Effect of Microglial Inflammation in Prion Disease

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Abstract

Prion diseases are a group of transmissible fatal neurodegenerative disorders. Neuropathological features of prion diseases include neuroinflammation featuring the infiltration of activated microglia in affected brain areas as well as the accumulation of an abnormal isoform of the cellular prion protein and neuronal loss. Recent studies have elucidated that inflammation in the brain induced by microglia plays an important role in the pathogenesis of neurodegenerative disorders including prion disease. Thus, the regulation of neuroinflammation is key in terms of therapeutic and preventative approaches. The functions of neuroinflammation and microglia in this disease are discussed in this article.

Background

Prion diseases are a group of transmissible fatal neurodegenerative disorders of bovine spongiform encephalopathy (BSE) in cattle, Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, and chronic wasting disease (CWD) in deer and elk. They are caused by the conversion of cellular prion protein (PrP^{C}) into the pathological isoform (PrP^{Sc}) through conformational changes (Prusiner, 1998; Wechselberger, 2002). PrP^{Sc} is protease-resistant, and has a higher proportion of β-sheet structure in place of the normal α-helix structure. The accumulation of abnormal forms of prion protein (PrP^{Sc}) is important for developing the disease. Prion disease is neuropathologically characterized by neuronal vacuolation, neuronal loss, astrogliosis, and accumulation of activated microglial cells in affected brain areas (Ironside, 1998). Microglia, the resident macrophages in the central nervous system, are exquisitely sensitive to pathological tissue alterations, undergoing morphological and phenotypic changes to adopt a so-called activated state and perform immunological functions

in response to pathophysiological brain insults (Ransohoff *et al.*, 2009; Perry *et al.*, 2010). Many studies have demonstrated that the microglia have very diverse effector functions, in line with macrophage populations in other organs (Graeber, 2010). Mounting evidence also indicates that microglial activation contributes to neuronal damage in several neurodegenerative diseases including Alzheimer's disease, prion diseases, Parkinson's disease, multiple sclerosis, and Huntington's disease (Perry *et al.*, 2009; Gonzalez *et al.*, 1999). In prion diseases and other neurodegenerative disorders, microglia can become overactivated and generate reactive oxygen species (ROS), nitric oxide ($NO^{\cdot}$), and cytokines, which can cause vascular damage in addition to neurodegeneration (Block *et al.*, 2007; Garção, *et al.*, 2006; Aguzzi *et al.*, 2006). Inflammation in the CNS accelerates the pathology of neurodegenerative disorders. Thus, neuroinflammation is an attractive target for novel therapeutic approaches. In this review, the roles of microglia in the neurodegenerative and in flammatory process mediated by prion infection are discussed.

Microglial inflammation in the central nervous system

Microglia are the resident macrophages in the central nervous system (CNS), in which they are ubiquitously distributed, accounting for approximately 10% of the adult brain cell population and representing the initial and primary immune response (Fig. 3.1A) (Kreutzberg, 1996). Recent studies revealed various functions of microglia and their crucial role in the upkeep of the brain environment. It is reported that microglia play an essential role in neurogenesis and in the extension of neuronal synapses. Microglia remove old synapses and promote neurons in learning-dependent synapse formation through brain-derived neurotrophic factor (BDNF) signalling (Parkhurst *et al.*, 2013).

Activated microglia are able to confer full immune effecter function, allowing them to eradicate the source of brain insult and to restore tissue integrity. This includes neuroprotective functions such as phagocytosis to remove the pathogen and cytotoxic effects via the release of pro-inflammatory mediators and ROS for swift removal of harmful pathogens

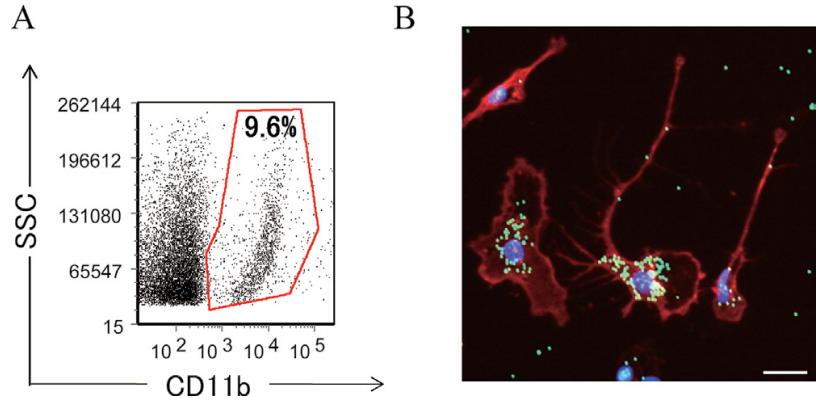

Figure 3.1 Population and phagocytosis of microglia. (A) the rate of microglia (cd11b positive cells) in the brain cells is approximate 10%. (B) microglia showed phagocytic activity and activated microglia released inflammatory cytokines. green shows latex beads, red shows cd11b, and blue shows nuclei. scale bar is 20μm.

(Fig. 3.1B) (van Rossum *et al.*, 2004; Neumann *et al.*, 2006; Imai *et al.*, 2007). Microglia activation occurs following exposure to CNS pathogen and detection of a variety of stimuli such as lipopolysaccharide (LPS), interferon-gamma (IFN-γ), amyloid-beta (Aβ) and other pro-inflammatory cytokines during injury and disease (Dheen *et al.*, 2007). Activated microglia can be identified and distinguished from their resting phenotype based on a combination of morphological and immunophenotypic changes. This includes a change from their typical ramified morphology to a reactive phenotype characterized by hypotrophy of the cell body, shortened and extensively branched processes (an amoeboid morphology), and significant upregulation of cytoplasmic and membrane molecules (Ransohoff *et al.*, 2009).

To initiate innate immune responses, microglia enhance the expressions of toll-like receptors (TLR) (Bsibsi *et al.*, 2002) and multiple pro-inflammatory mediators such as tumour necrosis factor-alpha (TNF-α) (Floden *et al.*, 2005), interleukin (IL)-1 (Hartlage-Rubsamen *et al.*, 1999), and IL-6 (Suzumura *et al.*, 1996). The release of various chemokines including macrophage inflammatory protein-1α (MIP-1α) and MIP-1β (Takami *et al.*, 1997), monocyte chemoattractant protein-1 (MCP-1) (Babcock *et al.*, 2003), and also those involved in lymphocyte recruitment suggest that microglial activation is a process that precedes peripheral immune cell recruitment and that it is the first line of innate immunity in the CNS. Meanwhile, microglial cytotoxic functions are increased due to cytokine stimulation by other immune cells with the release of $NO^{\bullet}$ (Banati *et al.*, 1993), such as superoxide (Chan *et al.*, 2007). It was also reported that microglia isolated from prion infected mice showed increased expression of IL-1β, TNF-α and CFS1, but not IL-6, IL-10 or TGF-β, which correlates with disease progression (Vincenti *et al.*, 2015). In addition, a recent study reported new inflammatory genes upregulated early in the prion brain, including genes involved in inflammation, monocyte recruitment and growth regulation (Carroll *et al.*, 2015).

Early post-mortem and histopathological investigations have reported the presence of large numbers of activated microglia in the CNS of patients with neurodegenerative disease including Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis (ALS), and prion disease (McGeer *et al.*, 1988a,b; Raine, 1994; Banati *et al.*, 1998; Li *et al.*, 1996; Sitte *et al.*, 2001; Sapp *et al.*, 2001), although it remains inconclusive whether they play a role in pathogenesis or simply appear as a consequence of the disease process. The role of microglia as contributors to the progression of neurodegenerative disease was first proposed in Alzheimer's disease (Griffin *et al.*, 1989), where long-term intake of non-steroidal anti-inflammatory drugs (NSAIDs) was associated with a reduced risk of developing the disease (Etminan *et al.*, 2003; Vlad *et al.*, 2008). Although a harmful role for microglia in neuroinflammation is a popular view, there is mounting evidence that points to the contrary, that is, that microglia are in fact neuroprotective in these diseases (Hines *et al.*, 2009; Power *et al.*, 2009).

CXCR3 accelerates prion replication but prolongs survival times after prion infection

It has recently been reported that the chemokine receptor CXCR3 prolongs the survival period in prion infection (Riemer *et al.*, 2008). In neurodegenerative disease, elevated chemokine expression levels have been observed in numerous pathologies of the brain. During prion infection in the CNS, the chemokines chemokine (C-C motif) ligand 2 (CCL2) (Felton *et al.*, 2005), CCL3 (Lu *et al.*, 2004; Riemer *et al.*, 2004), CCL5 (Lee *et al.*,

2005; Marella *et al.*, 2004), CCL6, CCL9, and CCL12 (Xiang *et al.*, 2004) have been found to be upregulated. Moreover, induction of the chemokines CXCL9 (Schultz *et al.*, 2004), CXCL10, and CXCL13 (Riemer *et al.*, 2000) is seen at the early, asymptomatic stages of scrapie infection and is sustained at high levels throughout the disease process, possibly indicating an involvement in disease progression. In the periphery, these two groups of chemokines are potent chemoattractants for T cells (Klein *et al.*, 1997) and B cells (Lewicki *et al.*, 2003).

The chemokine receptor CXCR3 is widely expressed in brain tissue and has been found on astrocytes (Biber *et al.*, 2002; Flynn *et al.*, 2003), microglia (Priller *et al.*, 2006), neurons (Omari *et al.*, 2005), and oligodendrocytes (Horuk *et al.*, 2001). Established CXCR3 ligands are CXCL9, CXCL10, and CXCL11. Other chemokines, namely, CCL21 and CXCL13, which are regular ligands of receptor CCR7 and CXCR5, respectively, are also thought to recognize CXCR3 (Jenh *et al.*, 2001; Rappert *et al.*, 2002). CXCR3 has been shown *in vitro* and *in vivo* to govern migration but not proliferation of microglia (Flynn *et al.*, 2003; Rappert *et al.*, 2004). In these studies, CXCR3-deficient mice infected with scrapie agents were characterized to determine the consequences of the impairment of microglial migration on disease development. CXCR3-deficient mice showed significantly prolonged survival time of up to 30 days on average. However, they displayed accelerated accumulation of misfolded proteinase K-resistant prion protein (PrP^{Sc}) and 20 times higher infectious prion titres than wild-type mice at the asymptomatic stage of the disease. In CXCR3-deficient animals, microglia activation was found to be reduced and quantitative analysis of gliosis-associated gene expression alterations demonstrated reduction in the number of proinflammatory factors (Riemer *et al.*, 2008). These findings suggested that inflammatory glial responses act in concern with PrP^{Sc} in disease development. CXCR3 is crucial for the recruitment of microglia to the inflammatory site of PrP^{Sc} deposition.

Neurotoxic prion peptide induces IL-1β production in microglia via inflammasome

The PrP fragment 106–126 (PrP106–126) is often used as PrP^{Sc} neurotoxic peptide in the research of prion disease, because PrP106–126 possesses similar physiochemical and pathological properties to PrP^{Sc}. PrP106–126 forms amyloid fibrils with a high β-sheet content, shows partial protease K resistance, and is neurotoxic *in vitro* (Henriques *et al.*, 2008; Forloni *et al.*, 1993; Selvaggini *et al.*, 1993).

Extensive research has indicated that the accumulation of aggregated PrP^{Sc} leads to activation of microglia, which in turn release superoxide, chemotactic factors, pro-inflammatory cytokines, and neurotoxic factors (Giese *et al.*, 1998; Marella *et al.*, 2004; Rock *et al.*, 2004). In addition, several studies have shown that multiple cytokines and chemokines, such as IL-1β, TNF-α, and CCL3, were upregulated in the brain from prion-infected mice (Tribouillard-Tanvier *et al.*, 2009). IL-1β plays a crucial role in the regulation of immune and inflammatory responses. It is produced as the inactive precursor pro-IL-1β in the cytosol, and a variety of stimuli lead to higher expression of pro-IL-1β (Dinarello, 2007). Pro-IL-1β is cleaved by caspase-1 into IL-1β, the active mature form. It has recently been reported that neurotoxic prions active mouse microglia and lead to IL-1β production (Peyrin *et al.*, 1999; Yang *et al.*, 2008; Garcao *et al.*, 2006; Crozet *et al.*, 2008). Shi *et al.*, reported that PrP106–126 leads to the formation of NALP3 inflammasome in activated microglia (Shi *et al.*, 2012).

Inflammasome is a cytosolic protein complex that serves as a platform for activating the pro-inflammatory cytokines IL-1β and IL-18 via caspase-1 cleavage (Lamkanfi *et al.*, 2009). The inflammasome plays an important role in innate immunity and is involved in inflammatory disorders. NALP3, one of the most widely researched inflammasomes, consists of NACHT, LRR, and PYD domain-containing proteins, and is a well-known member of the NOD-like receptor family (Mariathasan *et al.*, 2007; Tschopp *et al.*, 2003). In this research, primary microglia cells from neonatal mice were primed with LPS and treated with PrP106–126. PrP106–126 activates caspase-1 and induces IL-1β release in LPS-primed microglia. The expression of NALP3 and ASC was also upregulated by PrP106–126 stimulation, indicating that neurotoxic prion peptide induces IL-1β via NALP3 inflammasome systems. It appears that inflammasome is a crucial mediator of severe inflammation and neuronal damage induced by microglia infected with prion.

It has recently been reported that CD36 plays an important role in microglial activation and IL-1β production triggered by PrP106–126 stimulation (Kouadir *et al.*, 2012). Blocking CD36 receptor reduces the microglial activation; i.e. the enhanced production of IL-1β, TNF-α, and IL-6 associated with PrP106–126 treatment. The relationship of CD36 and inflammasome has not yet been elucidated; however, CD36 may be an important player in the inflammation caused by prion disease.

It has also been reported that NALP3 inflammasome is involved in the innate immune response to Aβ (Halle *et al.*, 2008). It is widely accepted that the extracellular accumulation of Aβ in senile plaques is a principal event in the pathogenesis of Alzheimer's disease (Weiner *et al.*, 2006; Meyer-Luehmann *et al.*, 2008). Microglia and invading bone marrow-derived mononuclear phagocytes are central to the initiation and progression of this disease. Microglia are activated by and recruited to senile plaques, whereupon they phagocytose Aβ and secrete cytokines after activation (Simard *et al.*, 2006). Moreover, systemic inhibition of inflammation or immunization against Aβ decreases plaque burden and delays disease onset (Weggen *et al.*, 2001; Schenk *et al.*, 1999). One prominent cytokine consistently found in diseased tissues at early stages is IL-1β, which has been detected in microglia cells surrounding Aβ plaques in patients with Alzheimer's disease and in animal models of this disease. Similarly to PrP106–126, IL-1β is released from activated microglia in Alzheimer's disease via NALP3 inflammasome. Amyloid protein including PrP^{Sc} and Aβ may activate the inflammasome via a common mechanism. However, it was also reported that the absence of NLRP3 did not significantly change levels of IL-1β in the brain at the terminal stage of the prion disease (Nuvolone *et al.*, 2015). Further studies are required to fully elucidate the detailed mechanism.

Antioxidant cellular prion protein might contribute to control inflammasome in microglia

Recently, it is reported ROS are essential secondary messengers that trigger NLRP3/NALP3 inflammasome activation (Dostert *et al.*, 2008; Zhou *et al.*, 2010). ROS production by H_2O_2, such as superoxide and hydroxyl radicals, activates the inflammasome, while knockdown of TRX, a cellular antioxidant protein, enhances IL-1β activation by silica, uric acid crystals, and asbestos (Dostert *et al.*, 2009). These findings suggest that oxidative stress could be sufficient to trigger NLRP3/NALP3 activation and the mechanism how NLRP3/NALP3 senses ROS. ATP-mediated ROS production has been shown to stimulate the PI3K

pathway, and pharmacological inhibition of PI3K inhibits ATP-mediated caspase-1 activation, suggesting that PI3K may be involved in inflammasome activation downstream of ROS (Cruz *et al.*, 2007). It has also been reported that the non-steroidal anti-inflammatory drug aspirin inhibits the cytotoxicity of prion peptide PrP106–126 to neuronal cells associated with microglia activation *in vitro* (Yang *et al.*, 2008). ROS are generated mainly via the NADPH oxidase pathway and mitochondria. It has recently been reported that mitochondria control the activation of NLRP3 inflammasome and that the inflammasome activation is negatively regulated by autophagy and positively regulated by ROS. In that study, mitophagy/autophagy blockade led to accumulation of damaged, ROS-generating mitochondria, which in turn activated the NLRP3 inflammasome (Zhou *et al.*, 2010). The putative pathway involved in activation of inflammasome is depicted in Fig. 3.2. It was also reported that NADPH oxidase NOX2 is involved in prion pathogenesis (Sorce *et al.*, 2014). NOX2 is strongly expressed on the microglia of CJD patients and, significantly, NOX2 is expressed around neuronal vacuoles. In this report, mice lacking NOX2 decreased the locomotors deficiency and survived longer after inoculation with PrP^{Sc} than those with NOX2.

Oxidation has been shown to be increased by prion infection. In brains from patients with Creutzfeldt–Jakob disease and from Syrian hamsters affected by scrapie, the amounts of glutamic and aminoadipic semialdehydes (products of metal-catalysed oxidation), malondialdehydelysine (a product of lipoxidation), N-epsilon-carboxyethyllysine (a product of glycoxidation), and N-epsilon-carboxymethyllysine (generated by lipoxidation and glycoxidation) were increased. The conversion of PrP^{C} into PrP^{Sc} was accompanied by alterations in fatty acid composition and increased phosphorylation of ERK(1/2) and p38, protein kinases known to respond to increased levels of ROS (Pamplona *et al.*, 2008).

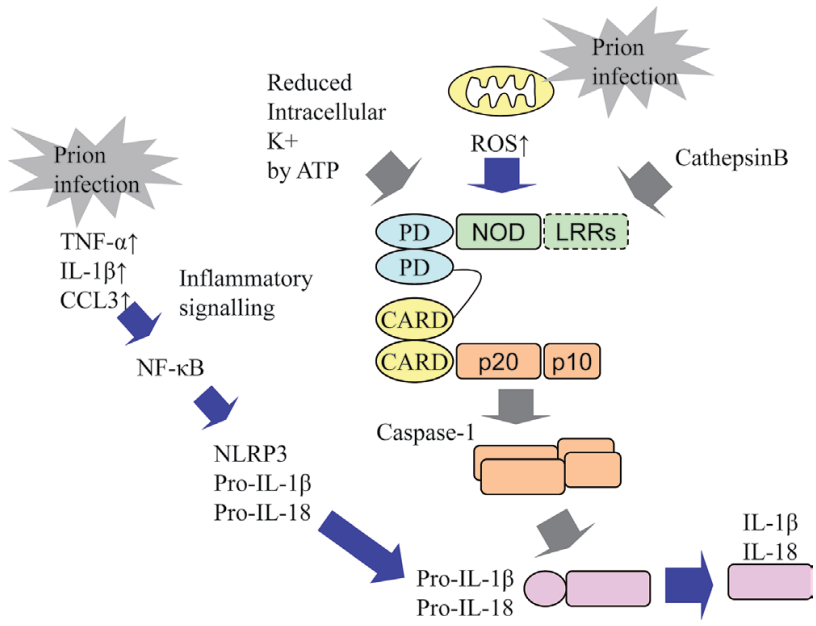

Figure 3.2 The schematic pathway of nlrp3 inflammasome activation. IL-1β is produced via nlrp3 inflammasome activation. nlrp activation is associated with ros production in mitochondria.

In the CNS, PrP^C a cell surface glycoprotein, is expressed mainly in neurons and also in glial cells, and it is an important factor in neurodegenerative prion diseases including bovine spongiform encephalopathy BSE, scrapie, and CJD. Kuwahara *et al.*, reported that PrP^C-deficient neuronal cells die via apoptosis in serum-free medium, which indicates that PrP^C protects against oxidative stress under conditions of serum deprivation (Kuwahara *et al.*, 1999). Sakudo *et al.*, revealed that PrP^C has superoxide dismutase activity and protects neurons from oxidative stress (Sakudo *et al.*, 2005). Antioxidant PrP^C may contribute to suppress inflammasome activation. The relationship between PrP^C and inflammasome remains to be fully elucidated.

Conclusion

In this review, the inflammatory reactions of microglia were examined in detail. Inflammatory responses have an important effect on progression of neurodegenerative disorders, but many aspects of the phenomena are not clear. The relationship between neurodegenerative disorders and microglial inflammasome has been elucidated in recent reports. These studies suggested that reactive astrocytes regulated by microglia are closely involved in the brain environment (Liddelow *et al.*, 2017). Further research will elucidate the complicated neuroinflammatory mechanism interacting with microglia, astrocytes and neurons in these neurodegenerative disorders. The regulation of chronic inflammation in the brain is beneficial for to the treatment of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, and prion disease.

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