

Chapter 3 from:

Prions

Current Progress in Advanced Research

Edited by Akikazu Sakudo

ISBN: 978-1-910190-95-1 (paperback)

ISBN 978-1-910190-96-8 (ebook)

Effect of Microglial Inflammation in Prion Disease

3

Yasuhisa Ano^{1*}, Akikazu Sakudo^{2,3} and Takashi Onodera⁴

¹Research Laboratories for Health Science and Food Technologies, Kirin Company Ltd., Yokohama, Japan.

²Laboratory of Biometabolic Chemistry, School of Health Sciences, University of the Ryukyus, Nishihara, Japan.

³Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan.

⁴Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan.

*Correspondence: Yasuhisa_Ano@kirin.co.jp

<https://doi.org/10.21775/9781910190951.03>

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 chapter.

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), 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 chapter, the roles of microglia in the neurodegenerative and inflammatory 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 effector 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

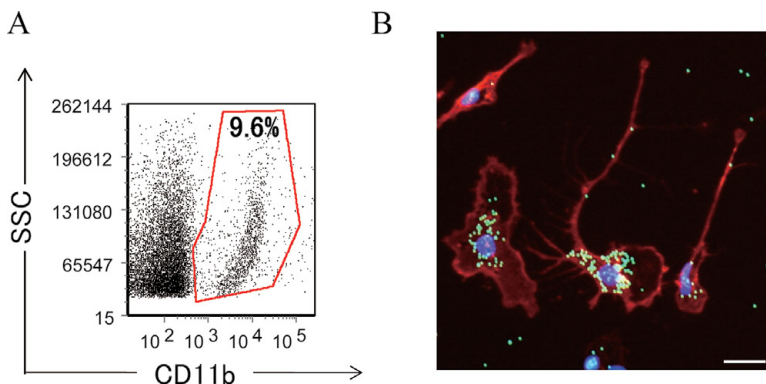


Figure 3.1 Population and phagocytosis of microglia. (A) the rate of microglia (cd11b positive cells) in the brain cells is approximate 10%. (B) microglia showed phagocytic activity and activated microglia released inflammatory cytokines. green shows latex beads, red shows cd11b, and blue shows nuclei.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

(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 (A β) 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-1 α (MIP-1 α) and MIP-1 β (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 \cdot (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-1 β , 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 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-1 β 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-1 β , TNF- α , and CCL3, were upregulated in the brain from prion-infected mice (Tribouillard-Tanvier *et al.*, 2009). IL-1 β plays a crucial role in the regulation of immune and inflammatory responses. It is produced as the inactive precursor pro-IL-1 β in the cytosol, and a variety of stimuli lead to higher expression of pro-IL-1 β (Dinarello, 2007). Pro-IL-1 β is cleaved by caspase-1 into IL-1 β , the active mature form. It has recently been reported that neurotoxic prions activate mouse microglia and lead to IL-1 β 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-1 β 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-1 β 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-1 β 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-1 β production triggered by PrP106–126 stimulation (Kouadir *et al.*, 2012). Blocking CD36 receptor reduces the microglial activation; i.e. the enhanced production of IL-1 β , 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 A β (Halle *et al.*, 2008). It is widely accepted that the extracellular accumulation of A β 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 A β and secrete cytokines after activation (Simard *et al.*, 2006). Moreover, systemic inhibition of inflammation or immunization against A β 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-1 β , which has been detected in microglia cells surrounding A β plaques in patients with Alzheimer's disease and in animal models of this disease. Similarly to PrP106–126, IL-1 β is released from activated microglia in Alzheimer's disease via NALP3 inflammasome. Amyloid protein including PrP^{Sc} and A β 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-1 β 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 H₂O₂, such as superoxide and hydroxyl radicals, activates the inflammasome, while knockdown of TRX, a cellular antioxidant protein, enhances IL-1 β 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 (Sorice *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 amino adipic semialdehydes (products of metal-catalysed oxidation), malondialdehydelysine (a product of lipoxidation), N-epsilon-carboxyethyllysine (a product of glycooxidation), and N-epsilon-carboxymethyllysine (generated by lipoxidation and glycooxidation) 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).

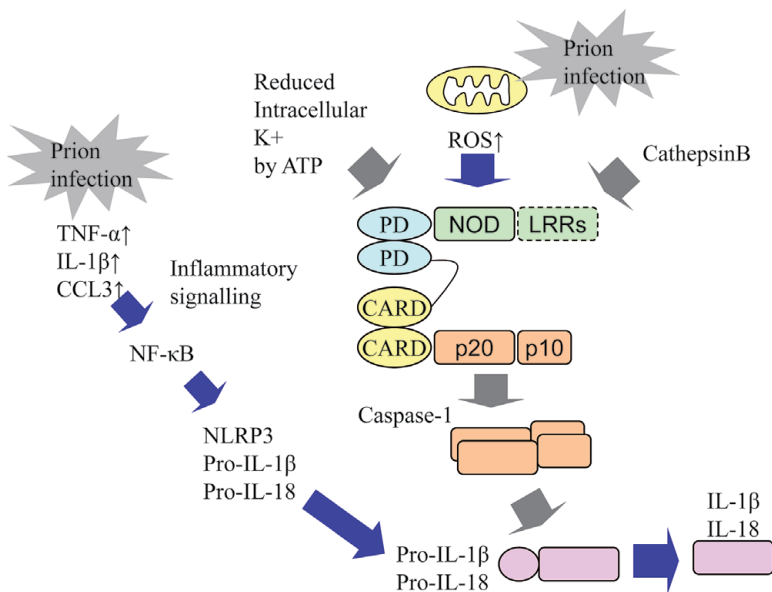


Figure 3.2 The schematic pathway of nlrp3 inflammasome activation. IL-1β is produced via nlrp3 inflammasome activation. nlrp activation is associated with ros production in mitochondria.

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.

References

- Aguzzi, A., and Heikenwalder, M. (2006). Pathogenesis of prion diseases: current status and future outlook. *Nat. Rev. Microbiol.* 4, 765–775.
- Babcock, A.A., Kuziel, W.A., Rivest, S., and Owens, T. (2003). Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J. Neurosci.* 23, 7922–7930.
- Banati, R.B., Daniel, S.E., and Blunt, S.B. (1998). Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. *Mov. Disord.* 13, 221–227. <https://doi.org/10.1002/mds.870130205>
- Banati, R.B., Gehrman, J., Schubert, P., and Kreutzberg, G.W. (1993). Cytotoxicity of microglia. *Glia* 7, 111–118. <https://doi.org/10.1002/glia.440070117>
- Biber, K., Dijkstra, I., Trebst, C., De Groot, C.J., Ransohoff, R.M., and Boddeke, H.W. (2002). Functional expression of CXCR3 in cultured mouse and human astrocytes and microglia. *Neuroscience* 112, 487–497.
- Block, M.L., Zecca, L., and Hong, J.S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69.
- Bsibsi, M., Ravid, R., Gveric, D., and van Noort, J.M. (2002). Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol.* 61, 1013–1021.
- Carroll, J.A., Striebel, J.F., Race, B., Phillips, K., and Chesebro, B. (2015). Prion infection of mouse brain reveals multiple new upregulated genes involved in neuroinflammation or signal transduction. *J. Virol.* 89, 2388–2404.
- Chan, W.Y., Kohsaka, S., and Rezaie, P. (2007). The origin and cell lineage of microglia: new concepts. *Brain Res. Rev.* 53, 344–354.
- Crozet, C., Beranger, F., and Lehmann, S. (2008). Cellular pathogenesis in prion diseases. *Vet. Res.* 39, 44. <https://doi.org/10.1051/vetres:2008021>
- Cruz, C.M., Rinna, A., Forman, H.J., Ventura, A.L., Persechini, P.M., and Ojcius, D.M. (2007). ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. *J. Biol. Chem.* 282, 2871–2879.

- Dheen, S.T., Kaur, C., and Ling, E.A. (2007). Microglial activation and its implications in the brain diseases. *Curr. Med. Chem.* 14, 1189–1197.
- Dinarello, C.A. (2007). A signal for the caspase-1 inflammasome free of TLR. *Immunity* 26, 383–385.
- Dostert, C., Guarda, G., Romero, J.F., Menu, P., Gross, O., Tardivel, A., Suva, M.L., Stehle, J.C., Kopf, M., Stamenkovic, I., *et al.* (2009). Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLOS ONE* 4, e6510. <https://doi.org/10.1371/journal.pone.0006510>
- Dostert, C., Pétrilli, V., Van Bruggen, R., Steele, C., Mossman, B.T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–7. <https://doi.org/10.1126/science.1156995>
- Etminan, M., Gill, S., and Samii, A. (2003). Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. *BMJ* 327, 128. <https://doi.org/10.1136/bmj.327.7407.128>
- Felton, L.M., Cunningham, C., Rankine, E.L., Waters, S., Boche, D., and Perry, V.H. (2005). MCP-1 and murine prion disease: separation of early behavioural dysfunction from overt clinical disease. *Neurobiol. Dis.* 20, 283–295.
- Floden, A.M., Li, S., and Combs, C.K. (2005). Beta-amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor alpha and NMDA receptors. *J. Neurosci.* 25, 2566–2575.
- Flynn, G., Maru, S., Loughlin, J., Romero, I.A., and Male, D. (2003). Regulation of chemokine receptor expression in human microglia and astrocytes. *J. Neuroimmunol.* 136, 84–93.
- Forloni, G., Angeretti, N., Chiesa, R., Monzani, E., Salmona, M., Bugiani, O., and Tagliavini, F. (1993). Neurotoxicity of a prion protein fragment. *Nature* 362, 543–546. <https://doi.org/10.1038/362543a0>
- Garção, P., Oliveira, C.R., and Agostinho, P. (2006). Comparative study of microglia activation induced by amyloid-beta and prion peptides: role in neurodegeneration. *J. Neurosci. Res.* 84, 182–193. <https://doi.org/10.1002/jnr.20870>
- Giese, A., Brown, D.R., Groschup, M.H., Feldmann, C., Haist, I., and Kretschmar, H.A. (1998). Role of microglia in neuronal cell death in prion disease. *Brain Pathol.* 8, 449–457.
- González-Scarano, F., and Baltuch, G. (1999). Microglia as mediators of inflammatory and degenerative diseases. *Annu. Rev. Neurosci.* 22, 219–240. <https://doi.org/10.1146/annurev.neuro.22.1.219>
- Graeber, M.B. (2010). Changing face of microglia. *Science* 330, 783–8. <https://doi.org/10.1126/science.1190929>
- Griiffin, W.S., Stanley, L.C., Ling, C., White, L., MacLeod, V., Perrot, L.J., White, C.L., and Araoz, C. (1989). Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* 86, 7611–7615.
- Halle, A., Hornung, V., Petzold, G.C., Stewart, C.R., Monks, B.G., Reinheckel, T., Fitzgerald, K.A., Latz, E., Moore, K.J., and Golenbock, D.T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat. Immunol.* 9, 857–865. <https://doi.org/10.1038/ni.1636>
- Hartlage-Rubsamen, M., Lemke, R., and Schilieb, R. (1999). Interleukin-1 beta, inducible nitric oxide synthase, and nuclear factor-kappaB are induced in morphologically distinct microglia after rat hippocampal lipopolysaccharide/interferon-gamma injection. *J. Neurosci. Res.* 57, 388–398.
- Henriques, S.T., Pattenden, L.K., Aguilar, M.I., and Castanho, M.A.R.B. (2008). PrP (106–126) does not interact with membranes under physiological conditions. *Biophys. J.* 95, 1877–1889.
- Hines, D.J., Hines, R.M., Mulligan, S.J., and Macvicar, B.A. (2009). Microglia processes block the spread of damage in the brain and require functional chloride channels. *Glia* 57, 1610–1618. <https://doi.org/10.1002/glia.20874>
- Horuk, R. (2001). Chemokine receptors. *Cytokine Growth Factor Rev.* 12, 313–335.
- Imai, F., Suzuki, H., Oda, J., Ninomiya, T., Ono, K., Sano, H., and Sawada, M. (2007). Neuroprotective effect of exogenous microglia in global brain ischemia. *J. Cereb. Blood Flow Metab.* 27, 488–500.
- Ironside, J.W. (1998). Prion diseases in man. *J. Pathol.* 186, 227–234.
- Jenh, C.H., Cox, M.A., Hipkin, W., Lu, T., Pugliese-Sivo, C., Gonsiorek, W., Chou, C.C., Narula, S.K., and Zavodny, P.J. (2001). Human B cell-attracting chemokine 1 (BCA-1; CXCL13) is an agonist for the human CXCR3 receptor. *Cytokine* 15, 113–121. <https://doi.org/10.1006/cyto.2001.0923>
- Klein, M.A., Frigg, R., Flechsig, E., Raeber, A.J., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R.M., and Aguzzi, A. (1997). A crucial role for B cells in neuroinvasive scrapie. *Nature* 390, 687–690. <https://doi.org/10.1038/37789>
- Kouadir, M., Yang, L., Tan, R., Shi, F., Lu, Y., Zhang, S., Yin, X., Zhou, X., and Zhao, D. (2012). CD36 participates in PrP(106-126)-induced activation of microglia. *PLOS ONE* 7, e30756. <https://doi.org/10.1371/journal.pone.0030756>

- Kreutzberg, G.W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312–318.
- Kuwahara, C., Takeuchi, A.M., Nishimura, T., Haraguchi, K., Kubosaki, A., Matsumoto, Y., Saeki, K., Matsumoto, Y., Yokoyama, T., Itoharu, S., *et al.* (1999). Prions prevent neuronal cell-line death. *Nature* 400, 225–226. <https://doi.org/10.1038/22241>
- Lamkanfi, M., and Dixit, V.M. (2009). The inflammasomes. *PLOS Pathog.* 5, e1000510.
- Lee, H.P., Jun, Y.C., Choi, J.K., Kim, J.I., Carp, R.I., and Kim, Y.S. (2005). The expression of RANTES and chemokine receptors in the brains of scrapie-infected mice. *J. Neuroimmunol.* 158, 26–33.
- Lewicki, H., Tishon, A., Homann, D., Mazarguil, H., Laval, F., Asensio, V.C., Campbell, I.L., DeArmond, S., Coon, B., Teng, C., *et al.* (2003). T cells infiltrate the brain in murine and human transmissible spongiform encephalopathies. *J. Virol.* 77, 3799–3808.
- Li, H., and Cuzner, M.L. (1996). Newcombe, J. Microglial-derived macrophages in early multiple sclerosis plaques. *Neuropathol. Appl. Neurobiol.* 22, 207–215.
- Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.S., Peterson, T.C., *et al.* (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481–487. <https://doi.org/10.1038/nature21029>
- Lu, Z.Y., Baker, C.A., and Manuelidis, L. (2004). New molecular markers of early and progressive CJD brain infection. *J. Cell. Biochem.* 93, 644–652. <https://doi.org/10.1002/jcb.20220>
- Marella, M., and Chabry, J. (2004). Neurons and astrocytes respond to prion infection by inducing microglia recruitment. *J. Neurosci.* 24, 620–627. <https://doi.org/10.1523/JNEUROSCI.4303-03.2004>
- Mariathasan, S., and Monack, D.M. (2007). Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat. Rev. Immunol.* 7, 31–40.
- McGeer, P.L., Itagaki, S., Boyes, B.E., and McGeer, E.G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* 38, 1285–1291.
- McGeer, P.L., Itagaki, S., and McGeer, E.G. (1988). Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* 76, 550–557.
- Meyer-Luehmann, M., Spire-Jones, T.L., Prada, C., Garcia-Alloza, M., de Calignon, A., Rozkalne, A., Koenigsnecht-Talboo, J., Holtzman, D.M., Bacskai, B.J., and Hyman, B.T. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature* 451, 720–724. <https://doi.org/10.1038/nature06616>
- Neumann, J., Gunzer, M., Gutzeit, H.O., Ullrich, O., Reymann, K.G., and Dinkel, K. (2006). Microglia provide neuroprotection after ischemia. *FASEB J.* 20, 714–716.
- Nuvolone, M., Sorce, S., Schwarz, P., and Aguzzi, A. (2015). Prion pathogenesis in the absence of NLRP3/ASC inflammasomes. *PLOS ONE* 10, e0117208. <https://doi.org/10.1371/journal.pone.0117208>
- Omari, K.M., John, G.R., Sealfon, S.C., and Raine, C.S. (2005). CXC chemokine receptors on human oligodendrocytes: implications for multiple sclerosis. *Brain* 128, 1003–1015.
- Pamplona, R., Naudí, A., Gavín, R., Pastrana, M.A., Sajani, G., Ilieva, E.V., Del Río, J.A., Portero-Otín, M., Ferrer, I., and Requena, J.R. (2008). Increased oxidation, glycooxidation, and lipoxidation of brain proteins in prion disease. *Free Radic. Biol. Med.* 45, 1159–1166. <https://doi.org/10.1016/j.freeradbiomed.2008.07.009>
- Parkhurst, C.N., Yang, G., Ninan, I., Savas, J.N., Yates, J.R., Lafaille, J.J., Hempstead, B.L., Littman, D.R., and Gan, W.B. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* 155, 1596–1609. <https://doi.org/10.1016/j.cell.2013.11.030>
- Perry, V.H., Nicoll, J.A., and Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat. Rev. Neurol.* 6, 193–201. <https://doi.org/10.1038/nrneuro.2010.17>
- Peyrin, J.M., Lasmézas, C.L., Haik, S., Tagliavini, F., Salmona, M., Williams, A., Richie, D., Deslys, J.P., and Dormont, D. (1999). Microglial cells respond to amyloidogenic PrP peptide by the production of inflammatory cytokines. *Neuroreport* 10, 723–729.
- Power, J.H., and Blumbergs, P.C. (2009). Cellular glutathione peroxidase in human brain: cellular distribution, and its potential role in the degradation of Lewy bodies in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol.* 117, 63–73. <https://doi.org/10.1007/s00401-008-0438-3>
- Priller, J., Prinz, M., Heikenwalder, M., Zeller, N., Schwarz, P., Heppner, F.L., and Aguzzi, A. (2006). Early and rapid engraftment of bone marrow-derived microglia in scrapie. *J. Neurosci.* 26, 11753–11762.
- Prusiner, S.B. (1998). Prions. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13363–13383.
- Raine, C.S. (1994). Multiple sclerosis: immune system molecule expression in the central nervous system. *J. Neuropathol. Exp. Neurol.* 53, 328–337.
- Ransohoff, R.M., and Perry, V.H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145. <https://doi.org/10.1146/annurev.immunol.021908.132528>

- Rappert, A., Bechmann, I., Pivneva, T., Mahlo, J., Biber, K., Nolte, C., Kovac, A.D., Gerard, C., Boddeke, H.W., Nitsch, R., *et al.* (2004). CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion. *J. Neurosci.* *24*, 8500–8509. <https://doi.org/10.1523/JNEUROSCI.2451-04.2004>
- Rappert, A., Biber, K., Nolte, C., Lipp, M., Schubel, A., Lu, B., Gerard, N.P., Gerard, C., Boddeke, H.W., and Kettenmann, H. (2002). Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl⁻ current and chemotaxis in murine microglia. *J. Immunol.* *168*, 3221–3226.
- Riemer, C., Neidhold, S., Burwinkel, M., Schwarz, A., Schultz, J., Krätzschmar, J., Mönning, U., and Baier, M. (2004). Gene expression profiling of scrapie-infected brain tissue. *Biochem. Biophys. Res. Commun.* *323*, 556–564. <https://doi.org/10.1016/j.bbrc.2004.08.124>
- Riemer, C., Queck, I., Simon, D., Kurth, R., and Baier, M. (2000). Identification of upregulated genes in scrapie-infected brain tissue. *J. Virol.* *74*, 10245–10248.
- Riemer, C., Schultz, J., Burwinkel, M., Schwarz, A., Mok, S.W., Gültner, S., Bamme, T., Norley, S., van Landeghem, F., Lu, B., *et al.* (2008). Accelerated prion replication in, but prolonged survival times of, prion-infected CXCR3^{-/-} mice. *J. Virol.* *82*, 12464–12471. <https://doi.org/10.1128/JVI.01371-08>
- Rock, R.B., Gekker, G., Hu, S., Sheng, W.S., Cheeran, M., Lokensgard, J.R., and Peterson, P.K. (2004). Role of microglia in central nervous system infections. *Clin. Microbiol. Rev.* *17*, 942–964, table of contents.
- Sakudo, A., Lee, D.C., Nishimura, T., Li, S., Tsuji, S., Nakamura, T., Matsumoto, Y., Saeki, K., Itoharu, S., Ikuta, K., *et al.* (2005). Octapeptide repeat region and N-terminal half of hydrophobic region of prion protein (PrP) mediate PrP-dependent activation of superoxide dismutase. *Biochem. Biophys. Res. Commun.* *326*, 600–606.
- Sapp, E., Kegel, K.B., Aronin, N., Hashikawa, T., Uchiyama, Y., Tohyama, K., Bhide, P.G., Vonsattel, J.P., and DiFiglia, M. (2001). Early and progressive accumulation of reactive microglia in the Huntington disease brain. *J. Neuropathol. Exp. Neurol.* *60*, 161–172.
- Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., *et al.* (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* *400*, 173–177. <https://doi.org/10.1038/22124>
- Schultz, J., Schwarz, A., Neidhold, S., Burwinkel, M., Riemer, C., Simon, D., Kopf, M., Otto, M., and Baier, M. (2004). Role of interleukin-1 in prion disease-associated astrocyte activation. *Am. J. Pathol.* *165*, 671–678.
- Selvaggini, C., De Gioia, L., Cantù, L., Ghibaudi, E., Diomedea, L., Passerini, F., Forloni, G., Bugiani, O., Tagliavini, F., and Salmons, M. (1993). Molecular characteristics of a protease-resistant, amyloidogenic and neurotoxic peptide homologous to residues 106-126 of the prion protein. *Biochem. Biophys. Res. Commun.* *194*, 1380–1386.
- Shi, F., Yang, L., Kouadir, M., Yang, Y., Wang, J., Zhou, X., Yin, X., and Zhao, D. (2012). The NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation. *J. Neuroinflammation* *9*, 73. <https://doi.org/10.1186/1742-2094-9-73>
- Simard, A.R., Soulet, D., Gowing, G., Julien, J.P., and Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* *49*, 489–502.
- Sitte, H.H., Wanschitz, J., Budka, H., and Berger, M.L. (2001). Autoradiography with [³H]PK11195 of spinal tract degeneration in amyotrophic lateral sclerosis. *Acta Neuropathol.* *101*, 75–78.
- Sorce, S., Nuvolone, M., Keller, A., Falsig, J., 7Varol, A., Schwarz, P., Bieri, M., Budka, H., and Aguzzi, A. (2014). The role of the NADPH oxidase NOX2 in prion pathogenesis. *PLOS Pathog.* *10*, e1004531. <https://doi.org/10.1371/journal.ppat.1004531>
- Suzumura, A., Sawada, M., and Marunouchi, T. (1996). Selective induction of interleukin-6 in mouse microglia by granulocyte-macrophage colony-stimulating factor. *Brain Res.* *713*, 192–198.
- Takami, S., Nishikawa, H., Minami, M., Nishiyori, A., Sato, M., Akaike, A., and Satoh, M. (1997). Induction of macrophage inflammatory protein MIP-1 alpha mRNA on glial cells after focal cerebral ischemia in the rat. *Neurosci. Lett.* *227*, 173–176.
- Tribouillard-Tanvier, D., Striebel, J.F., Peterson, K.E., and Chesebro, B. (2009). Analysis of protein levels of 24 cytokines in scrapie agent-infected brain and glial cell cultures from mice differing in prion protein expression levels. *J. Virol.* *83*, 11244–11253. <https://doi.org/10.1128/JVI.01413-09>
- Tschopp, J., Martinon, F., and Burns, K. (2003). NALPs: a novel protein family involved in inflammation. *Nat. Rev. Mol. Cell Biol.* *4*, 95–104. <https://doi.org/10.1038/nrm1019>
- van Rossum, D., and Hanisch, U.K. (2004). Microglia. *Metab. Brain. Dis.* *19*, 393–411.
- Vincenti, J.E., Murphy, L., Grabert, K., McColl, B.W., Cancellotti, E., Freeman, T.C., and Manson, J.C. (2015). Defining the microglia response during the time course of chronic neurodegeneration. *J. Virol.* *90*, 3003–17. <https://doi.org/10.1128/JVI.02613-15>

- Vlad, S.C., Miller, D.R., Kowall, N.W., and Felson, D.T. (2008). Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology* 70, 1672–1677. <https://doi.org/10.1212/01.wnl.0000311269.57716.63>
- Wechselberger, C., Wurm, S., Pfarr, W., and Höglinger, O. (2002). The physiological functions of prion protein. *Exp. Cell Res.* 281, 1–8.
- Weggen, S., Eriksen, J.L., Das, P., Sagi, S.A., Wang, R., Pietrzik, C.U., Findlay, K.A., Smith, T.E., Murphy, M.P., Bulter, T., *et al.* (2001). A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 414, 212–216. <https://doi.org/10.1038/35102591>
- Weiner, H.L., and Frenkel, D. (2006). Immunology and immunotherapy of Alzheimer's disease. *Nat. Rev. Immunol.* 6, 404–416.
- Xiang, W., Windl, O., Wunsch, G., Dugas, M., Kohlmann, A., Dierkes, N., Westner, I.M., and Kretzschmar, H.A. (2004). Identification of differentially expressed genes in scrapie-infected mouse brains by using global gene expression technology. *J. Virol.* 78, 11051–11060. <https://doi.org/10.1128/JVI.78.20.11051-11060.2004>
- Yang, L., Zhou, X., Yang, J., Yin, X., Han, L., and Zhao, D. (2008). Aspirin inhibits cytotoxicity of prion peptide PrP106-126 to neuronal cells associated with microglia activation in vitro. *J. Neuroimmunol.* 199, 10–7. <https://doi.org/10.1016/j.jneuroim.2008.04.028>
- Zhou, R., Tardivel, A., Thorens, B., Choi, I., and Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 11, 136–140. <https://doi.org/10.1038/ni.1831>
- Zhou, R., Yazdi, A.S., Menu, P., and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221–225. <https://doi.org/10.1038/nature09663>