Review

Activation of Innate Immunity and Autophagy in Brain Tissues with Prion Disease and Degradation of Abnormal PrPs in Cells — China's Studies

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ABSTRACT

Unlike infectious diseases caused by conventional microbes, there are no detectable specific humoral or cellular immunoresponses to prion infection. However, extensive and active gliosis is observable in affected brain regions along with significant deposits of scrapie-like prion protein (PrP^{Sc}). Here, we summarize our studies of vibrant activation of host non-specific immunocomponents and autophagy in the microenvironment of prion infected brains. Activation of the brain's innate immunity and autophagy upon prion infection reflect non-specific host defense systems attempt to dispose of accumulated prions. Vibrant elevation of neuroinflammation leads to neuron injury.

The neuropathology of prion disease (PrD) has two completely different scenarios — one is severe depression and death due to severe neuron loss, cell apoptosis, and extensive spongiform degeneration, and the other is activation, expressed as gliosis, activated autophagy, and non-specific immunity. These polarized neuropathological scenarios are continually observable, even at the terminal stage of most types of human and animal PrDs. Prion infection does not elicit any detectable specific humoral or cellular immunoresponse, but host innate immunity appears to be persistently activated in infected brains, possibly for clearance of prions. Accumulated scrapie-like prion protein (PrP^{Sc}) may further stimulate strong non-specific immunoresponse. However, overreactive brain immunoresponse may eventually deteriorate pathological processes of PrD. In this report, we summarize major findings of our studies on overreaction of innate immunity and inflammation.

MICROGLIOSIS AND ASTROGLIOSIS

Microglia are resident mononuclear phagocytes of the central nerve system (CNS), comprising approximately 10% of CNS cells. Based on scrapie-infected rodent models, substantial microgliosis is noticeable in infected brains, regardless of amounts (marked by Iba1) or activities (marked by CD68), and shows a time-dependent increase. Histologic distribution of activated microglia co-localize with PrP^{Sc} in various brain regions (*1*). Abnormal activation of microglia can also be detected in the cortex, thalamus, and cingulate gyrus regions of sporadic Creutzfeldt-Jakob disease (sCJD) and G114V genetic CJD (gCJD) patients with large amounts of PrP^{Sc} deposits, but are not detected in the brains of D178N fatal familial insomnia (FFI) patients with fewer PrP^{Sc} deposits (*2*–*4*). Brain CXC3L1 is a negative regulator for activation of microglia that is downregulated in prion infected rodent models, highlighting a possible regulating pathway of fractalkine signaling deficiency (*1*).

Exposure of supernatant from lipopolysaccharide (LPS)-treated microglia BV2 cells and tumor necrosis factor α (TNF-α) onto scrapie infected cell line SMB-S15 induces lower cell viability than its normal partner cell line SMB-PS. An activated phosphorylated form of mixed lineage kinase domain-like protein (p-MLKL, a marker of necroptosis) was observed both in SMB-S15 cells and in cortical tissues of patients with sCJD and gCJD. The decreased cell viability of SMB-S15 and the increased p-MLKL induced by TNF-α can be completely rescued by a necroptosis specific inhibitor necrostatin-1. Removal of PrP^{Sc} propagation in SMB-S15 cells by resveratrol partially rescues cellular tolerance to TNF-α. Overreactive microglia may have more influence on cells containing PrP^{Sc} via activation of the necroptosis pathway (*5*).

Glial fibrillary acidic protein (GFAP) distributes

exclusively in cytoplasm of mature astrocytes. One of the functions of GFAP is in the interaction of astrocytes with other cells that are required for the formation and maintenance of the insulating layer (myelin). Specific molecular interaction was found between prion protein (PrP) and GFAP, for both recombinant and native PrP (including PrP^{Sc}) (*6*). Overreactive proliferation of astrocytes during prion infection can lead to overexpression of a small heat shock protein, α B-crystallin (*7*).

ABERRANT ACTIVATION OF COMPLEMENT SYSTEM

Abnormally enhanced complement systems have been repeatedly identified in prion infected brains, including total complement activity levels and major components (C1q and C3), highlighting activation of the complement classical pathway (Figure 1). Membrane-attacking complexes (MAC) in infected brains also show remarkable time-dependent deposition during the incubation period, indicating persistently activated terminal complement components. MAC-specific signals overlap with neurons, while C3 distributed in astrocytes and microglia possibly associate with activation of those cells. (*8*). The alternative pathway (AP) for activation of the complement system in the CNS is also activated during prion infection. Key triggering elements and positive regulation of AP, complement factor B (CFB), and complement factor P (CFP) increase significantly in brain tissues of scrapie infected mice in a time dependent manner (*9*).

Unlike in brain tissues, complement components in cerebrospinal fluid (CSF) from sCJD patients are reduced or unchanged compared to non-CJD cases. Proteomic assays found that two components (C9 and CFB) are significantly decreased and three (C4b, C7, and C2) are non-significantly decreased in CSF samples of sCJD patients (*10*). Complement hemolytic activity (CH50) has significantly lower activity in CSF samples in various types of human prion diseases. Complement homeostasis happens differently in brains and in CSF of prion disease patients. Contrary to the wide range of CH50 values among sCJD patients, the CH50 values in genetic prion diseases, including T188K gCJD, E200K gCJD, and D178N FFI, are much narrower, which reflects distinct pathogeneses of sporadic and inherited prion diseases (*11*).

ABNORMAL ALTERATIONS OF CHEMOKINES AND CYTOKINES

Along with activation and proliferation of microglia and astrocytes during prion infection, levels of many chemokines and cytokines in infected brains are also

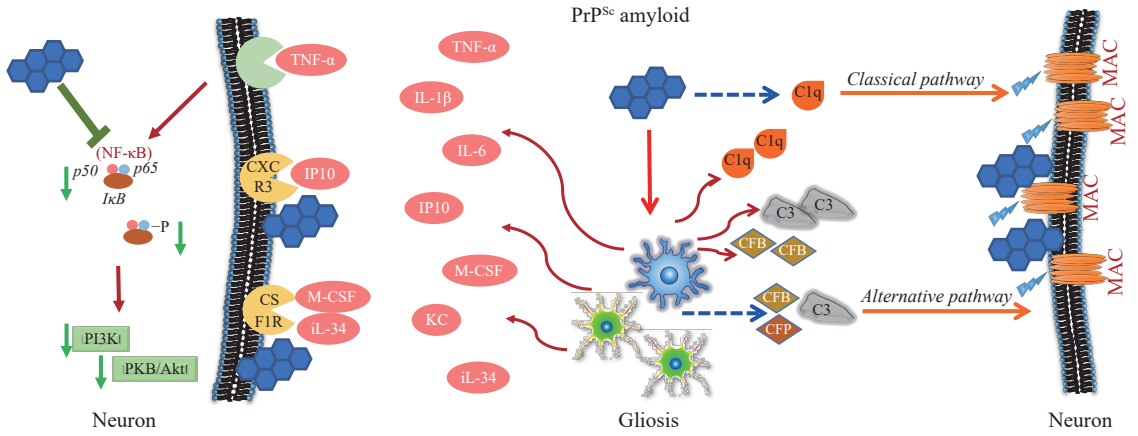

FIGURE 1. Schema of activation and level changes of components of innate immunity and neuroinflammation in the central nervous system during prion infection.
Note: PrP^{Sc} amyloid stimulates the activation of astrocytes and microglia, and secretes various complement components, chemokines, cytokines, and inflammatory factors. Complement components can form membrane-attacking complexes (MAC) on the surface of PrP^{Sc} infected neurons through classical and alternative pathways, subsequently inducing neuronal damage. Various chemokines, cytokines, and inflammatory factors can bind to receptors on the surface of neuronal membranes to activate a series of signaling pathways in neurons that induce neuronal death when infected with PrP^{Sc}.
Abbreviation: PrP^{Sc}=scrapie-like prion protein; MAC=membrane-attacking complexes; CFB=complement factor B; CFP=complement factor P.

increased — for example, interleukin (IL)-1β, IL-6, and TNF-α accompany marked PrP^{Sc} deposits (Figure 1) (*1*–*2*). Many chemokines in supernatant from cultured microglia increased significantly when stimulated with LPS, strongly implying a critical role for microglia activation in upregulated chemokines (*5*).

Transcriptional and translational levels of interferon gamma-induced protein 10 (IP10) and macrophage colony stimulating factor (M-CSF) were repeatedly upregulated in prion infected brains and prion infected cell lines. Slight but significant increases of IP10 levels were identified in the CSF samples of sCJD patients compared to non-CJD patients (*12*). Receptors CXCR3 for IP10 and CSF1R for M-CSF were increased in prion infected brains. Upregulated ligand signals were detected in neurons and microglia, while increased receptors are primarily distributed in neurons, which indicates that microglial cells are the main secretory cells for IP10 and M-CSF, with neurons as the major target cells. Neuropathologically, IP10 and CXCR3, as well as M-CSF and CSF1R, accumulate in brain regions with more PrP^{Sc} deposits. Molecular binding activities of PrP with IP10/CXCR3 and M-CSF/CSF1R were also addressed. Clearance of PrP^{Sc} replication in prion infected cells partially converts overexpression of IP10 and M-CSF.

ACTIVATION OF AUTOPHAGE

There are two main mechanisms for quality control of protein expression in eukaryotic cells: ubiquitin-proteasome and autophagy-lysosome pathways. Enhanced macroautophagic system can be clearly identified in the brains of prion disease patients and in prion infected cell lines or cells expressing PrP mutants. Deep repression of the mammalian target of the rapamycin (mTOR) pathway — an essential negative regulatory mechanism — has been shown to be closely associated with autophagy activation. Blockage of macroautophagic activity by a specific inhibitor (bafilomycin A) efficiently slows degradation of abnormal PrPs and PrP^{Sc} in cultured cells. Activation of the autophagic system begins at early stages of prion infection, likely attempting to resolve abnormal PrP aggregation. Neuron damage will occur once PrP^{Sc} replication and deposits exceed the capacity of the host's clearance system, including by autophagy (*13*).

Knockdown of ATG5 and treatment of three autophagic inhibitors induces a significant increase of PrP^{Sc} in prion infected cell lines and an increase of mTOR levels. F-box and WD repeat domains containing 7 (FBXW7) constitute one of the four subunits of the E3 ubiquitin protein ligase and targets mTOR for ubiquitination and degradation. Brain FBXW7 levels in scrapie infected animals and prion infected cells were upregulated at early stage. Knockdown of cellular FBXW7 remarkably inhibited autophagic flux and increased PrP^{Sc} accumulation. Enhanced expression of FBXW7 and subsequent activation of autophagy via downregulation of mTOR at early stage acts to clear invasive prions (*14*).

AMP-activated protein kinase (AMPK) is a serine/threonine kinase functioning as a positive regulator for autophagy by phosphorylating its downstream Unc-51-like autophagy activating kinase 1 (ULK1) at specific sites. Increases of brain AMPK and ULK1, as well as their phosphorylated forms AMPK-Thr172 and ULK1-Ser555, occurred at early stages of scrapie infected hamsters. Liver kinase B1 (LKB1), which mediates AMPK activation, is also increased in the infected brains at early and middle disease stage. Upregulation of activators in brains correlate with reduction of mTOR and activation of autophytic activity during prion infection. Upregulation of AMPK and ULK1 were seen in prion infected cell lines and knockdowns of cellular ULK1 reduced autophytic activity. The enhanced brain AMPK-ULK1 pathway reflects an active host response to prion infection (*15*).

Mitophagy is a special, selective autophagy process that maintains mitochondrial health and eliminates damaged mitochondria. Marked increases of Pink1 and Parkin were observed in prion infected cell lines. Activated Pink1/Parkin pathway modifies outer membrane proteins on damaged mitochondria via phospho-ubiquitin polyubiquitin chains, which reflects activated autophytic flux. Inhibition on the expressions of either Pink1 or Parkin in prion-infected cells can relieve autophagic flux. Aberrantly enhanced Pink1 and Parkin were also observable in different brain regions of scrapie-infected mice. Pink1- and Parkin-positive cells distributed more in the areas with amounts of PrP^{Sc} in scrapie infected mice, indicating an association between PrP^{Sc} deposits and activation of mitophagy (Figure 2) (*16*).

ENHANCEMENT OF OTHER ELEMENTS ASSOCIATED NEUROINFLAMMATION

α 1-antichymotrypsin (α 1-ACT) is an acute-phase inflammatory protein. α 1-ACT levels are significantly

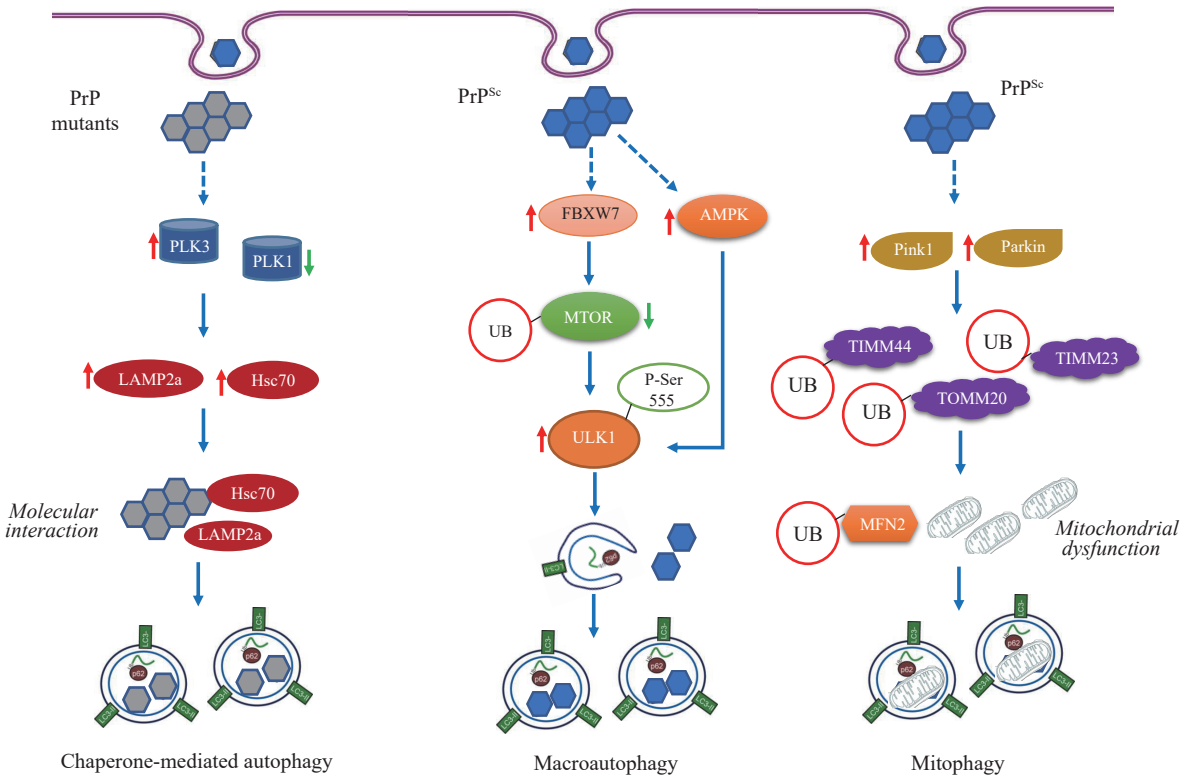

FIGURE 2. Schema of chaperone-mediated autophagy, macroautophagy, and mitophagy in cytoplasm to degrade PrP mutants and PrP^{Sc}.
Note: Abnormal changes in PLK family components can enhance the expression of cellular LaAMP2a and Hsc70, which can form molecular complexes with intracellular PrP mutants and induce autophagy. PrP^{Sc} activated FBXW7 and AMPK signaling pathways, thus induce macroautophagy. PrP^{Sc} can also activate Pink1-Parkin signaling pathways and induce mitochondrial dysfunction and mitophagy.
Abbreviation: PrP=prion protein; PrP^{Sc}=scrapie-like prion protein; PLK=polo-like kinase; LaAMP2a=lysosome-associated membrane protein type 2A; Hsc70=heat shock cognate protein 70; FBXW7=F-box and WD repeat domains containing 7; AMPK=AMP-activated protein kinase.

increased in brain tissues of scrapie-infected rodents in a time dependent manner. Increased α 1-ACT shows morphological co-localization with PrP^{Sc} deposits in infected brains and is identifiable in astrocytes, microglia, and neurons (*17*). Galectin-1 (Gal-1) is implicated in the regulation of innate and adaptive immunity. Remarkable increases in brain Gal-1 is observed in many scrapie-infected rodents at terminal disease stages. In postmortem brains of human prion diseases, Gal-1 levels were upregulated. More *S*-nitrosylated forms of Gal-1 were detected in infected brains (*18*).

The aquaporins (AQPs) are a family of 13 hydrophobic integral transmembrane water channel proteins involved in transcellular and transepithelial water movement and fluid transport. AQPs — especially AQP4 — are implicated as proinflammatory features of astrocytes. Notably, increased AQP1, AQP4, and AQP9 levels were found in the brain tissues of several scrapie infected mouse models in a time-dependent manner. AQP1 levels are increased in the cortex regions of some human PrDs. AQPs-positive cells are astrocyte-like morphologically and co-localize with GFAP positive proliferative astrocytes. Areas predominant with AQPs overlap with abundant PrP^{Sc} in brain tissues in scrapie murine models, strongly reflecting active neuroinflammation in prion disease (*19*).

DEGRADATION OF ABNORMAL PrP MUTATNS AND PrP^{Sc} IN CELLS

Hsp70 levels are increased in the brains of prion infected hamsters. Hsp70 can form complexes with abnormal Cyto-PrP and PG14-PrP that accumulates in cytoplasm, but not with wild-type PG5-PrP. Overexpression or activation of Hsp70 selectively mediates degradation of Cyto-PrP and PG14-PrP and

reduces cytotoxicity (*20*). Another heat shock protein, Hsp104, present in cellular mitochondrion of fungus, bacteria, and plants, has an effect on biochemical features of PrP *in vitro*. The recombinant yeast Hsp104 can inhibit fibril assembly of the synthetic PrP106-126 peptide and formation of fibril-like structure. Treatment of Hsp104 shifts secondary structures of PrP106-126 fibrils from β-sheet to random coil. Hsp104 disassembles mature PrP106-126 fibrils (*21*).

Protein p62/sequestosome 1 (SQSTM1) is a key cargo adaptor involved in autophagy-lysosome degradation. Overexpression of p62/SQSTM1 efficiently relieves cytosolic PrP (Cyto-PrP and PG14) aggregations and cell apoptosis. Inhibition of autophagy-lysosome blocks p62/SQSTM1-mediated degradation of abnormal PrPs. More complexes of p62/SQSTM1 and LC3 in cells expressing misfolded PrPs imply that p62/SQSTM1 plays an important role in the homeostasis of abnormal PrPs via an autophagy–lysosome-dependent way (*22*).

Polo-like kinase 3 (PLK3) has been proven to interact molecularly with PrP *in vitro* and *in vivo*. Overexpression of PLK3 significantly decreases levels of cytosolic mutated PrPs (Cyto-PrP and PG14) in cultured cells, but does not affect levels in wild-type PrP. The kinase domain of PLK3 appears to be responsible for clearance of abnormal PrPs independently of its kinase activity. Knockdown of PLK3 aggravates the accumulation of cytosolic PrPs. PLK3 overexpression in a scrapie infected cell line causes a notable reduction of PrP^{Sc} levels, but with little effect on PrP^{c} expression in its normal partner cell line, SMB-PS. Recovery of PLK3 at early stages of prion infection may help prevent toxic accumulation of PrP^{Sc} in brain tissues (*23*).

Overexpression of PLK3-mediated degradation of PrP mutants and PrP^{Sc} is repressed by lysosome inhibition. PrP mutants can interact with two major components of chaperone-mediated autophagy (CMA) effectors: lysosome-associated membrane protein type 2A (LAMP2a) and heat shock cognate protein 70 (Hsc70). Overexpression of PLK3 significantly enhances cellular levels of LAMP2a and Hsc70, accompanied with a reduction of accumulations of mutant PrP and PrP^{Sc}. Time-dependent reduction of LAMP2a and Hsc70 has been observed in brain tissues of prion infected hamsters, indicating impairment of CMA during prion infection (Figure 2) (*24*).

PERSPECTIVE

Our numerous studies propose a double-edged sword of activation of brain innate immunity and neuroinflammation in the progression of prion disease neuroprotective and neurotoxic outcomes. Although there are often conflicting results from different studies regarding specific immunoelements, it is generally considered that activation of innate immunity, probably at an early stage, facilitates clearance of pathogenic prions and maintenance of normal neuronal biology. Long-term overactivation of brain innate immunity may exceed neuron tolerance thresholds at some point in time, leading to persistent and irreversible damage. Precise intervention of local immunoresponses at correct time points is an important principle for guiding R&D of immunotherapy for prion diseases.

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