HHS Public Access

Author manuscript

Dev Neurobiol. Author manuscript; available in PMC 2019 June 01.

Published in final edited form as:

Dev Neurobiol. 2018 June ; 78(6): 604–617. doi:10.1002/dneu.22576.

Microglial Interaction with the Neurovascular System in Physiology and Pathology

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Abstract

Microglia as immune cells of the central nervous system (CNS) play significant roles not only in pathology but also in physiology, such as shaping of the CNS during development and its proper maintenance in maturity. Emerging research is showing a close association between microglia and neurovasculature that is critical for brain energy supply. In this review, we summarize the current literature on microglial interaction with the vascular system in the normal and diseased brain. First, we highlight data that indicate interesting potential involvement of microglia in developmental angiogenesis. Then we discuss the evidence for microglial participation with the vasculature in neuropathologies from brain tumors to acute injuries such as ischemic stroke to chronic neurodegenerative conditions. We conclude by suggesting future areas of research to advance the field in light of current technical progress and outstanding questions.

Keywords

Microglia; Neurovasculature; Angiogenesis; Stroke; Neurodegeneration

[1] Introduction

Microglia are innate immune cells of the brain that are fully engaged in central nervous system (CNS) functions in normal and pathological conditions (Hanisch and Kettenmann, 2007; Casano and Peri, 2015). Depending on various methods used and regions examined, microglia make up 5–15% of brain cells (Pelvig et al., 2008; Lyck et al., 2009) and many of their properties distinguish them as a unique cell type from other brain cells as confirmed by recent transcriptome studies (Hickman et al., 2013; Zhang et al., 2014; Bennett et al., 2016). Neurons and macroglia like astrocyte and oligodendrocytes are derived from the neuroectoderm, while microglia originate from the embryonic yolk sac, then migrate and colonize the neuroepithelium (Prinz and Priller, 2014; Reemst et al., 2016). In the mature brain, microglia are exquisitely tiled and share non-overlapping territories with a small central soma and multiple elaborate processes (Davalos et al., 2005; Nimmerjahn et al., 2005; Wu et al., 2007). Unlike peripheral macrophages and circulating monocytes, microglia

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Conflict of Interest: The authors declare no competing financial interests.

keep a fairly low turnover rate (Lawson et al., 1992; Askew et al., 2017; Tay et al., 2017) without contributions from peripheral sources such as circulating monocytes or blood-derived macrophages in the healthy condition (Ajami et al., 2007; Ajami et al., 2011; Gu et al., 2016).

One of the most notable features of microglia is their robust morphological plasticity by which they perform CNS surveillance (Davalos et al., 2005; Nimmerjahn et al., 2005). With remarkably dynamic processes, they frequently interact with neuronal elements including somata (Li et al., 2012; Eyo et al., 2014), axons (Baalman et al., 2015), dendrites and synapses (Wake et al., 2009; Tremblay et al., 2010; Eyo et al., 2015; Eyo et al., 2017) by which they sense and monitor neural activity and thus modulate neural circuit processing (Chen et al., 2010; Paolicelli and Gross, 2011; Parkhurst et al., 2013; Schafer et al., 2013; Squarzoni et al., 2014). Microglia are also the primary phagocytes of the brain and therefore engage and engulf excess neural material during early development (Marin-Teva et al., 2004; Petersen and Dailey, 2004; Wakselman et al., 2008; Svahn et al., 2013; Eyo et al., 2016) and in select neurogenic niches in the mature brain (Sierra et al., 2010; Sierra et al., 2014; Abiega et al., 2016; Fourgeaud et al., 2016). Thus, microglial roles in shaping brain development and function by regulating neuronal activity and phagocytic clearance have been well established and continue to be explored.

In addition to functioning in the healthy brain, microglia participate in aberrant neurological conditions from acute injury to neurodegenerative diseases (Ransohoff and Perry, 2009; Nayak et al., 2014; Peng et al., 2016; Eyo et al., 2017). Here, they are hotly debated to promote either neuroprotective or neurotoxic functions. They can ameliorate pathology as is the case with acute experimental seizures where seizure behaviors are worsened with microglial elimination or microglial P2Y12 receptor depletion (Mirrione et al., 2010; Eyo et al., 2014) and ischemic stroke where they are presumed to reduce infarct size and promote functional recovery (Lalancette-Hebert et al., 2007; Narantuya et al., 2010; Faustino et al., 2011). Conversely, they can also promote disease progression as in the case of neuropathic pain (Peng et al., 2016). It is noteworthy to point out that microglial functions are not always straight-forward in diseases. For example, while studies may suggest neuroprotective microglial functions in ischemia there is also evidence for neurotoxic activity in the disease (Wu et al., 2012; Tian et al., 2016). Interestingly, microglia have also been implicated in the pathogenesis of psychiatric disorders such as pathological grooming in mice which is suggested to mimic obsessive compulsive disorder (OCD) in humans (Chen et al., 2010). Together, these studies and many others indicate that microglia are significant cellular components in the brain orchestrating critical events during normal development, the maintenance of the mature CNS and during pathology.

It has been long recognized that microglia physically interact with the neurovasculature and interest in the physiological significance of these interactions are increasing (Arnold and Betsholtz, 2013; Dudvarski Stankovic et al., 2016). The brain vasculature, which includes a complex arrangement of arteries, veins and capillaries, distributes essential substances like oxygen and glucose to the brain while eliminating waste products like CO_2 (Daneman and Prat, 2015). The brain, though constituting only about 2% of the body's weight, demands about 20% of the body's energy (Magistretti and Allaman, 2015). This fact implies a need

for efficient and controlled delivery of blood to the brain, which is especially critical for optimal synaptic and thus cognitive function. While astrocytes are now established as critical regulators of the neurovasculature (MacVicar and Newman, 2015), microglia have increasingly been implicated in neurovascular development and complexity and thus indirectly regulate brain function. This review will summarize the current literature on the role for microglia in neurovascular function. Our assessment will begin with the relevant literature on the current conclusions regarding microglial roles in developmental angiogenesis and conclude with the insights provided by studies investigating microglial function in pathological conditions including cerebral tumors and neurodegenerative diseases.

While the focus of this review is not on microglial-astrocyte or microglial-pericyte interactions, it should be noted that there is growing evidence for interactions between microglia and these cells as components of the neurovascular unit. For example, microglia promote astrocyte differentiation (Nakanishi et al., 2007). However, astrocytes also regulate microglial phenotypes by paracrine signaling of released molecules (Rezaie et al., 2002; Bohlen et al., 2017) indicating that bidirectional regulation between microglia and astrocytes could have consequences on vascular development and integrity. Pericytes also serve as cytokine responsive cells and amplify microglia activation (Matsumoto et al., 2014) and are suggested to function as immune suppressors (Hurtado-Alvarado et al., 2014). This might imply a regulation by pericytes of the microglial phenotype on the uninjured brain. They have been recently reported to be a source of microglia especially following ischemia (Ozen et al., 2014; Sakuma et al., 2016). Moreover, activated microglia promote pericytes cell death via ROS production (Ding et al., 2017) and may thus compromise blood vessel integrity since pericytes make up the basement membranes of the vasculature. Therefore, although we don’t highlight the various interactions between these cellular components of the neurovascular unit and microglia in the following pages, these interactions cannot be ignored.

[2] Microglia and Blood Vessels in Normal Physiology

[2.1] Microglial Colonization of the CNS Precedes the Development of, but then Closely Associates with Blood Vessels

Several studies have documented that microglial emergence precedes the carefully orchestrated formation of the cerebral blood vessel network in the developing brain. This is the case in the mouse retina (Rymo et al., 2011) as well as hindbrain (Fantin et al., 2010). Consistent with these results from mice, avian data using chick-quail chimeras also revealed that yolk sac derived macrophages that give rise to microglia invade the CNS prior to and independent of neurovascular development (Cuadros et al., 1993; Kurz and Christ, 1998).

Although microglia and the neurovasculature do not appear in the developing CNS at the same time, when they do appear, they often display physical interactions. Early studies documented the localization of microglial cells with the vasculature in the developing rodent brain (Ashwell et al., 1989) and were sometimes referred to as “pericytic macrophages” (Thomas, 1999). This has been repeatedly confirmed in subsequent studies in the brain proper (Arnoux et al., 2013) and suggests relevant (perhaps bi-directional) communication

between microglia and the vasculature. Moreover, these observations provided support for an early hypothesis that microglia migrate into the brain via blood vessels (Perry et al., 1985). Subsequent results, however, suggest that circulating cells do not infiltrate the brain parenchyma in the healthy CNS (Ajami et al., 2007) dampening enthusiasm for this early hypothesis. The observation of microglial proximity to blood vessels is especially noticeable during development. For example, in both the developing human and mouse retina, microglia are closely opposed to blood vessels (Checchin et al., 2006; Rymo et al., 2011). Furthermore, "juxtavascular" microglia were observed by real time confocal imaging to migrate along the walls of cerebral blood vessels in brain slices (Grossmann et al., 2002). These microglia exhibited a greater likelihood of migration than non-juxtavascular microglia. Although such observations were determined in an excised tissue context, whether the observations are an artifact of tissue excision or representative of the native developmental brain environment will have to be elucidated using more robust *in vivo* imaging approaches. Moreover, the precise function of this migration along blood vessels is not clear.

Less work has been done to provide details on microglial-vascular interactions in the mature brain. Nevertheless, interest in functional interactions between microglia and the neurovasculature continues to mount. Our initial studies using *in vivo* two photon imaging in adult mice has revealed that microglia maintain robust physical contact with elements of the neurovasculature (Fig. 1). Work is underway to adequately characterize these interesting microglial interactions with the neurovascular system and determine their functional significance in physiology and pathology.

[2.2] Microglia Promote Angiogenesis

One of the increasingly recognized functions of microglia is that they participate in the formation of new blood vessels or angiogenesis. This has especially been documented in pathological contexts (see next section) and presumes prior mechanistic similarities in earlier development. We will now consider some of the evidence that have been provided for this from both genetic and pharmacological approaches.

Support for the involvement of microglia in developmental angiogenesis from genetic approaches was first provided by the genetic depletion of macrophage colony stimulating factor, otherwise known as mCSF. This gene controls the development and survival of cells of the monocytic lineage including brain microglia (Cecchini et al., 1994). In mice genetically deficient of mCSF, the development of microglia and other monocyte-derived cells are lacking. Interestingly, the complexity of the developing retinal vasculature in mCSF knockout mice was also reduced suggesting roles for microglial mCSF signaling in angiogenesis in the retina. The deficiency in vasculature complexity was transient as differences were only observed in development but not in adulthood. Therefore, there might be complementary non-mCSF or microglia-independent mechanisms for retinal angiogenesis (Kubota et al., 2009).

Similar to mCSF, the *PU.1* gene controls hematopoietic cell differentiation and genetic interruption of its function prevents microglial development (Scott et al., 1994; McKercher et al., 1996). Interestingly, PU.1 mutant mice also exhibit less elaborate blood vessel

complexity (Fantin et al., 2010). Of course, given the limitation that these genetic approaches target non-microglial cells, the deficiency in vascular complexity could result from contributions from non-microglial population. Therefore, more selective microglial elimination techniques will have to be employed in future studies to adequately determine microglia-specific roles in developmental angiogenesis. However, it is worth noting that the challenge of ablating microglia in early development is not trivial and has yet to be accomplished at the time of this writing.

Pharmacological evidence suggesting microglial contributions to developmental angiogenesis has been multiply documented. First, consistent with a role for mCSF, its pharmacological inhibition using neutralizing antibodies to mCSF also reduced blood vessel complexity (Kubota et al., 2009). This pharmacological approach also suffers from the limitation that mCSF could target non-microglial cells that express mCSF receptors. Second, selective depletion of microglia using clodronate liposomes by which microglia die upon their uptake liposomes, resulted in reduced vascularization in the developing mouse retina (Checchin et al., 2006). Yet, as with the genetic approach, future studies will have to specifically target microglia selectively and do so especially in an *in vivo* context. Together, these results consistently suggest that microglia promote developmental angiogenesis.

[3] Microglia and Blood Vessels in Pathology

Although microglial roles in blood vessel development and maintenance have not received intense research attention as discussed above, more research has been directed to its function in various pathologies from brain tumors to acute brain injuries like ischemia to chronic neurodegenerative diseases. Current data on these findings will be discussed in this section. The relevance of findings from such studies is that understanding microglial roles in these pathological contexts could (i) inform future therapies that can be developed in the treatment directed towards ameliorating the progression of these pathologies and (ii) inform researchers on candidate mechanisms to identify factors by which microglia may regulate developmental angiogenesis and neurovascular physiology.

[3.1] Microglia and Blood Vessels in Brain Tumors

In the last two decades of angiogenesis research, it has become clear that vascular endothelial growth factor (VEGF) is a predominant regulator in the development and patterning of blood vessels (Thomas, 1996). This has been confirmed in multiple systems including the mouse brain (Ruhrberg et al., 2002; Haigh et al., 2003; Raab et al., 2004), the mouse retina (Stalmans et al., 2002; Haigh et al., 2003) and the quail neural tube (James et al., 2009). As with developmental angiogenesis, the tumor environment is pro-angiogenic and thus seems to promote the development of new blood vessels (otherwise termed “neovascularization”), which is important for (i) the delivery of blood and its accompanying nutrients to the tumor and (ii) the metastasis of tumor cells from the original tumor site to novel sites (Lorger, 2012). On this account, brain tumors possess a characteristically increased density of blood vessels (Lopes, 2003) some of which could be malformed and potentially leaky. Factors that promote the neovascularization of tumors are therefore

generally considered detrimental to the outcome for the patient while those that conversely limit tumorigenic neovascularization are considered beneficial.

While astrocytes are considered to be a predominant cell type that secrete VEGF for angiogenesis (Pierce et al., 1995), selectively abrogating astrocyte-derived VEGF did not significantly alter the developmental angiogenesis (Scott et al., 2010; Weidemann et al., 2010) suggesting that either compensatory mechanisms are in play or astrocyte-derived VEGF is not critical for blood vessel development. Although microglial VEGF roles in developmental angiogenesis are not clear, microglia are now known to express some VEGF isoforms (Zhang et al., 2014) suggesting the capacity for regulating vascularization.

In human tumors, microglial/macrophage density was increased with a corresponding increase in tumorigenic neovascularization (Nishie et al., 1999; Brandenburg et al., 2016) and macrophages were increasingly associated with blood vessels (Leek et al., 1996; Brandenburg et al., 2016). Since microglia are the macrophages of the CNS, the implication that macrophages induce angiogenesis in tumor environments (Kobayashi et al., 1994; Sunderkotter et al., 1994; Polverini, 1997; Wang et al., 2013; Qin et al., 2015) could also apply to microglia (Wyckoff et al., 2004). Interestingly, despite the fact that it is difficult to distinguish brain resident microglia from infiltrated macrophages molecularly, recent evidence suggest that the pro-angiogenic function is predominantly carried out by microglia rather than macrophages in brain tumors (Brandenburg et al., 2016). Furthermore, factors derived from microglia are known to facilitate tumor progression (Sliwa et al., 2007). Specifically, microglia release tumor necrosis factor-alpha (TNFα) in the tumorigenic environment (Hattermann et al., 2014; Hwang et al., 2016), which in turn regulates the release of VEGF from glioma cell lines (Ryuto et al., 1996) that is critical for neovascularization. Thus either by the direct release of VEGF or the indirect release of other factors that increase its expression, microglia participate in the promotion of tumor angiogenesis. Finally, since VEGF serves as a chemoattractant to microglia (Forstreuter et al., 2002), its release in tumors could serve as an attractive signal to microglia towards blood vessels in an autocrine (if microglia are the source) or paracrine (if other cells are the source) manner.

In addition to microglial VEGF signaling, microglia release matrix metalloproteinases (MMPs) in vascularizing the tumor environment. Since MMPs function to degrade and remodel the extracellular matrix, their function is pro-angiogenic and thought to be recruited by the tumor for the promotion of tumor expansion (Egeblad and Werb, 2002; Rao, 2003). Microglia express several MMPs along with other cells of the brain (Hickman et al., 2013; Zhang et al., 2014; Holtman et al., 2015). MMP expression such as MT1-MMP (or MMP14), has been detected on human and murine microglia while tumor cells fail to express the protein (Markovic et al., 2009). Moreover, microglial depletion using either clodronate liposomes which eliminated MMP expression or genetic ablation both resulted in a reduction in tumor invasion (Markovic et al., 2009) confirming a pro-tumorigenic role for the microglial-derived MMP. Consistent with a role for microglial release of MT1-MMP in promoting tumor-induced angiogenesis, upregulation of the gene during tumor progression was correlated with increased neovascularization (Gabrusiewicz et al., 2011). These results

indicate that microglial MMP activity promotes tumor progression by facilitating vascularization in the tumor environment.

Other microglial factors have been identified that promote tumor growth. For example, the microglial Na(+)/H(+) exchanger isoform 1 (NHE1) was recently identified as a respectable target to ameliorate tumor progression (Zhu et al., 2016). The mechanisms investigated suggested a regulation of tumor cell migration and proliferation. However, it would be interesting to determine whether the exchanger promotes tumor invasiveness by angiogenic mechanisms as well. Recently, CXCL2, a cytokine which is predominantly expressed by microglia in the brain parenchyma (Hickman et al., 2013; Zhang et al., 2014; Holtman et al., 2015), was shown to promote tumor-induced angiogenesis (Brandenburg et al., 2016). These results indicate that microglia facilitate blood vessel formation in a tumorigenic environment and some of the regulatory factors include growth factors, proteases, transporters and cytokines.

[3.2] Microglia and Blood Vessels in Ischemic Stroke

Stroke is the fifth leading cause of mortality in United States and a leading cause of disability (Talwalkar and Uddin, 2015). Many aspects of the role of microglia in ischemic contexts have been extensively reviewed (Ma et al., 2016). Microglia respond earliest following an ischemic insult and serve as the first line of defense to the injury (Morioka et al., 1991; Weinstein et al., 2010). Microglial accumulation is also one of the earliest cellular signatures in cerebral ischemia (Gelderblom et al., 2009). Ischemia induced by photothrombosis revealed that microglial dynamic activity is closely associated with capillary blood flow around its cell body (Masuda et al., 2011). The dynamics of microglial processes is suppressed around the capillary with decreased blood flow, suggesting microglial surveillance is inhibited during ischemia, which is consistent with evidence from the developing brain (Eyo and Dailey, 2012).

Microglia become closely associated with blood vessel after ischemia by forming perivascular clusters and phagocytic structures (Jolivel et al., 2015). The accumulation of microglia around the vasculature subsequently led to the disintegration of the vessels which included their upregulation of phagocytic CD68 expression in the penumbra area. Accumulation of microglia with blood vessels also correlated with the invasion of blood-borne molecules during reperfusion (Jolivel et al., 2015). Interestingly, a selective inactivation of microglial CX3CR1 that has been reported to regulate microglial migration (Cardona et al., 2006; Liang et al., 2009) and significantly reduce blood extravasation (Jolivel et al., 2015). This may be a mechanism for neuroprotection in stroke since several studies have indicated reduced stroke pathology in CX3CR1-deficient mice (Denes et al., 2008; Jolivel et al., 2015).

Furthermore, perivascular microglia might also contribute to cerebral ischemia by releasing microglia-specific cytokines that are known to compromise vascular integrity in ischemia (Sprague and Khalil, 2009). For example, IL-1β and TNFα, both known to increase the permeability of the blood brain barrier (BBB) (Tsao et al., 2001; Mayhan, 2002; Sibson et al., 2002; Wang et al., 2014; Richter et al., 2017) are released by microglia early during ischemia (Lambertsen et al., 2012) to promote the compromise of the BBB. Later in the

progression of ischemia, microglia also release VEGF (Xie et al., 2013) known to promote angiogenesis in stroke (Zhang et al., 2000) suggesting that they could play some reparative functions by inducing neovascularization in later stages of stroke that contrasts with their earlier function. Of note, even IL-1β has been reported to have pro-angiogenic functions as well (Giulian et al., 1988) which may also be recruited in the latter repair following ischemic injury. Together, these results suggest that in the stroke context, microglia breakdown extant blood vessels, partially through cytokine insults and partially through phagocytic engulfment early on following the insult, and then contribute to building new vasculature later on. Future work will have to more precisely and adequately test this hypothesis.

[3.3] Microglia and Blood Vessels in Neurodegeneration

[3.3.1] Alzheimer's disease—Alzheimer's disease (AD) represents the most common neurodegenerative disease and is especially fatal in the aging population (Ballard et al., 2011). Characterized by amyloid beta (Aβ) deposits in the brain as a histopathological hallmark, microglial reactivity in the AD brain is also well known (Heneka et al., 2015; Yeh et al., 2017) but the specific contribution of microglia remain hotly debated (Gold and El Khoury, 2015; Malm et al., 2015). Here, we focus on some of the evidence that among other things, microglia in the AD context participates in vascular abnormalities that occur in the disease.

As with previous discussions, VEGF expression is increased in AD in response to Aβ deposition (Kalaria et al., 1998; Tarkowski et al., 2002). Since Aβ is deposited in and/or around blood vessels in addition to the parenchyma (Okamoto et al., 2009; Hickman and El Khoury, 2010), VEGF, among other chemoattractant, may mobilize microglia to surround blood vessels. This is consistent with a robust perivascular accumulation of microglia in AD (Ryu and McLarnon, 2009; Giannoni et al., 2016). In the 5xFAD model of AD, a longitudinal assessment of AD pathology using intravital two-photon microscopy revealed overlapping regions of neurovascular defects and Aβ plaques, which correlated with increased microglial activation (Giannoni et al., 2016). Whether the microglial reactivity in perivascular regions was a cause, consequence or an independent correlating factor with the histopathological vascular defects was not determined.

However, in an experimental model of AD where Aβ is injected into the hippocampus, there is a corresponding increase in neovascularization and microglial activation (Zand et al., 2005). Furthermore, in this condition, the BBB becomes leaky and this has been correlated with BBB-associated astrocytes and microglia (Ryu and McLarnon, 2006). Indeed, application of minocycline to inhibit microglial activation in this context significantly reduced the Aβ-induced defect in BBB integrity (Ryu and McLarnon, 2006). This result was further supported by an alternative approach using CD11b antibodies to block microglial function, which resulted in reduced neurovascular deficits from Aβ injection (Ryu and McLarnon, 2009). Although the precise mechanisms for microglial action remain to be elucidated, one of the mechanisms that may be proposed to be employed by microglia in promoting vascular pathology in Aβ pathology is through the purinergic P2X7 receptor (P2X7R) signaling (Ryu and McLarnon, 2008). Microglia are recognized as a predominant cell that expresses the P2X7R in the brain (Hickman et al., 2013; Zhang et al., 2014;

Holtman et al., 2015) despite the lingering controversy of neuronal P2X7R expression (Illes et al., 2017; Miras-Portugal et al., 2017). In this light, since pharmacological inhibition of P2X7Rs improved defects induced by Aβ treatment such as aberrant vascular function (Ryu and McLarnon, 2008), it is tempting to speculate that a primary cellular component of action occurred through microglia.

MMPs expressed by microglia and released in AD pathology have also been documented to contribute to the progression of AD (Kim and Joh, 2012). However, whether MMP action is specifically through microglia or has any specific effect on AD-induced vascular aberrations remains to be determined. Finally, microglia also release several cytokines including TNFα and IL-1β during AD pathology (Cameron and Landreth, 2010; Mandrekar-Colucci and Landreth, 2010; Wang et al., 2015) that can also compromise the vasculature as discussed above (see section 3.2 above). On the basis of the above, microglia are thus thought to promote vascular pathology including a breakdown of the BBB in Aβ pathology like AD.

[3.3.2] Multiple Sclerosis and Parkinson's disease—Multiple sclerosis (MS) is a progressive neurodegenerative autoimmune disease characterized by demyelination, brain atrophy and chronic inflammation (Lassmann et al., 2001; Vos et al., 2005). As with other conditions discussed above, angiogenesis has been reported in animal models of MS (Seabrook et al., 2010; Girolamo et al., 2014). In addition, BBB integrity is compromised in early stages of the disease leading to increased leukocyte infiltration and accumulation of blood products such as fibrinogen into the brain (Vos et al., 2005; van Horssen et al., 2007). Real time *in vivo* imaging revealed that leaked fibrinogen serves to attract microglia towards blood vessels in the early stages of animal models of MS. This attraction continues into more severe stages of the disease (Davalos et al., 2012). However, although microglia in general are thought to promote MS pathology (Heppner et al., 2005), it's precise role in vascular abnormalities of the disease such as BBB compromise and angiogenesis have not been clarified and should be a focus of future studies.

Parkinson's disease (PD) is a progressive neurodegenerative motor disease and is characterized by the loss of dopaminergic neurons in the substantia nigra (Kalia and Lang, 2015). Activation of the immune system and especially microglia is well known for disease progression (Whitton, 2007; Luo et al., 2017). As with the AD and MS, neovascularization (Barcia et al., 2005; Desai Bradaric et al., 2012) and a compromised BBB (Brochard et al., 2009; Gray and Woulfe, 2015) have been reported in PD/animal models of PD. However, whether and how microglia may be involved in regulating neurovascular changes that occur during PD has not been adequately explored.

[4] Outstanding Questions and Future Direction

The previous sections have documented the progress in understanding microglial engagement with the neurovascular system in health and disease (Fig. 2). However, at least three avenues of research should be employed to address outstanding questions. First is the question of molecular and mechanistic details of microglial regulation of vascular function in the developing and mature brain. While compelling evidence support an important role for microglia in maintaining vasculature function in the brain, the basis of communication

between microglia and the neurovasculature is still elusive at this moment. More basic research would lead to a better understanding of microglia-vascular interaction that can be harnessed to treat vascular pathologies of the brain. Precise roles of cytokines, free radicals, purines and proteases should be investigated in these various contexts. Relevant intracellular signaling pathways by which microglia receive signals from and send signals to the vasculature remain to be identified. Furthermore, future research should be directed to reveal the extent and details of microglial physical and dynamic interactions with elements of the neurovasculature and how they may differ between for example, capillaries, arteries and/or veins. The advent of real time two photon imaging can be used to accomplish this.

A second aspect that should guide future research is the question of specific microglial populations that may orchestrate neurovascular development and maintenance (or disintegration) in health and disease. RNA sequencing and transcriptional profiling data suggests a rich heterogeneity of microglia with distinctive molecular profiles (Zhang et al., 2014; Grabert et al., 2016; Keren-Shaul et al., 2017). In light of these results, it would be interesting to determine whether there is a microglial subtype specifically responsible for the maintenance/modulation of the neurovascular system in various brain regions. Morphological and molecular characterizations of such region specific microglia-vascular interaction would have to be determined. In addition, perivascular microglia have been identified in the literature but whether they are molecularly distinct from other resident microglia will need to be clarified to better understand their function.

Finally, while evidence is mounting for promising roles of microglia in angiogenesis and the maintenance of the BBB integrity, a lot of work remains to be done to determine precise microglial involvement in these processes especially in pathology. Prior work did not adequately ascertain microglial involvement because of the lack of specificity in the approaches. Approaches that potentially targeted microglia and peripheral monocytes/macrophages indiscriminately may have led to faulty conclusions in microglia-neurovascular interactions. Minocycline was mostly used to inhibit microglial activation in studying microglia-vascular interaction, however, it also has other non-microglial and perhaps direct neuromodulatory effects (Huang et al., 2010). Moreover, future studies are needed to differentiate the direct microglial interaction with neurovascular system from the indirect effect of microglia-neuron or microglia-astrocyte communication. Continual advancements in microglial-specific genetic and pharmacological tools in general cell ablation and specific protein deletion has now set the stage for better studies to adequately address these concerns.

Acknowledgments

This work is supported by the grants from the National Institute of Health (R01NS088627 and R21DE025689). We thank Dr. Junting Liu from Wu lab at Rutgers for the technical assistance in microglia-neurovascular imaging in our initial studies.

References

Abiega O, Beccari S, Diaz-Aparicio I, Nadjar A, Laye S, Leyrolle Q, Gomez-Nicola D, Domercq M, Perez-Samartin A, Sanchez-Zafra V, Paris I, Valero J, Savage JC, Hui CW, Tremblay ME, Deudero JJ, Brewster AL, Anderson AE, Zaldumbide L, Galbarriatu L, Marinas A, Vivanco MD, Matute C, Maletic-Savatic M, Encinas JM, Sierra A. Correction: Neuronal Hyperactivity Disturbs ATP

Microgradients, Impairs Microglial Motility, and Reduces Phagocytic Receptor Expression Triggering Apoptosis/Microglial Phagocytosis Uncoupling. PLoS Biol. 2016; 14:e1002554. [PubMed: 27649285]

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci. 2011; 14:1142–1149. [PubMed: 21804537]

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci. 2007; 10:1538–1543. [PubMed: 18026097]

Arnold T, Betsholtz C. Correction: The importance of microglia in the development of the vasculature in the central nervous system. Vasc Cell. 2013; 5:12. [PubMed: 23809768]

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

Ashwell KW, Hollander H, Streit W, Stone J. The appearance and distribution of microglia in the developing retina of the rat. Vis Neurosci. 1989; 2:437–448. [PubMed: 2487081]

Askew K, Li KZ, Olmos-Alonso A, Garcia-Moreno F, Liang YJ, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S, Sierra A, Molnar Z, Cragg MS, Garaschuk O, Perry VH, Gomez-Nicola D. Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. Cell Reports. 2017; 18:391–405. [PubMed: 28076784]

Baalman K, Marin MA, Ho TS, Godoy M, Cherian L, Robertson C, Rasband MN. Axon initial segment-associated microglia. J Neurosci. 2015; 35:2283–2292. [PubMed: 25653382]

Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer’s disease. Lancet. 2011; 377:1019–1031. [PubMed: 21371747]

Barcia C, Bautista V, Sanchez-Bahillo A, Fernandez-Villalba E, Faucheux B, Poza y Poza M, Fernandez Barreiro A, Hirsch EC, Herrero MT. Changes in vascularization in substantia nigra pars compacta of monkeys rendered parkinsonian. J Neural Transm (Vienna). 2005; 112:1237–1248. [PubMed: 15666038]

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. New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci U S A. 2016; 113:E1738–1746. [PubMed: 26884166]

Bohlen CJ, Bennett FC, Tucker AF, Collins HY, Mulinyawe SB, Barres BA. Diverse Requirements for Microglial Survival, Specification, and Function Revealed by Defined-Medium Cultures. Neuron. 2017; 94:759–773. e758. [PubMed: 28521131]

Brandenburg S, Muller A, Turkowski K, Radev YT, Rot S, Schmidt C, Bungert AD, Acker G, Schorr A, Hippe A, Miller K, Heppner FL, Homey B, Vajkoczy P. Resident microglia rather than peripheral macrophages promote vascularization in brain tumors and are source of alternative pro-angiogenic factors. Acta Neuropathol. 2016; 131:365–378. [PubMed: 26718201]

Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay JM, Duyckaerts C, Flavell RA, Hirsch EC, Hunot S. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest. 2009; 119:182–192. [PubMed: 19104149]

Cameron B, Landreth GE. Inflammation, microglia, and Alzheimer’s disease. Neurobiol Dis. 2010; 37:503–509. [PubMed: 19833208]

Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM. Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci. 2006; 9:917–924. [PubMed: 16732273]

Casano AM, Peri F. Microglia: multitasking specialists of the brain. Dev Cell. 2015; 32:469–477. [PubMed: 25710533]

Cecchini MG, Dominguez MG, Mocci S, Wetterwald A, Felix R, Fleisch H, Chisholm O, Hofstetter W, Pollard JW, Stanley ER. Role of colony stimulating factor-1 in the establishment and regulation

of tissue macrophages during postnatal development of the mouse. Development. 1994; 120:1357–1372. [PubMed: 8050349]

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

Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, Capecchi MR. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell. 2010; 141:775–785. [PubMed: 20510925]

Cuadros MA, Martin C, Coltey P, Almendros A, Navascues J. First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. J Comp Neurol. 1993; 330:113–129. [PubMed: 8468399]

Daneman R, Prat A. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015; 7:a020412. [PubMed: 25561720]

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 2005; 8:752–758. [PubMed: 15895084]

Davalos D, Ryu JK, Merlini M, Baeten KM, Le Moan N, Petersen MA, Deerinck TJ, Smirnoff DS, Bedard C, Hakozaki H, Gonias Murray S, Ling JB, Lassmann H, Degen JL, Ellisman MH, Akassoglou K. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun. 2012; 3:1227. [PubMed: 23187627]

Denes A, Ferenczi S, Halasz J, Kornyei Z, Kovacs KJ. Role of CX3CR1 (fractalkine receptor) in brain damage and inflammation induced by focal cerebral ischemia in mouse. J Cereb Blood Flow Metab. 2008; 28:1707–1721. [PubMed: 18575457]

Desai Bradaric B, Patel A, Schneider JA, Carvey PM, Hendey B. Evidence for angiogenesis in Parkinson's disease, incidental Lewy body disease, and progressive supranuclear palsy. J Neural Transm (Vienna). 2012; 119:59–71. [PubMed: 21748523]

Ding X, Zhang M, Gu R, Xu G, Wu H. Activated microglia induce the production of reactive oxygen species and promote apoptosis of co-cultured retinal microvascular pericytes. Graefes Arch Clin Exp Ophthalmol. 2017; 255:777–788. [PubMed: 28074262]

Dudvarski Stankovic N, Teodorczyk M, Ploen R, Zipp F, Schmidt MH. Microglia-blood vessel interactions: a double-edged sword in brain pathologies. Acta Neuropathol. 2016; 131:347–363. [PubMed: 26711460]

Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002; 2:161–174. [PubMed: 11990853]

Eyo U, Dailey ME. Effects of oxygen-glucose deprivation on microglial mobility and viability in developing mouse hippocampal tissues. Glia. 2012; 60:1747–1760. [PubMed: 22847985]

Eyo UB, Gu N, De S, Dong H, Richardson JR, Wu LJ. Modulation of microglial process convergence toward neuronal dendrites by extracellular calcium. J Neurosci. 2015; 35:2417–2422. [PubMed: 25673836]

Eyo UB, Miner SA, Weiner JA, Dailey ME. Developmental changes in microglial mobilization are independent of apoptosis in the neonatal mouse hippocampus. Brain Behav Immun. 2016; 55:49–59. [PubMed: 26576723]

Eyo UB, Murugan M, Wu LJ. Microglia-Neuron Communication in Epilepsy. Glia. 2017; 65:5–18. [PubMed: 27189853]

Eyo UB, Peng J, Murugan M, Mo M, Lalani A, Xie P, Xu P, Margolis DJ, Wu LJ. Regulation of Physical Microglia-Neuron Interactions by Fractalkine Signaling after Status Epilepticus. eNeuro. 2017:3.

Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ. Neuronal Hyperactivity Recruits Microglial Processes via Neuronal NMDA Receptors and Microglial P2Y12 Receptors after Status Epilepticus. J Neurosci. 2014; 34:10528–10540. [PubMed: 25100587]

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

Faustino JV, Wang X, Johnson CE, Klibanov A, Derugin N, Wendland MF, Vexler ZS. Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. J Neurosci. 2011; 31:12992–13001. [PubMed: 21900578]

Forstreuter F, Lucius R, Mentlein R. Vascular endothelial growth factor induces chemotaxis and proliferation of microglial cells. J Neuroimmunol. 2002; 132:93–98. [PubMed: 12417438]

Fourgeaud L, Traves PG, Tufail Y, Leal-Bailey H, Lew ED, Burrola PG, Callaway P, Zagorska A, Rothlin CV, Nimmerjahn A, Lemke G. TAM receptors regulate multiple features of microglial physiology. Nature. 2016; 532:240–244. [PubMed: 27049947]

Gabrusiewicz K, Ellert-Miklaszewska A, Lipko M, Sielska M, Frankowska M, Kaminska B. Characteristics of the alternative phenotype of microglia/macrophages and its modulation in experimental gliomas. PLoS One. 2011; 6:e23902. [PubMed: 21901144]

Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, Arumugam TV, Orthey E, Gerloff C, Tolosa E, Magnus T. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. Stroke. 2009; 40:1849–1857. [PubMed: 19265055]

Giannoni P, Arango-Lievano M, Neves ID, Rousset MC, Baranger K, Rivera S, Jeanneteau F, Claeysen S, Marchi N. Cerebrovascular pathology during the progression of experimental Alzheimer's disease. Neurobiol Dis. 2016; 88:107–117. [PubMed: 26774030]

Girolamo F, Coppola C, Ribatti D, Trojano M. Angiogenesis in multiple sclerosis and experimental autoimmune encephalomyelitis. Acta Neuropathol Commun. 2014; 2:84. [PubMed: 25047180]

Giulian D, Woodward J, Young DG, Krebs JF, Lachman LB. Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. J Neurosci. 1988; 8:2485–2490. [PubMed: 2470873]

Gold M, El Khoury J. beta-amyloid, microglia, and the inflammasome in Alzheimer's disease. Semin Immunopathol. 2015; 37:607–611. [PubMed: 26251237]

Grabert K, Michoel T, Karavolos MH, Clohisey S, Baillie JK, Stevens MP, Freeman TC, Summers KM, McColl BW. Microglial brain region-dependent diversity and selective regional sensitivities to aging. Nat Neurosci. 2016; 19:504–516. [PubMed: 26780511]

Gray MT, Woulfe JM. Striatal blood-brain barrier permeability in Parkinson's disease. J Cereb Blood Flow Metab. 2015; 35:747–750. [PubMed: 25757748]

Grossmann R, Stence N, Carr J, Fuller L, Waite M, Dailey ME. Juxtavascular microglia migrate along brain microvessels following activation during early postnatal development. Glia. 2002; 37:229–240. [PubMed: 11857681]

Gu N, Peng J, Murugan M, Wang X, Eyo UB, Sun D, Ren Y, DiCicco-Bloom E, Young W, Dong H, Wu LJ. Spinal Microgliosis Due to Resident Microglial Proliferation Is Required for Pain Hypersensitivity after Peripheral Nerve Injury. Cell Rep. 2016; 16:605–614. [PubMed: 27373153]

Haigh JJ, Morelli PI, Gerhardt H, Haigh K, Tsien J, Damert A, Miquerol L, Muhlner U, Klein R, Ferrara N, Wagner EF, Betsholtz C, Nagy A. Cortical and retinal defects caused by dosage-dependent reductions in VEGF-A paracrine signaling. Dev Biol. 2003; 262:225–241. [PubMed: 14550787]

Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007; 10:1387–1394. [PubMed: 17965659]

Hattermann K, Sebens S, Helm O, Schmitt AD, Mentlein R, Mehdorn HM, Held-Feindt J. Chemokine expression profile of freshly isolated human glioblastoma-associated macrophages/microglia. Oncol Rep. 2014; 32:270–276. [PubMed: 24859792]

Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015; 14:388–405. [PubMed: 25792098]

Heppner FL, Greter M, Marino D, Falsig J, Raivich G, Hovelmeyer N, Waisman A, Rulicke T, Prinz M, Priller J, Becher B, Aguzzi A. Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat Med. 2005; 11:146–152. [PubMed: 15665833]

Hickman SE, El Khoury J. Mechanisms of mononuclear phagocyte recruitment in Alzheimer's disease. CNS Neurol Disord Drug Targets. 2010; 9:168–173. [PubMed: 20205643]

Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK, El Khoury J. The microglial sensome revealed by direct RNA sequencing. Nat Neurosci. 2013; 16:1896–1905. [PubMed: 24162652]

Holtman IR, Noback M, Bijlsma M, Duong KN, van der Geest MA, Ketelaars PT, Brouwer N, Vainchtein ID, Eggen BJ, Boddeke HW. Glia Open Access Database (GOAD): A comprehensive gene expression encyclopedia of glia cells in health and disease. Glia. 2015; 63:1495–1506. [PubMed: 25808223]

Huang WC, Qiao Y, Xu L, Kacimi R, Sun X, Giffard RG, Yenari MA. Direct protection of cultured neurons from ischemia-like injury by minocycline. Anat Cell Biol. 2010; 43:325–331. [PubMed: 21267407]

Hurtado-Alvarado G, Cabanas-Morales AM, Gomez-Gonzalez B. Pericytes: brain-immune interface modulators. Front Integr Neurosci. 2014; 7:80. [PubMed: 24454281]

Hwang JS, Jung EH, Kwon MY, Han IO. Glioma-secreted soluble factors stimulate microglial activation: The role of interleukin-1beta and tumor necrosis factor-alpha. J Neuroimmunol. 2016; 298:165–171. [PubMed: 27609291]

Illes P, Khan TM, Rubini P. Neuronal P2X7 Receptors Revisited: Do They Really Exist? J Neurosci. 2017; 37:7049–7062. [PubMed: 28747388]

James JM, Gewolb C, Bautch VL. Neurovascular development uses VEGF-A signaling to regulate blood vessel ingression into the neural tube. Development. 2009; 136:833–841. [PubMed: 19176586]

Jolivel V, Bicker F, Biname F, Ploen R, Keller S, Gollan R, Jurek B, Birkenstock J, Poisa-Beiro L, Bruttger J, Opitz V, Thal SC, Waisman A, Bauerle T, Schafer MK, Zipp F, Schmidt MH. Perivascular microglia promote blood vessel disintegration in the ischemic penumbra. Acta Neuropathol. 2015; 129:279–295. [PubMed: 25500713]

Kalaria RN, Cohen DL, Premkumar DR, Nag S, LaManna JC, Lust WD. Vascular endothelial growth factor in Alzheimer's disease and experimental cerebral ischemia. Brain Res Mol Brain Res. 1998; 62:101–105. [PubMed: 9795165]

Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015; 386:896–912. [PubMed: 25904081]

Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell. 2017; 169:1276–1290. e1217. [PubMed: 28602351]

Kim YS, Joh TH. Matrix metalloproteinases, new insights into the understanding of neurodegenerative disorders. Biomol Ther (Seoul). 2012; 20:133–143. [PubMed: 24116286]

Kobayashi S, Nagaura T, Kimura I, Kimura M. Interferon-gamma-activated macrophages enhance angiogenesis from endothelial cells of rat aorta. Immunopharmacology. 1994; 27:23–30. [PubMed: 7515864]

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

Kurz H, Christ B. Embryonic CNS macrophages and microglia do not stem from circulating, but from extravascular precursors. Glia. 1998; 22:98–102. [PubMed: 9436792]

Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci. 2007; 27:2596–2605. [PubMed: 17344397]

Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. J Cereb Blood Flow Metab. 2012; 32:1677–1698. [PubMed: 22739623]

Lassmann H, Bruck W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med. 2001; 7:115–121. [PubMed: 11286782]

Lawson LJ, Perry VH, Gordon S. Turnover of Resident Microglia in the Normal Adult-Mouse Brain. Neuroscience. 1992; 48:405–415. [PubMed: 1603325]

Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res. 1996; 56:4625–4629. [PubMed: 8840975]

Li Y, Du XF, Liu CS, Wen ZL, Du JL. Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. Dev Cell. 2012; 23:1189–1202. [PubMed: 23201120]

Liang KJ, Lee JE, Wang YD, Ma W, Fontainhas AM, Fariss RN, Wong WT. Regulation of dynamic behavior of retinal microglia by CX3CR1 signaling. Invest Ophthalmol Vis Sci. 2009; 50:4444–4451. [PubMed: 19443728]

Lopes MB. Angiogenesis in brain tumors. Microsc Res Tech. 2003; 60:225–230. [PubMed: 12539176]

Lorger M. Tumor microenvironment in the brain. Cancers (Basel). 2012; 4:218–243. [PubMed: 24213237]

Luo C, Jian C, Liao Y, Huang Q, Wu Y, Liu X, Zou D, Wu Y. The role of microglia in multiple sclerosis. Neuropsychiatr Dis Treat. 2017; 13:1661–1667. [PubMed: 28721047]

Lyck L, Santamaria ID, Pakkenberg B, Chemnitz J, Schroder HD, Finsen B, Gundersen HJG. An empirical analysis of the precision of estimating the numbers of neurons and glia in human neocortex using a fractionator-design with sub-sampling. Journal of Neuroscience Methods. 2009; 182:143–156. [PubMed: 19520115]

Ma Y, Wang J, Wang Y, Yang GY. The biphasic function of microglia in ischemic stroke. Prog Neurobiol. 2016

MacVicar BA, Newman EA. Astrocyte regulation of blood flow in the brain. Cold Spring Harb Perspect Biol. 2015:7.

Magistretti PJ, Allaman I. A cellular perspective on brain energy metabolism and functional imaging. Neuron. 2015; 86:883–901. [PubMed: 25996133]

Malm TM, Jay TR, Landreth GE. The evolving biology of microglia in Alzheimer's disease. Neurotherapeutics. 2015; 12:81–93. [PubMed: 25404051]

Mandrekar-Colucci S, Landreth GE. Microglia and inflammation in Alzheimer's disease. CNS Neurol Disord Drug Targets. 2010; 9:156–167. [PubMed: 20205644]

Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M. Microglia promote the death of developing Purkinje cells. Neuron. 2004; 41:535–547. [PubMed: 14980203]

Markovic DS, Vinnakota K, Chirasani S, Synowitz M, Raguet H, Stock K, Sliwa M, Lehmann S, Kalin R, van Rooijen N, Holmbeck K, Heppner FL, Kiwit J, Matyash V, Lehnardt S, Kaminska B, Glass R, Kettenmann H. Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion. Proc Natl Acad Sci U S A. 2009; 106:12530–12535. [PubMed: 19617536]

Masuda T, Croom D, Hida H, Kirov SA. Capillary blood flow around microglial somata determines dynamics of microglial processes in ischemic conditions. Glia. 2011; 59:1744–1753. [PubMed: 21800362]

Matsumoto J, Takata F, Machida T, Takahashi H, Soejima Y, Funakoshi M, Futagami K, Yamauchi A, Dohgu S, Kataoka Y. Tumor necrosis factor-alpha-stimulated brain pericytes possess a unique cytokine and chemokine release profile and enhance microglial activation. Neurosci Lett. 2014; 578:133–138. [PubMed: 24993300]

Mayhan WG. Cellular mechanisms by which tumor necrosis factor-alpha produces disruption of the blood-brain barrier. Brain Res. 2002; 927:144–152. [PubMed: 11821008]

McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Baribault H, Klemsz M, Feeney AJ, Wu GE, Paige CJ, Maki RA. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. EMBO J. 1996; 15:5647–5658. [PubMed: 8896458]

Miras-Portugal MT, Sebastian-Serrano A, de Diego Garcia L, Diaz-Hernandez M. Neuronal P2X7 Receptor: Involvement in Neuronal Physiology and Pathology. J Neurosci. 2017; 37:7063–7072. [PubMed: 28747389]

Mirrione MM, Konomos DK, Gravanis I, Dewey SL, Aguzzi A, Heppner FL, Tsirka SE. Microglial ablation and lipopolysaccharide preconditioning affects pilocarpine-induced seizures in mice. Neurobiol Dis. 2010; 39:85–97. [PubMed: 20382223]

Morioka T, Kalehua AN, Streit WJ. The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. J Cereb Blood Flow Metab. 1991; 11:966–973. [PubMed: 1719009]

Nakanishi M, Niidome T, Matsuda S, Akaike A, Kihara T, Sugimoto H. Microglia-derived interleukin-6 and leukaemia inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. Eur J Neurosci. 2007; 25:649–658. [PubMed: 17328769]

Narantuya D, Nagai A, Sheikh AM, Masuda J, Kobayashi S, Yamaguchi S, Kim SU. Human microglia transplanted in rat focal ischemia brain induce neuroprotection and behavioral improvement. PLoS One. 2010; 5:e11746. [PubMed: 20668522]

Nayak D, Roth TL, McGavern DB. Microglia development and function. Annu Rev Immunol. 2014; 32:367–402. [PubMed: 24471431]

Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005; 308:1314–1318. [PubMed: 15831717]

Nishie A, Ono M, Shono T, Fukushi J, Otsubo M, Onoue H, Ito Y, Inamura T, Ikezaki K, Fukui M, Iwaki T, Kuwano M. Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. Clin Cancer Res. 1999; 5:1107–1113. [PubMed: 10353745]

Okamoto Y, Ihara M, Fujita Y, Ito H, Takahashi R, Tomimoto H. Cortical microinfarcts in Alzheimer's disease and subcortical vascular dementia. Neuroreport. 2009; 20:990–996. [PubMed: 19483658]

Ozen I, Deierborg T, Miharada K, Padel T, Englund E, Genove G, Paul G. Brain pericytes acquire a microglial phenotype after stroke. Acta Neuropathol. 2014; 128:381–396. [PubMed: 24848101]

Paolicelli RC, Gross CT. Microglia in development: linking brain wiring to brain environment. Neuron Glia Biol. 2011; 7:77–83. [PubMed: 22857738]

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd, Lafaille JJ, Hempstead BL, Littman DR, Gan WB. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell. 2013; 155:1596–1609. [PubMed: 24360280]

Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B. Neocortical glial cell numbers in human brains. Neurobiology of Aging. 2008; 29:1754–1762. [PubMed: 17544173]

Peng J, Gu N, Zhou L, UBE, Murugan M, Gan WB, Wu LJ. Microglia and monocytes synergistically promote the transition from acute to chronic pain after nerve injury. Nat Commun. 2016; 7:12029. [PubMed: 27349690]

Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience. 1985; 15:313–326. [PubMed: 3895031]

Petersen MA, Dailey ME. Diverse microglial motility behaviors during clearance of dead cells in hippocampal slices. Glia. 2004; 46:195–206. [PubMed: 15042586]

Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci U S A. 1995; 92:905–909. [PubMed: 7846076]

Polverini PJ. Role of the macrophage in angiogenesis-dependent diseases. EXS. 1997; 79:11–28. [PubMed: 9002218]

Prinz M, Priller J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nature Reviews Neuroscience. 2014; 15:300–312. [PubMed: 24713688]

Qin T, Wang C, Chen X, Duan C, Zhang X, Zhang J, Chai H, Tang T, Chen H, Yue J, Li Y, Yang J. Dopamine induces growth inhibition and vascular normalization through reprogramming M2-polarized macrophages in rat C6 glioma. Toxicol Appl Pharmacol. 2015; 286:112–123. [PubMed: 25818600]

Raab S, Beck H, Gaumann A, Yuce A, Gerber HP, Plate K, Hammes HP, Ferrara N, Breier G. Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. Thromb Haemost. 2004; 91:595–605. [PubMed: 14983237]

Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol. 2009; 27:119–145. [PubMed: 19302036]

Rao JS. Molecular mechanisms of glioma invasiveness: the role of proteases. Nat Rev Cancer. 2003; 3:489–501. [PubMed: 12835669]

Reemst K, Noctor SC, Lucassen PJ, Hol EM. The Indispensable Roles of Microglia and Astrocytes during Brain Development. Frontiers in Human Neuroscience. 2016:10. [PubMed: 26869898]

Rezaie P, Trillo-Pazos G, Greenwood J, Everall IP, Male DK. Motility and ramification of human fetal microglia in culture: an investigation using time-lapse video microscopy and image analysis. Exp Cell Res. 2002; 274:68–82. [PubMed: 11855858]

Richter F, Eitner A, Leuchtweis J, Bauer R, Lehmenkuhler A, Schaible HG. Effects of interleukin-1ss on cortical spreading depolarization and cerebral vasculature. J Cereb Blood Flow Metab. 2017; 37:1791–1802. [PubMed: 27037093]

Ruhrberg C, Gerhardt H, Golding M, Watson R, Ioannidou S, Fujisawa H, Betsholtz C, Shima DT. Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev. 2002; 16:2684–2698. [PubMed: 12381667]

Rymo SF, Gerhardt H, Wolfhagen Sand F, Lang R, Uv A, Betsholtz C. A two-way communication between microglial cells and angiogenic sprouts regulates angiogenesis in aortic ring cultures. PLoS One. 2011; 6:e15846. [PubMed: 21264342]

Ryu JK, McLarnon JG. Minocycline or iNOS inhibition block 3-nitrotyrosine increases and blood-brain barrier leakiness in amyloid beta-peptide-injected rat hippocampus. Exp Neurol. 2006; 198:552–557. [PubMed: 16480717]

Ryu JK, McLarnon JG. Block of purinergic P2X(7) receptor is neuroprotective in an animal model of Alzheimer's disease. Neuroreport. 2008; 19:1715–1719. [PubMed: 18852683]

Ryu JK, McLarnon JG. A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J Cell Mol Med. 2009; 13:2911–2925. [PubMed: 18657226]

Ryuto M, Ono M, Izumi H, Yoshida S, Weich HA, Kohno K, Kuwano M. Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1. J Biol Chem. 1996; 271:28220–28228. [PubMed: 8910439]

Sakuma R, Kawahara M, Nakano-Doi A, Takahashi A, Tanaka Y, Narita A, Kuwahara-Otani S, Hayakawa T, Yagi H, Matsuyama T, Nakagomi T. Brain pericytes serve as microglia-generating multipotent vascular stem cells following ischemic stroke. J Neuroinflammation. 2016; 13:57. [PubMed: 26952098]

Schafer DP, Lehrman EK, Stevens B. The "quad-partite" synapse: microglia-synapse interactions in the developing and mature CNS. Glia. 2013; 61:24–36. [PubMed: 22829357]

Scott A, Powner MB, Gandhi P, Clarkin C, Gutmann DH, Johnson RS, Ferrara N, Fruttiger M. Astrocyte-derived vascular endothelial growth factor stabilizes vessels in the developing retinal vasculature. PLoS One. 2010; 5:e11863. [PubMed: 20686684]

Scott EW, Simon MC, Anastasi J, Singh H. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science. 1994; 265:1573–1577. [PubMed: 8079170]

Seabrook TJ, Littlewood-Evans A, Brinkmann V, Pollinger B, Schnell C, Hiestand PC. Angiogenesis is present in experimental autoimmune encephalomyelitis and pro-angiogenic factors are increased in multiple sclerosis lesions. J Neuroinflammation. 2010; 7:95. [PubMed: 21176212]

Sibson NR, Blamire AM, Perry VH, Gauldie J, Styles P, Anthony DC. TNF-alpha reduces cerebral blood volume and disrupts tissue homeostasis via an endothelin- and TNFR2-dependent pathway. Brain. 2002; 125:2446–2459. [PubMed: 12390971]

Sierra A, Beccari S, Diaz-Aparicio I, Encinas JM, Comeau S, Tremblay ME. Surveillance, phagocytosis, and inflammation: how never-resting microglia influence adult hippocampal neurogenesis. Neural Plast. 2014; 2014:610343. [PubMed: 24772353]

Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell. 2010; 7:483–495. [PubMed: 20887954]

Sliwa M, Markovic D, Gabrusiewicz K, Synowitz M, Glass R, Zawadzka M, Wesolowska A, Kettenmann H, Kaminska B. The invasion promoting effect of microglia on glioblastoma cells is inhibited by cyclosporin A. Brain. 2007; 130:476–489. [PubMed: 17107968]

Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol. 2009; 78:539–552. [PubMed: 19413999]

Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, Bessis A, Ginhoux F, Garel S. Microglia Modulate Wiring of the Embryonic Forebrain. Cell Rep. 2014

Stalmans I, Ng YS, Rohan R, Fruttiger M, Bouche A, Yuce A, Fujisawa H, Hermans B, Shani M, Jansen S, Hicklin D, Anderson DJ, Gardiner T, Hammes HP, Moons L, Dewerchin M, Collen D, Carmeliet P, D'Amore PA. Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. J Clin Invest. 2002; 109:327–336. [PubMed: 11827992]

Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. J Leukoc Biol. 1994; 55:410–422. [PubMed: 7509844]

Svahn AJ, Graeber MB, Ellett F, Lieschke GJ, Rinkwitz S, Bennett MR, Becker TS. Development of ramified microglia from early macrophages in the zebrafish optic tectum. Dev Neurobiol. 2013; 73:60–71. [PubMed: 22648905]

Talwalkar A, Uddin S. Trends in emergency department visits for ischemic stroke and transient ischemic attack: United States, 2001–2011. NCHS Data Brief. 2015:1–8.

Tarkowski E, Issa R, Sjogren M, Wallin A, Blennow K, Tarkowski A, Kumar P. Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. Neurobiol Aging. 2002; 23:237–243. [PubMed: 11804709]

Tay TL, Mai D, Dautzenberg J, Fernandez-Klett F, Lin G, Sagar, Datta M, Drougard A, Stempfl T, Ardura-Fabregat A, Staszewski O, Margineanu A, Sporbert A, Steinmetz LM, Pospisilik JA, Jung S, Priller J, Grun D, Ronneberger O, Prinz M. A new fate mapping system reveals context-dependent random or clonal expansion of microglia. Nat Neurosci. 2017; 20:793–803. [PubMed: 28414331]

Thomas KA. Vascular endothelial growth factor, a potent and selective angiogenic agent. J Biol Chem. 1996; 271:603–606. [PubMed: 8557658]

Thomas WE. Brain macrophages: on the role of pericytes and perivascular cells. Brain Res Brain Res Rev. 1999; 31:42–57. [PubMed: 10611494]

Tian DS, Li CY, Qin C, Murugan M, Wu LJ, Liu JL. Deficiency in the voltage-gated proton channel Hv1 increases M2 polarization of microglia and attenuates brain damage from photothrombotic ischemic stroke. J Neurochem. 2016; 139:96–105. [PubMed: 27470181]

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

Tsao N, Hsu HP, Wu CM, Liu CC, Lei HY. Tumour necrosis factor-alpha causes an increase in blood-brain barrier permeability during sepsis. J Med Microbiol. 2001; 50:812–821. [PubMed: 11549183]

van Horssen J, Brink BP, de Vries HE, van der Valk P, Bo L. The blood-brain barrier in cortical multiple sclerosis lesions. J Neuropathol Exp Neurol. 2007; 66:321–328. [PubMed: 17413323]

Vos CM, Geurts JJ, Montagne L, van Haastert ES, Bo L, van der Valk P, Barkhof F, de Vries HE. Blood-brain barrier alterations in both focal and diffuse abnormalities on postmortem MRI in multiple sclerosis. Neurobiol Dis. 2005; 20:953–960. [PubMed: 16039866]

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci. 2009; 29:3974–3980. [PubMed: 19339593]

Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A. Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. J Neurosci. 2008; 28:8138–8143. [PubMed: 18685038]

Wang SC, Yu CF, Hong JH, Tsai CS, Chiang CS. Radiation therapy-induced tumor invasiveness is associated with SDF-1-regulated macrophage mobilization and vasculogenesis. PLoS One. 2013; 8:e69182. [PubMed: 23940516]

Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med. 2015; 3:136. [PubMed: 26207229]

Wang Y, Jin S, Sonobe Y, Cheng Y, Horiuchi H, Parajuli B, Kawanokuchi J, Mizuno T, Takeuchi H, Suzumura A. Interleukin-1beta induces blood-brain barrier disruption by downregulating Sonic hedgehog in astrocytes. PLoS One. 2014; 9:e110024. [PubMed: 25313834]

Weidemann A, Krohne TU, Aguilar E, Kurihara T, Takeda N, Dorrell MI, Simon MC, Haase VH, Friedlander M, Johnson RS. Astrocyte hypoxic response is essential for pathological but not developmental angiogenesis of the retina. Glia. 2010; 58:1177–1185. [PubMed: 20544853]

Weinstein JR, Koerner IP, Moller T. Microglia in ischemic brain injury. Future Neurol. 2010; 5:227–246. [PubMed: 20401171]

Whitton PS. Inflammation as a causative factor in the aetiology of Parkinson's disease. Br J Pharmacol. 2007; 150:963–976. [PubMed: 17339843]

Wu LJ, Vadakkan KI, Zhuo M. ATP-induced chemotaxis of microglial processes requires P2Y receptor-activated initiation of outward potassium currents. Glia. 2007; 55:810–821. [PubMed: 17357150]

Wu LJ, Wu G, Akhavan Sharif MR, Baker A, Jia Y, Fahey FH, Luo HR, Feener EP, Clapham DE. The voltage-gated proton channel Hv1 enhances brain damage from ischemic stroke. Nat Neurosci. 2012; 15:565–573. [PubMed: 22388960]

Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res. 2004; 64:7022–7029. [PubMed: 15466195]

Xie L, Mao X, Jin K, Greenberg DA. Vascular endothelial growth factor-B expression in postischemic rat brain. Vasc Cell. 2013; 5:8. [PubMed: 23601533]

Yeh FL, Hansen DV, Sheng M. TREM2, Microglia, and Neurodegenerative Diseases. Trends Mol Med. 2017; 23:512–533. [PubMed: 28442216]

Zand L, Ryu JK, McLarnon JG. Induction of angiogenesis in the beta-amyloid peptide-injected rat hippocampus. Neuroreport. 2005; 16:129–132. [PubMed: 15671861]

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci. 2014; 34:11929–11947. [PubMed: 25186741]

Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, Bruggen N, Chopp M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest. 2000; 106:829–838. [PubMed: 11018070]

Zhu W, Carney KE, Pigott VM, Falgoust LM, Clark PA, Kuo JS, Sun D. Glioma-mediated microglial activation promotes glioma proliferation and migration: roles of Na+/H+ exchanger isoform 1. Carcinogenesis. 2016; 37:839–851. [PubMed: 27287871]

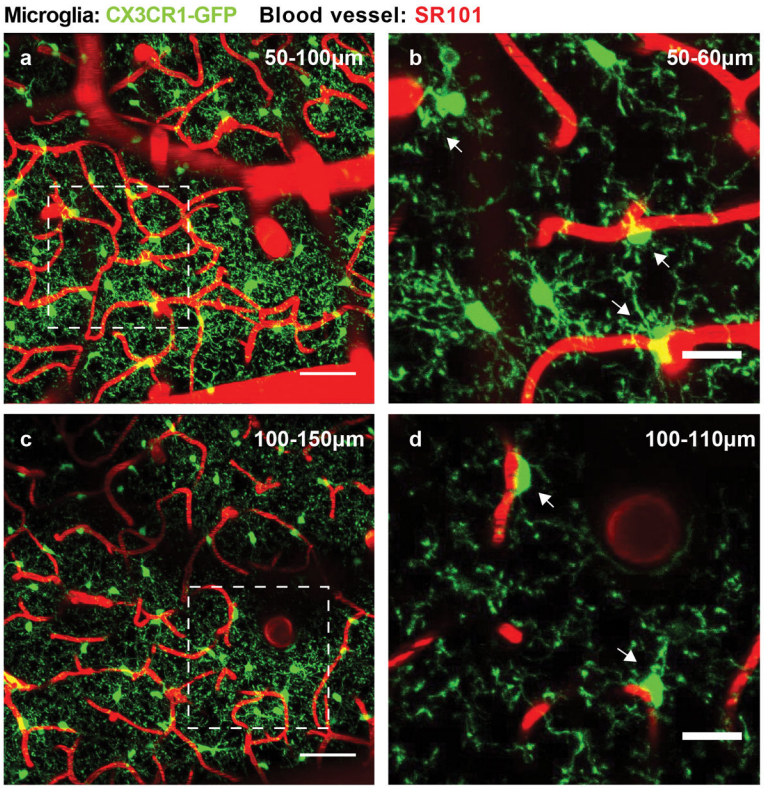

Figure 1. Microglia and blood vessels exist in close proximity in the adult brain

Z-stack images from the somatosensory cortex of a transgenic CX3CR1-GFP$^{+/-}$ mouse injected with SR101 (red) to label the neurovasculature. **a–b,** Low magnification images of ramified microglia and blood vessels in 50 μm z-stack images. Scale bar: 50 μm. **c–d,** High magnification images of ramified microglia and blood vessels in 10 μm z-stack images of the corresponding boxed regions in (**a**) and (**b**). Physical contact between microglia somata and capillaries are indicated with arrows. Scale bar: 20 μm.

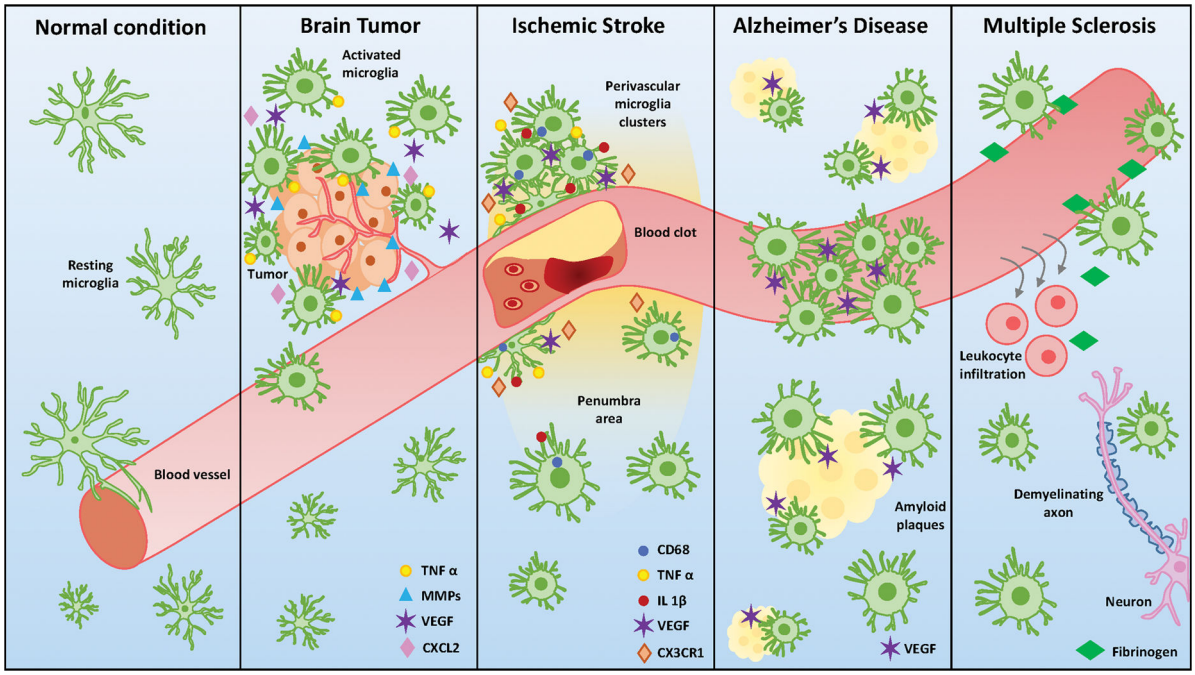

Figure 2. Schematic diagram of microglial interaction with the neurovascular system in physiology and pathology

Resting microglia, as depicted in normal condition, are characterized with ramified cellular processes and close proximity of blood vessel. Activated microglia under diseased conditions are featured with larger somata and enriched in the dysfunctional core of associated diseases. Molecules, like chemokines, cytokines & growth factors, included in interaction of microglia and neurovascular system are indicated in each panel as normal condition, brain tumor, ischemic stroke, Alzheimer's disease and multiple sclerosis.