Chapter 5
Roles in Immune Responses

Richa Hanamsagar, Sandra M. Cardona, Tammy Kielian, and Astrid E. Cardona

Abstract Microglia are best known as the mononuclear phagocytes of the central nervous system (CNS) parenchyma. As a resident glial cell population, microglia play key roles during the initiation, propagation, and/or resolution of inflammation. Recently, the discovery that microglial cells continuously survey their local CNS environment in vivo improved our understanding of their immune-surveillance properties in health and disease. Microglial interactions with other elements of the immune system and resident cells of the CNS define a fine balance between neuroprotection and irreparable tissue damage. In this chapter we highlight the innate immune properties of microglia, with a focus on events that initiate an inflammatory response within the brain proper including, Toll-like receptors, inflammasomes, cytokines, and chemokines, and their relationship to immune-mediated disease exacerbation or resolution.

Keywords Microglia • Phagocytosis • Toll-like receptor • Nod-like receptor • Inflammasome • Chemokine • Scavenging • Antigen presentation • Blood–brain barrier

R. Hanamsagar, B. Pharm., Ph.D.
Department of Psychology and Neuroscience, Duke University,
210 Research Dr, Room 3019, Durham, NC 27710, USA
e-mail: rmh45@duke.edu

S.M. Cardona, Ph.D.
Department of Biology, University of Texas at San Antonio,
West Campus/Tobin lab MBT 1.212, San Antonio, TX 78249, USA
e-mail: sandra.cardona@utsa.edu

T. Kielian, Ph.D. (✉)
Department of Pathology and Microbiology, University of Nebraska Medical Center,
985900 Nebraska Medical Center, Room 7029 DRCII, Omaha, NE 68198-5900, USA
e-mail: tkielian@unmc.edu

A.E. Cardona, Ph.D. (✉)
Department of Biology and South Texas Center for Emerging Infectious Diseases,
University of Texas at San Antonio, West Campus/Tobin lab MBT 1.216,
San Antonio, TX 78249, USA
e-mail: astrid.cardona@utsa.edu

M.-È. Tremblay, A. Sierra (eds.), *Microglia in Health and Disease*,
DOI 10.1007/978-1-4939-1429-6_5

Bullet Points

- Immune surveillance, antigen recognition, and phagocytosis are key functions of microglia during the initiation, propagation, and/or resolution of inflammation.
- Innate immune activation of microglia can be triggered by numerous pathways including pattern recognition receptors and chemokine receptors.
- Microglial cells participate actively during innate immune responses, but also activate pathways that contribute to adaptive immunity.
- The physiological importance of microglia in inflammasome activation during central nervous system (CNS) infection and neurodegeneration highlights potential venues for CNS pathogen clearance.
- Functions of microglia do not occur in isolation but are influenced by neighboring cells including astrocytes and neurons, and the blood–brain barrier.

5.1 Regulation of CNS Innate Immune Reactions

Microglia are the resident mononuclear phagocytes of the CNS, comprising approximately 10–15 % of the total cell population within the parenchyma. These cells share many phenotypic and functional characteristics with macrophages and, as such, are major players in the brain's innate immune responses. Developmental studies in mice have shown that microglia are derived from primitive yolk sac myeloid progenitors seeding the brain parenchyma between embryonic days 9 and 10 (Alliot et al. 1999; Ginhoux et al. 2010), indicating that microglia constitute a population of cells ontogenically distinct from tissue macrophages, which originate from bone marrow-derived monocytes (Fogg et al. 2006; Parwaresch and Wacker 1984).

5.1.1 Immune Surveillance

Historically, the CNS was considered an immune-privileged site based on its lack of lymphatic drainage, low levels of major histocompatibility complex (MHC) expression, which is essential for antigen presentation (see below), and restricted diffusion of molecules and cells from the periphery by the blood–brain barrier (BBB). However, the healthy CNS is now recognized as a site where immune surveillance occurs (Greenwood et al. 2011). Microglia share several markers with bone marrow-derived macrophages (Prinz et al. 2011), including the fractalkine receptor CX3CR1 and the lectin Siglec-H (Gautier et al. 2012; Harrison et al. 1998). It has been proposed that the expression of CX3CR1 and its ligand CX3CL1 (fractalkine or FKN) on microglia and neurons, respectively, assists in chemotactic migration of microglia to neurons during neuronal injury or inflammatory conditions (Chapman

et al. 2000; Harrison et al. 1998; Mizutani et al. 2012; Streit et al. 2005) (see Chap. 9 for further reading on their roles in the healthy brain). This highlights the use of traditional immune chemotactic signals to facilitate specific interactions between CNS cellular constituents.

Immune surveillance within the CNS occurs during normal physiological conditions and microglia play a critical role in this process. Although microglia were previously considered to exist in a "resting" state characterized by a ramified morphology, elegant studies have demonstrated that in the intact adult brain, microglia are highly active and continuously surveying their microenvironment by extending and retracting their motile processes (Davalos et al. 2005; Nimmerjahn et al. 2005) (see Chaps. 2 and 4 for further reading). This attribute may be essential to elicit immediate responses to neuronal injury or invading pathogens.

Microglia exist in at least two morphologically distinct states based exclusively on their appearance; namely, amoeboid and ramified. The amoeboid state refers to cells with larger cell bodies as well as shorter and thicker processes, a phenotype which has been correlated with microglial "activation" in response to neuronal injury and inflammatory conditions. Conversely, "quiescent" microglia are generally described as ramified, exhibiting longer and thinner processes. Interestingly, microglia possess an amoeboid morphology during early stages of CNS development, transitioning into a ramified form during the late fetal and early neonatal periods (Boya et al. 1979). Ramified microglia transform again into the amoeboid-like morphology after CNS insults, such as injury or inflammation (Streit 2002; Xiang et al. 2006) (Fig. 5.1). However, in some models of inflammation the correlation between microglial morphology and function is not as strong. For instance, low doses of systemic lipopolysaccharide (LPS, a major component of the outer membrane of gram-negative bacteria) can induce the production of pro-inflammatory cytokines without any apparent changes in morphology (Sierra et al. 2007) (Table 5.1). Among other mechanisms, microglial remodeling, swelling, and process extension were shown to be controlled by chloride channels (Ducharme et al. 2007; Zierler et al. 2008) and the phosphorylation/dephosphorylation of cofilin, a member of the actin depolymerizing factor (ADF) family (Hadas et al. 2012), while the inflammatory response is regulated by the transcription factor NFκB (Wilms et al. 2003). Although it remains unclear whether these two pathways interact, disruption of the actin cytoskeleton has been shown to activate NFκB in cultured human intestinal epithelial cells, which supports the notion that cytoskeletal disruption may occur upstream of NFκB (Nemeth et al. 2004).

5.1.2 Antigen Recognition in the CNS

Microglial responses are often measured based on the array of cytokines, chemokines, and complement components produced during specific pathologies (Tables 5.1 and 5.2). Cytokines are small signaling molecules that circulate within and among organs, affecting nearly every biological process by orchestrating the degree of

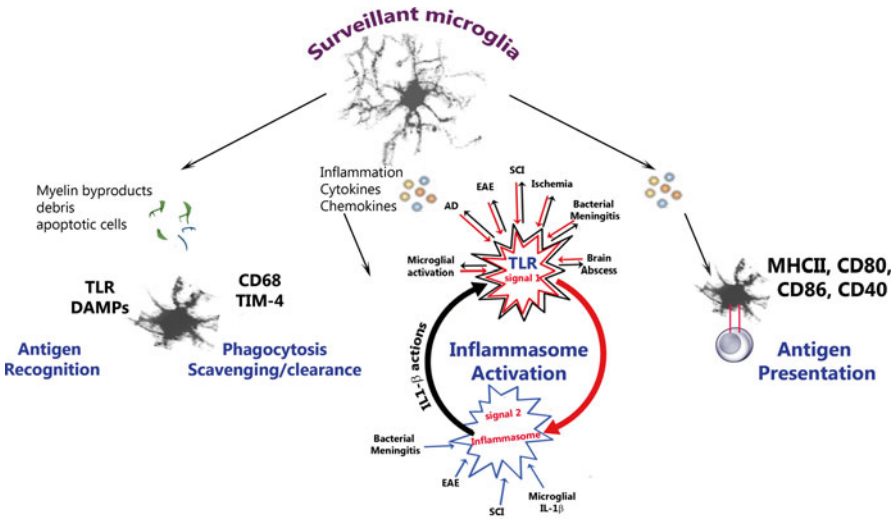

Fig. 5.1 Microglia and innate immunity. Antigen recognition, phagocytosis, inflammatory mediator release, and antigen presentation are among the most recognized functions of microglia in health and disease. Their Toll-like receptors (TLR) and danger-associated molecular patterns (DAMPs) receptors play an important role in recognizing antigens such as myelin byproducts, debris, or amyloid-beta (Aβ), making their engagement critical for sensing cellular damage. Membrane receptors such as CD68 and T cell immunoglobulin mucin-4 (TIM-4) are involved in the processes of phagocytosis and clearance of damaged cells. In general, antigens presented through MHC class II lead to adaptive responses that elicit Th1, Th2, or Th17 responses. In parallel, antigen recognition activates the inflammasome. The pro-inflammatory cytokine interleukin (IL)-1β is implicated in the pathophysiology of numerous neurodegenerative diseases, including multiple sclerosis/experimental autoimmune encephalomyelitis (EAE) and Alzheimer's disease (AD), and a number of central nervous system (CNS) infections ranging from bacterial meningitis, brain abscess, and human immunodeficiency virus (HIV)-associated dementia. The initial response upon TLR activation (signal 1) in certain pathologies with further inflammatory cues leads to IL-1β release and inflammasome activation (signal 2) that in turns activates TLR components creating a positive feedback regulation of the inflammatory cascade. Although the roles of inflammasomes are becoming increasingly well-defined, insights into inflammasome biology in the CNS are only just emerging. Interactions between TLRs and the inflammasome mediate responses to both pathogen-associated and endogenous triggers during CNS pathology

inflammatory reaction. Cytokines are broadly categorized as pro-inflammatory (e.g., interleukin (IL)-1β, IL-6, and tumor necrosis factor α (TNFα)) and anti-inflammatory (e.g., transforming growth factor β (TGFβ) and IL-4), and further stratified based on their capacity to promote (1) cellular immunity (i.e., the activation of phagocytes and antigen-specific T lymphocytes, such as helper (Th)1 cells, which produce mainly interferon γ (IFNγ) and IL-12); (2) antibody-mediated responses (mediated by Th2 cells and associated with cytokines such as IL-4, IL-10, and IL-13); and (3) responses mediated by Th17 cells (distinguished by their release of IL-17, associated with pathogen clearance and tissue inflammation in autoimmune diseases) (Dinarello 2007) (see Chap. 16 for further reading on their roles in multiple sclerosis). Chemotactic cytokines (or chemokines) participate in cellular

Table 5.1 Microglial cytokines[a]

Cytokine	Condition	References
IL-1α	In vitro, viral nucleocapsid, LPS	Lee et al. (1993), Lokensgard et al. (2001)
IL-1β	In vitro, viral nucleocapsid, LPS, Aβ	Lee et al. (1993), Lokensgard et al. (2001), Lue et al. (2001b)
IL-3	In vitro, IFNγ	Hanisch (2002)
IL-6	In vitro, viral nucleocapsid, LPS, Aβ, cytomegalovirus	Lee et al. (1993), Lokensgard et al. (2001), Lue et al. (2001b), Pulliam et al. (1995)
IL-8	In vitro, IFNγ, HIV	Hanisch (2002), Renner et al. (2012)
IL-10	In vitro, IFNγ/LPS,	Hanisch (2002), Ledeboer et al. (2002), Williams et al. (1996)
IL-12	In vitro, IFNγ/LPS, IL-12	Aloisi et al. (1997), Becher et al. (1996), Hanisch (2002), Stalder et al. (1997)
IL-15	In vitro, IFNγ	Hanisch (2002)
IL-16	In vivo, fetal tissues	Schwab et al. (2001)
IL-23	In vitro, IFNγ/LPS	Li et al. (2003)
M-CSF	In vitro, β-amyloid	Lue et al. (2001b)
TGFβ	In vitro, IL-1	da Cunha et al. (1997)
TNFα	In vitro, viral nucleocapsid, LPS, HIV	Koka et al. (1995), Lee et al. (1993), Lokensgard et al. (2001), Pulliam et al. (1995)

[a]Selected reports, influenced by activation stimuli, model, age, and species from which microglial cells were derived

activation by arresting and positioning relevant cells with spatiotemporal precision (Ransohoff 2009). The complement system also constitutes one of the first defense mechanisms by coating the surface of cells and pathogens, thereby assisting in the process of phagocytosis (see Chap. 9 for more information on its involvement with synaptic pruning). Therefore, the coordinated control of soluble mediators by microglial cells is considered a key determinant in the processes of antigen recognition in the CNS (Tables 5.1 and 5.2).

Innate immune activation in the CNS can be triggered by numerous pathways upon recognition of invading pathogens and/or tissue damage by pattern recognition receptors (PRRs). Within the last 10 years, much attention has been focused on Toll-like receptor (TLR) activation in several models of CNS infection, neurodegenerative disease, and injury (Hanamsagar et al. 2012). TLRs are stimulated by pathogen- or danger-associated molecular patterns of pathogen or host origin (PAMPs and DAMPs), respectively, leading to NFκB and MAPK activation, and the subsequent release of pro-inflammatory mediators. A total of 13 TLRs have been identified in human, all of which are expressed at the cell surface except TLRs 3, 7, and 9, which are exclusive to the endosomal compartments.

Microglia express a wide range of TLRs to varying degrees depending on their phenotype (Olson and Miller 2004). In the resting state, many TLRs cannot be detected in microglia; however, TLRs are rapidly induced upon cellular activation (Bsibsi et al. 2002) (Fig. 5.1). In microglia, TLRs are considered a crucial first line

Table 5.2 Microglial chemokines[a]

Cytokine	Condition	References
CCL1	Ex vivo, *S. aureus*	Kielian et al. (2001), Kielian et al. (2002), Kielian (2004)
CCL2	In vitro, viral Tat protein	D'Aversa et al. (2004), Kielian (2004)
CCL3	In vitro, LPS,	Kielian (2004), Kremlev et al. (2004)
CCL4	In vitro, viral Tat protein	D'Aversa et al. (2004), Kielian (2004)
CCL5	In vitro, LPS	Kielian (2004), Kremlev et al. (2004)
CCL7	In vitro, TLR2 signal	Aravalli et al. (2005)
CCL8	In vitro, TLR2 signal	Aravalli et al. (2005)
CCL9	In vitro, TLR2 signal	Aravalli et al. (2005)
CCL11	In vitro, neuropeptide	Wainwright et al. (2008)
CXCL1	In vitro, TLR2 signal	Aravalli et al. (2005)
CXCL2	In vitro, ATP	Kielian (2004), Shiratori et al. (2010)
CXCL4	In vitro, TLR2 signal	Aravalli et al. (2005)
CXCL5	In vitro, TLR2 signal	Aravalli et al. (2005)
CXCL8	IFNγ/sCD40L, viral Tat protein, *S. aureus*	D'Aversa et al. (2004), D'Aversa et al. (2008), Kielian (2004)
CXCL9	In vitro, TLR2 signal, IFNγ	Aravalli et al. (2005), Ellis et al. (2010)
CXCL10	In vitro, LPS, TLR2 signal, IFNγ, viral Tat protein, *S. aureus*	Aravalli et al. (2005), D'Aversa et al. (2004), Ellis et al. (2010), Kielian (2004), Kremlev et al. (2004)
CXCL16	Ex vivo, glioma	Ludwig et al. (2005)

[a]Selected reports, influenced by activation stimuli, model, age, and species from which microglia were derived

of defense against bacteria and viruses, as well as recognition of endogenous danger signals (Kielian et al. 2005; Ravindran et al. 2010; Zhou et al. 2006). Resultant pro-inflammatory mediator and reactive oxygen species (ROS) production triggered by TLR ligands can be either beneficial or detrimental to the host. For example, TLR9 activation can enhance microglial phagocytosis of amyloid-beta (Aβ) as reported in primary neuron-microglia cocultures (Doi et al. 2009), and TLR2 signaling is pivotal for nitric oxide (NO) production and anti-bacterial responses following *Streptococcus* challenge, leading to neuronal cell death (Lehnardt et al. 2006). Conversely, persistent TLR activation may cause exaggerated immune responses, resulting in damage to neurons and oligodendrocytes (Lehnardt et al. 2002; Lehnardt et al. 2003). Microglial activation is evident following a single systemic LPS injection, which also triggers the expression of numerous pro-inflammatory cytokines and chemokines (Chen et al. 2012; Lehnardt et al. 2002; Rivest 2009). Although microglial activation was originally thought to result from the action of systemically induced cytokines elicited by TLR4 engagement, strong evidence suggests that TLR4 expression within the CNS is essential for eliciting central inflammation either via direct activation of resident microglia or indirectly through cerebral vascular endothelial cells (Chen et al. 2012; Lehnardt et al. 2002). In addition,

injection of LPS directly into the brain induces robust and transient expression of numerous inflammatory mediators, including IL-1, TNFα, IL-6, the subunit p40 of IL-12 (IL-12p40), and TGFβ through TLR4 and its adapter, MyD88 (Glezer et al. 2007b). Although astrocytes, endothelial cells, and neurons have been shown to express TLR4 (Bowman et al. 2003; Liu and Kielian 2011; Rolls et al. 2007; Zhou et al. 2006), their role in immunity in response to TLR4 ligands is considered to be limited as compared to microglia (Glezer et al. 2006; Glezer et al. 2007a).

Chronic neurodegeneration also leads to microglial activation (Dheen et al. 2007; Nakajima and Kohsaka 2001) (see Chap. 18 about microglial involvement in neurodegenerative diseases). Aβ, the principal component of Alzheimer's disease (AD)-associated senile plaques, has been shown to induce production of IL-1β, IL-6, TNFα, and IL-18 in white and gray matter microglia cultured from human autopsy tissue (Lue et al. 2001a; Rogers and Lue 2001). IL-1β and IL-18 are produced after a series of signaling events that involve Nod-Like receptors (NLRs), inducing the formation of a multi-cellular complex known as the inflammasome (see below). It was shown that Aβ can activate microglia through the NLRP3 inflammasome pathway (Blasko et al. 2004; Halle et al. 2008) and the subsequent production of IL-1β has been proposed to worsen neurodegeneration (Lindberg et al. 2005). In Parkinson's disease (PD), α-synuclein aggregates are considered central to the pathology, associated with a progressive neuronal loss in the substantia nigra pars compacta (SNpc). In fact, it has been shown that microglial activation is widespread in the SNpc (McGeer et al. 1988), but also in the hippocampus, putamen, and cingulate cortex of PD patient brains (Imamura et al. 2003). A correlation has also been demonstrated between microglial activation and the loss of dopaminergic terminals in the postmortem midbrain of PD patients (Imamura et al. 2003). Indeed, microglia exposed to α-synuclein in vitro express high levels of IL-1β, TNFα, and IL-6 (Su et al. 2008) and a recent study has reported that α-synuclein can also activate the inflammasome (Codolo et al. 2013).

5.1.3 Phagocytosis: Scavenging Functions/Clearance

Phagocytosis is an evolutionarily conserved process serving to remove cellular debris and dying cells or aggregated proteins that can be toxic and trigger exaggerated immune responses. It refers to the recognition, engulfment, and degradation of different types of cargo including dead cells, debris, bacteria, Aβ, dendritic spines, etc. (Sierra et al. 2013). From *Drosophila* (Kurant 2011) to higher eukaryotes, efforts have been directed at understanding the molecular basis underlying the phagocytosis of endogenous debris and pathogens. Understanding antigen uptake and the pathways that lead to phagocytosis and antigen presentation for proper T-cell activation has also been a topic of study for many years. Microglia are considered a first line of defense against invading pathogens and they respond rapidly to inflammatory stimuli. A primary function of microglia is to eliminate harmful exogenous insults as well as endogenous proteins or cellular debris not only during

CNS injury, but also during development and adult neurogenesis (Sierra et al. 2013). There is ample evidence that microglia can phagocytose neurons during neuroinflammatory conditions. Generally, neuronal debris and degenerating axons are readily engulfed by microglia utilizing the scavenging receptor CD68 (Fraser et al. 2010; Tanaka et al. 2009). It is thought that the nucleotide ATP released from dying cells is not only an important trigger for microglial migration to sites of injury, via purinergic receptor and inflammasome activation, but also regulates the phagocytic process itself (Koizumi et al. 2007). Upon axonal degeneration, it was also shown that microglial release of type I interferons (IFNα and IFNβ, which are important for protection against many viral infections) can provide additional inflammatory feedback to maintain phagocytic activity, while creating a more permissive environment for axonal outgrowth in vitro (Hosmane et al. 2012). Interestingly, activated microglia have been shown to phagocytose viable neurons (Fricker et al. 2012), an in vitro observed phenomenon termed "phagoptosis" which is triggered by inflammation and the transient and reversible exposure of membrane phosphatidylserine (PS) on stressed cells. However, the physiological implications of this process remain unclear (Brown and Neher 2012). Other work has shown that microglia play a neuroprotective role during CNS injury by their rapid engulfment of apoptotic polymorphonuclear neutrophils (PMN) (Neumann et al. 2008), in addition to inducing T-cell apoptosis (Magnus et al. 2001)—the latter representing a potential mechanism to subvert autoimmunity. Besides the role of microglia in the phagocytosis of bacterial and viral particles during CNS infections, several studies have highlighted the importance of microglial phagocytosis in response to Aβ deposition in the brain. For example, microglia have been shown to clear Aβ plaques through Fc receptor-mediated phagocytosis and peptide degradation (Bard et al. 2000), although they do not seem to reduce overall the Aβ load in the AD brain. Other surface receptors implicated in fibrillar Aβ engulfment include the scavenger receptor CD36, $\alpha_6\beta_1$ integrin, and the integrin and thrombospondin-1 receptor CD47 (Koenigsknecht and Landreth 2004).

Another interesting area in the process of phagocytosis relates to the pathways activated during physiological versus inflammatory conditions. Initial approaches using opsonized beads (i.e., polystyrene microspheres) in vivo proposed two mechanisms of phagocytosis. Inflammatory microglial phagocytosis is accompanied by the release of TNFα, IL-1β, ROS, and NO. In contrast, homeostatic phagocytosis, which is important to clear apoptotic cells or myelin debris, correlates with the induction of anti-inflammatory factors such as IL-10, TGF-β2, prostaglandin E2, and platelet-activating factor (PAF) (Ryu et al. 2012). Moreover, the uptake of apoptotic cells by microglia can downregulate pro-inflammatory cytokines, including TNFα, IL-12, and IL-1β (Ryu et al. 2012). Of relevance are the differences in lysosomal attributes between various cell types, including microglia, macrophages, monocyte-derived dendritic cells (DCs), and neutrophils. For example, a comparison of primary mouse microglia and J774 macrophages revealed that microglia contain higher levels of many lysosomal proteases than macrophages (Majumdar et al. 2007). However, the microglial lysosomes were less acidic with a pH ~ 6, compared to pH ~ 5 in macrophages, suggesting a decreased lysosomal enzymatic activity.

Interestingly, treatment with macrophage colony-stimulating factor (M-CSF) and IL-6 correlated with lysosomal acidification, enabling microglial cells to degrade Aβ fibrils effectively (Majumdar et al. 2007). Immunohistochemical analyses of primary microglia also showed that the CIC-7 chloride transporter, which acidifies lysosomes via cotransporting protons and chloride ions, is not delivered efficiently to microglial lysosomes, appearing mistargeted and mobilized to the ER for degradation. In this study, M-CSF was also shown to induce CIC-7 trafficking to the lysosomes, revealing an important pathway for the phagocytosis of different types of cargo upon microglial activation through M-CSF (Majumdar et al. 2011). As mentioned above, the relationship between M-CSF microglial activation and lysosome acidification suggests a protective role in AD, by promoting degradation of Aβ fibrils and hence reducing plaques deposition. However, these in vitro findings may need to be interpreted with caution, as conflicting results that microglial phagocytosis is the primary cause of neuronal cell death in the presence of low levels of extracellular Aβ were also obtained in vitro (Neniskyte et al. 2011). In mouse models of AD it was additionally shown that CX3CR1 deletion is associated with an increased phagocytic ability, leading to greater Aβ contents inside of microglial phagolysosomes (Liu et al. 2010b). Therefore, cell–cell interactions between microglia and neurons, notably through CX3CR1-CX3CL1 communication, and the cytokine environment may collectively determine whether microglia are equipped to initiate phagocytosis.

Microglia express a wide range of TLRs, which makes them effective defenders against invading pathogens. For example, peptidoglycan (PGN) recognition is mediated by TLR2 in microglia (Kielian et al. 2005), TLR4 is necessary for *Citrobacter koseri*-mediated microglial activation and response to LPS (Lehnardt et al. 2002; Liu and Kielian 2009; Olson and Miller 2004), while TLR stimulation is important for eliciting the release of inflammatory mediators following *Streptococcus pneumonia* (Ribes et al. 2010) and *Staphylococcus aureus* challenge (Kochan et al. 2012). Studies with cultured macrophages have demonstrated that several TLRs localize to phagosomes (i.e., the vesicles formed around bacteria once engulfed by phagocytosis) following bacterial uptake (Husebye et al. 2010; Underhill et al. 1999), although similar findings have not yet been reported in microglia.

Recognition of dying cells is a key mechanism in the process of cellular digestion. It is clear that the classical "eat me" signal PS, apparently specific to apoptotic cells, is recognized by the phagocytic receptor TIM-4 (T cell immunoglobulin mucin-4) (Kobayashi et al. 2007; Santiago et al. 2007; Savill and Gregory 2007). The phagocytic "synapse" is now considered as a complex structure, which involves PS and the receptors TIM-1 and TIM-4 on phagocytic cells. The seven transmembrane G-protein-coupled receptor BAL1 (B aggressive lymphoma 1) mediates PS binding on the extracellular surface, and their intracytoplasmic portions form complexes with modular proteins engulfment and motility (ELMO)-Dock180-Rac involved in phagocytic signaling (Kobayashi et al. 2007; Santiago et al. 2007). This information brings us closer to understanding the mechanisms by which cellular debris in the form of exosomes regulate immune responses (Sokolowski and Mandell 2011).

5.1.4 Microglia and Inflammasome Activation

Microglia are capable of recognizing a diverse array of infectious agents, including bacteria, fungi, and viruses (Esen and Kielian 2006; Kaushik et al. 2011; Liu and Kielian 2009; Rambach et al. 2010), by expressing various PRRs, namely TLRs and NLRs (Hanamsagar et al. 2011; Jack et al. 2005b; Lee et al. 2013; Regnier-Vigouroux 2003). The recognition of PAMPs by TLRs makes it nearly impossible for a pathogen to go undetected, as it is difficult to evade the immune system by mutating an essential pathogenic motif. Microglia respond to pathogens by producing a wide range of pro-inflammatory mediators, including ROS and nitrogen species, cytokines, and chemokines (Aloisi 2001; Tambuyzer et al. 2009). In a similar manner, microglia are activated in response to endogenous danger signals such as the nucleotide ATP or misfolded proteins, which also culminate into pro-inflammatory cytokine secretion. It is thought that recognition of these DAMPs occurs via the activation of NLRs within the cytoplasm of host cells. NLR inflammasome activation leads to the processing and release of pro-inflammatory cytokines, including IL-1β and IL-18 which have been implicated in the pathophysiology of numerous CNS neurodegenerative diseases, including AD and PD (Benzing et al. 1999; Koprich et al. 2008). Acute brain injuries such as stroke, trauma, and hemorrhage are also characterized by neuroinflammation and IL-1 release, a process which is particularly linked to disease exacerbation (Denes et al. 2011; Lu et al. 2005; Masada et al. 2003). A role for IL-1β and IL-18 has also been described in several CNS infection models, including bacterial meningitis, brain abscess, and human immunodeficiency virus (HIV)-associated dementia (Ghorpade et al. 2003; Iannello et al. 2010; Kielian et al. 2004; Saukkonen et al. 1990; Xiong et al. 2012) (see Chap. 15 for further reading on HIV). IL-1β and its receptor IL-1R play a crucial role in the amplification of cytokine/chemokine networks, and bacterial clearance during acute *S. aureus* infection (Kielian et al. 2004; Xiong et al. 2012). IL-1β and IL-18 also regulate the induction of adaptive immunity, and both cytokines have been shown to influence disease development in experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), an inflammatory-mediated demyelinating disease of the human CNS (Gris et al. 2010; Sutton et al. 2006) (Fig. 5.1) (see Chap. 16 for further reading on MS). The clinical course of MS is variable but usually initiates with reversible episodes of neurological disability that transform into a disease of continuous and irreversible neurological decline. Axonal damage and neurodegeneration are a major cause of irreversible neurological disability in MS. Inflammation and demyelination are additional hallmarks of the disease that manifest in clinical symptoms such as numbness, muscle spasms, optic neuritis, neuropathic pain, and paralysis (Dutta and Trapp 2011; Trapp et al. 1999; Trapp and Nave 2008).

In AD, extracellular Aβ triggers activation of the NLRP3 inflammasome in microglia, due to lysosomal damage and cathepsin B release following fibril uptake (Halle et al. 2008). Enhanced caspase-1 expression has been reported in the brains of AD patients, while double transgenic APP/PS1 mice harboring the human amyloid precursor protein (APP) and the presenilin 1 (PS1) mutation for familial AD

demonstrated reduced caspase-1, IL-1β, and Aβ burdens when lacking NLRP3 or caspase-1 (Heneka et al. 2013). NLRP3 deficiency has been associated with a skewing of microglial activation towards an M2-like anti-inflammatory state, suggesting an important regulatory role for the inflammasome in microglial-induced inflammation. It is not clear whether other types of NLR inflammasomes could serve as platforms for IL-1β processing in response to Aβ, as their contribution has not yet been tested in AD animal models. However, a recent study that examined NLRP1 gene polymorphisms in a cohort of AD patients revealed an association of four non-synonymous polymorphisms in NLRP1 with AD, suggesting that these mutations may be involved in the predisposition to AD (Pontillo et al. 2012). Nevertheless, a direct cause-and-effect relationship remains to be defined.

The EAE animal model commonly requires injection of *Mycobacterium* along with the myelin peptides, to render the endogenous protein immunogenic. It is thought that the initial detection of danger signals (which remain ill-defined) within the CNS is mediated by microglia through their ability to present antigen and secrete immune molecules that can recruit peripheral immune cells into the CNS. Infiltrating macrophages and T cells can damage neurons, which provides signals to perpetuate microglial activation. If not tightly regulated, this can lead to progressive and irreversible tissue damage (Jack et al. 2005a). Microglia can phagocytose myelin debris, which triggers IL-1β release (Williams et al. 1994); however, additional studies are needed to better define the role of microglial inflammasomes during MS development and/or progression. In general, NLRP3 inflammasome activation has been reported as detrimental to the host in the context of EAE. For example, NLRP3-deficient mice displayed reduced neuroinflammation and delayed myelin loss in a model of demyelination induced by cuprizone, i.e., a dietary chelator commonly used to induce demyelinating lesion in the corpus callosum of rodents as a model of MS (Jha et al. 2010). Similarly, in myelin-oligodendrocyte glycoprotein (MOG)-induced EAE, NLRP3 deficiency reduced disease severity by inhibiting inflammatory cell infiltrates into the spinal cord (Gris et al. 2010). There is also evidence that the inflammasome adaptor protein Apoptosis-associated Speck-like protein containing CARD (ASC) contributes to the progression of EAE, independently from its inflammasome activity; although no association with NLRP3 expression was found in that study (Shaw et al. 2010). Nonetheless, in all cases, the cellular basis of inflammasome activation remains to be determined.

Recently, it has been demonstrated that activated microglia are critical for the maintenance of chronic pain following traumatic spinal cord injury (SCI) (Hains and Waxman 2006). Additionally, microglia are among the first responders to neuronal damage and injury and produce a vast range of pro-inflammatory mediators, including IL-1β, within hours following SCI (Pineau and Lacroix 2007). The NLRP1 inflammasome may be involved in IL-1β and IL-18 processing following SCI through the activation of $P2X_4$ receptor in neurons (de Rivero Vaccari et al. 2008). Currently, it is thought that the majority of IL-1β release that follows SCI originates from neurons (Bernier 2012), and additional studies are needed to determine the role of microglia in inflammasome activation during SCI and chronic pain (see Chaps. 11 and 19 for further reading on these topics).

Although the CNS is usually well-protected from microbial invasion, in many instances are bacterial, fungal, viral, parasitic, and prion infections known to be life-threatening. The role of microglia in the rapid recognition and responses to pathogens has been extensively studied (Cosenza et al. 2002; Hanisch et al. 2001; Kielian et al. 2002; Lee et al. 1995). However, the physiological importance of inflammasome activation in the context of infectious diseases has only recently begun to be investigated. In particular, it was shown that cultured microglia respond to live *S. aureus* by producing IL-1β in a NLRP3- and ASC-dependent manner (Hanamsagar et al. 2011). The same study demonstrated the existence of redundant mechanisms for microglial IL-1β release, including bacterial pore-forming toxins and extracellular ATP, as well as caspase-1 and cathepsin B activity. A recent study with *Legionella pneumophila* demonstrated that flagellin recognition by the NLRC4 inflammasome may be important in restricting bacterial replication within microglia (Jamilloux et al. 2013). Neurotoxin prion infection can also lead to NLRP3 inflammasome activation in microglia (Shi et al. 2012), defining a novel trigger for the NLRP3 inflammasome apart from the traditional stimuli identified to date. Viral infections of the CNS were also shown to activate microglia, and a recent study further suggested that ROS production and subsequent NLRP3 inflammasome activation are critical during West Nile virus infection (Kaushik et al. 2012). Other inflammasomes such as HIN200 or AIM2, which are known to respond directly to viral nucleic acids, may also be involved; however, their role in microglia remains to be investigated.

5.2 Microglia in the Regulation of Adaptive Immunity

As demonstrated in the previous section, microglia, the main immune responsive glial cell population of the CNS, do not only produce a myriad of inflammatory cytokines and chemokines, but also receive inflammatory signals from nearby astrocytes, neurons, endothelial cells, and infiltrating leukocytes. Although chemokines and chemokine receptors are key players in the innate immune response, there are also pathways that directly link chemokine receptor signaling on microglia to the generation of effector T cells. In addition, alternative molecular mechanisms further highlight the role of microglial phagocytosis and antigen processing in effector T-cell responses in the CNS. Therefore, the type of CNS insult, the local inflammatory response, and the molecular program of microglial cells altogether contribute to the contextual regulation of neuroprotective versus neurotoxic pathways.

5.2.1 Antigen Presentation

T cells can recognize antigens only in the form of a peptide bound to MHC on the surface of antigen-presenting cells (APCs), in a process called antigen presentation. Professional APCs (e.g., macrophages, DCs identified by the expression of CD11c,

and B lymphocytes) express MHC class II, while non-professional APCs (e.g., fibroblast, thymic epithelial cells, glial cells, pancreatic beta cells, and vascular endothelial cells) express MHC class I. Only professional APCs can activate a helper T cell that has never encountered the antigen before. Therefore, it is well established that adaptive immune responses depend on the function of professional APCs in the CNS. In addition to the peptide-MHC molecule interactions, contacts between the T cell and APCs via costimulatory molecules (such as CD86, intercellular adhesion molecule 1 (ICAM-1), and CD40) are required for full T-cell activation. Without this costimulatory signal, T cells will arrest in a nonactivated state, referred to as anergy. Considering that the immune system can be detrimental or beneficial to brain function, either by causing tissue damage or contributing to repair (Shaked et al. 2004; Wraith and Nicholson 2012), microglial ability to present antigens to T cells through MHC class II could allow these normally quiescent cells to play a yet undescribed role in shaping the outcome of certain neurological diseases where antigen presentation is critically involved such as MS and EAE (O'Keefe et al. 2002). Some studies have demonstrated that treatment of microglia with granulocyte macrophage colony-stimulating factor (GM-CSF) can transform these cells into a DC-like phenotype in vitro (Ponomarev et al. 2005). Usually identified as $CD11c^+$ cells, brain DCs are found in perivascular regions of the CNS and in peripheral blood but might be derived from a microglial precursor as well (Prodinger et al. 2011). However, it remains unresolved whether APCs have the capacity to leave the CNS and transport CNS-derived antigens to the draining lymph nodes. Given the observations that the cerebrospinal fluid (CSF) and CNS parenchyma lack naïve T cells under physiological conditions, it is commonly accepted that adaptive immune responses against CNS antigens are initiated in the periphery, and subsequently propagated to the CNS by circulating memory T cells, which are restimulated by antigens within the CNS (Ransohoff and Engelhardt 2012). The involvement of T cell–APC interactions in EAE has been evaluated using adoptive transfer experiments with activated T cells, either myelin-specific or ovalbumin-specific. In these experiments, the ovalbumin-specific T cells remained in the subarachnoid space (SAS), whereas myelin-specific T cells readily invaded the parenchyma. Ovalbumin-specific T cells were only detected in the brain parenchyma when ovalbumin-pulsed APCs were placed in the SAS (Bartholomaus et al. 2009). Therefore, these data suggest that antigen-specific interactions between T cells and APCs occurring within the SAS are essential for the development of EAE (Ransohoff and Engelhardt 2012). The fact that most studies exploring microglial interactions with T cell activation were performed in vitro prompts careful interpretation, as it was shown that microglia that develop in mixed glial cultures display a more activated phenotype than microglia in pure cultures (Carson et al. 1998). In vivo microglia express low levels of accessory molecules required for efficient antigen presentation and have weak antigen-presenting activity (Ransohoff and Engelhardt 2012). Although adult microglia can express costimulatory molecules under certain conditions (Carson et al. 1998; Garcia et al. 2013; Zhang et al. 2002), they failed to present a peptide antigen to naïve T cells in vitro (Carson et al. 1998). Furthermore, adult microglia were less efficient, when compared to $CD11c^+$ DCs, to

restimulate primed T cells and to induce T cell proliferation in vitro (Ford et al. 1995; Garcia et al. 2013). Similarly, during Theiler's murine encephalomyelitis virus-induced demyelinating disease, CNS-infiltrating macrophages were more highly activated based on their MHC class II expression than resident microglia. However, microglia isolated at the onset of disease were as efficient as infiltrating macrophages at inducing Th1 proliferation and IFNγ production (Mack et al. 2003). Although it was reported that EAE disease severity is ameliorated when microglia are selectively depleted before disease induction via systemic administration of ganciclovir (an antiviral nucleotide analogue) to mice engineered to express the thymidine kinase of herpes simplex virus (HSVTK) under the CD11b promoter (Heppner et al. 2005) (see Chap. 16 for further reading about this model), the exact mechanism linking microglia to APC functions in vivo remains unsolved.

Although microglia display enhanced clearance of myelin debris in CNS autoimmunity models (Nielsen et al. 2009), compared with naïve/physiological conditions, it is still unclear how the scavenging mechanism relating to antigen processing and presentation differs between microglia and professional CD11c-positive DCs. Recently it was shown that oligodendrocytes secrete small membrane vesicles containing myelin antigens, called exosomes, which are efficiently taken up by microglia both in vitro and in vivo. However, these exosomes are preferentially internalized by microglia devoid of antigen presenting capacity (Fitzner et al. 2011). Therefore, it was proposed that a decreased exosome internalization by MHC class II-positive microglia might allow myelin antigens to be transported with the interstitial fluid to the meningeal macrophages, which could then present myelin-derived antigen to myelin-specific T cells present in the cerebrospinal fluid (Fitzner et al. 2011; Ransohoff and Engelhardt 2012). It has been demonstrated, however, that IFNγ enables brain CD11c-expressing cells to become effective APCs and to induce antigen-specific T cells expressing the CD4 glycoprotein at their surface (CD4+) to proliferate and secrete cytokines specific to the differentiated Th1/Th17 effector T cell subtypes, which are considered as encephalitogenic (Gottfried-Blackmore et al. 2009). In an entorhinal cortex lesion model, the fact that microglia do not upregulate MHC class II also indicates that they could represent a DC population capable of producing immunological tolerance, i.e., a condition where the immune system does not attack an antigen, under particular circumstances. However, evidence is still lacking to validate a role for microglia expressing the CD11c marker in transporting antigens from the brain to lymphoid organs (Prodinger et al. 2011). Not all microglia express CD11c, only a subpopulation of about 1.1 ± 0.1 % of microglia in naïve controls, and 4 ± 0.8 % at 3 weeks after cuprizone treatment expressed CD11c (Remington et al. 2007). It was shown that CD11c-positive microglial cells in culture are approximately three times better at stimulating T cell activation than CD11c-negative cells, indicative of enhanced antigen presentation capacity associated with this phenotype. Also, resident brain microglia appeared to be key functional players during adaptive responses in the sub-chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, dopaminergic neurotoxin) mouse model of PD that is associated with T-cell recruitment into the CNS (Depboylu et al. 2012). Using bone marrow chimeric mice to distinguish the

resident microglial population from infiltrating leukocytes, in combination with retrograde neuronal tracing, it was shown that resident microglia upregulate MHC class II after MPTP administration. Importantly, microglia also contained neuronal tracer and appeared in close contact with CD4+ T cells in the lesion area. Therefore, resident microglia represent likely candidates for the presentation of antigens to infiltrating activated T lymphocytes during dopaminergic cell death.

More than a decade ago, when the concept of attenuating Aβ accumulation in the brain by eliciting adaptive immune responses emerged, it was the first time that a self-peptide was introduced as a vaccine. Preclinical studies were successful, but the initial clinical trials were halted because of the development of severe inflammatory reactions in the vaccinated AD patients (Nicoll et al. 2003; Schenk 2008). While a number of reports suggested that T cells were activated in the patients and could be found both in the periphery and brain tissues, the question on how those T cells gained access to the brain has been investigated by some groups. In this regard, microglia were proposed to be indirectly involved in the transendothelial migration and activation of T cells. Their involvement with the regulation of adaptive T cells effector responses was evidenced by their high levels of TNFα release in response to Aβ application in vitro, thus resulting in over-expression of the chemokine receptor CXCR2 (mouse equivalent of the human IL-8 receptor) on peripheral T cells derived from AD patients (Liu et al. 2010a). During EAE, disease severity was significantly exacerbated in hypermyelinated PLP-Akt-DD transgenic mice compared to wild-type animals with "normal" myelin content (Jaini et al. 2013). PLP-Akt-DD transgenic animals have been engineered to drive, under the proteolipid PLP promoter, expression of the constitutively active Akt-1 which harbors aspartic acid (D) at positions Thr308 and Ser473, two sites where phosphorylation leads to full activation of Akt. In these mice, expression of Akt-DD dramatically increased myelin production, correlating with increased neurological score and exacerbated neuronal damage. There was also an increased number of resident microglia in the hypermyelinated mice, but their individual capacity to present antigens was not increased, as revealed by the levels of IL-17 (a pro-inflammatory cytokine inducing and mediating pro-inflammatory responses) secreted by T cells isolated from these mice in the presence of microglia (Jaini et al. 2013). This work suggests that the load of self-antigens imposed on the APCs could have an important influence on the disease onset and progression (Fig. 5.1). Therefore, the drastic differences between microglia versus professional DCs, as APCs, support the notion that microglial activity, if well controlled, is a crucial step in determining neuronal survival.

5.2.2 Chemokine Receptors

Microglia express most chemokine receptors in vivo, including CCR3, CCR5, CCR8, CXCR3, CXCR4, and CX3CR1, depending on the CNS microenvironment that shapes their effector phenotype (Biber et al. 2002; Cardona and Ransohoff 2007; Glabinsk and Ransohoff 2001; Prinz and Priller 2010; Ransohoff 2002; Trebst

et al. 2001; Trebst et al. 2003), with actions that promote either tissue damage and neurotoxicity or neuroprotection and tissue repair (Cardona et al. 2006; Garcia et al. 2013; Prinz et al. 2011) (also see Chap. 6). Several studies support the concept that microglia are a heterogeneous population based on the selective expression of chemokine receptors. For example, transcriptome analyses in combination with protein validation revealed that nearly all microglial cells express high levels of complement C1q, while only subsets of microglia express the CCR1 ligand, CXCL14, and TREM2 (triggering receptor expressed on myeloid cells 2; which regulates microglial activation and phagocytosis) (Schmid et al. 2009). Adding another layer of complexity, the regulation of microglial function is also influenced by the other CNS resident cells. For example, several studies in PD models suggest a scenario in which astrocyte-microglial communication impacts T cell responses. Astrocytes exposed in vitro to neuron-derived α-synuclein induce expression of transcripts for chemokines such as CCL3-7, CCL12, 19 and 20, and CXCL1,2,4,5,9,10, 11, 12, and 16, and CX3CL1 (Harms et al. 2013). Microglial expression of the chemokine receptors CCR3, CCR5, CXCR3 and CX3CR1 makes them highly responsive to their respective ligands produced by astrocytes (Cowell et al. 2006; Flynn et al. 2003; Gautier et al. 2012). Chemokine receptor-ligand interactions also correlated with morphological changes in microglia and increased expression of MHC class II in vivo (Garcia et al. 2013). Most importantly, microglial responses to aggregated α-synuclein induced antigen processing and presentation, driving T cell proliferation and cytokine release, as well as neurodegeneration in a mouse model of PD in vivo (Harms et al. 2013), suggesting that antigen presentation could promote an inflammatory environment detrimental to neuronal survival. A similar role for microglia/MHC class II was also demonstrated in a facial nerve axotomy model, in which the physical distance between the injury to the facial nerve and the neuronal localization in the brainstem provides a good model to avoid direct CNS trauma and/or disruption of the BBB (Byram et al. 2004). Therefore, these results implicate a central role for microglial MHC class II in the activation of adaptive immune responses, indicating that this process could be viewed as a neuroprotective target.

Microglia represent important components of the CNS immune response in MS and EAE, alongside peripherally derived macrophages and DCs. The mechanism by which these cell types repopulate during acute and chronic brain inflammation have been explored. The chemokine receptor CCR2 that signals in response to several monocyte chemoattractant molecules (known as CCL2, CCL8, and CCL16) is crucial for the accumulation of macrophages and DCs to the sites of inflammation, although its role in mobilizing microglia is still controversial. There is evidence suggesting that $CD11b^{+}$ $Ly6C^{hi}CCR2^{+}CD62L^{+}$ (or adhesion molecule L-selectin+) monocytes are mobilized from the bone marrow into the bloodstream, via a GM-CSF-dependent pathway before EAE relapses (King et al. 2009). However, microglia are recognized by a $CD11b^{+}$ $Ly6C^{-}CCR2^{-}CD62L^{+}$ phenotype (Carson et al. 1998), and the lack of microglial CCR2 upregulation in CCR2-RFP reporter mice supports a selective role for CCR2 in the trafficking of macrophages (King et al. 2009; Saederup et al. 2010). However, it was reported that over-expression of

CCL2 also leads to morphological activation of microglia and pro-inflammatory cytokine release in the CNS (Selenica et al. 2013), although a direct interaction between exogenous CCL2 and CCR2 on microglia is uncertain. CCR2 also targets the recruitment of $CCR2^{+}CD11c^{+}$ DCs. As expected, the expression of multiple chemokine receptors by myeloid cells may account for the synergistic effects observed when analyzing DC trafficking or microglial mobilization within the CNS.

The pathways that influence chemokine receptor expression are complex and cytokines are the most studied candidates in this process. However, the highly conserved cell–cell communication pathway Notch, initially identified as a pleiotropic mediator of cell fate in invertebrates, has emerged as an important regulator of immune cell development and function. It was recently shown that microglia express the Notch ligand DLL4 and that DLL4 blockade reduces the neurological symptoms of EAE, correlating with a downregulation of CCR2 and CCR6 expression on T cells. Although microglia are not the only cell type expressing DLL4 in the brain (Benedito and Duarte 2005; Shutter et al. 2000), microglia–T cells interactions mediated by DLL4 and Notch receptors could contribute to the regulation of T cells chemokine receptors expression, and their responses to organ-specific chemokine production (Reynolds et al. 2011).

In addition to microglia–T cell interactions, microglia-neuron communication is now recognized as a key mechanism in the regulation of microglial function. Several studies have confirmed the predominant microglial expression of CX3CR1 (Cardona et al. 2006; Gautier et al. 2012; Jung et al. 2000), whose ligand CX3CL1 is expressed in neuronal membranes (Bazan et al. 1997; Mizoue et al. 1999; Rossi et al. 1998). The chemokine module is released by action of proteases including A Disintegrin and metalloproteinase domain-containing protein 10 and 17 (ADAM10 and ADAM10) and cathepsin S (Clark et al. 2009; Garton et al. 2001; Hundhausen et al. 2003). Binding of CX3CL1 to CX3CR1 on microglia has been viewed as neuroprotective because this interaction inhibits microglial activation in selected models of neurodegeneration (Bachstetter et al. 2011; Cardona et al. 2006; Rogers et al. 2011). Although some studies suggest that CX3CR1 deficiency could be neuroprotective and/or anti-inflammatory in various contexts of disease such as AD (Fuhrmann et al. 2010; Lee et al. 2010; Mattison et al. 2013), in EAE, the absence of CX3CR1 correlated with an enhanced neurological disease and increased microglial activation, reflected by their increased MCH class II expression and proliferation. These features of microglia also correlated with an increased T cell proliferation. Furthermore, higher IFNγ and IL-17 levels were detected in cerebellar and spinal cord tissues of CX3CR1-deficient mice (Garcia et al. 2013).

Overall chemokine receptors regulate microglial mobilization within the CNS, but the observation of higher frequencies of IFNγ- and IL-17-producing T cells in the lymphoid tissues of CX3CR1-deficient mice, and the enhanced T cell proliferation induced by CX3CR1-deficient DCs, demonstrate that besides their role in chemoattraction, some chemokine receptors such as CX3CR1 may contribute to the establishment of adaptive immune responses via regulation of antigen presentation function.

5.3 Microglia and the BBB

The BBB is the interface between the CNS and the periphery, fulfilling two main functions, acting as a physical barrier and a selective exchange barrier aimed to maintain the proper CNS microenvironment for optimal neuronal function. This mechanical separation of the CNS is accomplished by the presence of specialized endothelial cells tightly attached one to another via tight junctions and adherent junctions (Hawkins and Davis 2005; Hermann and ElAli 2012). These junctions are formed by the tight junction proteins occludins and claudins, as well as the junctional adhesion molecules E-cadherin, P-cadherin, and N-cadherin, whose ultimate role is to restrict blood-borne molecules and peripheral cells from entering the CNS (Pardridge 2003; Wilson et al. 2010). Extensive work in the last decade has unraveled the presence of a specialized intrinsic innate immune system in the CNS, which also involves the BBB as an active contributor, as opposed to being an immunological neutral and passive barrier (Muldoon et al. 2013). It has been observed not only that peripheral immune cells can cross an intact BBB (Carson et al. 2006), but that the BBB modulates the differentiation of infiltrating CD14+ monocytes into DCs through the influence of BBB-secreted TGFβ and GM-CSF. These data confer a more active role of the BBB in the intrinsic innate immunity of the CNS. Despite the limited infiltration of peripheral immune cells into the CNS under physiological conditions, it is well known that neutrophils, eosinophils, T lymphocytes, monocytes, and other immune cells can infiltrate the CNS parenchyma after injuries, infections, and chronic diseases such as MS (Wilson et al. 2010).

Although it is unclear whether BBB dysfunction during pathological conditions is due to a loss of signals provided by the CNS, or to breakdown signals produced during pathology, recent work has shown that communication between the BBB and microglia is one of the early events involved in EAE. In particular, disruption of the barrier and subsequent leakage of the plasma protein fibrinogen has been identified as a signal triggering microglial motility towards perivascular regions, microglial activation and clustering, as well as axonal damage, using two-photon in vivo imaging. Importantly, these changes in microglia were shown to occur prior to the onset of neurological changes, suggesting their relevance to the disease pathogenesis (Davalos et al. 2012) (also see Chap. 4 for more information on these findings).

Activated microglia and infiltrating macrophages secrete a wide range of pro-inflammatory signals, including NO and ROS, which have been categorized as a major mechanism leading to axonal damage in MS (Lassmann 2010), through the initiation of mitochondrial dysfunction which, in turn, leads to the production of mitochondrial-derived ROS (Haider et al. 2011). Indeed, increased ROS generation has been observed in close proximity to fibrin-mediated microglial clusters at the peak of EAE, compared to distal areas with normal microglial density, or healthy controls (Davalos et al. 2012). Since fibrin is formed from fibrinogen through the action of the protease thrombin, it represents a molecular marker for BBB disruption and fibrinogen leakage into the CNS parenchyma. Interestingly, in vitro treatment of microglia with fibrinogen induced a 5.1-fold increase of H_2O_2 and a 7.4-fold

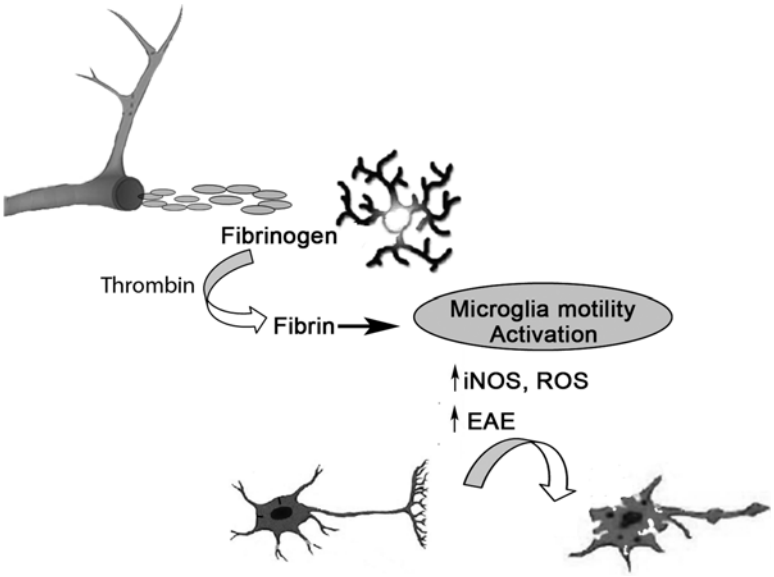

Fig. 5.2 Microglia and the interface to peripheral communication. Fibrinogen appears to be a key modulator of microglial activity as a result of early blood–brain barrier (BBB) damage. Once extravasated, it is lysed into fibrin by thrombin and interacts with parenchymal microglia, leading to an increased expression of inducible nitric oxide synthase (iNOS) and reactive oxygen species (ROS) that accounts for neuronal damage associated with certain neuroinflammatory conditions such as EAE

increase in inducible nitric oxide synthase (iNOS) gene expression (Davalos et al. 2012), strongly suggesting that fibrinogen might contribute to ROS generation during inflammatory demyelinating lesions. These data highlight the importance of fibrin deposition for the development of axonal degeneration within microglial clusters. Postmortem analysis of MS brains has reinforced this finding of microglial clustering within normal-appearing white matter (van Horssen et al. 2012), although microglial association with the disrupted BBB remains a challenge to assess, due to a limited effectiveness of molecular probes to identify microglia at their different states of "activation" in heavily fixed human specimens, which also complicates investigation of their association with BBB leakiness.

This aggregation of microglial cells at perivascular areas before disease onset may constitute an early indicator of new lesion formation, as demyelination and axonal damage are exacerbated at the peak of EAE, coincident with rapid microglial process extension and retraction at the sites of BBB leakage showing fibrin deposition (Fig. 5.2). Despite the strong correlation between perivascular fibrinogen deposition and microglial clusters formation at the early stages of neuroinflammation in EAE, the role of fibrinogen or other blood products within the CNS and their interaction with microglial cells in the context of other CNS pathologies remains to be investigated.

Both in vivo and in vitro studies support the idea that microglia release inflammatory mediators that regulate BBB permeability. Coculture models of microvascular endothelial cells showed that LPS-activated microglia induce BBB leakiness by producing ROS through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Matsumoto et al. 2012; Sumi et al. 2010) and TNFα release (Nishioku et al. 2010). Microglial activation is evident in the aged CNS and breakdown of the BBB and blood–retinal barrier in the aging brain suggests that microglial actions at the vasculature interface may contribute not only to neuroinflammatory conditions, but also to the pathogenesis of age-related neurological disorders (see Chap. 13 for additional information on aging). Further understanding of the mechanisms involved in the coupling between the BBB and microglia in innate immunity will require to investigate the role of microglia in the regulation of BBB dynamics during normal physiological conditions, considering that primary microglial cells in culture express a recently discovered tight junction protein, tricellulin (Mariano et al. 2011), whose role in the CNS and immune function remains to be elucidated.

5.4 Summary

It is now well accepted that microglia do not only participate in the genesis of immune responses during CNS infection/injury, but also play key roles in maintaining homeostasis. During CNS development and inflammatory conditions, microglia display the ability to phagocytose debris and apoptotic cells, release cytokines, and upregulate MHC class II to modulate effector T cell responses. These functions of microglia do not occur in isolation but are influenced by neighboring cells, including astrocytes, neurons, and the BBB. Microglia are capable of producing a vast array of cytokines and chemokines and are therefore able to influence the CNS environment in a very effective manner. Microglia can also respond to soluble mediators produced by neurons and astrocytes, and a current challenge is to dissect the signals that enable such an orchestrated network to sustain optimal CNS function. The most recognized function of microglia as phagocytes and scavengers is temporally regulated to allow for appropriate antigen recognition and presentation during adaptive immune responses. As our knowledge of microglial functions during homeostasis and pathology continues to expand, it may be possible to consider harnessing microglial activity to benefit CNS integrity and improve outcomes following neuroinflammatory injury. For many years researchers have been studying the mechanisms that lead to microglial activation and enhance their phagocytic properties. Another interesting venue involves the use of microglia as shuttles of anti-inflammatory molecules. Although the issue of identifying microglial origins is still a moving target, progress has been made to show that microglia are a distinct myeloid subset with unique properties, making them distinct from DCs and macrophages. Balancing neuroprotective versus neurotoxic properties of microglia has been and will continue to be a topic of arduous investigation, with the goal of modulating CNS inflammation due to autoimmunity, infection, or neurodegeneration.

References

Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res 117:145–152

Aloisi F (2001) Immune function of microglia. Glia 36:165–179

Aloisi F, Penna G, Cerase J et al (1997) IL-12 production by central nervous system microglia is inhibited by astrocytes. J Immunol 159:1604–1612

Aravalli RN, Hu S, Rowen TN et al (2005) Cutting edge: TLR2-mediated proinflammatory cytokine and chemokine production by microglial cells in response to herpes simplex virus. J Immunol 175:4189–4193

Bachstetter AD, Morganti JM, Jernberg J et al (2011) Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. Neurobiol Aging 32:2030–2044

Bard F, Cannon C, Barbour R et al (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 6:916–919

Bartholomaus I, Kawakami N, Odoardi F et al (2009) Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. Nature 462:94–98

Bazan JF, Bacon KB, Hardiman G et al (1997) A new class of membrane-bound chemokine with a CX3C motif. Nature 385:640–644

Becher B, Dodelet V, Fedorowicz V et al (1996) Soluble tumor necrosis factor receptor inhibits interleukin 12 production by stimulated human adult microglial cells in vitro. J Clin Invest 98:1539–1543

Benedito R, Duarte A (2005) Expression of Dll4 during mouse embryogenesis suggests multiple developmental roles. Gene Expr Patterns 5:750–755

Benzing WC, Wujek JR, Ward EK et al (1999) Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. Neurobiol Aging 20:581–589

Bernier LP (2012) Purinergic regulation of inflammasome activation after central nervous system injury. J Gen Physiol 140:571–575

Biber K, Dijkstra I, Trebst C et al (2002) Functional expression of CXCR3 in cultured mouse and human astrocytes and microglia. Neuroscience 112:487–497

Blasko I, Stampfer-Kountchev M, Robatscher P et al (2004) How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. Aging Cell 3:169–176

Bowman CC, Rasley A, Tranguch SL et al (2003) Cultured astrocytes express toll-like receptors for bacterial products. Glia 43:281–291

Boya J, Calvo J, Prado A (1979) The origin of microglial cells. J Anat 129:177–186

Brown GC, Neher JJ (2012) Eaten alive! Cell death by primary phagocytosis: 'phagoptosis'. Trends Biochem Sci 37:325–332

Bsibsi M, Ravid R, Gveric D et al (2002) Broad expression of Toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol 61:1013–1021

Byram SC, Carson MJ, DeBoy CA et al (2004) CD4-positive T cell-mediated neuroprotection requires dual compartment antigen presentation. J Neurosci 24:4333–4339

Cardona AE, Ransohoff RM (2007) Chemokine receptors in neuroinflammation. In: Harrison JK and Lukas NW (eds) The chemokine receptors. Humana Press, Totowa, NJ, pp 351–370

Cardona A, Pioro EP, Sasse ME et al (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9:917–924

Carson MJ, Reilly CR, Sutcliffe JG et al (1998) Mature microglia resemble immature antigen-presenting cells. Glia 22:72–85

Carson MJ, Doose JM, Melchior B et al (2006) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65

Chapman GA, Moores K, Harrison D et al (2000) Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. J Neurosci 20:RC87

Chen Z, Jalabi W, Shpargel KB et al (2012) Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4. J Neurosci 32:11706–11715

Clark AK, Yip PK, Malcangio M (2009) The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. J Neurosci 29:6945–6954

Codolo G, Plotegher N, Pozzobon T et al (2013) Triggering of inflammasome by aggregated alpha-synuclein, an inflammatory response in synucleinopathies. PLoS One 8:e55375

Cosenza MA, Zhao ML, Si Q et al (2002) Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. Brain Pathol 12: 442–455

Cowell RM, Xu H, Parent JM et al (2006) Microglial expression of chemokine receptor CCR5 during rat forebrain development and after perinatal hypoxia-ischemia. J Neuroimmunol 173:155–165

D'Aversa TG, Yu KO, Berman JW (2004) Expression of chemokines by human fetal microglia after treatment with the human immunodeficiency virus type 1 protein Tat. J Neurovirol 10: 86–97

D'Aversa TG, Eugenin EA, Berman JW (2008) CD40-CD40 ligand interactions in human microglia induce CXCL8 (interleukin-8) secretion by a mechanism dependent on activation of ERK1/2 and nuclear translocation of nuclear factor-kappaB (NFkappaB) and activator protein-1 (AP-1). J Neurosci Res 86:630–639

da Cunha A, Jefferson JJ, Tyor WR et al (1997) Transforming growth factor-beta1 in adult human microglia and its stimulated production by interleukin-1. J Interferon Cytokine Res 17:655–664

Davalos D, Grutzendler J, Yang G et al (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8:752–758

Davalos D, Ryu JK, Merlini M et al (2012) Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun 3:1227

de Rivero Vaccari JP, Lotocki G, Marcillo AE et al (2008) A molecular platform in neurons regulates inflammation after spinal cord injury. J Neurosci 28:3404–3414

Denes A, Pinteaux E, Rothwell NJ et al (2011) Interleukin-1 and stroke: biomarker, harbinger of damage, and therapeutic target. Cerebrovasc Dis 32:517–527

Depboylu C, Stricker S, Ghobril JP et al (2012) Brain-resident microglia predominate over infiltrating myeloid cells in activation, phagocytosis and interaction with T-lymphocytes in the MPTP mouse model of Parkinson disease. Exp Neurol 238:183–191

Dheen ST, Kaur C, Ling EA (2007) Microglial activation and its implications in the brain diseases. Curr Med Chem 14:1189–1197

Dinarello CA (2007) Historical insights into cytokines. Eur J Immunol 37(suppl 1):S34–S45

Doi Y, Mizuno T, Maki Y et al (2009) Microglia activated with the toll-like receptor 9 ligand CpG attenuate oligomeric amyloid {beta} neurotoxicity in in vitro and in vivo models of Alzheimer's disease. Am J Pathol 175:2121–2132

Ducharme G, Newell EW, Pinto C et al (2007) Small-conductance Cl- channels contribute to volume regulation and phagocytosis in microglia. Eur J Neurosci 26:2119–2130

Dutta R, Trapp BD (2011) Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Prog Neurobiol 93:1–12

Ellis SL, Gysbers V, Manders PM et al (2010) The cell-specific induction of CXC chemokine ligand 9 mediated by IFNgamma in microglia of the central nervous system is determined by the myeloid transcription factor PU.1. J Immunol 185:1864–1877

Esen N, Kielian T (2006) Central role for MyD88 in the responses of microglia to pathogen-associated molecular patterns. J Immunol 176:6802–6811

Fitzner D, Schnaars M, van Rossum D et al (2011) Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. J Cell Sci 124:447–458

Flynn G, Maru S, Loughlin J et al (2003) Regulation of chemokine receptor expression in human microglia and astrocytes. J Neuroimmunol 136:84–93

Fogg DK, Sibon C, Miled C et al (2006) A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 311:83–87

Ford AL, Goodsall AL, Hickey WF et al (1995) Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. Phenotypic differences defined and direct ex vivo antigen presentation to myelin basic protein-reactive CD4+ T cells compared. J Immunol 154:4309–4321

Fraser DA, Pisalyaput K, Tenner AJ (2010) C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. J Neurochem 112:733–743

Fricker M, Neher JJ, Zhao JW et al (2012) MFG-E8 mediates primary phagocytosis of viable neurons during neuroinflammation. J Neurosci 32:2657–2666

Fuhrmann M, Bittner T, Jung CK et al (2010) Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. Nat Neurosci 13:411–413

Garcia J, Pino PA, Mizutani M et al (2013) Regulation of adaptive immunity by the fractalkine receptor during autoimmune inflammation. J Immunol 191:1063–1072

Garton KJ, Gough PJ, Blobel CP et al (2001) Tumor necrosis factor-alpha-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). J Biol Chem 276: 37993–38001

Gautier EL, Shay T, Miller J et al (2012) Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol 13:1118–1128

Ghorpade A, Holter S, Borgmann K et al (2003) HIV-1 and IL-1 beta regulate Fas ligand expression in human astrocytes through the NF-kappa B pathway. J Neuroimmunol 141:141–149

Ginhoux F, Greter M, Leboeuf M et al (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845

Glabinsk AR, Ransohoff RM (2001) Targeting the chemokine system for multiple sclerosis treatment. Curr Opin Investig Drugs 2:1712–1719

Glezer I, Lapointe A, Rivest S (2006) Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. FASEB J 20:750–752

Glezer I, Chernomoretz A, David S et al (2007a) Genes involved in the balance between neuronal survival and death during inflammation. PLoS One 2:e310

Glezer I, Simard AR, Rivest S (2007b) Neuroprotective role of the innate immune system by microglia. Neuroscience 147:867–883

Gottfried-Blackmore A, Kaunzner UW, Idoyaga J et al (2009) Acute in vivo exposure to interferon-gamma enables resident brain dendritic cells to become effective antigen presenting cells. Proc Natl Acad Sci U S A 106:20918–20923

Greenwood J, Heasman SJ, Alvarez JI et al (2011) Review: leucocyte-endothelial cell crosstalk at the blood-brain barrier: a prerequisite for successful immune cell entry to the brain. Neuropathol Appl Neurobiol 37:24–39

Gris D, Ye Z, Iocca HA et al (2010) NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. J Immunol 185:974–981

Hadas S, Spira M, Hanisch UK et al (2012) Complement receptor-3 negatively regulates the phagocytosis of degenerated myelin through tyrosine kinase Syk and cofilin. J Neuroinflammation 9:166

Haider L, Fischer MT, Frischer JM et al (2011) Oxidative damage in multiple sclerosis lesions. Brain 134:1914–1924

Hains BC, Waxman SG (2006) Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J Neurosci 26:4308–4317

Halle A, Hornung V, Petzold GC et al (2008) The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol 9:857–865

Hanamsagar R, Torres V, Kielian T (2011) Inflammasome activation and IL-1beta/IL-18 processing are influenced by distinct pathways in microglia. J Neurochem 119:736–748

Hanamsagar R, Hanke ML, Kielian T (2012) Toll-like receptor (TLR) and inflammasome actions in the central nervous system. Trends Immunol 33:333–342

Hanisch UK (2002) Microglia as a source and target of cytokines. Glia 40:140–155
Hanisch UK, Prinz M, Angstwurm K et al (2001) The protein tyrosine kinase inhibitor AG126 prevents the massive microglial cytokine induction by pneumococcal cell walls. Eur J Immunol 31:2104–2115
Harms AS, Cao S, Rowse AL et al (2013) MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. J Neurosci 33:9592–9600
Harrison JK, Jiang Y, Chen S et al (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 95:10896–10901
Hawkins BT, Davis TP (2005) The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev 57:173–185
Heneka MT, Kummer MP, Stutz A et al (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493:674–678
Heppner FL, Greter M, Marino D et al (2005) Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat Med 11:146–152
Hermann DM, ElAli A (2012) The abluminal endothelial membrane in neurovascular remodeling in health and disease. Sci Signal 5:re4
Hosmane S, Tegenge MA, Rajbhandari L et al (2012) Toll/interleukin-1 receptor domain-containing adapter inducing interferon-beta mediates microglial phagocytosis of degenerating axons. J Neurosci 32:7745–7757
Hundhausen C, Misztela D, Berkhout TA et al (2003) The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion. Blood 102:1186–1195
Husebye H, Aune MH, Stenvik J et al (2010) The Rab11a GTPase controls Toll-like receptor 4-induced activation of interferon regulatory factor-3 on phagosomes. Immunity 33:583–596
Iannello A, Samarani S, Debbeche O et al (2010) Potential role of IL-18 in the immunopathogenesis of AIDS, HIV-associated lipodystrophy and related clinical conditions. Curr HIV Res 8:147–164
Imamura K, Hishikawa N, Sawada M et al (2003) Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol 106:518–526
Jack C, Ruffini F, Bar-Or A et al (2005a) Microglia and multiple sclerosis. J Neurosci Res 81:363–373
Jack CS, Arbour N, Manusow J et al (2005b) TLR signaling tailors innate immune responses in human microglia and astrocytes. J Immunol 175:4320–4330
Jaini R, Popescu DC, Flask CA et al (2013) Myelin antigen load influences antigen presentation and severity of central nervous system autoimmunity. J Neuroimmunol 259:37–46
Jamilloux Y, Pierini R, Querenet M et al (2013) Inflammasome activation restricts Legionella pneumophila replication in primary microglial cells through flagellin detection. Glia 61: 539–549
Jha S, Srivastava SY, Brickey WJ et al (2010) The inflammasome sensor, NLRP3, regulates CNS inflammation and demyelination via caspase-1 and interleukin-18. J Neurosci 30: 15811–15820
Jung S, Aliberti J, Graemmel P et al (2000) Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol Cell Biol 20:4106–4114
Kaushik DK, Gupta M, Basu A (2011) Microglial response to viral challenges: every silver lining comes with a cloud. Front Biosci 16:2187–2205
Kaushik DK, Gupta M, Kumawat KL et al (2012) NLRP3 inflammasome: key mediator of neuroinflammation in murine Japanese encephalitis. PLoS One 7:e32270
Kielian T (2004) Microglia and chemokines in infectious diseases of the nervous system: views and reviews. Front Biosci 9:732–750

Kielian T, Barry B, Hickey WF (2001) CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. J Immunol 166:4634–4643
Kielian T, Mayes P, Kielian M (2002) Characterization of microglial responses to Staphylococcus aureus: effects on cytokine, costimulatory molecule, and Toll-like receptor expression. J Neuroimmunol 130:86–99
Kielian T, Bearden ED, Baldwin AC et al (2004) IL-1 and TNF-alpha play a pivotal role in the host immune response in a mouse model of Staphylococcus aureus-induced experimental brain abscess. J Neuropathol Exp Neurol 63:381–396
Kielian T, Esen N, Bearden ED (2005) Toll-like receptor 2 (TLR2) is pivotal for recognition of S. aureus peptidoglycan but not intact bacteria by microglia. Glia 49:567–576
King IL, Dickendesher TL, Segal BM (2009) Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. Blood 113:3190–3197
Kobayashi N, Karisola P, Pena-Cruz V et al (2007) TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. Immunity 27:927–940
Kochan T, Singla A, Tosi J et al (2012) Toll-like receptor 2 ligand pretreatment attenuates retinal microglial inflammatory response but enhances phagocytic activity toward Staphylococcus aureus. Infect Immun 80:2076–2088
Koenigsknecht J, Landreth G (2004) Microglial phagocytosis of fibrillar beta-amyloid through a beta1 integrin-dependent mechanism. J Neurosci 24:9838–9846
Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K et al (2007) UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446:1091–1095
Koka P, He K, Zack JA et al (1995) Human immunodeficiency virus 1 envelope proteins induce interleukin 1, tumor necrosis factor alpha, and nitric oxide in glial cultures derived from fetal, neonatal, and adult human brain. J Exp Med 182:941–951
Koprich JB, Reske-Nielsen C, Mithal P et al (2008) Neuroinflammation mediated by IL-1beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. J Neuroinflammation 5:8
Kremlev SG, Roberts RL, Palmer C (2004) Differential expression of chemokines and chemokine receptors during microglial activation and inhibition. J Neuroimmunol 149:1–9
Kurant E (2011) Keeping the CNS clear: glial phagocytic functions in Drosophila. Glia 59: 1304–1311
Lassmann H (2010) Axonal and neuronal pathology in multiple sclerosis: what have we learnt from animal models. Exp Neurol 225:2–8
Ledeboer A, Breve JJ, Wierinckx A et al (2002) Expression and regulation of interleukin-10 and interleukin-10 receptor in rat astroglial and microglial cells. Eur J Neurosci 16:1175–1185
Lee SC, Liu W, Dickson DW et al (1993) Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1 beta. J Immunol 150:2659–2667
Lee SC, Kress Y, Dickson DW et al (1995) Human microglia mediate anti-Cryptococcus neoformans activity in the presence of specific antibody. J Neuroimmunol 62:43–52
Lee S, Varvel NH, Konerth ME et al (2010) CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. Am J Pathol 177:2549–2562
Lee HM, Kang J, Lee SJ et al (2013) Microglial activation of the NLRP3 inflammasome by the priming signals derived from macrophages infected with mycobacteria. Glia 61:441–452
Lehnardt S, Lachance C, Patrizi S et al (2002) The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. J Neurosci 22:2478–2486
Lehnardt S, Massillon L, Follett P et al (2003) Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci U S A 100:8514–8519
Lehnardt S, Henneke P, Lien E et al (2006) A mechanism for neurodegeneration induced by group B streptococci through activation of the TLR2/MyD88 pathway in microglia. J Immunol 177:583–592

Li J, Gran B, Zhang GX et al (2003) Differential expression and regulation of IL-23 and IL-12 subunits and receptors in adult mouse microglia. J Neurol Sci 215:95–103
Lindberg C, Chromek M, Ahrengart L et al (2005) Soluble interleukin-1 receptor type II, IL-18 and caspase-1 in mild cognitive impairment and severe Alzheimer's disease. Neurochem Int 46:551–557
Liu S, Kielian T (2009) Microglial activation by Citrobacter koseri is mediated by Citrobacter koseri is mediated by TLR4- and MyD88-dependent pathways. J Immunol 183:5537–5547
Liu S, Kielian T (2011) MyD88 is pivotal for immune recognition of Citrobacter koseri and astrocyte activation during CNS infection. J Neuroinflammation 8:35
Liu YJ, Guo DW, Tian L et al (2010a) Peripheral T cells derived from Alzheimer's disease patients overexpress CXCR2 contributing to its transendothelial migration, which is microglial TNF-alpha-dependent. Neurobiol Aging 31:175–188
Liu Z, Condello C, Schain A et al (2010b) CX3CR1 in microglia regulates brain amyloid deposition through selective protofibrillar amyloid-beta phagocytosis. J Neurosci 30:17091–17101
Lokensgard JR, Hu S, Sheng W et al (2001) Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus. J Neurovirol 7:208–219
Lu KT, Wang YW, Yang JT et al (2005) Effect of interleukin-1 on traumatic brain injury-induced damage to hippocampal neurons. J Neurotrauma 22:885–895
Ludwig A, Schulte A, Schnack C et al (2005) Enhanced expression and shedding of the transmembrane chemokine CXCL16 by reactive astrocytes and glioma cells. J Neurochem 93:1293–1303
Lue LF, Rydel R, Brigham EF et al (2001a) Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia 35:72–79
Lue LF, Walker DG, Rogers J (2001b) Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. Neurobiol Aging 22:945–956
Mack CL, Vanderlugt-Castaneda CL, Neville KL et al (2003) Microglia are activated to become competent antigen presenting and effector cells in the inflammatory environment of the Theiler's virus model of multiple sclerosis. J Neuroimmunol 144:68–79
Magnus T, Chan A, Grauer O et al (2001) Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation. J Immunol 167:5004–5010
Majumdar A, Cruz D, Asamoah N et al (2007) Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils. Mol Biol Cell 18:1490–1496
Majumdar A, Capetillo-Zarate E, Cruz D et al (2011) Degradation of Alzheimer's amyloid fibrils by microglia requires delivery of ClC-7 to lysosomes. Mol Biol Cell 22:1664–1676
Mariano C, Silva SL, Pereira P et al (2011) Evidence of tricellulin expression by immune cells, particularly microglia. Biochem Biophys Res Commun 409:799–802
Masada T, Hua Y, Xi G et al (2003) Overexpression of interleukin-1 receptor antagonist reduces brain edema induced by intracerebral hemorrhage and thrombin. Acta Neurochir Suppl 86: 463–467
Matsumoto J, Dohgu S, Takata F et al (2012) Lipopolysaccharide-activated microglia lower P-glycoprotein function in brain microvascular endothelial cells. Neurosci Lett 524:45–48
Mattison HA, Nie H, Gao H et al (2013) Suppressed pro-inflammatory response of microglia in CX3CR1 knockout mice. J Neuroimmunol 257:110–115
McGeer PL, Itagaki S, Boyes BE et al (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38:1285–1291
Mizoue LS, Bazan JF, Johnson EC et al (1999) Solution structure and dynamics of the CX3C chemokine domain of fractalkine and its interaction with an N-terminal fragment of CX3CR1. Biochemistry 38:1402–1414
Mizutani M, Pino PA, Saederup N et al (2012) The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. J Immunol 188:29–36
Muldoon LL, Alvarez JI, Begley DJ et al (2013) Immunologic privilege in the central nervous system and the blood-brain barrier. J Cereb Blood Flow Metab 33:13–21

Nakajima K, Kohsaka S (2001) Microglia activation and their significance in the central nervous system. J Biochem 130:169–175
Nemeth ZH, Deitch EA, Davidson MT et al (2004) Disruption of the actin cytoskeleton results in nuclear factor-kappaB activation and inflammatory mediator production in cultured human intestinal epithelial cells. J Cell Physiol 200:71–81
Neniskyte U, Neher JJ, Brown GC (2011) Neuronal death induced by nanomolar amyloid beta is mediated by primary phagocytosis of neurons by microglia. J Biol Chem 286:39904–39913
Neumann J, Sauerzweig S, Ronicke R et al (2008) Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. J Neurosci 28:5965–5975
Nicoll JA, Wilkinson D, Holmes C et al (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med 9:448–452
Nielsen HH, Ladeby R, Fenger C et al (2009) Enhanced microglial clearance of myelin debris in T cell-infiltrated central nervous system. J Neuropathol Exp Neurol 68:845–856
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318
Nishioku T, Matsumoto J, Dohgu S et al (2010) Tumor necrosis factor-alpha mediates the blood-brain barrier dysfunction induced by activated microglia in mouse brain microvascular endothelial cells. J Pharmacol Sci 112:251–254
O'Keefe GM, Nguyen VT, Benveniste EN (2002) Regulation and function of class II major histocompatibility complex, CD40, and B7 expression in macrophages and microglia: implications in neurological diseases. J Neurovirol 8:496–512
Olson JK, Miller SD (2004) Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 173:3916–3924
Pardridge WM (2003) Blood-brain barrier drug targeting: the future of brain drug development. Mol Interv 3(90–105):51
Paloneva J, Kestilä M, Wu J, Salminen A, Böhling T, Ruotsalainen V, Hakola P, Bakker AB, Phillips JH, Pekkarinen P, Lanier LL, Timonen T, Peltonen L (2000) Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat Genet 25(3):357–61, PMID: 10888890
Parwaresch MR, Wacker HH (1984) Origin and kinetics of resident tissue macrophages. Parabiosis studies with radiolabelled leucocytes. Cell Tissue Kinet 17:25–39
Pineau I, Lacroix S (2007) Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500:267–285
Ponomarev ED, Novikova M, Maresz K et al (2005) Development of a culture system that supports adult microglial cell proliferation and maintenance in the resting state. J Immunol Methods 300:32–46
Pontillo A, Catamo E, Arosio B et al (2012) NALP1/NLRP1 genetic variants are associated with Alzheimer disease. Alzheimer Dis Assoc Disord 26:277–281
Prinz M, Priller J (2010) Tickets to the brain: role of CCR2 and CX(3)CR1 in myeloid cell entry in the CNS. J Neuroimmunol 224:80–84
Prinz M, Priller J, Sisodia SS et al (2011) Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. Nat Neurosci 14:1227–1235
Prodinger C, Bunse J, Kruger M et al (2011) CD11c-expressing cells reside in the juxtavascular parenchyma and extend processes into the glia limitans of the mouse nervous system. Acta Neuropathol 121:445–458
Pulliam L, Moore D, West DC (1995) Human cytomegalovirus induces IL-6 and TNF alpha from macrophages and microglial cells: possible role in neurotoxicity. J Neurovirol 1:219–227
Rambach G, Dum D, Mohsenipour I et al (2010) Secretion of a fungal protease represents a complement evasion mechanism in cerebral aspergillosis. Mol Immunol 47:1438–1449
Ransohoff RM (2002) The chemokine system in neuroinflammation: an update. J Infect Dis 186(suppl 2):S152–S156

Ransohoff RM (2009) Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. Immunity 31:711–721
Ransohoff RM, Engelhardt B (2012) The anatomical and cellular basis of immune surveillance in the central nervous system. Nat Rev Immunol 12:623–635
Ravindran C, Cheng YC, Liang SM (2010) CpG-ODNs induces up-regulated expression of chemokine CCL9 in mouse macrophages and microglia. Cell Immunol 260:113–118
Regnier-Vigouroux A (2003) The mannose receptor in the brain. Int Rev Cytol 226:321–342
Remington LT, Babcock AA, Zehntner SP et al (2007) Microglial recruitment, activation, and proliferation in response to primary demyelination. Am J Pathol 170:1713–1724
Renner NA, Sansing HA, Morici LA et al (2012) Microglia activation by SIV-infected macrophages: alterations in morphology and cytokine secretion. J Neurovirol 18:213–221
Reynolds ND, Lukacs NW, Long N et al (2011) Delta-like ligand 4 regulates central nervous system T cell accumulation during experimental autoimmune encephalomyelitis. J Immunol 187:2803–2813
Ribes S, Ebert S, Regen T et al (2010) Toll-like receptor stimulation enhances phagocytosis and intracellular killing of nonencapsulated and encapsulated Streptococcus pneumoniae by murine microglia. Infect Immun 78:865–871
Rivest S (2009) Regulation of innate immune responses in the brain. Nat Rev Immunol 9: 429–439
Rogers J, Lue LF (2001) Microglial chemotaxis, activation, and phagocytosis of amyloid beta-peptide as linked phenomena in Alzheimer's disease. Neurochem Int 39:333–340
Rogers JT, Morganti JM, Bachstetter AD et al (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J Neurosci 31:16241–16250
Rolls A, Shechter R, London A et al (2007) Toll-like receptors modulate adult hippocampal neurogenesis. Nat Cell Biol 9:1081–1088
Rossi DL, Hardiman G, Copeland NG et al (1998) Cloning and characterization of a new type of mouse chemokine. Genomics 47:163–170
Ryu KY, Cho GS, Piao HZ et al (2012) Role of TGF-beta in survival of phagocytizing microglia: autocrine suppression of TNF-alpha production and oxidative stress. Exp Neurobiol 21: 151–157
Saederup N, Cardona AE, Croft K et al (2010) Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One 5:e13693
Santiago C, Ballesteros A, Martinez-Munoz L et al (2007) Structures of T cell immunoglobulin mucin protein 4 show a metal-ion-dependent ligand binding site where phosphatidylserine binds. Immunity 27:941–951
Saukkonen K, Sande S, Cioffe C et al (1990) The role of cytokines in the generation of inflammation and tissue damage in experimental gram-positive meningitis. J Exp Med 171:439–448
Savill J, Gregory C (2007) Apoptotic PS to phagocyte TIM-4: eat me. Immunity 27:830–832
Schenk D (2008) Current challenges for the successful treatment and prevention of Alzheimer's disease: treating the pathologies of the disease to change its clinical course. Alzheimers Dement 4:S119–S121
Schmid CD, Melchior B, Masek K et al (2009) Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. J Neurochem 109(suppl 1): 117–125
Schwab JM, Schluesener HJ, Seid K et al (2001) IL-16 is differentially expressed in the developing human fetal brain by microglial cells in zones of neuropoesis. Int J Dev Neurosci 19:93–100
Selenica ML, Alvarez JA, Nash KR et al (2013) Diverse activation of microglia by chemokine (C-C motif) ligand 2 overexpression in brain. J Neuroinflammation 10:86
Shaked I, Porat Z, Gersner R et al (2004) Early activation of microglia as antigen-presenting cells correlates with T cell-mediated protection and repair of the injured central nervous system. J Neuroimmunol 146:84–93

Shaw PJ, Lukens JR, Burns S et al (2010) Cutting edge: critical role for PYCARD/ASC in the development of experimental autoimmune encephalomyelitis. J Immunol 184:4610–4614
Shi F, Yang L, Kouadir M et al (2012) The NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation. J Neuroinflammation 9:73
Shiratori M, Tozaki-Saitoh H, Yoshitake M et al (2010) P2X7 receptor activation induces CXCL2 production in microglia through NFAT and PKC/MAPK pathways. J Neurochem 114: 810–819
Shutter JR, Scully S, Fan W et al (2000) Dll4, a novel Notch ligand expressed in arterial endothelium. Genes Dev 14:1313–1318
Sierra A, Gottfried-Blackmore AC, McEwen BS et al (2007) Microglia derived from aging mice exhibit an altered inflammatory profile. Glia 55:412–424
Sierra A, Abiega O, Shahraz A et al (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. Front Cell Neurosci 7:6
Sokolowski JD, Mandell JW (2011) Phagocytic clearance in neurodegeneration. Am J Pathol 178:1416–1428
Stalder AK, Pagenstecher A, Yu NC et al (1997) Lipopolysaccharide-induced IL-12 expression in the central nervous system and cultured astrocytes and microglia. J Immunol 159:1344–1351
Stevens B, Allen NJ, Vazquez LE et al (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131:1164–1178
Streit WJ (2002) Microglia and the response to brain injury. Ernst Schering Res Found Workshop (39):11–24
Streit WJ, Davis CN, Harrison JK (2005) Role of fractalkine (CX3CL1) in regulating neuron-microglia interactions: development of viral-based CX3CR1 antagonists. Curr Alzheimer Res 2:187–189
Su X, Maguire-Zeiss KA, Giuliano R et al (2008) Synuclein activates microglia in a model of Parkinson's disease. Neurobiol Aging 29:1690–1701
Sumi N, Nishioku T, Takata F et al (2010) Lipopolysaccharide-activated microglia induce dysfunction of the blood-brain barrier in rat microvascular endothelial cells co-cultured with microglia. Cell Mol Neurobiol 30:247–253
Sutton C, Brereton C, Keogh B et al (2006) A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J Exp Med 203: 1685–1691
Tambuyzer BR, Ponsaerts P, Nouwen EJ (2009) Microglia: gatekeepers of central nervous system immunology. J Leukoc Biol 85:352–370
Tanaka T, Ueno M, Yamashita T (2009) Engulfment of axon debris by microglia requires p38 MAPK activity. J Biol Chem 284:21626–21636
Trapp BD, Nave KA (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269
Trapp BD, Ransohoff R, Rudick R (1999) Axonal pathology in multiple sclerosis: relationship to neurologic disability. Curr Opin Neurol 12:295–302
Trebst C, Sorensen TL, Kivisakk P et al (2001) CCR1+/CCR5+ mononuclear phagocytes accumulate in the central nervous system of patients with multiple sclerosis. Am J Pathol 159:1701–1710
Trebst C, Staugaitis SM, Kivisakk P et al (2003) CC chemokine receptor 8 in the central nervous system is associated with phagocytic macrophages. Am J Pathol 162:427–438
Underhill DM, Ozinsky A, Hajjar AM et al (1999) The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 401:811–815
van Horssen J, Singh S, van der Pol S et al (2012) Clusters of activated microglia in normal-appearing white matter show signs of innate immune activation. J Neuroinflammation 9:156
Wainwright DA, Xin J, Sanders VM et al (2008) Differential actions of pituitary adenylyl cyclase-activating polypeptide and interferon gamma on Th2- and Th1-associated chemokine expression in cultured murine microglia. J Neurodegener Regen 1:31–34
Williams K, Ulvestad E, Waage A et al (1994) Activation of adult human derived microglia by myelin phagocytosis in vitro. J Neurosci Res 38:433–443
Williams K, Dooley N, Ulvestad E et al (1996) IL-10 production by adult human derived microglial cells. Neurochem Int 29:55–64

Wilms H, Rosenstiel P, Sievers J et al (2003) Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease. FASEB J 17:500–502

Wilson EH, Weninger W, Hunter CA (2010) Trafficking of immune cells in the central nervous system. J Clin Invest 120:1368–1379

Wraith DC, Nicholson LB (2012) The adaptive immune system in diseases of the central nervous system. J Clin Invest 122:1172–1179

Xiang Z, Chen M, Ping J et al (2006) Microglial morphology and its transformation after challenge by extracellular ATP in vitro. J Neurosci Res 83:91–101

Xiong J, Burkovetskaya M, Karpuk N et al (2012) IL-1RI (interleukin-1 receptor type I) signalling is essential for host defence and hemichannel activity during acute central nervous system bacterial infection. ASN Neuro 4:e00085

Zhang GX, Li J, Ventura E et al (2002) Parenchymal microglia of naive adult C57BL/6J mice express high levels of B7.1, B7.2, and MHC class II. Exp Mol Pathol 73:35–45

Zhou H, Lapointe BM, Clark SR et al (2006) A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. J Immunol 177:8103–8110

Zierler S, Frei E, Grissmer S et al (2008) Chloride influx provokes lamellipodium formation in microglial cells. Cell Physiol Biochem 21:55–62