Microglia: the physiology and its role in the aging brain

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Microglia are resident immune cells of the CNS and have a distinct phenotype in the healthy brain, induced by 'off' signals expressed by neurons and astrocytes. In reaction to disturbance in the CNS, microglia change their morphology and phenotype towards a beneficial or detrimental state, thereby regulating the disease pathology. Microglial activation is very diverse and dynamic, regulated by 'on' signals, also released by neurons and astrocytes. Normal aging of the brain is accompanied with increased microglial activation and enlarged microglial subtypes, increasing the risk of neurogenerative diseases. A better comprehension of microglial behavior will be necessary in understanding the pathogenesis of ischemia and neurogenerative diseases, in order to develop effective therapies.

Keywords; microglia, phenotypes, activation, signaling, neurodegenerative disease, aging.

Introduction

Microglia are resident tissue macrophages of the central nervous system (CNS), including the spinal cord and represent a part of the innate immune response (Mariani *et al.,* 2009). They are abundant within the brain and represent around 5-20% of the total glial population (Yang *et al.,* 2010). The highest density is found in areas including hypothalamus, hippocampus and basal ganglia (Figure. 1). The lowest concentrations are found in areas such as cerebellum and the brain stem (Savchenko *et al.,* 1997).

Microglia recognize a wide array of infectious pathogens in the CNS, partly which their Toll-like receptors (TLRs), that sense highly conserved microbial pathogen associated molecular patterns (PAMPs) (Kielian *et al.,* 2006). In contrast to PAMPs produced by microorganisms, only a few proteins, like Heat shock protein (HSP) 60, HSP70 and fibrogen, are believed to act as ligand for TLRs during neural damage. Recognition of these endogenous ligands generates an immune response, thereby creating an environment that is more permissive of restricting the neural damage and facilitating repair (Soulet & Rivest, 2008).

The phenotype of microglia is tightly regulated in a normal healthy CNS. During infection, microglia can rapidly change their morphology and expression of various molecules because of neural damage and the presence of endogenously produced toxic proteins, (Kreutzberg *et al.,* 1996; Hanisch & Kettenmann, 2007). Their phenotype depends on the sequence and duration of their exposure to diverse stimuli in different pathologies (Perry *et al.,* 2010). In the 'resting' state microglia are highly branched, also called ramified. Microglia seem to lose their ramifications and become more amoeboid during chronic immune challenges (Kreutzberg *et al.,* 1996).

Once activated, microglia upregulate their major histocompatibility complex (MHC) class I and class II expression and costimulatory molecules, that contribute to both CD4-specific and CD8-specific T cell responses (Aloisi *et al.,* 1999). There are two types of microglia activity: neurotoxic and neuroprotectitive. Neurotoxic activity is caused by TLR ligands, including

bacterial lipopolysacharides (LPS) and might occur after excessive and uncontrolled stimulation of microglia or when microglia function is declined (Badoer *et al.,* 2010). Neuroprotective activity, on the other hand, might be induced by interleukin (IL)-4, which stimulates cell renewal (Kim & de Vellis, 2005; Badoer *et al.,* 2010).

In disorders like Alzheimer and Parkinson's disease, microglia activation is prominent around areas of neurodegeneration (Glass *et al.,* 2010). Microglia participate in initiation and progression in neurodegenerative diseases by releasing cytotoxic molecules such as pro-inflammatory cytokines, reactive oxygen species (ROS), proteinases and complement proteins (Aloisi *et al.,*1999). There is also evidence that neurons inform microglia about their status and that neurons are capable of controlling microglia function (Biber et *al.,* 2007).

In this review I highlight the activation and deactivation mechanism of microglia and its communication with other cells, and draw attention to the fact that microglia are highly plastic and have different phenotypes. I also focus on microglia activity in the aging brain in understanding its relationship with neurogenerative diseases.

Microglial phenotypes

The phenotype of microglia is different from other tissue macrophages. For this reason it took very long to establish the myeloid origin of microglia (Ransohoff & Perry, 2009). Both, perivascular and parenchymal microglial cells derive from myeloid progenitors orginate from the yolk sac (Figure 2). The progenitors establish themselves in the developing brain and in retina during embryonic stage, where they develop in immature microglia (Herbomel *et al.,* 2001). Adult microglia might be maintained by self replication, or by division of progenitor cells which are present in the brain. Another explanation is that circulating progenitor cells are able to pass the blood-brain barrier (BBB) and once in the CNS the progenitors differentiate into microglia cells (Ransohoff & Perry, 2009).

Several years ago it was found that there are various subtypes of macrophages. These can generally be divided into M1 (classically activated via toll-like receptor or via interferon-gamma (IFN-γ)) and M2 (alternatively activated macrophages), which further subdivided in M2a (induced by IL-4 or IL-13), M2b (induced by immune complexes in combination with IL-1β or LPS) and M2c (induced by IL-10, TGFβ or glucocorticoids). It is known that these subtypes have different effects within the immune system. M1 have pro-inflammatory properties, whereas M2 have anti-inflammatory properties (Martinez *et al,* 2008; Raes *et al.,* 2002; Song *et al.,* 2000). However, evidence for intrinsic specialisation of microglia is very rare. It seems that microglia can have different forms, each syntheses different subsets of cytokines en express different receptors. Microglial diversity depends on the durance and intensity of the exposure to external signals, which might lead to a phenotype, but not necessarily to a M1 or a M2 subtype (Hanisch en Kettenmann, 2007; Perry, 2010).

The micro-environment of the CNS plays an important role in the differentiation of the microglial phenotype (Figure 3). In the healthy brain microglia are highly ramified cells, also called 'resting' state. Many receptors on ramified microglia are expressed at low levels or even not at all (Pocock & Kettenmann, 2007). Research using 2-Photon microscopy showed that ramified microglia actively screen their micro-environment searching for 'danger'-signals, as well changes in these signals (Nimmerjahn *et al.,* 2005). Ramified microglia are therefore no 'resting' cells and should be renamed as 'surveying' cells (Hanisch and Kettenmann, 2007).

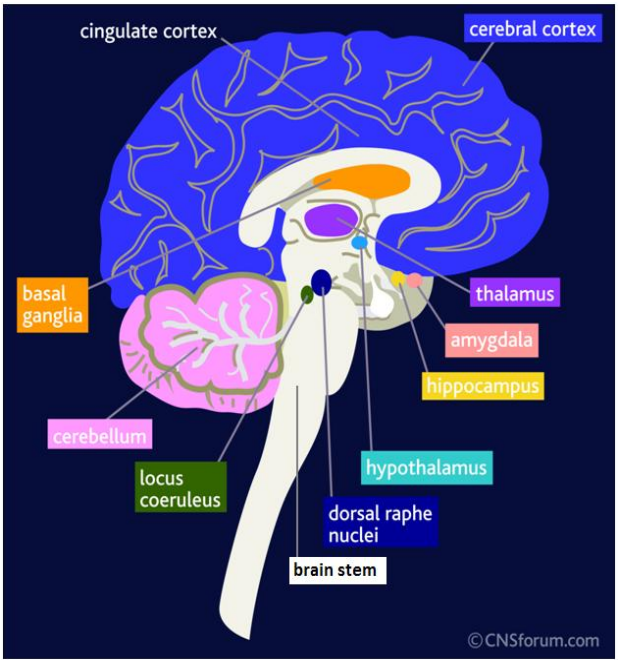

Figure 1. Brain regions and microglial expression. High microglial expression in hypothalamus, hippocampus and basal ganglia. Low microglial expression in cerebellum and brain stem (altered figure from www.cnsforum.com).

In response to disturbance of nervous system homeostasis and neural injury, microglia can proliferate. Activated microglial cells highly increase the expression of receptors, including glutamate, gamma-aminobutyric acid (GABA) cytokine, and purine receptors (Pocock & Kettenmann, 2007). Microglia also seem to become more phagocytic, macrophage like cells and move towards the site of the injury (Kreutzberg *et al.*, 1996). This response depends on purinoreceptor stimulation and may also involve assistance from astrocytes (Haynes *et al.*, 2006; Hanisch & Kettenmann, 2007).

Microglial activation leads to different response phenotypes. During bacterial invasion the microglia release inflammatory mediators and are involved in het phagocytosis of bacteria. Interestingly, when apoptotic cells or myelin debris are removed, microglia release anti-inflammatory mediators. Two important signaling pathways are involved in microglial responsiveness. First one is the sudden appearance of factors, such as TLRs, that includes induced receptor signaling (Kielian *et al.*, 2006). Second is constructive signaling, such as ligand pairs CX3CL1-CX3CR1 and CD200-CD200R (discussed below), which have a calming effect (Wang *et al.*, 2007; Hoek et al., 2000). Microglia also express neurotransmitter receptors, like GABA or adrenalin. Its activation causes an anti-inflammatory response (Pocock & Kettenmann 2007).

Microglial activation by IFN-γ and IL-4 induces a T helper type 1 and 2 (TH1 and TH2) response, respectively. High IL-4 and low IFN-γ levels support microglia-induced adult oligodendrogenesis and neurogenesis (Butovsky *et al.*, 2006). It also offers neuroprotection by the regulation of insulin-like growth factor (IGF)-I and tumor necrosis factor (TNF)-α. Treatment with high concentrations of IFN-γ or LPS treatment, on the other hand, reduces the neutrophilic and TH1 attracting signals, and might thereby reduce the microglia-induced cell renewal. IL-4 activation of microglia can reverse this reaction. This shows that microglia actions are diverse and stimulus-related, depending on the stimulus intensity and context. Microglia also communicate via adaptive immunity by exchanging soluble messengers, including cytokines, and by physical contact, including MHC-II, that present antigens (Butovsky *et al.*, 2006; Hanisch & Kettenmann, 2007).

Protective and detrimental activity

Microglial activation can lead to detrimental or protective activity. Inflammation can induce brain damage and might lead to the activation of microglia, which is a protective value. Inflammatory cytokines may be neuroprotective via direct activation of NF-κβ in neurons, which initiate an innate immune response (Glezer *et al.*, 2007). By contrast, fully activated microglia are believed to be toxic, by releasing ROS, nitric oxide (NO) and cytokines like IL-1β, TNF-α and IFN-γ that can damage

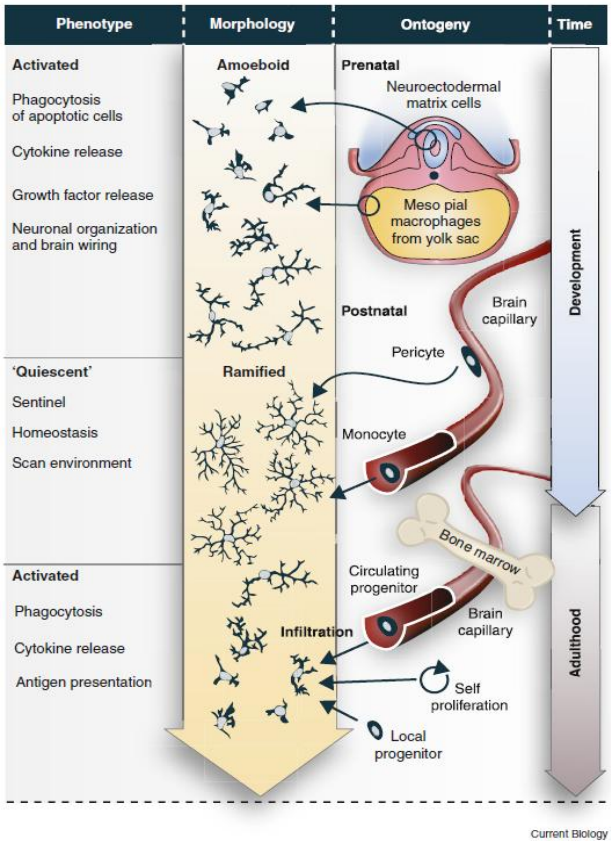

Figure 2. Various phenotypes and morphologies of microglia during development and adulthood under both, normal and inflammatory conditions (Soulet & Rivest, 2008).

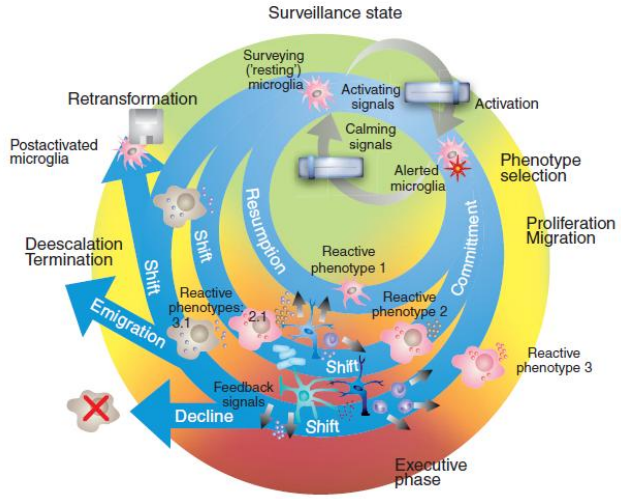

Figure 3. Activation process of microglia during different stages of activation. Microglial cells in the surveillance state ('resting' state) actively scan the environment for 'danger'-signals. The appearance of these 'danger'-signals (e.g., in infection, ischemia or trauma) leads to transition of microglia to an alert state. The signals and their context are interpreted and can initiate an 'activation' response. Microglia differentiate into different phenotypes, depending on the transcription factors and enter their exclusive phase. Reactive phenotypes may shift into a different phase (more than the three phases that are shown). Some of the microglial cells may emigrate to the bloodsystem or die, while other microglia may return to a surveying state. Some microglia may not return to a complete 'resting' state and may remain postactivated, as 'memory' cells (Hanisch & Kettenmann, 2007).

the brain tissue. Microglial toxic activity often includes stimuli eliciting defence-oriented reactions like LPS (Block *et al.*, 2007; Kim & de Vellis, 2005).

In demyelinating disorders, like multiple sclerosis (MS), reduction of microglial activation can attenuate the disease development. Experimental autoimmune encephalitis, the primary animal model of MS, is repressed by ganciclovir-induced microglial paralysis (Greter *et al.*, 2005; Heppner *et al.*, 2005). According to the authors microglial paralysis might limit the CD4+ T cells autoreactivity which might reduce inflammation lesions and limit demyelinisation. Suppression of microglia activation can impair remyelination by facilitating oligodendrocyte progenitor cells (OPCs) recruitment and altered growth factor expression, promoting the myeline formation (Kotter *et al.*, 2005).

Neural damage might also be limited by another microglial mechanism. Glutamate is a neurotoxic substance, which is involved in neurogenerative diseases and ischemia. LPS activated microglia can express glutamate uptake protein (GLT-1), induced by TNF-α stimulation (Persson *et al.*, 2005). This implies that microglia could be important for controlling the glutamate levels and could thereby improve neural survival (Hanisch & Kettenmann, 2007).

In Parkinson's disease (PD), microglial activation seems to cause neural damage. Profound loss of dopaminergic neurons is often found in the substantia nigra accompanied by

high numbers of microglia in this area (Amor *et al.,* 2010). The IFN-γ plasma levels of these patients are increased. High IFN-γ concentration induces dopaminergic neural death, by regulating microglial activity. This effect can be reduced by the immunosuppresive cytokine IL-10. In the study of Qian et al, IL-10 significantly decreased LPS-induced degeneration of dopaminergic neurons, by inhibiting the microglial production of TNF-α and the microglial induced oxidative stress (Qian *et al.,* 2006). This study shows that in PD activation of microglia induces neural damage.

Microglia have a dual role in Alzheimer disease. They can be neuroprotective by degradating beta-amyliod (Aβ)-plaques via secreted nonmatrix matalloprotease (Qui *et al.,* 1997, Yan *et al.,* 2003). Microglia and macrophages have different abilities to phagocyte Aβ-plaques. Microglial lysosomes are less acid compared to those of macrophages, which impairs the fibrillary Aβ degradation (Simard *et al.,* 2004). Proinflammatory cytokines decrease the microglial phagocytic activity, suggesting that microglia have reduced phagocytotic capacity during inflammatory response (Koenigsknecht-Talboo & Landreth, 2005; Hanisch & Kettenmann, 2007). Minocycline, an anti-inflammatory drug, improved the behavioral performance, by reducing the numbers of activated microglia (Fan *et al.,* 2007). Reactive microglia in AD brain also produce highly cytotoxic substances, like proinflammatory cytokines, reactive oxygen intermediates, proteinases, and complement proteins, contributing to neural dysfuntion and cell death (Griffin *et al.,* 1998). Anti-inflammatory mediators may partly antagonize the neuroprotective functioning of microglia, contributing to the disease progression and chronicity (Henneka & 0'Banion, 2007).

Microglia seem to be involved in neurodegeneration, as well as neuro-regeneration. Inflammation associated microglia reduces neurogenesis, whereas T-cell activated microglia promote the neurogenesis (Butovsky *et al,* 2006). Enhanced microglial production of TNF-α can lead to neural apoptosis (Gonz'alez-Scarano & Baltuch, 1999). Microglial cells can remove denditic structures, probably through microglial phagocytosis. In CXCR-3 deficient mice no microglial migration towards lesion site and no removal of dendritic cells is found (Rappert *et al.,* 2004). It was suggested that removal of non-functional neural structures is beneficial to the brain. It creates space for new connections, which might help to the generation of neural structures (Hanisch & Kettermann, 2007).

Ion channels and microglial activity

A wide variety of ion channels has been identified and characterized in microglia. Most studies have been performed in cultured primary cells or in cell lines, only are carried out in brain slices. Microglia can express ion channels, including K+, H+, Na+, Ca2+ and Cl^-, depending on the functional state of microglia (Eder *et al.,* 1998). For example, the expression of voltage K+ channels is increased in activated microglia (Eder *et al.,* 1996). Voltage- and/or Ca2+ activated K+ channels regulate the proliferation, migration and the cytokine production of microglia. Delayed rectifier (DR) K+ channels might be involved in repolarizing the microglial membrane potential after depolarizing events. Membrane hyper-polarisation might initiate microglial proliferation, migration, cytokine secretion or phagocytosis (Gallin, 1991; Lewis & Cahalan, 1995; Zhou *et al.,* 1998). A negative membrane potential is essential for maintaining the driving force for Ca2+ influx through calcium release activated Ca21 (CRAC) channels, which eventually leads to the regulation of the microglial gene expression. So K+ channels are involved in the regulation of the functional state of microglia (Lewis & Cahalan, 1995; Eder *et al.,* 1998).

Two types of Ca2+ channels are detected in microglia, CRAC channels and voltage-gated Ca2+ channels (Colton *et al.,* 1994; Nörenberg et al., 1997). Voltage-gated Ca2+ channels seem to be involved in microglial superoxide formation. In lymphocytes the elevated Ca2+ levels are essential for proliferation and gene expression (Lewis & Cahalan, 1988). Chronic increased basal Ca2+ levels reduces the receptor-triggerd Ca2+ signaling. Besides, high Ca2+ levels are required for the release of NO and cytokines. This could have an important role in some executive functions in activated microglia (Hoffmann *et al.,* 2003, Farber & Kettenmann, 2005).

The expression of microglial Na+ channels is regulated by astrocytes. These channels are mainly expressed in ramified microglia (Sievers *et al.,* 1994). Sodium channel activation results in rapid membrane depolarization, which seems to trigger signaling cascades for microglial activation. This might lead to an immune response, such as microglial phagocytic activity, migration and cytokine release (Eder, 2010).

Proton channels in microglia are modulated by cytoskeletal disruptive agents (Eder *et al.,* 1998). Modulation of H+ channels due to cytoskeletal reorganization leads to a decrease in current density and an increase in activation time constant after LPS stimulation. Therefore H+ channel activity is also required de-ramplification of microglia, leading to a more activated state (Schilling *et al.,* 2004). During phagocytosis, proton channels are involved in H+ extrusion. Proton channels may help to maintain the membrane potential of microglia and pH by charge compensation during NADPH oxidase mediated respiratory burst (Eder and DeCoursey, 2001). By compensating the electron-efflux and withstand internal acidification, proton channels might also help to maintain the superoxide formation (Henderson *et al.,* 1993).

Chloride channels have an important role in ROS-formation, phagocytosis and ramification of microglia (Eder, 1998). Furthermore, Cl^- channels are involved in microglial proliferation and migration (Hines *et al.,* 2009). Microglial Cl^- channels might be related to shift in resting brain potential. This shift to more positive potential, by Ca2+ influx, near the Cl^- reversal potential might regulate several microglial functions (Eder, 1998). One of these functions might be microglia chemotaxis towards the injury site, contributing to the reduction of brain lesions (Rappert *et al.,* 2002; Hines *et al.,* 2009).

Signaling of microglia

Microglia can sense neural activity based on neural transmitter concentrations, like NO, TNF-α and IL-6, by expressing neural transmitter receptors (van Rossum & Hanisch, 2004). As mentioned above, ion channel activity is closely related to the functional state of microglia. In the healthy brain the activation of microglia is restricted. Once activated, microglial immune functions are rapidly turned down to prevent secondary neural damage (Galea *et al.,* 2007). This immune function is regulated by so-called ‘On’ and ‘Off’ signals. Off signals are found in the healthy brain and their disappearance causes a microglial response. On signals act and operate by appearance and initiate a pro- or anti-inflammatory microglial immune response (van Rossum & Hanisch, 2004). On and off signal act in a different way, but have not necessary different functions (Figure 4 & 5; Biber *et al.,* 2007). For example, microglial phagocytosis can be induced by absence of off signals, as well as in presence of on signals (discussed below). These two classes of signals contribute to both, protective and damaging effects of microglial activation.

Off signals

Members of the immunoglobulin superfamily (IgSF) suppress microglial immune

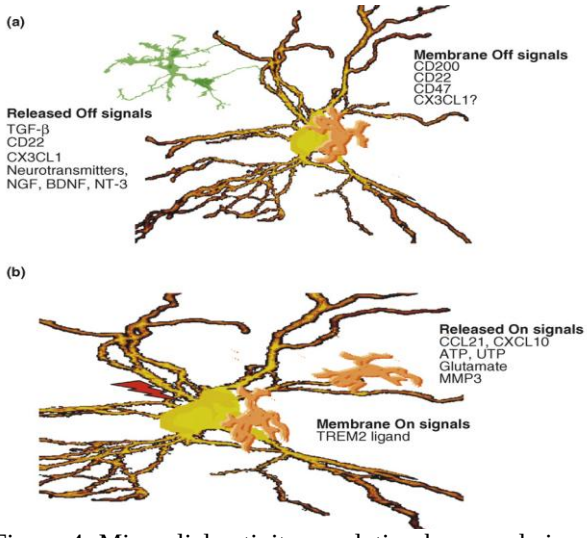

Figure 4. Microglial activity regulation by neural signals. (a) Off signals, contrittutively found in healthy neurons, reduce microglial activity under inflammatory conditions (red). Released Off signals can be CX3CL1, TGF-β and various neuro-transmitters. Membrane bound Off signals, like IGSF molecules, are probably involved in the interaction between activated microglia and neurons at the injury site. Whether synaptic activity is involved in the release of Off signals is still unclear. (b) On signals are expressed by impaired or damaged neurons (red lightning bolt). Releases On signals, including chemokines, purines and glutamate, can initiate both microglial protective or detrimental activity. The release site for neural signals is still unknown. The membrane bound On signals, like TREM2, expressed by apoptotic neurons intiate microglia phagocytosis (Biber *et al.*, 2007).

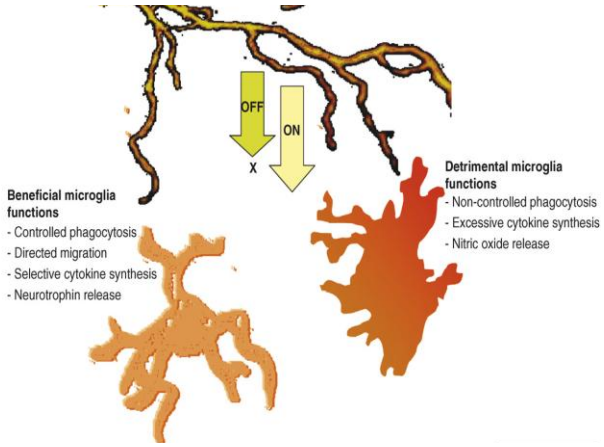

Figure 5. On and Off signals act in a different way, but do not have necessary different functions. Off signals are constitutively released by neurons, act by disappearance. On signals, expressed by overactive and damaged neurons and act by appearance. Both signal classes contribute to beneficial or detrimental functions of microglia (Biber *et al.*, 2007).

function (Figure 4a) and might work under inflammatory conditions. CD200 and CD47, both members of IgSF, are highly expressed at neural membrane surface. Microglial cells contain CD200 receptors. In CD200-deficient mice microglia are less ramified, they have disordered arrangement and a shorter glial processes (Hoek *et al.*, 2000).

The increased microglial response is confirmed by upregulation of MHCII antigen expression, iNOS and TNF-α production (Broderick *et al.*, 2002, Deckert *et al.*, 2006). These findings suggest that CD200 has an inhibitory function in brain inflammation (Hoek *et al.*, 2000). In response to neural damage, the neural membranes release CD22, which inhibits microglial proinflammatory cytokine production. Treatment with CD22 antibodies increased the microglial activation, suggesting that CD22 mediates, at least in part, the reduction of microglial TNF-α production (Mott *et al.*, 2004). CD47 is expressed by astrocytes, endothelial cells, and neurons, whereas its receptor CD172a (SIRPa or SHPS-1) is present on microglia and neurons but not on astrocytes (Reinhold *et al.*, 1995). Activation of CD172a downregulates microglial phagocytosis, TNF-α expression and inhibited migration of neutrophils across the BBB (Latour *et al.*, 2001; Tan *et al.*, 2000; de Vries *et al.*, 2002), leading to the downregulation of the microglial inflammatory response.

Complement regulators, including CD31 and CD46, are expressed on neurons, astrocytes oligodendrocytes and microglia (Elward & Gasque, 2003). In apoptotic cells, CD46 was released from the membrane surface, making the cell a target for phagocytosis (Elward *et al.*, 2005). CD31 and CD46 provide a detachment signal. Therefore, the presence of CD31 and CD46 on neural cells inhibits their phagocytosis by microglia. (Brown *et al.*, 2002; Elward &

Gasque, 2003; Elward *et al.,* 2005). This 'don't eat me' signaling and inhibition of the inflammatory response that can promote tissue repair.

CX3CL is a chemokine that is constitutively expressed by healthy neurons and astrocytes, respectively (Harrison *et al.,* 1998). Its receptor is found in microglia and CX3CL is involved in microglia activation. Neural damage induces CX3CL upregulation in microglia (Zhuang *et al.,* 2007). CX3CL registration activates p38 MAPK in spinal cord (Clark *et al.,* 2007) and injection with antibody against CX3CL reduced p38 activation (Zhuang *et al.,* 2007). Furthermore, CX3CL deficiency in mice is associated with increased neural death and microglial activity (Rappert *et al.,* 2004). These results indicate that CX3CL reduces the inflammatory neural death, by suppressing microglial activity by activating p38MAPK.

Neurotransmitters, except glutamate, inhibit the release of various proinflammatory molecules, including IL-1β, TNF-α and chemokines, depending on the type of neurotransmitter. These findings imply that microglia can sense neuronal activity by local neurotransmitter levels (Faber & Kettenmann, 2005). This off signaling reduces the microglial release of proinflammatory molecules. Transforming growth factor (TGF)-β, an anti-inflammatory cytokine, is also primary expressed by neurons. Microglial activity was found to be increased in TGF-β-deficient mice, indicating that TGF-β might contribute to off-signaling in the brain (Brionne *et al.,* 2003; Biber *et al.,* 2007).

On signals

Purines are potential on signals (Figure 4b) and are released by damaged or overactive neurons (Wang *et al.,* 2004). P2Y12 receptor senses purine release at an early fase after neural injury. P2X12 blocker Pertussis toxin (PTX) inhibits the ruffling. Small G-protein Rac activated by ATP and ADP stimulation, is also found to be down-regulated after PTX treatment (Fumagalli *et al.,* 2004). Extracellular ATP acts as a chemo-attractant for microglia (Haynes et al., 2006). Taken together, it seems that ruffling and chemotaxis of microglia, regulated by ATP or ADP, are mediated by P2X12. P2X7R activation also contributes to the activation of multiple microglia responses induced by ATP. Activation of P2X7R, induces secretion of pro-inflammatory cytokines and chemokines, leading to the formation of membrane pores in microglial cells (Ferrari *et al.,* 1997). This results in apoptotic death of microglia. Interestingly, low activation of P2X7R increased microglial proliferation (Bianco *et al.,* 2006). So P2X7R might have an important role in the regulation of microglial proliferation and cell death. The P2X6 receptor is upregulated in kainic acid induced neural injury. Kainic induced neural injury results in increased extracellular ATP, which is immediately metabolized into UDP. In damaged neurons, UTP leak causes phagocytosis (Koizumi *et al.,* 2007). ATP/UDP might act as a chemo-attractant for microglia, which remove the necrotic cells. ATP induced activation of p38 via P2X4 receptor leads to microglial release of brain-derived neurothropic factor (BDNF) (Trang *et al.,* 2009). BDNF induced central sensitization via dis-inhibiton (Coull *et al.,* 2005). Inhibition of ATP or P2X4 in mice prevented tactile allodynia, which is an abnormal hypersensitive reaction to harmless stimuli (Tsuda *et al.,* 2003; Tsuda *et al.,* 2005). Therefore P2X4 could be a therapeutic target for the prevention of neuropathic pain.

Excessive release of glutamate is associated with neurodegeneration (Farber & Kettenmann, 2005). Its release causes neural death and it act as an activation signal for microglia cells. Activation of glutamate receptor triggers the release of TNF-α, IL-6 and IL–β, via activation of p38 MAPK (Ji & Suter, 2007). TNF-α binding to microglial-derived Fas ligand leads to

neurotoxity (Taylor *et al.,* 2005). TNF-α enhances excitatory synaptic transmission by increased frequency of spontaneous excitatory postsynaptic currents and the amplitude of AMPA- or glutamate NMDA-induced currents, leading to central sensitization. Neuropathic pain sensitization can also manifest as a decrease in inhibitory synaptic transmission by IL-6 (Coull *et al.,* 2005; Kawasaki *et al.,* 2008). IL-1β can enhance excitatory synaptic transmission, as well as decreasing inhibitory synaptic transmission (Coull *et al.,* 2005; Kawasaki *et al.,* 2008).

In neuronal cells, matrix metalloproteinase (MMP)-3 participates in apoptotic signaling in response to cellular stress. MMP-3 triggers the microglial production of proinflammatory and cytotoxic molecules as well as MMP-3, which in turn contribute to neuronal damage. MMP-3 is upregulated in PD and AD (Cauwe *et al*., 2007; Horstmann *et al*,. 2010). It has been shown that MMP-2, MMP-3 and MMP-9 mediate the BBB disruption, which allows infiltration of neutrophils into the brain (Gasche *et al*,. 2001; Gurney *et al.,* 2006). This can lead to neural inflammation and neural death.

Activation of triggering receptors expressed on myeloid cells (TREM)-2 increases phagocytotic activity of microglia. Resent studies have shown that activation of the TREM2 receptor induced phosphorylation of DNAX activating protein of 12 kDa (DAP12), as well as activation of microglial phagocytosis by triggering protein tyrosine kinase ERK (Bouchon et al., 2001; Hamerman *et al.,* 2006). Knock down of microglail TREM2 reduced phagocytosis of apoptotic neurons, but also increased the proinflammatory cytokine production of TNF-α and NO synthase-2 (Hamerman *et al.,* 2006). It was suggested that TREM2-DAP12 signaling mediate CNS homeostasis by reducing microglial inflammatory activity (Biber *et al.,* 2007). Furthermore, TREM2 regulates the migratory capacity of microglia by changing actin polymerization and cytoskeleton organization (Takahashi *et al.,* 2005).

Chemokines have an important role in the pathogenesis of neurodegenerative diseases like AD and MS, as well as in neurological disorders, like stroke and ischemia (Savarin-Vuaillat & Ransohoff, 2007). Under physiological conditions, the chemokines CXCL1, CXCL8, and CXCL12 modulate neurotransmitter release and regulate ion channel activity (Bertollini *et al.,* 2006). Chemokines are released by endangered neurons. Stimulation of TREM-2 increased the expression of chemokine receptor CCR7 (Bouchon *et al.,* 2001) and promoted migration towards its ligands, CCL19 and CCL21 (Takahashi *et al.,* 2005). CCL-21 activates microglia and induces neuropathic pain (Zhao *et al.,* 2007). CCL21 activates both CCR7 and CXCR3. CXCR3 and its ligand CXCL10 are expressed in neurons and astrocytes, in AD and MS brain (Xia et al., 2000; Filipovic et al., 2003). CXCR3 deficiency is strongly related to impaired microglial migration compared to wild type cells (de Jong *et al.,* 2005; Rappert *et al.,* 2004). These findings suggest that chemokines have an important role in the migration of microglia. Furthermore, CCL2 (or MCP-1) can be up-regulated in response to injury or degeneration. Its receptor activation on microglial cells induces chemotaxis. CCL2 deficiency is associated with decreased microglial activity, accompanied by transient improvement of neural survival (Muessel *et al.,* 2002). It is recently shown that intra-spinal CCL2 induced extensive microglial reaction in the ipsilateral spinal horn (Thacker *et al.,* 2009). These findings suggest that CCL2 is critical for spinal microglial activation and neurotoxic microglial activity.

Microglia in the aging brain

In recent study of Streit et al, dystrophic microglia were found in the human brain (Streit

et al., 2004). These microglia have abnormalities in their cytoplasmatic structure, such as deramification, atrophy, spheroid formation and fragmentation of processes (Figure 6). The amount of dystrophic microglia is increased in the older brain, suggesting that dystrophy is related to cell aging (Streit *et al.*, 2004). Under neuropathological conditions, such as schizophrenia and AD, microglia degeneration and death are upregulated, supporting the idea that microglia are involved in the pathogenesis of neurogenerative disease (Streit, 2006).

Normal aging of the brain is accompanied by increased activation of neuroglial cells. Electron microscopy showed progressive increase in microglial activation with age, while there is little change in the activation of astrocytes and oligidendrocytes (Vaughan & Peters, 1974). It is unclear why microglial activation significantly differs during aging. One possibility is inhibition of microglia and astrocyte proliferation at young age by TNF-β1, a strong anti-inflammatory cytokine. Proliferation was enhanced across the life span, suggesting that aging promotes a proliferative microenvironment of the brain (Rozovsky *et al.*, 1998; Vaughan & Peters, 1974). Age-related increased microglial activation was also consistent with decreased sensitivity to TGF-β1 (Rozovsky *et al.,* 1998), that might contribute to neurogenerative diseases.

Another possibility is induction of IL-1β and IL-10 in the aged brain. The imunnosuppresive cytokine IL-10 significantly reduced the microglial production of TNF-α, NO, ROS formation after LPS stimulation (Qian *et al.,* 2006). In contrast, IL-1β is a pro-inflammatory cytokine. Decreased IL-10 expression of aged related microglial activation, may lead to increased IL-β expression (Ye and Johnson, 2001; Chen *et al.,* 2008). Expression of MHC-II was upregulated in aged microglia and the most promentent induction of IL-β was found in these MHC-II+ microglia. Furthermore, LPS injection caused excessive microglial activation and protein induction of both IL-1β and IL-10 in aged microglia. Taken together, these findings show microglial hyperactivity in the aged brain and that this contributes to excessive neuroinflammation (Henry *et al.,* 2009).

Neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrathydropyridine (MPTP), that damages dopaminergic cells in PD brain, might also be involved in age-related microglial activation. MPTP is upregulated in the aged brain (Sugama *et al.,* 2003). Direct neural damage by MPTP leads to reactive gliosis. In return, microglial activation enhances the neurodegeneration, leading to the progression of diseases such as PD and AD (Liberatore *et al.,* 1999; Wu *et al.,* 2002). Age-related MPTP activation may be relevant to ROS formation upregulated in the aged brain, suggesting that ROS-formation might be related to microglial activation (Blum *et al.,* 2001, Sugama *et al.,* 2003). Anti-oxidants, including N-acetyl-O-methyldopamine (NAMDA), downregulated microglial activation (Gao et al., 2003). These findings imply that endogenous anti-oxidants is decreased in the aged brain (Sugama *et al.,* 2003). This might lead to enhanced microglial activity and progression of neurodegeneration at old age.

Dystrophic microglia are related to neurodegenerative changes in AD. Non-activated, ramified microglia, are colonized with degenerating neuronal structures positive for tau (neurophil threads, neuritic plaques, neurofibrillary tangles) in AD brain, suggesting that microglial activation is not induced by Aβ (Lopes *et al.,* 2008; Streit *et al,* 2009). These findings imply that age-related microglial dysfunctioning, rather than induced microglial activation contributes to neurodegeneration in AD. Dysregulation of proliferative response and age-related change in morphology of resting microlia is found in the aging brain of rodents. Morphological changes and reduced regulated proliferation might reflect microglial

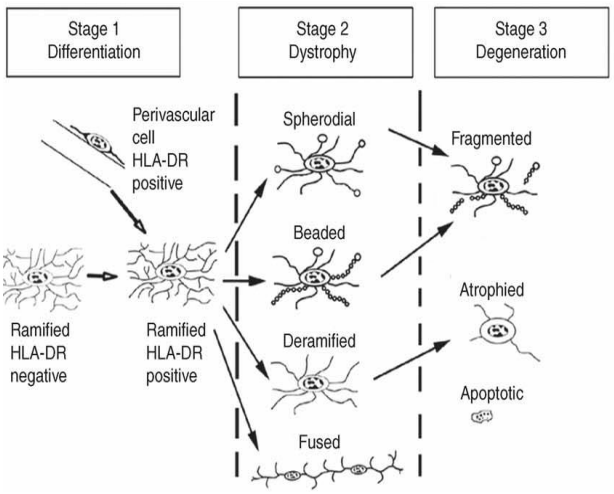

Figure 6. Age-related microglial deterioration. In the first phase microglia are HLA-DR-positive and differentiate to HLA-DR-positive ramified microglia or HLA-DR-negative microglia become HLA-DR-positive. In stage two, the HLA-DR-positive ramplified microglia develop spheroidal swellings and beaded or deramified processes. In stage three, degenerative changes such as fragmentation, atrophy (loss of processes) and apoptosis can occur. There are many possibilities how microglia may differentiate from one stage to another, but the exact way is still unknown. A likely theory is altered interaction among microglia (Streit *et al.*, 2006).

senescence, which is the loss of mitotic ability after repeated round of replication (Conde & Streit, 2006). Together these observations confirm the dysfunction hypothesis of AD, namely that development of age-associated neurodegeneration is caused by age-related decrease in microglia neuroprotection which occurs because microglia are subject to cellular senescence (Streit *et al.*, 2004).

Discussion

The brain is isolated from the adaptive immune system by the BBB. Resident cells like microglia, participate in the innate immune system by initiating apoptosis of infected or damaged cells and removing apoptotic cells in the CNS. Recent findings show that microglia are very dynamic, even under non-pathological conditions. Therefore the concept of 'activated' versus 'resting' microglia should be reconsidered. Microglia should be considered as very active and flexible neural cells.

In the past microglia was associated with detrimental activity. Contrary, microglia also have beneficial functions with might lead to neural protection. One of the protective functions is degrading Aβ- plaques in the AD brain. Furthermore, microglia-induced stimulation of growth factors and removal of myelin debris in MS creates a microenvironment for regeneration and neural repair. More research on the neurotropic effect of microglia and identification of such protective immune pathways is needed to design novel therapies for neural disease in the future.

Recent evidence suggests that ion channels are important in microglial activation. There is also reason to believe that microglial ion channels participate in several ways in the regulation of chemotaxis, proliferation and ramification. Thus, ion channels of microglia might be good candidates for a therapeutic targets in neurological disease. It is not well understood why the ion channel expression changes during activation and differentiation of microglia. Improved knowledge of these expression patterns may contribute to the understanding of the function and regulation of microglial ion channels, and thereby the behavior of microglia.

Neurons seem to have an important role in the control of microglial functioning. Off signals, released by neurons, are important to maintain the neural system homeostasis, restricting microglial activity. On signals are released by impaired or damaged neurons, which activate the neuroprotective or neurotoxic function of microglia. Identification of these signals may lead to better comprehension of the dual role of microglial functioning.

Microglia become more active during aging, this is clearly recognized by the increased numbers of enlarged, and especially phagocytic, microglial subtypes. This age related microglial activation leads to enhanced cytokine

production, such as IL-10 which might lead to increased risk of AD. Microglial hyperactivity in the aging brain is also associated with enhanced induction of Aβ and IL-10. In AD, downregulation of Aβ promoting genes contribute to neurogeneration. Increased microglial activation is accompanied with atrophy in the aged brain, which is associated with reduced cognitive functioning and memory, indicating impaired cellular function. So it seems that age-related increased microglial activation leads to aging of the brain.

Concluding, microglia are very important in the immune regulation of the CNS, repairing neural damage and also in developing and promoting neurodegenerative diseases. A better knowledge of microglial behavior will be necessary in understanding the pathogenesis of ischemia and neural diseases, in order to develop effective therapies.

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References

1. Aloisi, F., Ria, F., Columba-Cabezas, S., Hess, H., Penna, G., Aldorini, L., 1999. Relative efficiency of microglia, astrocytes, dendritic cells and B cells in naive CD4+ T cell priming and Th1/Th2 cell restimulation. Eur. J. Immunol. 29, 2705-2714.
2. Aloisi, F., Ria, F., Adorini, L., 2000. Regulation of T-cell responses by CNS antigen- presenting cells: different roles for microglia and astrocytes. Immunol. Today 21, 141-147.
3. Amor, S., Puentes, F., Baker, D., van der Valk, P., 2010. Inflammation in neurodegenerative diseases. Immunology 129, 154-169.
4. Badoer, E., 2010. Microglia: Activation in acute and chronic inflammatory states and in response to cardiovascular dysfunction.The International Journal of Biochemistry & Cell Biology 42, 1580–1585.
5. Bertollin,i C., Ragozzino. D., Gross, C., Limatola, C., Eusebi, F., 2006. Fractalkine/CX3CL1 depresses central synaptic transmission in mouse hippocampal slices. Neuropharmacology 51:816–821.
6. Bianco, F., Ceruti, S., Colombo, A., Fumagalli, M., Ferrari, D., Pizzirani, C., Matteoli, M., Di Vigillio, F., Abbracchio, M.P., Verderio, C., 2006. A role for P2X7 in microglial proliferation. J. Neurochem. 99,745–758.
7. Biber K., Neumann H., Inoue, K., Boddeke, H.W.G.M., 2007. Neuronal 'On' and 'Off' signals control microglia. Trends in Neurosciences 30, 596-613.
8. Blum, D., Torrch, S., Lambeng, N., Nissou, M., Benabid, A.L., Sadoul, R., Verna, J.M., 2001. Molecular pathways involved in the neuro toxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease, Prog. Neurobiol. 65, 135-172.
9. Block, M.L., Zecca, L., Hong, J.S., 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat. Rev. Neurosci. 8, 57–69.
10. Bouchon, A., Hernádez-Maunain, C., Cella, M., Colonna, M., 2001. A DAP12-mediated pathway regulates expression of Cc chemokine receptor 7 and maturation of human dendritic cells. J. Exp. Med. 194, 1111-1122.
11. Brionne, T.C., Tesseur, I., Masliah, E, Wyss-Coray, T., 2003. Loss of TGF-beta 1 leads to increased neuronal cell death and microgliosis in mouse brain. Neuron 40, 1133–1145.
12. Broderick, C., Hoek, R.M., Forrester, J.V., Liversidge, J., Sedwick, J.D., Dick, A.D., 2002. Constitutive retinal CD200 expression regulates resident microglia and activation state of inflammatory cells during experimental autoimmune uveoretinitis. Am. J. Pathol.161, 1669–1677.
13. Brown, S.B., Heinisch, I., Ross, E., Shaw, K., Buckley, C.D., Savill, J., 2002. Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. Nature 418, 200-203.
14. Butovsky, O., Talpalar, A.E., Ben-Yaakov, K. & Schwartz, M., 2005. Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-γ and IL-4 render them protective. Mol. Cell. Neurosci. 29, 381–393 .
15. Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A.E., Pluchino, S., Martino, G., Schwartz M., 2006. Microglia activated by IL-4 or IFN-ądifferentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol. Cell. Neurosci. 31, 149–160.
16. Cauwe, B., Van den Steen, P.E., Opdenakker G., 2007. The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases. Crit. Rev. Biochem. Mol. Biol. 42, 113-185.
17. Chen, Q., Smith, G.M., Shine, H.D., 2008. Immune activation is required for NT-3-induced axonal plasticity in chronic spinal cord injury. Exp. Neurol. 209, 497–509.
18. Clark, A.K., Yip, P.K., Grist, J., Gentry, C., Staniland, A.A., Marchand, F., Dehvari, M., Wotherspoon, G., Winter, J., Ullah, J., Bevan, S., Malcangio, M., 2007. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. Proc. Natl. Acad. Sci. USA 104, 10655–10670.
19. Colton, C.A., Jia, M., Li, M.X., Gilbert, D.L., 1994. K+ modulation of microglial superoxide production: involvement of voltage-gated Ca21 channels. Am. J. Physiol. 266, C1650–C1655.
20. Conde, J.R., Streit, W.J., (2006). Effect of aging on the microglial response to peripheral nerve injury. Neurobiol. Aging 27, 1451-1461.
21. Coull J.A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M.W., De Koninck, Y., 2005. BDNF from microglia causes the shift in

neuronal anion gradient underlying neuropathic pain. Nature 438, 1017–1021.
22. Deckert, M., Sedgwick, J.D., Fischer, E., Schlüter, D., 2006. Regulation of microglial cell responses in murine Toxoplasma encephalitis by CD200/CD200 receptor interaction. Acta Neuropathol. (Berl.) 111, 548–559.
23. de Jong, E.K., 2005. Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation for microglia activation remote from a primary lesion. J. Neurosci. 25, 7548–7557.
24. de Vries H, Hendriks J, Honing H, De lavalette, C.R., van der Pols, S.M., Hooijberg, E., Dijkstra, C.D., van den Berg, T.K., 2002. Signal-regulatory protein alpha-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. J. Immunol. 168, 5832-5899.
25. Eder, C., Klee, R., Heinemann U., 1996. Blockade of voltage-gated outward K+ currents of ramified murine microglia by scorpion peptide toxins. Neurosci. Lett. 219, 29–32.
26. Eder, C., 1998. Ion channels in microglia (brain macrophages). Am. J. Physiol. 275, 327-342.
27. Eder, C., DeCoursey, T.E., 2001. Voltage-gated proton channels in microglia. Prog. Neurobiol. 64, 277–305.
28. Eder, C., 2010. Ion channels in monocytes and microglia / brain macrophages: Promising therapeutic targets for neurological diseases. Journal of Neuroimmunology 224, 51–55.
29. Elward. K., Gasque P.B., 2003. " Eat me" and "don't eat me" signals govern the innate immune response and tissue repair in the CNS: Emphasis on the critical role of the complement system. Mol. Immunol. 40, 85-94.
30. Elward, K., Griffiths, M., Mizuno ,M., Harris, C.L., Neal, J.W., Morgan, B.P., Gasque P., 2005. CD46 plays a key role in tailoring innate immune recognition of apoptotic and necrotic cells. J. Biol. Chem. 280, 36342-36354.
31. Fan, R., Xu, F., Previtj, M.L., Davis, J., Grande, A.M., Robinson, J.K., van Nostrand, W.E., 2007. Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid. J. Neurosci. 27, 3057–3063.
32. Farber, K., Kettenmann, H., 2005. Physiology of microglia cells. Brain Res. Brain Res. Rev. 48, 133–143.
33. Ferrari, D., Chiozzi, P., Falzoni, S., Dal Susino, M., Collo, G., Buell, G., Di Vigilio, F., 1997. ATP-mediated cytotoxicity in microglial cells. Neuropharmacology 36,1295– 1301.
34. Filipovic, R., Jakovcevski, I., Zecevic, N., 2003. GRO-alpha and CXCR2 in the human fetal brain and multiple sclerosis lesions. Dev. Neurosci. 25,279–290.
35. Fumagalli, M., Brambilla, R., Dámbrosi, N., Volonté, C., Matteoli, M., Verderio, C., Abbracchio, M.P., 2003. Nucleotide-mediated calcium signaling in rat cortical astrocytes: Role of P2X and P2Y receptors. Glia 43, 218-230.
36. Galea, I., Beckmann, I., Perry, V.H., 2007. What is immune privilege (not)? Trends Immunol. 28, 12–18.
37. Gallin, E. K., 1991. Ion channels in leukocytes. *Physiol. Rev*. 71, 775–811, 1991.
38. Gasche, Y., Copin, J.C., Sugawara, T., Fujimura, M., Chan P.H., 2001. Matrix metalloproteinase inhibition prevents oxidative stress-associated blood-brain barrier disruption after transient focal cerebral ischemia. J. Cereb. Blood Flow Metab. 21, 1393–1400.
39. Gao, H.M., Liu, B., Zhang, W., Hong, J.S., 2003. Critcal role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease. FASEB J. 17, 1954-1965.
40. Glass, C.K., Saijo, K., Winner, B., Marchetto, M.C., Gage, F.H., 2010. Mechanisms underlying inflammation in neurodegeneration. Cell. 140, 918–34.
41. Glezer, I., Simard, A.R., Rivest, S., 2007. Neuroprotective role of the innate immune system by microglia. Neuroscience 147, 867–883.
42. Gonz´alez-Scarano, F., Baltuch, G., 1999. Microglia as mediators of inflammatory and degenerative diseases. Annu. Rev. Neurosci. 22, 219-240.
43. Greter, M., Heppner, F.L., Lemos, M.P., Odermatt, B., Goebels, N., Laufer, T., 2005. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nature medicine 11, 328-334.
44. Griffin, W.S., Sheng, J.G., Royston, M.C., Gentleman, S.M., McKenzie, J.E., Graham, D.I., Roberts, G.W., Mrak, R.E., 1998. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. Brain Pathol. 8, 65–72.
45. Gurney, K.J., Estrada, E.Y., Rosenberg G.A. 2006. Blood-brain barrier disruption by stromelysin-1 facilitates neutrophil infiltration in neuroinflammation. Neurobiol. Dis. 23, 87–96.
46. Hamerman, J.A. Jarjoura, J.R., Humphrey, M.B., Nakamura M.C., Seaman, W.E., Lanier, L.L., 2006. Cutting edge: inhibition of TLR and FcR responses in macrophages by triggering receptor expressed on myeloid cells (TREM)-2 and DAP12. J. Immunol. 177, 2051–2055.
47. Hanisch, U.K., and Kettenmann, H., 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. *10*, 1387–1394.
48. Harrison, J.K., Jiang, Y., Chen, S., Xia, Y., Macjejewski, D., McNamara, R.K., Streit, W.J., Salafranca, M.N., Adhikari, S., Thompson, D.A., Botti, P., Bacon, K.B., Feng, L., 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.
49. Haynes, S.E., Hollopeter, G., Yang G., Kurpius, D., Dailey, M.E., Gan, W.B., Julius, D., 2006 The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat. Neurosci. 9, 1512–1519.
50. Henderson, L.M., Moule, S.K., Chappell, J.B., 1993. The immediate activator of the NADPH oxidase is arachidonate not phosphorylation. Eur. J. Biochem. 211: 157–162.
51. Henneka, M.T., O'Banion, M.K., 2007. Inflammatory processes in Alzheimer's disease. J. Neuroimmunology 184, 69-91.
52. Henry, C.J., Huang, Y., Wynne, A.M., Godbout, J.P., 2009. Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines. Brain Behav. Immun. 23, 309-317.
53. Heppner, F.L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hövermeyer, N., Waisman, A., Rülicke, T., Prinz, M., Priller, J., Becher, B., Aguzzi, A., 2005 . Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat. Med. 11, 146–152.
54. Herbomel P., Thisse B., Thisse C., 2001. Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-

CSF receptor-dependent invasive process. Dev. Biol. 238, 274–88.
55. Hines, D.J., Hines, R.M., Mulligan, S.J., MacVicar, B.A., 2009. Microglia processes block the spread of damage in the brain and require functional chloride channels. Glia 57, 1610–1618.
56. Hoek, R.M., Ruuls, S.R., Murphy, C.A., Wright, G.J., Goddard, R., Zurawski, S.M., Streit, W.J., Brown, M.H., Barclay, A.N., Sedgwick, J.D., 2000. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290, 1768–1771.
57. Hoffmann, A., Kann, O., Ohlemeyer, C., Hanisch, U.K., Kettenmann, K., 2003. Elevation of basal intracellular calcium as a central element in the activation of brain macrophages (microglia): suppression of receptor-evoked calcium signaling and control of release function. J. Neurosci. 23, 4410-4419.
58. Horstmann, S., Budig, L., Gardner, H., Koziol, J., Deuschle, M., Schilling, C., Wagner, S., 2010. Matrix metalloproteinases in peripheral blood and cerebrospinal fluid in patients with Alzheimer's disease. Int. Psychogeriatr. 22, 966-972.
59. Ji, R.R., Suter, M.R., 2007. p38 MAPK, microglial signaling, and neuropathic pain. Mol. Pain. 2007, 3:33.
60. Kawasaki, Y., Zhang, L., Cheng, J.K., Ji, R.R., 2008. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J. Neurosci. 28:5189–5194.
61. Kielian T., 2006. Toll-like receptors in central nervous system glial inflammation and homeostasis. J. Neurosci. Res. 83(5), 711–730.
62. Kim, S.U., de Vellis, J., 2005. Microglia in health and disease. Journal of Neuroscience Research 81, 302–313.
63. Koenigsknecht-Talboo, J., Landreth, G.E., 2007. Microglial phagocytosis induced by fibrillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines. J. Neurosci. 25, 8240–8249.
64. Koizumi, S., Shigemotto-Mogami, Y., Nasu-Tada, K., Shinozalki, Y., Ohsawa, K., Tsuda, M., Joshi, B.V., Jakobson, K.A., Kohsaka, S., Inoue, K., 2007. UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446, 1091-1095.
65. Kotter, M.R., Zhao, C., van Rooijen, N., Franklin, R.J.M., 2005. Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. Neurobiol. Dis. 18, 166–175.
66. Kreutzberg, G.W., 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19, 312–318.
67. Latour, S.H., Tanaka, H., Demeure, C., Mateo, V., Rubio, M., Brown., Maliszewski, C., Lindberg, F.P., Oldenborg, A., Ullrich, A., Delespesse, G., Sarfati, M., 2001. Bidirectional negative regulation of human T and dendritic cells by CD47 and its cognate receptor signal regulator proteinYalpha: Downregulation of IL-12 responsiveness and inhibition of dendritic cell activation. J. Immunol. 167, 2547-2554.
68. Lewis, R. S., Cahalan, M.D., 1988. Subset-specific expression of potassium channels in developing murine T lymphocytes. Science 239, 771–775.
69. Lewis, R. S., Cahalan. M.D., 1995. Potassium and calcium channels in lymphocytes. Annu. Rev. Immunol. 13, 623–653.
70. Liberatore, G.T., Jackson-Lewis, V., Vukosavic, S., Mandir, A.S., Vila, M., McAuliffe, W.G., Dawson, V.L., Dawson, T.M., Przedborski, S., 1999. Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat. Med. 5, 1403-1409.
71. Lopes, K.O., Sparks, D.L., Streit, W.J., 2008. Microglial dystrophy in the aged and Alzheimer's disease brain is associated with ferritin immunoreactivity. Glia 56, 1048-1060.
72. Mariani, M.M., Kielian, T., 2009. Microglia in infectious diseases of central nervous system. J. Neuroimmune Pharmacol. 4, 448–461.
73. Martinez, F.O., Sica, A., Mantovani, A., Locati, M., 2008. Macrophage activation and polarization. Front Biosci. 1, 453-461.
74. Mott, R.T., Ait-Ghezala, G., Town, T., Mori, T., Vendrame, M., Zeng, J., Ehrhart, J., Mullan, M., Tan, J., 2004. Neuronal expression of CD22: Novel mechanism for inhibitory microglial preinflammatory cytokine production. Glia 46, 369–379.
75. Muessel, M.J., Klein, R.M., Wilson A.M., Berman, N.E., 2002. Ablation of the chemokine monocyte chemoattractant protein-1 delays retrograde neuronal degeneration, attenuates microglial activation, and alters expression of cell death molecules. Brain Res. Mol. Brain Res. 103, 12–27.
76. Nimmerjahn, A., Kirchhoff, F., and Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318.
77. Nörenberg, W., Cordes, A., Blöhbaum, G., Fröhlich, R., Illes, P., 1997. Coexistence of purino- and pyrimidino receptors on activated rat microglial cells. Br. J. Pharmacol. 121, 1087–1098.Perry, H.V., Nicoll, J.A.R., Holmes C., 2010. Microglia in neurodegenerative disease. Nat. Rev. Neurol. 6, 193-201.
78. Perry, V.H., Nicoll, J.A.R., Holmes, C., 2010. Microglia in neurodegenerative disease. Nat. Rev. Neurol. 6, 193-201.
79. Persson, M., Brantefjord, M., Hansson, E., Ronnback, L., 2005. Lipopolysaccharide increases microglial GLT-1 expression and glutamate uptake capacity in vitro by a mechanism dependent on TNF-α. Glia 51, 111–120.
80. Pocock, J.M., Kettenmann, H., 2007. Neurotransmitter receptors on microglia. Trends Neurosci. 30, 527-535.
81. Qian, L., Hong, S-J., Flood, P.M., 2006. Role of microglia in inflammation-mediated degeneration of dopaminergic neurons: neuroprotective effect of Interleukin 10. J Neural Transm. 70, 367-371.
82. Qiu, W.Q., Walsh, D.M., Ye, Z., Vekrellis, K., Zhang, J., Podlisny, M.B., Rosner, M.R., Safavi, A., Hersh, L.B., Selkoe, D.J., 1998. Insulin degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. J. Biol. Chem. 273, 32730–32738.
83. Raes, G., De Baetselier, P., Noel, W., Beschin, A., Brombacher F., Hassanzadeh, G., 2002. Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. J. Leukocyte Biol. 71, 597-602.
84. Ransohoff, R.M., Perry, V.H., 2009. Microglial Physiology: Unique Stimuli, Specialized Responses Annu. Rev. Immunol. 27:119–45.

85. Rappert, A., Biber, K., Nolte, C., Lipp, M., Schubel, A., Lu, B., Gerard, N.P., Gerard, C., Boddeke, H.W., Kettenmann, H., 2002. Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl- current and chemotaxis in murine microglia. J. Immunol. 168, 3221–3226.
86. Rappert, A., Beckmann, I., Pivneva, T., Mahlo, J., Biber, K., Nolte., Kovac, A.D., Gerard, A.D., Boddeke, H.W., Nitsch, R., Kettenmann, H., 2004. CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion. J. Neurosci. 24, 8500–8509.
87. Reinhold, M.I., Lindberg, F.P., Plas, D., Reynolds, S., Peters, M.G., Brown, E.J., 1995. In vivo expression of alternatively spliced forms of integrin-associated protein (CD47). J. Cell. Sci.108, 3419-3425.
88. Rosovsky, I., Finch, C.E., Morgan, T.E., 1998. Age-related activation of microglia and astrocytes: In vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation. Neurobiology of aging 19, 97-103.
89. Savarin-Vuaillat, C., Ransohoff, R.M., 2007. Chemokines and chemokine receptors in neurological disease: raise, retain, or reduce? Neurotherapeutics 4, 590–601.
90. Savchenko V.L., Nikonenko R., Skibo G.G., McKanna J.K., 1997. Distribution of microglia and astrocytes in different regions of the normal adult rat brain. Neurophysiology 29, 343–51.
91. Schilling, T., Lehmann, F., Rückert, B., Eder, C., 2004. Physiological mechanisms of lysophosphatidylcholine-induced de-ramification of murine microglia. J. Physiol. 557, 105-200.
92. Sievers, J., Schmidtmayer, J., Parwaresch, R., 1994. Blood monocytes and spleen macrophages differentiate into macrophage-like cells. Anat. Anz. 176, 45-51.
93. Simard, A.R., Rivest, S., 2004. Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. FASEB J. 18, 998–1000.
94. Simard, A., Soulet, D., Gowing, G., Julien, J.P., Rivest, S., 2006. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 49, 489–502.
95. Song, E., Ouyang, N., Hörbelt, M., Antus, B., Wang, M., Exton, M.S., 2000. Influence of Alternatively and Classically Activated macrophages on fibrogenic activities of human fibroblasts. Cell. Immunol. 204, 19–28.
96. Soulet, D., Rivest, S., 2008. Microglia. Curr. Biol. 18, 506-508.
97. Streit, W.J. Sammons, N.W., Kuhns, A.J., Sparks, D.L., 2004. Dystrophic microglia in the aging human brain. Glia 45, 208–212.
98. Streit, W.J., 2006. Microglial senescene: does the brain's immune system have an expiration date? Trends Neurosci. 29, 506-510.
99. Streit, W.J., Braak, H., Xue, Q.S., Beckmann, I., 2009. Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. Acta. Neuropathol. 118, 475–485.
100. Sugama, S., Yang, I., Cho, B.P., DeGiorgio, L.A.., Lorenzl, S., Albers, D.S., Beal, M.F., Volpe, B.T., Joh, T.H., 2003. Age-related microglial activation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. Brain Res. 964, 288-294.
101. Tan, J., Town, T., Mullan, M., 2000. CD45 inhibits CD40L-induced microglial activation via negative regulation of the Src/p44/42 MAPK pathway. J. Biol. Chem. 275, 37224–37231.
102. Takahashi, K., Richford, C.D., Neumann, H., 2005. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J. Exp. Med. 201, 647-657.
103. Taylor, D.L., Jones, F., Kubota, E.S., Pocock, J.M., 2005. Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha induced neurotoxicity in concert with microglial-derived Fas ligand. J. Neurosci. 25, 2952–2964.
104. Thacker, M.A., Clark, A.K., Bishop, T., Grist, J., Yip, P.K., Moon, L.D., Thompson, S.W., Marchand, F., McMahon, S.B., 2009. CCL2 is a key mediator of microglia activation in neuropathic pain states. Eur. J. Pain 13, 263–272.
105. Trang, T., Beggs, S., Wan, X., Salter, M.W., 2009. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. J. Neurosci. 29, 3518-3528.
106. Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M.W., Inoue, K., 2003. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 424, 778–783.
107. Tsuda, M., Inoue, K., Salter, M.W., 2005. Neuropathic pain and spinal microglia: a big problem from molecules in ''small'' glia. Trends Neurosci. 28, 101–107.
108. van Rossum, D., Hanisch, U.K. 2004. Microglia. Metab. Brain Dis. 19, 393–411.
109. Vaughan, D.W., Peters, A., 1974. Neural cells in the cerebral cortex of rats from young adulthood to old age: an electron microscope study. J. Neurocytol. 3, 405-429.
110. Wang, X.., Arcuino, G., Takano, T., Lin, L., Peng, W.G., Wan, P., Li., P., Xu, Q.S., Goldman, S.A., Nedergaard, M., 2004. P2X7 receptor inhibition improves recovery after spinal cord injury. Nat. Med. 10, 821–827.
111. Wang, X.J., Ye, M., Y.H. Zhang S.D., 2007. CD200–CD200R regulation of microglia activation in the pathogenesis of parkinson's disease. J Neuroimmune Pharmacol. 2, 259–264.
112. Wu, D.C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., Choi, D.K., Ischiropoulos, H., Przedborski, S., 2002. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrapyridine mouse model of Parkinson disease. Neurosci. 22, 1763-1771.
113. Xia, M.Q., Bacskai, B.J., Knowles, R.B., Qin, S.X., Hyman, B.T., 2000. Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: in vitro ERK1/2 activation and role in Alzheimer's disease. J. Neuroimmunol. 108, 227–235.
114. Yan, Q., Zhang, J., Liu, H., Babu-Khan, S., Vassar, R., Biere, A.L., Citron, M., Landreth, G., 2003. Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer's disease. J. Neurosci. 23, 7504–7509.
115. Yang, I., Han, S.J., Kaur, G., Crane, C., Parsa, A.T., 2010. The role of microglia in central nervous system immunity and glioma immunology. Journal of Clinical Neuroscience 17, 6–10.
116. Ye, S.M., Johnson, R.W., 2001. An age-related decline in interleukin-10 may contribute to the incresed

expression of interleukin-6 in brain of aged mice. Neuroimmunomodulation. 9, 183-192.

117. Zhao, P., Waxman, S.G., Hains, B.C., 2007. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. J. Neurosci. 27, 8893–8902.
118. Zhou, W., Cayabyab, F.S., Pennefather, P.S., Schlichter, L.C., DeCoursey, T.E., 1998. HERG-like K+ channels in microglia. J. Gen. Physiol. 111, 781-794.
119. Zhuang, Z.Y., Kawasaki, Y., Tan, P.H., Wen, Y.R., Ji, R.R.., 2007. Role of CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following injury-induced cleavage of fractalkine. Brain Behav. Immun. 21, 642–651.

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