasome activity was assayed from AD patients, inhibition of proteasome activity was reduced in the hippocampus and parahippocampal gyrus to 48%, the superior and middle

gyri (38%) and inferior parietal lobule (28%) compared to controls (Keller et al., 2000). Ubiquitin is present in neurofibrillary tangles (NFT), and senile plaque (SP) neurites of Alzheimer's disease brains (Perry et al., 1987). Ubiquitin is covalently associated with the insoluble neurofibrillary material of NFT and SP suggesting that the ubiquitin-mediated degradative pathway may be ineffective in removing NFT and SP in Alzheimer's disease brains.

In the liver, MDBs form as cytokeratin-covalently bound to a string of ubiquitin molecules, UBB⁺¹, transglutaminase and the proteasome subunits $\beta 5$ and Tbp7 in the 26 proteasome as well as heat shock proteins 70 and 90. (Fig 3). This complex of proteins was detected by confocal immunohistochemistry localization and Western blot analysis located in the high molecular weight ubiquitylated protein smear when proteolysis by the 26S proteasome failed to occur (Bardag-Gorce et al., 2007). MDB formation occurs in mice by inhibition of the proteasome *in vivo* by using the b5 subunit chymotrypsin inhibitor PS341 (Yuan et al., 2000). The results suggest that the proteasomes bind to the ubiquitylated protein complex and then move to accumulate at the edges of the MDB along with other proteins including HSP70 and 90 and transglutaminase. The presence of UBB⁺¹ in the ubiquitin smear and in the MDB is similar to that seen in AD (van Leeuwen et al., 1998). The mechanism of MDB formation is probably similar to the mechanism involved in aggresome formation in Alzheimer's disease where UBB⁺¹ prevents deubiquitination of the polyubiquitinated UBB⁻¹ proteasome complex (Bence et al, 2001) and causes neuronal death (de Urij et al., 2001).

What are the roles of heat shock proteins (HSP) in MDB formation and Alzheimer's disease?

Heat shock proteins play a key role in preventing protein misfolding and aggregation. Heat shock protein-90 (Hsp-90) is a ubiquitous molecular chaperone potentially involved in AD pathogenesis. Hsp-90 regulates the activity of the transcription factor, heat shock protein-1 (Hsp-1), the master regulator of the heat shock response. In AD, Hsp-90 may redirect neuronal aggregate formation and protect toxicity by activating Hsp-1 and the subsequent induction of heat shock proteins such as Hsp 70 (Ou et al., 2014). Hsp-90, as molecular chaperone, is capable of suppressing protein aggregation, solubilizing protein aggregates and targeting proteins for degradation. Kakimura et al (2002) reported that extracellular heat shock proteins such as Hsp-90, Hsp 70 and 32 could facilitate A β clearance by activation of microglial phagocytosis and A β degradation by NF- κ B, p38 MAPK activation and the Toll-like receptor (TLR4) pathway. Also, high-affinity Hsp-90-CHIP complex selectively degrades phosphorylated tau client proteins in AD. A critical mediator of this mechanism is Hsp-70-interacting protein, a tau ubiquitin ligase. The complex of Hsp 90 and Hsp 70/40 can inhibit A β formation and slow the rate of aggregation. In the case of Hsp 90 and Tau, the accumulation of tau leads to the formation of the toxic insoluble NFT inside the neuronal cytoplasm in AD. Tau phosphorylation and aggregation result in conformational changes that lead to neurodegeneration. Akt and CHIP co-regulate tau degradation (Dickey et al, 2008). Akt is ubiquitylated and degraded by the tau ubiquitin ligase CHIP, which largely depends on the Hsp 90 complex, while CHIP binds with both HSP 70 and 90, interacting with and degrading a number of proteins. The most important regulators of Hsp 90

machinery are the co-chaperones and posttranslational modifications of Hsp 90 itself, i.e., acetylation, nitrosylation and phosphorylation (Wandinger et al., 2008).

In the case of MDB formation, it has been shown in the DDC primed mouse model, that heat shock induced MDB formation over a 7 day period *in vivo* (Yuan et al., 1995). Hsp 25 was induced on day 1 through 6 after heat shock whereas Hsp 70 was induced on day 3 and 5 after heat shock. However, Hsp 90 was not induced. Human MDBs show colocalization of ubiquitin with Hsp 90 and 70 and mouse MDBs show colocalization of Hsp 70 with ubiquitin. (Fig 3). Both Hsp 70 and 90 were covalently bound, like ubiquitin, to proteins present in anti cytokeratin immunoprecipitates of MDB complexes. The MDB complexes were isolated from livers of mice treated to induce MDB formation (Bardag-Gorce et al., 2002). Chymotrypsin in the proteasome, when inhibited by PS-341, markedly increases cytokeratin aggregation in the liver of mice. Hsp 70 and 25 with cytokeratin 8, are markedly increased at the same time. Normal mouse hepatocytes cultured for 6 days, then transfected with a CK-18 plasmid, formed MDBs of CK18, indicating that normal liver cells making excess CK8 or CK18 form MDBs (Bardag-Gorce et al., 2004).

What are the roles that UBB+1 play in MDB, A β and AD pathogenesis

A mutant form of ubiquitin (UBB⁺¹) is found in the aggresomes in both MDBs and AD aggresomes (Riley et al., 2003). (Fig 3. In a recent review by Gentier and van Leeuwen (2015), they emphasized the importance of the DNA-RNA sequence differences in several genes including ubiquitin (UBB⁺¹) that occur in AD. The resulting misframed protein accumulates in the neurofibrillary tangles (NFTs). This suggests a link between the neurodegeneration of the AD type and loss of protein quality control by the ubiquitin proteasome system (UPS). UBB⁺¹ accumulates in the plaque and tangles of sporadic AD. AD starts in the brainstem causing changes in the central regulation of respiration. Gentier et al. (2015) mapped UBB⁺¹ in a mutant mouse model that was over expressing UBB⁺¹. They compared the distribution of UBB+1 with that seen in human AD.

The accumulation of proteins in AD and A β is caused by a lack of cellular clearance. The degradation of these proteins may be by autophagy. The A β and tau are turned over, not by the 26S proteasome, but instead, are likely to cause impairment of the function of the 26S proteasome. The formation of A β plaques in the transgenic model of AD can be modulated by UBB⁺¹ expression via secretase (Gentier et al., 2015). In the case of Tau, hyperphosphorylation of tau is the primary causative factor in AD development leading to neurotoxicity based on the observation that NFT density and distribution correlates with the clinical state of the disease. When tau is hyperphosphorylated, the ability to bind microtubules is reduced and tau becomes insoluble and accumulates. Microtubules become disrupted, dysregulating axonal transport. Tau is sequestered and self assembles. The 3 different forms that accumulate appear to coincide with the appearance of UBB⁺¹. UBB⁺¹ accumulates specifically in tau and polyglutamylopathies. It is too short to be degraded by the proteasome. It lacks the C-terminal glycine residue that ubiquitin has. At high levels it acts as an inhibitor of the proteasome. The UPS inhibition increases the accumulation of UBB⁺¹ and also increases the deposition of hyperphosphorylated tau in NFT (Ho et al., 2005). UBB⁺¹ causes neuritic beading, impairment of mitochondrial movements,

mitochondrial stress and degeneration of primary neurons (Tan et al., 2007). UBB+1 plays a role in the basic mechanisms of homeostasis and breathing (Gentier RJ and Leeuwen 2015).

We first showed that UBB+1 was also manifested in MDB formation in the mice treated the 26S proteasome inhibitor PS341. It co-localized with ubiquitin in the MDB aggresomes that formed (French et al., 2001) In human liver biopsies and autopsy livers where MDBs were stained with antibodies to UBB+1 and UB showed that the MDBs stained positive for colocalization by both antibodies. No amyloid was detected in any of the livers. Western blot analysis showed a UBB+1 band at 11 kilodaltons. It was concluded that UBB+1 was only found in the MDBs and therefore may act by interfering with the degradation of the MDBs because UBB+1 may inhibit the proteolytic function of the proteasome (McPhaul et al., 2002). (Fig 3). Electron microscopic viewing of immunogold UBB+1 antibody staining of isolated MDBs showed the presence of UBB+1 bound to the MDB filaments *in vitro*. In *in vitro* studies, we induced MDB formation by adding UBB+1 or PS341, a proteasome inhibitor to the tissue culture (Bardag-Gorce et al., 2003). An immunoprecipitate with a monoclonal antibody to cytokeratin 8 (CK8) was incubated for 24 h in the presence of different conditions involved in aggresome formation including ubiquitin, UBB+1, PS341, an ATP generating energy source, a deubiquitinating enzyme inhibitor, a purified proteasome fraction, and an E1-3 conjugating enzyme fraction. MDB protein aggresomes formed in the presence of ubiquitin, plus UBB+1, or PS341. These aggregates stained positive for CK8, UBB+1 and a proteasome subunit Tbp7 as demonstrated by Western blots. A second approach was used to form MDBs *in vitro* in cultured hepatocytes transfected with UBB+1 protein using Chariot. The cells were double-stained using CK8 and ubiquitin antibodies. The two proteins were colocalized in MDB-like aggregates. These results supported the conclusion that aggresomes form by a complex multifunctional process which is favored by the inhibition of the proteasome due to the presence of UBB+1.

Role of the inflammasome in AD and MDB formation

The formation of β amyloid plaques in AD stimulates the production of inactive IL-1 β an inflammatory cytokine (Prinz et al., 2011). The question is, what initiates the IL-1 β processing in AD. Caspase-1 processing of IL-1 β is mediated by the inflammasome (Kolliputi et al., 2012). Inflammasomes, such as the NLRP3 inflammasome, detect the inflammatory aggregates of β amyloid and inactive IL-1 β and respond by secreting caspase-1 to activate IL-1 β (Heneka et al., 2013). This leads to the creation of the inflammatory reaction around the plaque, which down regulates amyloid precursor protein (APP) degradation, as well as decreases the destruction of β -amyloid plaques by microglia. NLRP3 inflammasome activation by β -amyloid in microglia is necessary for maturation of IL-1 β and subsequent inflammatory events when Caspase-1 expression develops in the APP/ Presenilin-1 (PS1) mouse model of AD (Heneka et al., 2013). APP/PS1 mice had decreased hippocampal synaptic plasticity. Heneka et al (2013) concluded that the NLRP3 inflammasome leads to pathologic deposition and ineffective clearing of β amyloid in AD. They suggested that NLRP3 enhances AD and may be involved in synaptic dysfunction, cognitive impairment and the restriction of microglial clearance of amyloid.

The role of the inflammasome in the pathogenesis of the MDB in patients with alcoholic hepatitis (ASH) was investigated comparing liver biopsies from ASH patients with controls using immunohistochemistry staining and quantitative morphometrics (Peng et al., 2014). Antibodies used included NOD-1, NLRP3, NAIP, Mavs, ASC, Caspase-1, IL-1 β , IL-18, IFN γ , IL-10, IL-6, STAT3, p65, and MTCO2 for mitochondria. The biopsies were double stained for ubiquitin to correlate with the liver cells making MDBs. Mavs, caspase-1, IL-1 β and TNF α showed increased expression in ASH compared with controls ($p < 0.05$). NAIP expression markedly increased in ASH (0.01). (Fig 5). There was a trend in the levels of NLRP3, ASC, caspase-1, IL-18, IL-10 and p65 expression which correlated with the number of MDBs formed in the same fields that were used for quantitation of protein expression (correlation coefficients were between 0.62 and 0.93, $p < 0.04$). It was concluded that activation of the inflammasome was present in ASH and suggested that MDB formation may be involved in the activation of the inflammasome similar to that seen with β amyloid in AD.

What about the protein quality control system pathways in neurodegenerative diseases and MDB pathogenesis

Three protein quality control pathways IPOD, JUNQ and autophagy are actively involved in MDB and AD pathogenesis by removing or refolding misfolded proteins to prevent retention and aggregation of proteins (Kaganovich et al., 2008). In the case of IPOD and JUNQ pathways, compartmentalized sequestration of misfolded proteins are involved in neurodegenerated diseases. After misfolding most proteins are recognized and ubiquitinated, which directs them to the JUNQ pathway, a region that contains chaperones and the 26S proteasome. JUNQ concentrates soluble misfolded proteins which may be proteasomally degraded or misfolded by the chaperones. The insoluble aggregated proteins which may not be ubiquitinated can be targeted to the IPOD pathway (Takalo et al., 2013). It does not contain proteasomes but colocalizes with autophagy-associated proteins, such as Atg8.

Aggresomes also contain chaperones and components of the ubiquitin-proteasome system (UPS). Targeting to the aggresome, IPOD or JUNQ pathways involves active retrograde transport of cargo by the motor proteins on the microtubules to the microtubule organizing center (MTOC) next to the nucleus. The cargo colocalizes with ubiquitin and p62 and concentrates the soluble proteins targeted for clearance by the UPS or autophagy. Soluble misfolded proteins that are targeted to the USP for degradation are typically concentrated at a structure called the juxtanuclear quality control (JUNQ), which contains chaperones and proteasomal subunits. Here proteins may be refolded or degraded by the proteasome.

The insoluble aggregated proteins, such as are found in Huntington or prion proteins, can be directed to IPODs. These colocalize with autophagy-associated proteins suggesting that the IPODs may be disposed of by autophagy. Enhanced ubiquitination of the protein may redirect it to JUNQ. Accumulating evidence implies that sequestration of potentially harmful, misfolded or aggregated proteins into specific compartments and formation of intracellular inclusions is a cytoprotective event. Therefore, the compartmentalization of harmful misfolded proteins and protein aggregates may enhance their clearance and prevent them from blocking the USP or the autophagosome-lysosomal pathway (ALP) and occupying the cellular chaperones.

p62 is a major receptor for autophagic degradation of ubiquitinated targets. It binds to Atg8 on the autophagosomes and is itself substrate for ALP-mediated degradation. p62 is found in neuronal and glial inclusions in AD and Parkinson's disease (PD) dementia with Lewy bodies. The early accumulation of p62 in NFTs in AD suggests that p62 may be involved in NFT formation (Kuusisto et al., 2002).

The chaperone Hsp 104 is another quality control component that interacts with misfolded and aggregated proteins and co-localizes with both JUNQ and IPOD, mostly around the IPOD component, often in an arrangement around the protein inclusion. HSP 104 plays a role in preventing inheritance of oxidatively damaged proteins. At JUNQ, Hsp 104 serves to keep proteins soluble for either refolding or degradation (Kaganovich et al., 2008).

Autophagy has been shown to be involved in the clearance of protein aggregates. The IPOD, but not the JUNQ, colocalize with the autophagy marker Atg8 (Kaganovich et al., 2008). The association of the IPOD with an autophagic marker provides a link between aggregated proteins in the IPOD and the autophagic pathway.

We investigated the role of IPOD, JUNQ and the autophagosome in MDB formation in alcoholic hepatitis (Mendoza et al., 2015) because, in a recent study, elevated metacaspase 1 (Mca1) expression counteracted aggregation and accumulation of misfolded proteins and extended the life span of yeast (Hill et al., 2014). Mca1 may be associated with Ssa1 and HSP104 in disaggregation and fragmentation of aggregated proteins and their subsequent degradation through the ER-associated degradation (ERAD) pathway. If degradation is not available, protection of the cellular environment from a misfolded protein can be done by its sequestration into two distinct inclusion bodies, JUNQ and IPOD (Kaganovich et al., 2008). Mca1, Hsp104, Hsp40, Ydj1, Ssa1, VCP/p97 and p62 all play an important role in protein quality control systems. These protein concentrations were measured by fluorescent microscopic fluorescent intensity measurement using a microscopic morphometric assay in liver biopsy sections from patients with alcoholic hepatitis ASH where MDB were numerous. Mca1, Hsp104, Ydj1 and p62 were significantly up regulated compared to control liver sections (Fig 6). Hsp40 and VCP/97 tended to be increased in(ASH). Ssa1 levels tended to be increased in ASH. Recruitment of Mca1, Hsp104, Ydj1 and p62 may indicate that autophagy, the ERAD, JUNQ and IPOD systems are activated in ASH. Unreported results have shown that Atg6 is also markedly increased in alcoholic hepatitis. These studies indicate that the JUNQ, IPOD and autophagy systems are partially compensating for the inhibition of the activity of the 26S proteasome in ASH when MDBs are formed in large numbers.

CONCLUSION

The literature was reviewed to highlight the similarities and differences in the mechanisms involved in AD and other neurodegenerative diseases compared to the mechanisms involved in MDB formation. Common ground was found in oxidative stress, adduct formation, p62 expression, the ubiquitin-proteasome system, UBB⁺¹, HSPs, the protein quality control system, the inflammasome, IPOD, JUNQ and autophagy. The successful prevention of MDB formation by inhibiting FAT10 induction and loss of the 26S proteasome activity by feeding

SAMe or betaine was emphasized. It is recommended that SAMe or betaine be tried to test the hypothesis that these methyl donors taken daily in the diet might prevent the development of AD or PD.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4. NAIP was up-regulated in AH livers

A. Immunohistochemical analysis of liver biopsies obtained from two representative patients with alcoholic hepatitis and two representative control patients. The specimens were stained for the presence of NAIP (green, the first column) and ubiquitin (red, the second column). There was no co-localization seen with the tri-color filter (the third column). The fourth column demonstrated the fluorescence intensity measurement of NAIP stain. Screen snips were obtained from the morphometric screen to visualize and compare the intensity of staining in MDB forming hepatocytes, neighboring non-MDB forming cells, and control cells. The fluorescence intensity was traced along the yellow line in the top picture and shown as a green tracer in the bottom picture. B) Fluorescent intensity bar graph of MDB forming hepatocytes, non-MDB forming hepatocytes, and control cells from three AH specimens and three control specimens. The results were shown as Mean $\pm$ S.D.. The comparisons showing statistical significance ($p < 0.01$) were indicated.

Fig. 5.

Autophagy, the ER-associated degradation (ERAD) pathway, the JUXta Nuclear Quality (JUNQ) control compartment, and the Insoluble Protein Deposit (IPD) systems are cellular mechanisms responsible for protein maintenance. These systems are dependent on many chaperones and transport proteins for successful protein management. Mca1, Hsp104, Hsp40, Ydj1, Ssa1, VCP/p97, and p62 are some of the important chaperones in protein quality control systems. Most misfolded/aggregated proteins are initially processed in the ERAD and autophagy systems. The chaperones are very active in these protein quality control systems. But once proteins become terminally aggregated, the IPD and JUNQ systems are utilized by the cell as alternate pathways for damage control. The upregulation of Mca1, Hsp104, Ydj1, p62, Ssa1, Hsp40 and VCP/p97 in ASH may indicate that autophagy, the ERAD, the JUNQ, and the IPD systems are active in ASH.