Title
Microglia and Neonatal Brain Injury

Authors
Mallard, Carina
Tremblay, Marie-Eve
Vexler, Zinaida S

Publication Date
2019-05-01

DOI
10.1016/j.neuroscience.2018.01.023

Peer reviewed

HHS Public Access

Author manuscript

Neuroscience. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:

Neuroscience. 2019 May 01; 405: 68–76. doi:10.1016/j.neuroscience.2018.01.023.

Microglia and Neonatal Brain Injury

Carina Mallard[a], **Marie-Eve Tremblay**[b], **Zinaida S. Vexler**[c,*]

[a]Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden

[b]Axe Neurosciences, Centre de recherche du CHU de Québec-Université Laval, 2705 Boulevard Laurier, Québec, QC, Canada

[c]Department of Neurology, University California San Francisco, United States

Abstract

Microglial cells are now recognized as the “gate-keepers” of healthy brain microenvironment with their disrupted functions adversely affecting neurovascular integrity, neuronal homeostasis, and network connectivity. The perception that these cells are purely toxic under neurodegenerative conditions has been challenged by a continuously increasing understanding of their complexity, the existence of a broad array of microglial phenotypes, and their ability to rapidly change in a context-dependent manner to attenuate or exacerbate injuries of different nature. Recent studies have demonstrated that microglial cells exert crucial physiological functions during embryonic and postnatal brain development, some of these functions being unique to particular stages of development, and extending far beyond sensing dangerous signals and serving as antigen presenting cells. In this focused review we cover the roles of microglial cells in regulating embryonic vasculogenesis, neurogenesis, and establishing network connectivity during postnatal brain development. We further discuss context-dependent microglial contribution to neonatal brain injuries associated with prenatal and postnatal infection and inflammation, in relation to neurodevelopmental disorders, as well as perinatal hypoxia–ischemia and arterial focal stroke. We also emphasize microglial phenotypic diversity, notably at the ultrastructural level, and their sex-dependent influence on the pathophysiology of neurodevelopmental disorders.

Keywords

inflammation; perinatal stroke; hypoxia-ischemia; Toll-like receptors; electron microscopy; synapse

INTRODUCTION

The microglial field exploded over the past 6–8 years (Tremblay et al., 2015). Literature keeps growing identifying microglial cells (microglia) as “gate-keepers” of a healthy brain microenvironment and cell–cell communications under physiological conditions. The perception of these cells as purely toxic under neurodegenerative conditions has been

*Corresponding author. Address: University California San Francisco, Department of Neurology, 675 Nelson Rising Lane, San Francisco, CA 94158-0663, United States. Fax: +1-(415)-502-5821. Zena.Vexler@ucsf.edu(Z. S. Vexler).

challenged by demonstration of the existence of a broad array of microglial phenotypes having the ability to rapidly change in a context-dependent manner to attenuate or exacerbate injuries of different nature. Recently developed tools have allowed for discrimination between microglia and monocytes and demonstration of respective markedly different gene “signatures” (Butovsky et al., 2014; Bennett et al., 2016). Together with accumulating data on the presence of microglial sexual dimorphism (Hanamsagar et al., 2017) and the modulatory role of microglial homeostasis (Sipe et al., 2016; Mildner et al., 2017; Zrzavy et al., 2017), these new findings are major milestones for reconsideration of the physiological role of microglia and their contribution to neurodegenerative diseases.

Recent studies demonstrated that microglia have a broad range of effects in embryonic and postnatal physiological brain development, including regulation of embryonic vasculogenesis, secretion of trophic factors, immuno-surveillance, oligodendrogenesis and neurogenesis. They play key roles in establishing brain connectivity network during postnatal brain development (Paolicelli et al., 2011; Arnold and Betsholtz, 2013; Butovsky et al., 2014; Hagberg et al., 2015). Microglial cells are now known to undergo a major phenotypic transformation during physiological postnatal brain development (Butovsky et al., 2014), confirming the different morphological and ultrastructural phenotypes that were previously identified (Murabe and Sano, 1982; Dalmau et al., 1998), and to develop distinctly between male and female brains (Schwarz et al., 2012). Pharmacologic depletion of microglial cells or deficiency/malformation of individual signaling pathways in these cells were shown to play context-dependent roles in perinatal infection (Baburamani et al., 2014), hypoxia–ischemia (H–I) (Hagberg et al., 2015), arterial stroke (Faustino et al., 2011; Fernandez-Lopez et al., 2016), and developmental disorders (Derecki et al., 2012; Hagberg et al., 2012). In this review we will focus on new or recently revisited concepts pertaining to structural and functional aspects of microglia in the immature brain, effects that can lead to the age- and context-dependent microglial behavior under physiological and pathophysiological conditions.

MICROGLIAL CELLS GUIDE BLOOD-BRAIN BARRIER DEVELOPMENT IN EMBRYONIC BRAIN

In mice, the origin for microglial and monocyte pools is distinct (Ginhoux et al., 2010; Schulz et al., 2012) and the establishment of local brain microglial pool temporally occurs before blood–brain barrier (BBB) development, which begins mid-gestation and before pericytes and astrocytes take their respective positions surrounding the vessels (Cahoy et al., 2008; Armulik et al., 2010; Daneman et al., 2010; Zhang and Barres, 2010). Microglia have direct effects on vasculogenesis and vascular sprouting in embryonic brain (Checchin et al., 2006; Kubota et al., 2009). They guide endothelial sprouts in fetal brain, largely via VEGF-dependent mechanisms. Microglial deficiency adversely affects vasculature development (Fantin et al., 2010). Disrupted PU.1 or SDF-1 signaling in microglia/macrophages leads to distorted vasculature and embryonic lethality (Fantin et al., 2010). Mice deficient in VEGF/VEGF receptor 2 fail vessel formation, leading to embryonic lethality (Shalaby et al., 1995). Even lack of a single VEGF allele leads to embryonic lethality despite partial blood vessel development (Carmeliet et al., 1996). Disrupted integrin and chemokine signaling was

shown to adversely affect embryonic angiogenesis and BBB formation. For example, excessive vascular sprouting underlies cerebral hemorrhage in mice that lack αVβ8-integrin/transforming growth factor beta (TGFβ) signaling in the brain, whereas integrin/TGFβ signaling suppresses vascular branching/sprouting and prevents germinal matrix hemorrhage (Arnold et al., 2014). In early postnatal rat and mouse brain following stroke, pharmacological inhibition of the TGFβ1/TGFbR2 pathway selectively in microglia disrupts the BBB, which triggers hemorrhages in injured regions (Fernandez-Lopez et al., 2016). Importantly, monocytes are unable to substitute for the microglial roles in vasculogenesis in the developing brain (Checchin et al., 2006). The latter finding is consistent with different origins of microglia and monocytes and distinct signaling in these cell subtypes (Ginhoux et al., 2010; Butovsky et al., 2014). Together, these data suggest a principle role of microglia in vascular development and integrity and key role of microglial TGFβ1 signaling in modulating BBB integrity during brain development.

MICROGLIA, POSTNATAL BRAIN DEVELOPMENT AND BRAIN CONNECTIVITY

In human brain, the presence of ameboid CD68-positive microglia (i.e., feature of microglia with activated lysosomal component) has been reported as early as 13 weeks of gestation, with the highest number observed between 13 and 18 gestational weeks in the germinal matrix (Hutchins et al., 1990) and white matter (Monier et al., 2006). Topographic relationship of CD68-positive cells with growing axons was demonstrated (Cho et al., 2013). In the infant brain, ramified microglial cells were reported as the predominant type (Fujimoto et al., 1989; Cho et al., 2013).

Echoing these findings in human brain, in rodents, ameboid (considered “activated”) morphological phenotype is seen during the embryonic period and in newborn rodents when physiological death of overproduced neurons is high, followed by gradual microglial ramification during the first week of rodent’s life. Morphological and ultrastructural analyses in the neonatal rat brain conducted during the first week provided insights into this transformation of ameboid microglia into ramified cells, among the rat subventricular zone, corpus callosum, and cerebral cortex. Different stages of microglial maturation were thus described, ranging from an “ameboid” morphology in the white matter, to a “round” morphology with a few pseudopodia but devoid of processes located in the subventricular zone and nearby blood vessels, to a “primitive” morphology harboring a few processes in the cerebral cortex, hippocampus, and hypothalamus (Murabe and Sano, 1982; Dalmau et al., 1998). These “nascent” phenotypes showed microglia-distinctive long stretches of endoplasmic reticulum, numerous ribosomes, and an abundant accumulation of vacuoles and large phagocytic inclusions (Ferrer and Sarmiento, 1980; Murabe and Sano, 1982).

Microglia have been shown to regulate brain circuit connectivity in multiple ways. During embryonic neurogenesis they regulate stem cell pool via secretion of trophic factors and phagocytosis (Cunningham et al., 2013). Microglial involvement with the outgrowth of dopaminergic axons and proper positioning of interneurons in the forebrain was shown using cell-depletion approaches, as well as Cx3Cr1, CR3, and DAP1 mutants (Squarzoni et al.,

2014). Findings in mouse co-culture indicate that neurogenesis does not occur without microglia (Walton et al., 2006). Microglia provide trophic support to neurons and endothelial cells, notably via their production of BDNF, IGF-1/2, TGFβ. Disrupted growth factor production in microglia interrupts cortical layer formation (Ueno et al., 2013) and synaptic refinement during the neonatal to adolescent period (Bialas and Stevens, 2013; Butovsky et al., 2014). Microglia contribute to somatosensory cortex development during the "critical period" in mice (Arnoux et al., 2013). Functional analysis of thalamocortical synapses showed that Cx3Cr1 deficiency delays maturation of postsynaptic glutamate receptors (normally occurs in postnatal day 6–8, P6-P8 mice) (Arnoux et al., 2013). Cx3cr1 knockout mice transiently have fewer microglia in the hippocampus, higher dendritic spine density and less mature physiological responses and long-term depression (LTD) (Paolicelli et al., 2011; Zhan et al., 2014). Microglia also have morphological alterations and diminished response to the migratory and activating signal ATP (Pagani et al., 2015). Phagocytosis of overproduced neurons and weak synapses by microglia, processes that govern brain connectivity, plays key role in organizing brain circuits during the postnatal period (Paolicelli et al., 2011; Hoshiko et al., 2012; Schafer et al., 2012; Bialas and Stevens, 2013; Zhan et al., 2014). Direct microglial contacts with axon terminals and dendritic spines were revealed by electron microscopy, with our quantitative analyses showing that almost all (~94%) microglial processes appose synaptic elements in adolescent mouse cerebral cortex (Tremblay et al., 2010b). 3D reconstruction also revealed that a single microglial process can make multiples contacts with multiple synaptic elements at multiple synapses, sometimes with finger-like protrusions wrapping around spines and terminals (Tremblay et al., 2010a). Clathrin-coated pits are observed inside of microglial processes or synaptic elements, at their sites of contact, suggesting reciprocal exchange of molecular signals through clathrin-mediated endocytosis of membrane-bound receptors and their ligands. The two types of morphological specializations indicated that surveilling microglia do interact functionally with excitatory synapses (Tremblay and Majewska, 2011). While dynamic microglial contacts with axon terminals and dendritic spines were also observed in vivo (Tremblay et al., 2010a), live evidence for their engulfment is currently lacking. Functional studies nevertheless demonstrate that surveilling microglia contribute to activity-dependent synaptic pruning, particularly via the classical complement cascade. Disruption of microglia-specific CR3/C3 complement signaling leads to sustained deficits of synaptic connectivity during adolescence and adulthood (Bialas and Stevens, 2013).

MICROGLIAL CELLS MEDIATE DISTINCT RESPONSES IN THE INFECTIOUS AND STERILE INFLAMMATORY SETTINGS

The fetus and newborn infant are highly sensitive to infection and chorioamnionitis (inflammation of fetal membranes) are linked to both preterm birth and later neurological deficits in the newborn (Shatrov et al., 2010; Salas et al., 2013; Soraisham et al., 2013; Shankaran et al., 2014). Similarly, infants with early or late neonatal sepsis have an increased risk of neurological morbidity. Children born very preterm with a proven bacteremia in the first weeks of life perform worse on neurocognitive tests (Bright et al., 2017) and demonstrate neurological dysfunction at school age (Kavas et al., 2017). Postmortem studies show expression of several pro-inflammatory cytokines in the brains of

infants with proven infection and white matter injury, suggesting that the systemic inflammation also spreads to the brain (Kadhim et al., 2001). Thus, there is significant clinical evidence to suggest that inflammation is an important factor in neonatal brain disorders. Exactly how the brain inflammation arises, however, remains unclear. Trafficking of peripheral immune cells may contribute to the CNS inflammatory condition, but activation of resident microglia is likely to play an important role.

Based on comparisons of the timing of neurogenesis, synaptogenesis, gliogenesis, and myelination, together with age-dependent molecular and biochemical changes in rodents and humans, the rodent brain at P1–P5 corresponds to 23–32 weeks of gestation in humans, making it suitable for studies of preterm brain injury, whereas the rodent brain at P7–P10 resembles more 36–40 weeks of gestation in humans, and, thus, is better suited for studying brain injury at term (Semple et al., 2013). Brain injury and/or immune stimuli are associated with a marked induction of innate immunity via Toll-like receptors (TLRs) (Mallard et al., 2009; Stridh et al., 2011). Activation of TLRs by administration of synthetic ligands or microorganisms known to stimulate the receptors has been used extensively to study effects on microglia in the fetus or newborn. In early studies, it was reported that although expression of cytokines was increased in the brain of rat pups that had been exposed to the TLR4 agonist LPS (endotoxin) in utero, microglial numbers were either reduced or not affected (Cai et al., 2000; Bell and Hallenbeck, 2002). However, in later studies, using different markers to assess microglia (CD68), E. coli bacteria (which activate TLR-4) inoculation of uteri in rats at E17 demonstrated marked activation/morphologic transformation of microglia in pups up to two weeks after birth (Pang et al., 2005). In fetal sheep, LPS given at a gestational age which is comparable to the preterm human infant with respect to brain development, either to the fetus (Duncan et al., 2002; Mallard et al., 2003; Dean et al., 2011) or into the amniotic fluid (Nitsos et al., 2006), results in CNS inflammation, including the overall increased numbers of microglia and those with activated morphologic phenotype. Furthermore, intrauterine LPS to pregnant rabbits showed increased brain retention of (11)C-(R)-PK11195, a marker of neuroinflammation, on P1. Immunohistochemical assessment of these brains demonstrated increased numbers of microglia and morphological changes associated with an activation state in the periventricular region and hippocampus (Kannan et al., 2007). In the same model, the authors recently demonstrated that glutamate carboxypeptidase II (GCPII) is significantly increased in ionized calcium binding adaptor molecule 1 (Iba1)-positive microglia with morphological features of activation, suggesting that maternal intrauterine LPS exposure may mediate glutamate dysregulation in a microglia-dependent fashion (Zhang et al., 2016). Along the same line, intrauterine injection of LPS to E17 mice showed increased number of Iba1-positive cells with an ameboid shape in the offspring at P45, which was associated with increased synaptic strength, due in part to an increase in the probability of glutamate release from presynaptic CA3 axon terminals (Kelley et al., 2017). Thus, microglial activation following immune stimulation may not only play a role in inducing CNS inflammation, but also directly affect neurotransmission in the developing brain. Maternal immune activation by injection of Poly(I:C) at 15 was shown to lead to sustained microglial transcriptome reprograming through the adulthood (Mattei et al., 2017).

Systemic inflammation results in increased levels of cytokines and chemokines in the blood, including TNFα and IL-1β, pleiotropic cytokines known to also be produced by cells in the CNS, including microglia (Saliba and Henrot, 2001). Although these cytokines normally have important physiological and endocrine functions in the brain, when produced in excess they can have microglia-mediated cytotoxic effects. As an example, repeated systemic administration of IL-1β in P1-P5 mice results in a transient increase in microglial density and long-term myelination deficits, which are accompanied by cognitive defects (Favrais et al., 2011). Neonatal systemic activation of innate immune receptors like TLRs is also associated with brain dysfunction and can further activate microglia. We showed that administration of LPS at P5 leads to a transient increase in absolute number and cell density of Iba1-positive microglia, as well as a persistent alteration in hippocampal inflammatory status in microglia, but without driving infiltration of peripheral monocyte-derived macrophages (Smith et al., 2014). Further, repeated injections of the TLR2 agonist Pam(3)CSK (4) at P3-P11 decreased the volume of cerebral gray and white matter in association with elevated levels of several pro-inflammatory cytokines and chemokines in the brain as well as increased microglial density (Du et al., 2011). It was subsequently found that systemic TLR2 activation, but not TLR4, results in infiltration of peripheral immune cells into the brain, demonstrating that monocytes and neutrophils may contribute to the inflammatory response in the brain under specific conditions (Mottahedin et al., 2017).

It is clear from these studies that microglia are responders following antenatal and postnatal systemic infectious (e.g. TLR activation), as well as non-infectious (e.g. IL-1β) inflammation. However, the mechanisms that transfer peripheral signals to activate microglia remain obscure. Microglia are the predominant source of TLR2 expression during normal postnatal brain development (Lalancette-Hebert et al., 2017). Our recent comparison of TLR2 activation between systemic (i.p.) LPS stimulation and intracerebral IL-1β injection in P9-P10 dual-reporter luc/GFP-TLR2 mice, has demonstrated that LPS triggers strong TLR2-mediated microglial activation and increase in production of pro-inflammatory cytokines and chemokines (Lalancette-Hebert et al., 2017). In contrast, following IL-1β injection, the in vivo TLR2-luc signal is reduced and the inflammatory response is distinct and overall lessened (Lalancette-Hebert et al., 2017), suggesting that the microglia response and underlying mechanisms of inflammation may be different depending on the nature of initial systemic infectious/inflammatory stimuli.

MICROGLIA, INFECTIONS AND NEURODEVELOPMENTAL DISORDERS

Emerging clinical and experimental evidence suggests that the immune system in children with autism spectrum disorders (ASD) is altered, affecting the child's brain development and function (Ashwood et al., 2011; Hsiao, 2013; Lucchina and Depino, 2013), and that TLR2/3/4 activation is exacerbated and contributes to the immunological component of ASD (Enstrom et al., 2010). In mice, changes in microglial gene expression were demonstrated to lead to neurodevelopmental disorders and a primary deficit in microglia to be sufficient to induce autistic ASD-like behavior (Zhan et al., 2014). Mice lacking Cx3cr1 exhibit a transient reduction of microglial number during the early postnatal period and associated deficit in synaptic pruning, as mentioned above, leading to decreased functional brain connectivity, deficits in social interaction, and increased repetitive-behavior

phenotypes (Zhan et al., 2014; Fernandez de Cossio et al., 2017). A viral mimetic Poly(I:C) in mice and monkeys used as an immune challenge (Giulivi et al., 2013; Naviaux et al., 2013; Bauman et al., 2014) produced immune dysfunction and long-lasting ASD-like behavior (Giulivi et al., 2013). Compared to the offspring males from saline-injected mothers, those from immune challenged mothers displayed decreased preference for the social chamber and extremely high repetitive behavior in the marble burying test (Malkova et al., 2012). A link between the nucleotides ATP and ADP, which, when released to the extracellular space, affect innate immunity and inflammation and autistic behavior, was also established in the Poly(I:C) mouse model (Naviaux et al., 2013), suggesting the possibility that maternal immune activation mediates ASD specifically by reprograming microglial signaling. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring (Fernandez de Cossio et al., 2017). Literature is growing linking disruptions in microglia-mediated synaptic pruning to various neurodevelopmental and neuropsychiatric disorders including schizophrenia (reviewed in (Salter and Beggs, 2014; Neniskyte and Gross, 2017). Not only maternal immune activation, but also early life stress, have been shown to perturb the maturation of microglia in developing hippocampus (Delpech et al., 2016).

MICROGLIAL ROLE IN PERINATAL FOCAL ARTERIAL STROKE AND HYPOXIA–ISCHEMIA

Neuroinflammation is a characteristic feature of stroke progression in the adult and is a major contributor to brain injury (Iadecola and Anrather, 2011). Parenchymal, perivascular and peripheral circulating cells can contribute independently and in concert to stroke-induced production of inflammatory mediators and neuroinflammation (Iadecola and Anrather, 2011) as well as activation of endothelial cells (Osborn et al., 1989; Stanimirovic et al., 1997). Historically, in adult stroke, microglia were viewed as purely injurious, in part due to production of inflammatory mediators, reactive oxidant species (ROS) and other toxic molecules (reviewed in (Iadecola and Anrather, 2011)). Reconsideration of the microglial role in stroke is on-going following recent discoveries of the heterogeneity of the microglial pool (Lucin and Wyss-Coray, 2009), the microglial role in supporting neuronal health and neurogenesis (Walton et al., 2006), the distinct and even opposite roles of microglia and monocytes in stroke (Lambertsen et al., 2009), and the demonstration that lack of microglia disrupts neuronal network activity and exacerbates injury after stroke (Szalay et al., 2016). Furthermore, the importance of timing after stroke as a factor influencing microglial effects is being increasingly acknowledged, while the usefulness of classifying microglia into M1/M2A/2B phenotypes is being challenged as oversimplified and, as such, misleading to the in vivo situation (Martinez and Gordon, 2014; Murray et al., 2014; Ransohoff, 2016).

There is now ample evidence that the mechanisms of ischemic injury differ greatly between immature versus adult brain (reviewed in (Yager and Ashwal, 2009; Fernandez-Lopez et al., 2014)), including neuroimmune responses (Hagberg et al., 2015). Several lines of investigation have pointed to markedly different, and even opposite, effects of microglia to similar insults in neonatal as compared to adult brain (Cho et al., 2005; Lalancette-Hebert et al., 2009, 2017; Woo et al., 2012). For example, the scavenger receptor CD36 contributes to

acute injury after transient middle cerebral artery occlusion (tMCAO) by enhancing ROS production in microglia with activated morphologic phenotype (Cho et al., 2005) whereas CD36 attenuates acute injury after tMCAO in P9 mice (Woo et al., 2012). Another example is ~20-fold increase in TLR2 expression measured 24 h following tMCAO in adult luc/GFP-TLR2 mice (Lalancette-Hebert et al., 2009), whereas no increase in TLR2 expression, which is predominantly seen in microglial cells, is detected 24–72 h after tMCAO in P9 luc/GFP-TLR2 mice (Lalancette-Hebert et al., 2017).

In the neonatal brain, microglial cells undergo morphologic transformation after both H–I and focal arterial stroke. Activated microglia/macrophages are thought to contribute to H–I (McRae et al., 1995; Ivacko et al., 1996; Bona et al., 1999; Xu et al., 2001; Cowell et al., 2003) and excitotoxic injury (Dommergues et al., 2003). The demonstration that genetic deletion of IL-18 or caspase-1, which are produced predominately by activated microglia, attenuates brain injury also supports the notion that microglia exert injurious effects early after H–I (Hedtjarn et al., 2002). The initial demonstration that the tetracycline derivative minocycline could markedly protect the immature brain from H–I (Arvin et al., 2002) was also attributed to suppression of toxic microglial activation. However, minocycline's actions are not specific to microglia and its effects after neonatal H–I, either protective or injurious, were later shown to be species-dependent (Tsuji et al., 2004). After tMCAO induced in P7 rats minocycline provides only modest and short-term protection (Fox et al., 2005).

Contrary to the notion of microglial toxicity in neonatal brain following H–I, in acute neonatal stroke we demonstrated a protective role of microglia by showing that selective pharmacologic depletion of these cells by intracerebral injection of clodronate-liposomes two days before inducing tMCAO in P7 rats (Faustino et al., 2011) or P9 mice (Fernandez-Lopez et al., 2016) changes balance between caspase-3-dependent and -independent neuronal death (Faustino et al., 2011), limits engulfment and removal neuronal debris (Woo et al., 2012) and exacerbates injury (Faustino et al., 2011; Woo et al., 2012). Phagocytosis is particularly important in neonatal post-ischemic brain because the magnitude of apoptotic neuronal death is markedly higher in neonatal than in adult post-ischemic brain (Hu et al., 2000). A lack of microglia further increases the cytokine and chemokine levels induced by tMCAO in neonatal rats (Faustino et al., 2011), consistent with the notion of protective role of apoptotic debris removal. Another line of evidence for the vital role of microglial phagocytosis of dying neurons as a part of protection from neonatal stroke comes from the data that genetic deletion of the scavenger receptor CD36, receptor that contributes to several phagocytotic steps, makes acute injury worse (Woo et al., 2012; Li et al., 2015). The lack of the CD36 substantially reduces engulfment of neurons that express cleaved caspase-3 and increases production of inflammatory cytokines. Interestingly, in contrast to the observed superoxide accumulation in activated microglia after stroke and attenuation of superoxide accumulation in injured adult CD36 knockout mice, no significant increase in superoxide accumulation is detected in microglia from wild-type and CD36 knockout mice subjected to tMCAO at P9 (Woo et al., 2012). Cumulatively, these data show that CD36 may function differently in the neonate.

Another important aspect of microglial function in the neonatal brain is preservation of the neurovascular integrity and protection against hemorrhagic transformation after neonatal

stroke (Fernandez-Lopez et al., 2016). Microglial depletion triggers BBB leakage and induces hemorrhages, reduces microglia-derived TGFβ1 levels and the phosphorylation and intracellular distribution of SMAD2/3, but does not significantly alter expression or intracellular redistribution of several tight junction proteins. Selective inhibition of TGFbR2/ALK5 signaling in microglia via intra-cerebral liposome-encapsulated SB-431542 delivery triggers hemorrhages after tMCAO, demonstrating that TGFβ1/TGFbR2/ALK5 signaling in microglia protects from hemorrhages. Consistent with these observations in neonatal rats, depletion of microglia before tMCAO in P9 $Cx3cr1^{GFP/+}/Ccr2^{RFP/+}$ mice exacerbates injury and induces hemorrhagic transformation at 24 h (Fernandez-Lopez et al., 2016). Altogether these data suggest an array of microglial effects after neonatal brain ischemia related injury. While phagocytosis of neuronal debris and the newly discovered ability of microglial cells to protect neurovascular integrity serve as protective mechanisms, the inflammatory and ROS responses can contribute to injury. It is not clear whether the divergent results relate to different models of neonatal brain injury (i.p., the presence of systemic hypoxia in H–I model) or to time-resolved differing microglial phenotypes that participate in an acute and chronic injury. It is also essentially unknown whether a particular microglial subpopulation provides endogenous cerebrovascular protection.

SEXUAL DIMORPHISM AND MICROGLIA IN HEALTHY BRAIN DEVELOPMENT AND IN DISEASED IMMATURE BRAIN

Perinatal stroke and cerebral palsy are much more common in boys than in girls (Johnston and Hagberg, 2007; Nunez, 2012). Males are also at significantly higher risk than females of developing ASD and early onset forms of schizophrenia. Importantly, sex and sex hormones may have independent effects in stroke in children (Normann et al., 2009; Vannucci and Hurn, 2009) and juvenile mice (Herson et al., 2013). Although multiple sex-dependent mechanisms have been demonstrated, pertaining to neuronal death following neonatal H–I and focal ischemia, PARP-1 versus caspase-3 dependent (Hagberg et al., 2004; Renolleau et al., 2007, 2008), the response to anti-inflammatory therapy after perinatal brain injury (Nijboer et al., 2007; Fleiss et al., 2012) and divergent roles of innate immune receptors (Pimentel-Coelho et al., 2013), the underlying sex-based responses in injured neonatal brain continue to be insufficiently understood. Several studies revealed that microglia are sexually dimorphic (Schwarz et al., 2012; Lenz et al., 2013; Hanamsagar et al., 2017) and that the number of microglial cells is significantly higher in males than in females among several brain regions, including the hippocampus and parietal cortex (Schwarz et al., 2012; Lenz et al., 2013). Injury in Cx3Cr1 knockouts was shown to be affected by sex (Pimentel-Coelho et al., 2013) and sex-specific microglial functions were implicated in ASD (Derecki et al., 2012). Thus, the existence of intrinsic sex-specific differences in the neonatal period warrants studies in both sexes.

SUMMARY AND PERSPECTIVE

The fast progressing microglial field shows that microglial phenotypes rapidly evolve with brain maturation and that their interactions with neurons, the neurovasculature and other glial cells change dynamically. Literature is growing on the role of homeostatic microglia,

their receptor $P2RY_{12}R$ in particular, in overall brain development and synaptic plasticity (Sipe et al., 2016). Microglial cells are also recognized as being sexually dimorphic, and now believed to act as a neuropathology sensor in both the developing and mature brain. However, microglial contribution to injury markedly depends on brain maturation at the time of injury, injury context and sex. The frequently used term “activated microglia” is widely applied to both injurious and beneficial functions of these cells. While evidence is growing that the “gene signature” of microglial cells differs substantially from that of monocytes and other immune cells (Butovsky et al., 2014), the lack of readily available tools to reliably distinguish microglia from differentiating monocytes in injured brain as well as dynamic changes in the microglial phenotypes in response to changed local brain microenvironment, remains a big hurdle in separating the roles of microglia from those of other immune cells in the diseased brain. It is unknown whether ultrastructural features of microglial cells in injured neonatal brain bear resemblance with dysfunctional microglia demonstrated in adult or ageing brain (Bisht et al., 2016) and whether ultrastructural morphological features are different between male and female developing brains. Such knowledge would be important for an improved understanding of neurodevelopmental disorders as well as brain and neurovascular disorders that manifest much later in life.

ACKNOWLEDGMENTS

The authors acknowledge the funding sources that include Swedish Research Council (VR2012–2992; CM), Government grant in Public Health Service (ALFGBG-142881; CM), Åhlén Foundation (CM), Swedish Brain Foundation (FO2014-0080, FO2015-0190; CM), Torsten Söderberg (M98/15; CM), Wilhelm and Martina Lundgren Scientific Foundation (CM), Canada Research Chair (Tier 2) in Neuroimmune Plasticity in Health and Therapy (MET), RO1 NS44025 (ZSV), RO1 NS76726 (ZSV), R21NS098514 (ZSV), Boninger P2CHD086843- AR3T (ZSV), AHA17IRG33430004 (ZSV).

Abbreviations:

ASD	autism spectrum disorders
BBB	blood–brain barrier
Iba1	ionized calcium binding adaptor molecule 1
ROS	reactive oxidant species
TGFβ	transforming growth factor beta
TLRs	Toll-like receptors
tMCAO	transient middle cerebral artery occlusion

REFERENCES

Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C (2010) Pericytes regulate the blood–brain barrier. Nature 468:557–561. [PubMed: 20944627]

Arnold T, Betsholtz C (2013) The importance of microglia in the development of the vasculature in the central nervous system. Vasc Cell 5:4. [PubMed: 23422217]

Arnold TD, Niaudet C, Pang MF, Siegenthaler J, Gaengel K, Jung B, Ferrero GM, Mukouyama YS, Fuxe J, Akhurst R, Betsholtz C, Sheppard D, Reichardt LF (2014) Excessive vascular sprouting

underlies cerebral hemorrhage in mice lacking alphaVbeta8-TGFbeta signaling in the brain. Development 141:4489–4499. [PubMed: 25406396]

Arnoux I, Hoshiko M, Mandavy L, Avignone E, Yamamoto N, Audinat E (2013) Adaptive phenotype of microglial cells during the normal postnatal development of the somatosensory “Barrel” cortex. Glia 61:1582–1594. [PubMed: 23893820]

Arvin KL, Han BH, Du Y, Lin SZ, Paul SM, Holtzman DM (2002) Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. Ann Neurol 52:54–61. [PubMed: 12112047]

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J (2011) Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 25:40–45. [PubMed: 20705131]

Baburamani AA, Supramaniam VG, Hagberg H, Mallard C (2014) Microglia toxicity in preterm brain injury. Reprod Toxicol 48:106–112. [PubMed: 24768662]

Bauman MD, Iosif AM, Smith SE, Bregere C, Amaral DG, Patterson PH (2014) Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring. Biol Psychiatry 75:332–341. [PubMed: 24011823]

Bell MJ, Hallenbeck JM (2002) Effects of intrauterine inflammation on developing rat brain. J Neurosci Res 70:570–579. [PubMed: 12404511]

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A, Weissman IL, Chang EF, Li G, Grant GA, Hayden Gephart MG, Barres BA (2016) New tools for studying microglia in the mouse and human CNS. PNAS 113:E1738–E1746. [PubMed: 26884166]

Bialas AR, Stevens B (2013) TGF-beta signaling regulates neuronal C1q expression and developmental synaptic refinement. Nat Neurosci 16:1773–1782 (PMCID: PMC3973738). [PubMed: 24162655]

Bisht K, Sharma KP, Lecours C, Sanchez MG, El Hajj H, Milior G, Olmos-Alonso A, Gomez-Nicola D, Luheshi G, Vallieres L, Branchi I, Maggi L, Limatola C, Butovsky O, Tremblay ME (2016) Dark microglia: A new phenotype predominantly associated with pathological states. Glia 64:826–839. [PubMed: 26847266]

Bona E, Andersson AL, Blomgren K, Gilland E, Puka-Sundvall M, Gustafson K, Hagberg H (1999) Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. Pediatr Res 45:500–509. [PubMed: 10203141]

Bright HR, Babata K, Allred EN, Erdei C, Kuban KCK, Joseph RM, O’Shea TM, Leviton A, Dammann O, Investigators ES (2017) Neurocognitive outcomes at 10 years of age in extremely preterm newborns with late-onset bacteremia. J Pediatr 187:43–49. e41. [PubMed: 28526224]

Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE, Fanek Z, Liu L, Chen Z, Rothstein JD, Ransohoff RM, Gygi SP, Antel JP, Weiner HL (2014) Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci 17:131–143 (PMCID: 4066672). [PubMed: 24316888]

Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ, Barres BA (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28:264–278. [PubMed: 18171944]

Cai Z, Pan ZL, Pang Y, Evans OB, Rhodes PG (2000) Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res 47:64–72. [PubMed: 10625084]

Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 380:435–439. [PubMed: 8602241]

Checchin D, Sennlaub F, Levavasseur E, Leduc M, Chemtob S (2006) Potential role of microglia in retinal blood vessel formation. Invest Ophthalmol Vis Sci 47:3595–3602. [PubMed: 16877434]

Cho KH, Cheong JS, Kim JH, Abe H, Murakami G, Cho BH (2013) Site-specific distribution of CD68-positive microglial cells in the brains of human midterm fetuses: a topographical relationship with growing axons. Biomed Res Int 2013:762303. [PubMed: 24459672]

Cho S, Park EM, Febbraio M, Anrather J, Park L, Racchumi G, Silverstein RL, Iadecola C (2005) The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. J Neurosci 25:2504–2512. [PubMed: 15758158]

Cowell RM, Plane JM, Silverstein FS (2003) Complement activation contributes to hypoxic-ischemic brain injury in neonatal rats. J Neurosc 23:9459–9468.

Cunningham CL, Martinez-Cerdeno V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33:4216–4233 (PMCID: 3711552). [PubMed: 23467340]

Dalmau I, Finsen B, Zimmer J, Gonzalez B, Castellano B (1998) Development of microglia in the postnatal rat hippocampus. Hippocampus 8:458–474. [PubMed: 9825958]

Daneman R, Zhou L, Kebede AA, Barres BA (2010) Pericytes are required for blood–brain barrier integrity during embryogenesis. Nature 468:562–566. [PubMed: 20944625]

Dean JM, van de Looij Y, Sizonenko SV, Lodygensky GA, Lazeyras F, Bolouri H, Kjellmer I, Huppi PS, Hagberg H, Mallard C (2011) Delayed cortical impairment following lipopolysaccharide exposure in preterm fetal sheep. Ann Neurol 70:846–856. [PubMed: 22002627]

Delpech JC, Wei L, Hao J, Yu X, Madore C, Butovsky O, Kaffman A (2016) Early life stress perturbs the maturation of microglia in the developing hippocampus. Brain Behav Immun 57:79–93. [PubMed: 27301858]

Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SB, Guyenet PG, Kipnis J (2012) Wild-type microglia arrest pathology in a mouse model of Rett syndrome. Nature 484:105–109 (PMCID: 3321067). [PubMed: 22425995]

Dommergues MA, Plaisant F, Verney C, Gressens P (2003) Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection. Neuroscience 121:619–628. [PubMed: 14568022]

Du X, Fleiss B, Li H, D'Angelo B, Sun Y, Zhu C, Hagberg H, Levy O, Mallard C, Wang X (2011) Systemic stimulation of TLR2 impairs neonatal mouse brain development. PLoS One 6:e19583. [PubMed: 21573120]

Duncan JR, Cock ML, Scheerlinck JP, Westcott KT, McLean C, Harding R, Rees SM (2002) White matter injury after repeated endotoxin exposure in the preterm ovine fetus. Pediatr Res 52:941–949. [PubMed: 12438674]

Enstrom AM, Onore CE, Van de Water JA, Ashwood P (2010) Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav Immun 24:64–71. [PubMed: 19666104]

Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C (2010) Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 116:829–840. [PubMed: 20404134]

Faustino J, Wang X, Jonhson C, Klibanov A, Derugin N, Wendland M, Vexler ZS (2011) Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. J Neurosci 31:12992–13001 (PMCID: PMC3539822). [PubMed: 21900578]

Favrais G, van de Looij Y, Fleiss B, Ramanantsoa N, Bonnin P, Stoltenburg-Didinger G, Lacaud A, Saliba E, Dammann O, Gallego J, Sizonenko S, Hagberg H, Lelievre V, Gressens P (2011) Systemic inflammation disrupts the developmental program of white matter. Ann Neurol 70:550–565. [PubMed: 21796662]

Fernandez de Cossio L, Guzman A, van der Veldt S, Luheshi GN (2017) Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. Brain Behav Immun 63:88–98. [PubMed: 27697456]

Fernandez-Lopez D, Faustino J, Klibanov AL, Derugin N, Blanchard E, Simon F, Leib SL, Vexler ZS (2016) Microglial cells prevent hemorrhage in neonatal focal arterial stroke. J Neurosci 36:2881–2893 (PMCID: PMC4783493). [PubMed: 26961944]

Fernandez-Lopez D, Natarajan N, Ashwal S, Vexler ZS (2014) Mechanisms of perinatal arterial ischemic stroke. J Cereb Blood Flow Metab 34:921–932 (PMCID: 4050239). [PubMed: 24667913]

Ferrer I, Sarmiento J (1980) Nascent microglia in the developing brain. Acta Neuropathol 50:61–67. [PubMed: 7376828]

Fleiss B, Nilsson MK, Blomgren K, Mallard C (2012) Neuroprotection by the histone deacetylase inhibitor trichostatin A in a model of lipopolysaccharide-sensitised neonatal hypoxic-ischaemic brain injury. J Neuroinflammation 9:70 (PMCID: 3420244). [PubMed: 22512781]

Fox C, Dingman A, Derugin N, Wendland MF, Manabat C, Ji S, Ferriero DM, Vexler ZS (2005) Minocycline confers early but transient protection in the immature brain following focal cerebral ischemia-reperfusion. J Cereb Blood Flow Metab 25:1138–1149. [PubMed: 15874975]

Fujimoto E, Miki A, Mizoguti H (1989) Histochemical study of the differentiation of microglial cells in the developing human cerebral hemispheres. J Anat 166:253–264. [PubMed: 2621143]

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845 (PMCID: 3719181). [PubMed: 20966214]

Giulivi C, Napoli E, Schwartzer J, Careaga M, Ashwood P (2013) Gestational exposure to a viral mimetic poly(i:C) results in long-lasting changes in mitochondrial function by leucocytes in the adult offspring. Med Inflamm 2013:609602.

Hagberg H, Gressens P, Mallard C (2012) Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. Ann Neurol 71:444–457. [PubMed: 22334391]

Hagberg H, Mallard C, Ferriero D, Vannucci S, Levison S, Vexler Z, Gressens P (2015) The role of inflammation in perinatal brain injury. Nat Rev Neurol 11:192–208 (PMID: 25686754). [PubMed: 25686754]

Hagberg H, Wilson MA, Matsushita H, Zhu C, Lange M, Gustavsson M, Poitras MF, Dawson TM, Dawson VL, Northington F, Johnston MV (2004) PARP-1 gene disruption in mice preferentially protects males from perinatal brain injury. J Neurochem 90:1068–1075. [PubMed: 15312162]

Hanamsagar R, Alter MD, Block CS, Sullivan H, Bolton JL, Bilbo SD (2017) Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. Glia 65:1504–1520. [PubMed: 28618077]

Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C, Hagberg H (2002) Interleukin-18 involvement in hypoxic-ischemic brain injury. J Neurosci 22:5910–5919. [PubMed: 12122053]

Herson PS, Bombardier CG, Parker SM, Shimizu T, Klawitter J, Quillinan N, Exo JL, Goldenberg NA, Traystman RJ (2013) Experimental pediatric arterial ischemic stroke model reveals sex-specific estrogen signaling. Stroke 44:759–763. [PubMed: 23349190]

Hoshiko M, Arnoux I, Avignone E, Yamamoto N, Audinat E (2012) Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. J Neurosci 32:15106–15111. [PubMed: 23100431]

Hsiao EY (2013) Immune dysregulation in autism spectrum disorder. Int Rev Neurobiol 113:269–302. [PubMed: 24290389]

Hu BR, Liu CL, Ouyang Y, Blomgren K, Siesjo BK(2000) Involvement of caspase-3 in cell death after hypoxia-ischemia declines during brain maturation. J Cereb Blood Flow Metab 20:1294–1300. [PubMed: 10994850]

Hutchins KD, Dickson DW, Rashbaum WK, Lyman WD (1990) Localization of morphologically distinct microglial populations in the developing human fetal brain: implications for ontogeny. Brain Res Dev Brain Res 55:95–102. [PubMed: 2208643]

Iadecola C, Anrather J (2011) The immunology of stroke: from mechanisms to translation. Nat Med 17:796–808. [PubMed: 21738161]

Ivacko JA, Sun R, Silverstein FS (1996) Hypoxic-ischemic brain injury induces an acute microglial reaction in perinatal rats. Pediatr Res 39:39–47. [PubMed: 8825384]

Johnston MV, Hagberg H (2007) Sex and the pathogenesis of cerebral palsy. Dev Med Child Neurol 49:74–78. [PubMed: 17209983]

Kadhim H, Tabarki B, Verellen G, De Prez C, Rona AM, Sebire G (2001) Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. Neurology 56:1278–1284. [PubMed: 11376173]

Kannan S, Saadani-Makki F, Muzik O, Chakraborty P, Mangner TJ, Janisse J, Romero R, Chugani DC (2007) Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: detection with 11C-(R)-PK11195 and small-animal PET. J Nucl Med 48:946–954. [PubMed: 17504871]

Kavas N, Arisoy AE, Bayhan A, Kara B, Gunlemez A, Turker G, Oruc M, Gokalp AS (2017) Neonatal sepsis and simple minor neurological dysfunction. Pediatr Int 59:564–569. [PubMed: 27935218]

Kelley MH, Wu WW, Lei J, McLane M, Xie H, Hart KD, Pereira L, Burd I, Maylie J (2017) Functional changes in hippocampal synaptic signaling in offspring survivors of a mouse model of intrauterine inflammation. J Neuroinflammation 14:180. [PubMed: 28874190]

Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, Shibuya M, Saya H, Suda T (2009) M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. J Exp Med 206:1089–1102 (PMCID: 2715025). [PubMed: 19398755]

Lalancette-Hebert M, Faustino J, Thammisetty SS, Chip S, Vexler ZS, Kriz J (2017) Live imaging of innate immune response in neonates reveals differential TLR-2 dependent activation patterns in sterile inflammation and infection. Brain Behav Immun 65:312–327. [PubMed: 28579520]

Lalancette-Hebert M, Phaneuf D, Soucy G, Weng YC, Kriz J (2009) Live imaging of Toll-like receptor 2 response in cerebral ischaemia reveals a role of olfactory bulb microglia as modulators of inflammation. Brain.

Lambertsen KL, Clausen BH, Babcock AA, Gregersen R, Fenger C, Nielsen HH, Haugaard LS, Wirenfeldt M, Nielsen M, Dagnaes-Hansen F, Bluethmann H, Faergeman NJ, Meldgaard M, Deierborg T, Finsen B (2009) Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. J Neurosci 29:1319–1330. [PubMed: 19193879]

Lenz KM, Nugent BM, Haliyur R, McCarthy MM (2013) Microglia are essential to masculinization of brain and behavior. J Neurosci 33:2761–2772. [PubMed: 23407936]

Li F, Faustino J, Woo M, Derugin N, Vexler ZS (2015) Lack of the scavenger receptor CD36 alters microglial phenotypes after neonatal stroke. J Neurochem 13:445–452 (PMID: 26223273).

Lucchina L, Depino AM (2013) Altered peripheral and central inflammatory responses in a mouse model of autism. Autism Res.

Lucin KM, Wyss-Coray T (2009) Immune activation in brain aging and neurodegeneration: too much or too little? Neuron 64:110–122. [PubMed: 19840553]

Malkova NV, Yu CZ, Hsiao EY, Moore MJ, Patterson PH (2012) Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav Immun 26:607–616. [PubMed: 22310922]

Mallard C, Wang X, Hagberg H (2009) The role of Toll-like receptors in perinatal brain injury. Clin Perinatol 36:763–772. v–vi. [PubMed: 19944834]

Mallard C, Welin AK, Peebles D, Hagberg H, Kjellmer I (2003) White matter injury following systemic endotoxemia or asphyxia in the fetal sheep. Neurochem Res 28:215–223. [PubMed: 12608695]

Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime Rep 6:13. [PubMed: 24669294]

Mattei D, Ivanov A, Ferrai C, Jordan P, Guneykaya D, Buonfiglioli A, Schaafsma W, Przanowski P, Deuther-Conrad W, Brust P, Hesse S, Patt M, Sabri O, Ross TL, Eggen BJL, Boddeke E, Kaminska B, Beule D, Pombo A, Kettenmann H, Wolf SA (2017) Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment. Transl Psychiatry 7:e1120. [PubMed: 28485733]

McRae A, Gilland E, Bona E, Hagberg H (1995) Microglia activation after neonatal hypoxic-ischemia. Brain Res Dev Brain Res 84:245–252. [PubMed: 7743644]

Mildner A, Huang H, Radke J, Stenzel W, Priller J (2017) P2Y12 receptor is expressed on human microglia under physiological conditions throughout development and is sensitive to neuroinflammatory diseases. Glia 65:375–387. [PubMed: 27862351]

Monier A, Evrard P, Gressens P, Verney C (2006) Distribution and differentiation of microglia in the human encephalon during the first two trimesters of gestation. J Comp Neurol 499:565–582. [PubMed: 17029271]

Mottahedin A, Smith PL, Hagberg H, Ek CJ, Mallard C (2017) TLR2-mediated leukocyte trafficking to the developing brain. J Leukoc Biol 101:297–305. [PubMed: 27493242]

Murabe Y, Sano Y (1982) Morphological studies on neuroglia. VI. Postnatal development of microglial cells. Cell Tissue Res 225:469–485. [PubMed: 6290069]

Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA (2014) Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 41:14–20. [PubMed: 25035950]

Naviaux RK, Zolkipli Z, Wang L, Nakayama T, Naviaux JC, Le TP, Schuchbauer MA, Rogac M, Tang Q, Dugan LL, Powell SB (2013) Antipurinergic therapy corrects the autism-like features in the poly (IC) mouse model. PLoS One 8:e57380. [PubMed: 23516405]

Neniskyte U, Gross CT (2017) Errant gardeners: glial-cell-dependent synaptic pruning and neurodevelopmental disorders. Nat Rev Neurosci 18:658–670. [PubMed: 28931944]

Nijboer CH, Groenendaal F, Kavelaars A, Hagberg HH, van Bel F, Heijnen CJ (2007) Gender-specific neuroprotection by 2-iminobiotin after hypoxia-ischemia in the neonatal rat via a nitric oxide independent pathway. J Cereb Blood Flow Metab 27:282–292. [PubMed: 16736041]

Nitsos I, Rees SM, Duncan J, Kramer BW, Harding R, Newnham JP, Moss TJ (2006) Chronic exposure to intra-amniotic lipopolysaccharide affects the ovine fetal brain. J Soc Gynecol Invest 13:239–247.

Normann S, de Veber G, Fobker M, Langer C, Kenet G, Bernard TJ, Fiedler B, Strater R, Goldenberg NA, Nowak-Gottl U (2009) Role of endogenous testosterone concentration in pediatric stroke. Ann Neurol 66:754–758. [PubMed: 20033984]

Nunez J (2012) Sex and steroid hormones in early brain injury. Rev Endocr Metab Disord 13:173–186. [PubMed: 22565659]

Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R (1989) Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 59:1203–1211. [PubMed: 2688898]

Pagani F, Paolicelli RC, Murana E, Cortese B, Di Angelantonio S, Zurolo E, Guiducci E, Ferreira TA, Garofalo S, Catalano M, D'Alessandro G, Porzia A, Peruzzi G, Mainiero F, Limatola C, Gross CT, Ragozzino D (2015) Defective microglial development in the hippocampus of Cx3cr1 deficient mice. Front Cell Neurosci 9:111. [PubMed: 25873863]

Pang Y, Rodts-Palenik S, Cai Z, Bennett WA, Rhodes PG (2005) Suppression of glial activation is involved in the protection of IL-10 on maternal E. coli induced neonatal white matter injury. Brain Res Dev Brain Res 157:141–149. [PubMed: 15878785]

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456–1458. [PubMed: 21778362]

Pimentel-Coelho PM, Michaud JP, Rivest S (2013) Evidence for a gender-specific protective role of innate immune receptors in a model of perinatal brain injury. J Neurosci 33:11556–11572. [PubMed: 23843525]

Ransohoff RM (2016) A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 19:987–991. [PubMed: 27459405]

Renolleau S, Fau S, Charriaut-Marlangue C (2008) Gender-related differences in apoptotic pathways after neonatal cerebral ischemia. Neuroscientist 14:46–52. [PubMed: 17971506]

Renolleau S, Fau S, Goyenvalle C, Charriaut-Marlangue C (2007) Sex, neuroprotection, and neonatal ischemia. Dev Med Child Neurol 49:477 [Author reply 477–478]. [PubMed: 17518938]

Salas AA, Faye-Petersen OM, Sims B, Peralta-Carcelen M, Reilly SD, McGwin G Jr, Carlo WA, Ambalavanan N (2013) Histological characteristics of the fetal inflammatory response associated with neurodevelopmental impairment and death in extremely preterm infants. J Pediatr 163:652–657. e651-e652. [PubMed: 23664630]

Saliba E, Henrot A (2001) Inflammatory mediators and neonatal brain damage. Biol Neonate 79:224–227. [PubMed: 11275656]

Salter MW, Beggs S (2014) Sublime microglia: expanding roles for the guardians of the CNS. Cell 158:15–24. [PubMed: 24995975]

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an

activity and complement-dependent manner. Neuron 74:691–705 (PMICD: 3528177). [PubMed: 22632727]

Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336:86–90. [PubMed: 22442384]

Schwarz JM, Sholar PW, Bilbo SD (2012) Sex differences in microglial colonization of the developing rat brain. J Neurochem 120:948–963 (PMCID: 3296888). [PubMed: 22182318]

Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol 106–107:1–16.

Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC (1995) Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376:62–66. [PubMed: 7596435]

Shankaran S, Laptook AR, Pappas A, McDonald SA, Das A, Tyson JE, Poindexter BB, Schibler K, Bell EF, Heyne RJ, Pedroza C, Bara R, Van Meurs KP, Grisby C, Huitema CM, Garg M, Ehrenkranz RA, Shepherd EG, Chalak LF, Hamrick SE, Khan AM, Reynolds AM, Laughon MM, Truog WE, Dysart KC, Carlo WA, Walsh MC, Watterberg KL, Higgins RD, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2014) Effect of depth and duration of cooling on deaths in the NICU among neonates with hypoxic ischemic encephalopathy: a randomized clinical trial. JAMA 312:2629–2639. [PubMed: 25536254]

Shatrov JG, Birch SC, Lam LT, Quinlivan JA, McIntyre S, Mendz GL (2010) Chorioamnionitis and cerebral palsy: a meta-analysis. Obstet Gynecol 116:387–392. [PubMed: 20664400]

Sipe GO, Lowery RL, Tremblay ME, Kelly EA, Lamantia CE, Majewska AK (2016) Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. Nat Commun 7:10905 (PMCID: 4786684). [PubMed: 26948129]

Smith PL, Hagberg H, Naylor AS, Mallard C (2014) Neonatal peripheral immune challenge activates microglia and inhibits neurogenesis in the developing murine hippocampus. Dev Neurosci 36:119–131. [PubMed: 24642725]

Soraisham AS, Trevenen C, Wood S, Singhal N, Sauve R (2013) Histological chorioamnionitis and neurodevelopmental outcome in preterm infants. J Perinatol 33:70–75. [PubMed: 22555781]

Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, Bessis A, Ginhoux F, Garel S (2014) Microglia modulate wiring of the embryonic forebrain. Cell Rep 8:1271–1279. [PubMed: 25159150]

Stanimirovic DB, Wong J, Shapiro A, Durkin JP (1997) Increase in surface expression of ICAM-1, VCAM-1 and E-selectin in human cerebromicrovascular endothelial cells subjected to ischemia-like insults. Acta Neurochir Suppl 70:12–16. [PubMed: 9416264]

Stridh L, Smith PL, Naylor AS, Wang X, Mallard C (2011) Regulation of Toll-like receptor 1 and −2 in neonatal mice brains after hypoxia-ischemia. J Neuroinflammation 8:45. [PubMed: 21569241]

Szalay G, Martinecz B, Lenart N, Kornyei Z, Orsolits B, Judak L, Csaszar E, Fekete R, West BL, Katona G, Rozsa B, Denes A (2016) Microglia protect against brain injury and their selective elimination dysregulates neuronal network activity after stroke. Nat Commun 7:11499 (PMCID: 4857403). [PubMed: 27139776]

Tremblay ME, Lecours C, Samson L, Sanchez-Zafra V, Sierra A (2015) From the Cajal alumni Achucarro and Rio-Hortega to the rediscovery of never-resting microglia. Front Neuroanat 9:45. [PubMed: 25926775]

Tremblay ME, Lowery RL, Majewska AK (2010a) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8:e1000527. [PubMed: 21072242]

Tremblay ME, Majewska AK (2011) A role for microglia in synaptic plasticity? Commun Integr Biol 4:220–222. [PubMed: 21655446]

Tremblay ME, Riad M, Majewska A (2010b) Preparation of mouse brain tissue for immunoelectron microscopy. J Visualized Exp.

Tsuji M, Wilson MA, Lange MS, Johnston MV (2004) Minocycline worsens hypoxic-ischemic brain injury in a neonatal mouse model. Exp Neurol 189:58–65. [PubMed: 15296836]

Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci 16:543–551. [PubMed: 23525041]

Vannucci SJ, Hurn PD (2009) Gender differences in pediatric stroke: is elevated testosterone a risk factor for boys? Ann Neurol 66:713–714 (PMCID: 2888285). [PubMed: 20035500]

Walton NM, Sutter BM, Laywell ED, Levkoff LH, Kearns SM, Marshall GP 2nd, Scheffler B, Steindler DA (2006) Microglia instruct subventricular zone neurogenesis. Glia 54:815–825. [PubMed: 16977605]

Woo MS, Wang X, Faustino J, Derugin N, Wendland MF, Zhou P, Iadecola C, Vexler ZS (2012) Genetic deletion of CD36 enhances injury after acute neonatal stroke. Ann Neurol 72:961–970 (PMID: 23280844). [PubMed: 23280844]

Xu H, Barks JD, Schielke GP, Silverstein FS (2001) Attenuation of hypoxia-ischemia-induced monocyte chemoattractant protein-1 expression in brain of neonatal mice deficient in interleukin-1 converting enzyme. Brain Res Mol Brain Res 90:57–67. [PubMed: 11376856]

Yager JY, Ashwal S (2009) Animal models of perinatal hypoxic-ischemic brain damage. Pediatr Neurol 40:156–167. [PubMed: 19218028]

Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F, Vyssotski AL, Bifone A, Gozzi A, Ragozzino D, Gross CT (2014) Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 17:400–406. [PubMed: 24487234]

Zhang Y, Barres BA (2010) Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr Opin Neurobiol 20:588–594. [PubMed: 20655735]

Zhang Z, Bassam B, Thomas AG, Williams M, Liu J, Nance E, Rojas C, Slusher BS, Kannan S (2016) Maternal inflammation leads to impaired glutamate homeostasis and up-regulation of glutamate carboxypeptidase II in activated microglia in the fetal/newborn rabbit brain. Neurobiol Dis 94:116–128. [PubMed: 27326668]

Zrzavy T, Hametner S, Wimmer I, Butovsky O, Weiner HL, Lassmann H (2017) Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. Brain 140:1900–1913. [PubMed: 28541408]