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Microglia During Development and Aging

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Abstract

Microglia are critical nervous system-specific cells influencing brain development, maintenance of the neural environment, response to injury, and repair. They contribute to neuronal proliferation and differentiation, pruning of dying neurons, synaptic remodeling and clearance of debris and aberrant proteins. Colonization of the brain occurs during gestation with an expansion following birth with localization stimulated by programmed neuronal death, synaptic pruning, andaxonal degeneration. Changes inmicroglia phenotype relate to cellular processes including specific neurotransmitter, pattern recognition, or immune-related receptor activation. Upon activation, microglia cells have the capacity to release a number of substances, e.g., cytokines, chemokines, nitric oxide, and reactive oxygen species, which could be detrimental or beneficial to the surrounding cells. With aging, microglia shift their morphology and may display diminished capacity for normal functions related to migration, clearance, and the ability to shift from a pro-inflammatory to an anti-inflammatory state to regulate injury and repair. This shift in microgliapotentially contributes to increased susceptibility and neurodegeneration as a function of age. In the current review, information is provided on the colonization of the brain by microglia, the expression of various pattern recognition receptors to regulate migration and phagocytosis, and the shift in related functions that occur in normal aging.

Keywords

microglia; P2X receptors; aging; development; synapse stripping

1. Introduction

Microglia are resident cells of the brain involved in regulatory processes critical for development, maintenance of the neural environment, response to injury, and subsequent repair. These cells were included in the early mid-nineteenth century description of neuroglia by Virchow (1856). Later, they were classified as a third element in the central nervous system (CNS) morphologically distinct from neurons, astrocytes (Cajal, 1913), and oligodendrocytes (del Rio Hortega, 1932). Microglia sense pathological events in the CNS and serve as brain immune cells to orchestrate innate immune responses. They share phenotypic characteristics and innate immunological functions with other mononuclear phagocytes and express major histocompatibility complex (MHC) antigens, as well as T and

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B cell markers such as various cluster of differentiation (CD) proteins (Perry et al., 1985; McGeer and McGeer, 1995; Williams et al., 1994). However, they are distinct from other tissue macrophages due to their relative quiescent phenotype and tight regulation by the CNS microenvironment. They often provide the first line of defense against invading microbes and via interactions with neurons can be the first to detect critical changes in neuronal activity and health. In the mature brain, microglia are capable not only of actively monitoring but also controlling the extracellular environment, walling off areas of the CNS from non-CNS tissue, and removing dead or damaged cells. Work by Schmid et al. (2009) suggested that the CNS environment contributes to differentiation of monocytes to a neural specific phenotype, separating microglia from mononuclear phagocytes while maintaining many similar features. This “nervous system specificity” is demonstrated in microglia in their functions associated with synaptic pruning (Bruce-Keller, 1999; Paolicelli et al., 2011; Stevens et al., 2007), phagocytosis of apoptotic neurons and reorganization of neuronal circuitry (Sierra et al., 2010; Tremblay et al., 2010), pruning of axonal collaterals (Gehrmann et al., 1995; Wake et al., 2009), facilitation of axonal sprouting (Nagamoto-Combs et al., 2010), and remyelination of central axons (Olah et al., 2012).

Much of the research on microglia is based upon functions within the adult brain; yet, data from developmental and aging studies suggest that the nature of these functions changes over the lifespan. During development, it is thought that microglia contribute to the formation of the final neural network by stimulating vascularization and assisting in pruning of excess neurons and synapses as well as facilitating cell differentiation. As sentinels in the adult brain, microglia maintain homeostasis and possibly contribute to the neural network by assisting in synaptic remodeling and plasticity (Trapp et al., 2007; Svensson et al., 1993; Moran and Graeber, 2004; Stevens, 2007; Wake, 2009; Perry and O’Connor, 2010), as well as removing excess aberrant proteins and debris accumulating in the brain. With aging, multiple physiological changes occur that are associated with DNA damage, oxidative stress, and telomere shortening (Vijg and Campisi, 2008). Within the brain, this is accompanied by a decrease in synaptic structures (Duan et al., 2003; Hof and Morrison, 2004; Jacobs et al., 1997) and a deficit in synaptic plasticity. With aging, the ability of microglia to express pro-inflammatory cytokines tumor necrosis factor (TNF)α, TNFβ, interleukin (IL)-1α, IL-1β, and IL-6, as well as cytokine receptors (Table 1) is thought to contribute to the mild chronic inflammatory condition that develops. This elevation in pro-inflammatory cytokines is accompanied by a decrease in anti-inflammatory cytokines, such as IL-10 (Ye and Johnson, 2001). These results suggest that aging is associated with an increase in microglia activation and a decreased regulatory status. It has been hypothesized that the active monitoring functions of microglia become compromised due to an onset of cell senescence that hinders the cell’s ability to sense changes in their environment, and thereby limit performance of normal functions. In this case, one might expect that a loss of phagocytic ability of the cells would result in the accumulation of aberrant proteins, such as amyloid beta (Aβ), α synuclein, apolipoprotein E (ApoE), that are known to be associated with neurodegenerative diseases. Senescence, or any mechanism that diminishes the function of microglia, could also have an impact on synaptic plasticity and associated cognitive function. Additionally, with aging, microglia may shift their functional phenotype such that they either express a higher level of proinflammatory factors creating an environment facilitating oxidative stress or thus, be neurotoxic to the neuronal or glial population. Such a shift could also occur in microglia that would inhibit the regulatory aspects or repair capabilities of microglia to downregulate inflammation and promote recovery and tissue remodeling.

While characteristic features of microglia structure and function have been identified as they relate to brain development and as they change with aging, there are limited experimental data to fully demonstrate the altered function of microglia cells across the lifespan.

However, based upon what we know about the general signaling of the cells for migration and phagocytosis, it could be hypothesized that microglia might change with regard to how they detect changes in the environment and how they respond to those changes, including the severity and duration and nature of the response. The wealth of data on the normal adult brain in response to injury provides a framework for asking critical questions to determine how the cells may function during development and change with aging and the impact of such changes. It is highly likely that a temporal gain or loss of function contributes to defining the nature and function microglia at different stages of the lifecycle. This includes their contribution to brain development and maintaining a healthy neural environment, and possibly the susceptibility of these cells to factors associated with aging. A thorough examination of the existing literature on the physiology of microglia is outside of the current review; rather, the present chapter will focus on data available for microglia during these two ends of the lifestage spectrum. This chapter attempts to set a framework for evaluating multiple aspects of microglia dynamic neural-specific cells as they may apply across the life span.

2. Microglia During Brain Development

Mononuclear phagocytes (macrophages, dendritic cells, monocytes) are hematopoetic cells belonging to the myeloid lineage (van Furth et al., 1979). A major breakthrough in the understanding of the origin of these cells came with the identification of a clonotypic bone marrow precursor by using adoptive transfer monocytes from CX3CR1 green fluorescent protein (GFP) reporter mouse (Varol et al., 2007). These cells are released into the peripheral circulation and transported from the bone marrow to eventually become tissue specific macrophages. The lack of unique expression of cell surface markers between mononuclear phagocytes that infiltrating the brain and activated resident microglia has hindered the ability to discriminate between the two and originally suggested a common origin. This led to the proposal that microglia were like mononuclear phagocytes and thus derived from the bone marrow and seeded to become tissue specific macrophages. This may still be the case for the few circulating blood progenitors penetrating the vascular wall in the neonatal and adult brain or those cells repopulating the mononuclear phagocytes associated with the CNS such as perivascular macrophages. Work by Priller et al. (2001) and others demonstrated GFP-expressing bond marrow cells in the brain several weeks after irradiation and bone marrow transplantation (Eglitis and Mezey, 1997; Massengale et al., 2005). When the head was shielded during irradiation or parabolic mice were examined, a much lower level of engraftment was observed, if at all (Ajami et al., 2011; Ginhoux et al., 2010; Matsumoto and Fujiwara, 1987). Work from Ginhoux et al., (2010) suggested that only approximately 10–20% of donor origin microglia were seen in the parenchyma of bone marrow chimeria mice 10–21 months after transplantation. This low level of engraftment was also observed in a busulfan/CX3CR1GFP+ chimeric mouse (Katzmarski et al., 2013). While parabiotic mice show a lower rate of chimerism, the number of engrafted cells was decreased to only 5% at 1 and 12 months post-parabiosis (Bogunovic et al., 2006). These findings suggested that postnatal microglia are maintained independently of blood-borne hematopoietic progenitors. It is now thought that a large proportion of microglia colonization of the brain occurs prior to birth.

Microglia differentiate from a primitive macrophage population produced by the yolk sac (Alliot et al., 1999; Ginhoux et al., 2010). In colonization of the brain, microglial recruitment and differentiation is suggested to occur in hematopoietic waves during the embryonic and postnatal periods (Chan et al., 2007; Ransohoff and Perry 2009). In using reconstituted sublethally irradiated mice, Ginhoux et al., (2010) were able to identify that only a small proportion (5%) of adult microglia were derived from the transplanted cells with 95% remaining of host origin. Myeloid cells expression the hematopoietic marker

CD45 and adult macrophage markers CD11b, F4/80, and CX3CR1 can be detected in the mouse brain starting from E9.5 and by E10.5 could be observed in the cephalic mesenchyme and neuroepithelium (Ginhoux et al., 2010). In the rat, macrophages in the head were observed at E11 infiltrating the neurepithelium at E12 (Sorokin et al., 1992). Using single cell suspensions of mouse brain rudiment, no cells with properties of a microglial progenitor could be detected at E7.5, this increased to a single progenitor per brain rudiment at E8, to three cells at E8.5 followed by a sharp proliferation to reach an average of 1200 cells at E10.5 (Alliot et al., 1999). These cells remained highly proliferative throughout the embryonic period serving to populate the brain parenchyma. In the mouse, approximately 95% of the microglia are generated within the first two post-natal weeks (Alliot et al., 1999). Using a Cre-reporter mouse expressing the runt-related transcription factor 1 (*Runx1*), Ginhoux et al., (2010) furthered their evaluation of the origin of microglia during development. By fate mapping, reporter expression suggested a yolk sac origin for embryonic microglia and a migration of Runx1+ progenitors through the blood vessels after E8.5 with cells appearing in the brain rudiment at E9.5 (Ginhoux et al., 2010). Additional investigation identified macrophage colony stimulating factor receptor (MCSF-R)-expressing yolk sac precursors present at E8.5–9.5 as the origin for microglia (Schulz et al., 2012). In examining an earlier time in the process, Kierdorf et al (2013) identified cells by flow cytometry expressing cell surface markers characteristic of erythromyeloid progenitors Cd45-/c-kit+. These cells were able to differentiate into Iba-1+/CX3CR1-GFP+ cells and morphologically displayed shorter processes than the more mature microglia. These investigators confirmed the embryonic source of the cells and excluded any contribution from maternal macrophages to the microglia pool.

Colonization of the brain occurs with embryonic vascularization (Ajami et al., 2007) and relies heavily on blood vessels and an active process of blood circulation (Ginhoux et al., 2010). The peri-neural vascular plexus initiates vascularization of the CNS along a caudal-cephalic gradient commencing at the myelencephalon and ascending to the telencephalon (Marin-Padilla, 1985). During this time, cell-cell communication continues between microglia and vascular sprouts allowing for microglia migration towards developing vessels and stimulation of angiogenic activity (Rymo et al., 2011). While the vascular system is a primary avenue for transport, microglia appear in regions of the developing CNS devoid of vascularization (Hurley and Streit, 1995) with brain ventricles or para–meningeal transport serving as alternative routes of entry (review Cuadros and Navascues, 1998). Using immunoelectron microscopy of the rat cerebrum and spinal cord, Lassmann et al. (1991) demonstrated contact between parenchymal microglial process end-feet and the basal lamina of microvessels. This specific class of microglia has been referred to a juxtavascular microglia as distinguished from components of the vascular wall, the perivascular cells (pericytes) and from those microglia within the parenchyma that do not contact blood vessels. Using time-lasped microscopy of brain-slices from immature rodents, Grossmann et al. (2002) demonstrated that juxtavascular microglia are preferentially recruited to blood vessels and migrate along parenchymal surfaces upon stimulation following traumatic injury. Parenchyma microglia tracking along vessels showed a flattened morphology over the vessel surface and extended a leading process parallel to the vessel. A second group of blood vessel-associated microglia remained stationary and apparently anchored to the blood vessel but motile and actively extending processes into the surrounding parenchymal tissue (Grossmann et al., 2002). The heterogeneity of microglia is maintained with regards to brain location and relative distance to the vasculature. In the brain areas receiving the best blood supply, microglia display a more complex process ramification pattern (Rowan and Maxwell, 1981; Vela et al., 1995). Under conditions that decrease capillary flow the normal maturation of microglia to a ramified morphology is delayed (Masuda et al., 2011).

In the human brain, microglia colonization follows a tightly orchestrated process with distinct patterns of microglia migration occurring along radial glia, white matter tracts, as well as the vasculature (Rezaie and Male, 1999). By mid-to-late first trimester, a limited number of amoeboid microglia are present (Andjelkovic et al., 1998; Choi, 1981; Fujimoto et al., 1989) and by 24 weeks of gestation a large accumulation of cells is observed in the germinal matrix (Hutchins et al. 1990). Fetal microglia are located at highly vascularized sites in proximity to the parenchymal wall of radial blood vessels (Rezaie et al., 2005). With further development, microglia align within the subplate region of the telencephalon coinciding with the appearance of neurons, early synaptogenesis, and the establishment of thalamocortical projection fibers (Kostovic and Judas, 2002). As neurons populate and become established in the brain, the necessity for clearance of excess neurons is reduced and with it comes a morphological shift in microglia from a predominant amoeboid morphology normally associated with phagocytic actions. The number of nascent round and amoeboid microglia decreases and an increase is seen in highly ramified cells bearing long, thin, branched processes (Monier et al., 2006). As the cells reach their terminal location, they begin to take on a ramified morphology (Kostovic and Judas, 2002) and by 35 weeks of gestation microglia display a fully developed ramified morphology (Esiri et al., 1991). By birth and during the first few postpartum weeks, microglia disseminate throughout all parts of the brain, occupying defined spatial territories without substantial overlap.

In the mouse, microglia colonization follows a similar pattern to that observed in the human (Fig. 1). Between GD 10–15, when capillary sprouts perforate the CNS, focal degeneration and disintegration of subadjacent glial endfeet stimulate the penetration of endothelial cells and attract monocytes and macrophages from the blood (Marin-Padilla, 1985). Precursors of ramified brain-resident microglia expressing the adult myeloid cell marker, F4/80, are found in the vicinity of CNS capillaries and are characterized by a round or irregular-shaped cell body, with or without pseudopodia (Imamoto and Leblond, 1978). In rats, influx of $F4/80^+$ cells occurs at GD 15–16 with ramified $F4/80^+$ cells appearing in the brain parenchyma by GD 18–19 and becoming more ramified as they fully differentiate (Kaur and Ling, 1991). These cells maintain a highly proliferative state throughout embryonic life (Ginhoux et al., 2010) expressing myeloid cell markers, including cluster of differentiation (CD) 34 and CD45, and protein tyrosine phosphatase receptor type C (PTPRC) (Davoust et al., 2006). In the subcortical white matter, this proliferative state extends into the first week following birth (Hristova et al, 2010).

During the first postnatal month of life, microglia continue their development with a transition from a prominent phagocytic amoeboid morphology to one of more process bearing non-phagocytic features (Imamoto and Leblond, 1978; Orlowski et al., 2003). This transition is dependent upon the expression of Runt-related transcription factor 1 (Runx1) to inhibit cell proliferation (Zusso et al., 2010). Peak expansion of microglia coincides with increased expression of colony stimulating factor (CSF)-1 receptor (CSF-1R) as a receptor for IL-34 and essential for appropriate brain development, especially in the cortex and olfactory bulb (Erblich et al., 2011). By PND15, the entire parenchyma is filled with ramified microglia within defined territories and no cell overlapping (Lawson et al., 1990; Perry et al., 1985). It was initially thought that during the first 3 weeks of life, only 30% of the cells transform into ramified microglia, while the other 70% degenerate (Imamoto and Leblond, 1978; Wu et al., 1992). Thus, it was thought that the balance between cell proliferation and cell death would serve to regulate the number of cells surviving from the microglial lineage. However, further studies from Dalmau et al. (2003) using double-labeling techniques for microglia and apoptosis, demonstrated that there is a low proportion of microglia undergoing apoptosis during brain development. In the light of a high level of proliferation of microglia, the decrease in microglia density with brain development (Wu et al., 1992), if not due to apoptosis, may simply reflect the dramatic changes of volume

expansion in the CNS during development (Dalmau et al., 2003). During these high expansion period in the first two weeks of postnatal life, the maturational progression of microglia can be influenced and thus in turn could alter normal processes of neuronal and glia development. For example, microglia express thyroid hormone (TH) receptors (TRα1 and TRα1) and any shift in thyroid status significantly alters their maturation (Lima et al., 2001). During these first two weeks, hypothyroid animals show a deficit in the density of microglia and a delay in process extension; while, hyperthyroidism accelerates the extension of microglial processes and increases the density of cortical microglia. In addition to altered maturation, microglia can be depleted during this early period. Exposure to ethanol between days 3 and 5 of postnatal life significantly depletes microglia numbers in the cerebellum with a loss of Purkinje cells (Kane et al., 2011).

In the amoeboid state, microglial cells are able to migrate within the brain and serve a critical role in the clearance of excess debris generated during the course of neuronal and glia proliferation (Perry et al., 1985). The arrival of microglia in the brain parenchyma correlates with the presence of apoptotic cells (Rigato et al., 2011; Wakselman et al., 2008). In the postnatal brain this association is considered as a response towards programmed neuronal death or axonal degeneration (Bessis et al., 2007; Mallat et al., 2005; Perry et al., 1985; Rakic and Zecevic, 2000; Schlegelmilch et al., 2011; Wakselman et al., 2008). In addition to a secondary clearance function, an active role for microglia in pruning excess neurons has been suggested. In slice cultures, a direct role for microglia in the cell death of supernumerary Purkinje cells has been demonstrated (Marin-Teva et al., 2004). A similar process has been reported with the death of early postnatal hippocampal neurons (Wakselman et al., 2008).

While clearance of excess cellular debris and aberrant proteins from programmed cell death is critical, the distribution of microglia in the developing mammalian brain suggests a more heterogeneous role (Rezaie et al., 2005). Based upon the body of data, microglia can be considered as critical players in brain development and neural circuitry formation (Erblich et al., 2011; Pont-Lezica et al., 2011). They provide important functions associated with vascularization of the brain, neuronal proliferation, and cell differentiation to a neuronal or astrocyte lineage (Mallat et al., 2005; Ni et al., 2007; Schafer et al., 2010). Examples of these functions are demonstrated in the ability of microglia to facilitate neurogenesis of embryonic cortical cells (Aarum et al., 2003); differentiation of embryonic neural precursor cells to astrocytes (Antony et al., 2011); and differentiation of basal forebrain progenitors into cholinergic neurons (Jonakait et al., 2000). As the neuronal population is established, the demands on microglia shift and the cells take on duties associated with promoting axonal elongation, synaptogenesis, and myelination. Axon pathfinding is facilitated by microglia via modification of the extracellular matrix by thrombospondin (Chamak et al., 1994). Neurotrophic factors such as neuronal growth factor (NGF; Heese et al., 1998), brain derived neurotrophic factor (BDNF; Batchelor et al., 1999; Coull et al., 2005), neurotrophin-3 (NT3), glial derived neurotrophic factor (GDNF) and cytokines with neurotrophic activity (reviewed in Garden and Moller, 2006; Kim and deVellis, 2005) are provided by microglia to the developing brain. Macrophage colony stimulating factor (M-CSF) can promote the proliferation of microglia but it also acts as a neurotrophic factor supporting neuronal survival and neurite outgrowth indirectly through microglia (Michaelson et al., 1996).

The physical association of microglia with synapses implicates microglia with synaptic refinement during development. Process bearing “activated” or “reactive” microglia have been reported in the thalamus, cerebellum, olfactory bulb, and hippocampus during postnatal synaptic remodeling (Fiske and Brunjes, 2000; Perry et al., 1985). The co-localization of neuronal postsynaptic protein within microglia cytoplasm suggests an active process of

microglia to engulf and remove dendritic spines during development (Paolicelli et al., 2011). Synapses form as a function of sensory input and with input during the early postnatal days (PND 8–10) an increase in contact of microglia with immature dendritic spines is observed. This leads to a shift in the subsequent loss of these synapses (Tremblay et al., 2010). During postnatal remodeling of the visual cortex, visual experience regulates not only microglia contact but also morphology with cells progressing to a ramified state faster in animals monocular from birth (Rochefort et al., 2002). With synapse maturation or elimination, neurons up-regulate expression of the chemokine, fractalkine (CX3CL1) (Mody et al., 2001), that serves to signal microglia through activation of CX3CR1. Deficits in this receptor (Hughes et al., 2002; Nishiyori et al., 1998) are associated with a delay in synaptic pruning and spine elimination resulting in seizure susceptibility and a reduction in synapse efficiency (Paolicelli et al, 2011). The classical complement cascade has also been implicated in synapse elimination by microglia (Chu et al., 2010; Stevens et al., 2007). Complement receptor 3 (CR3) expression stimulates phagocytosis and spatially associates with complement factor, C1q, at synapses (Gasque et al., 2002). The correlation of complement expression with peak synaptic remodeling in the dorsal lateral geniculate nucleus (Stevens et al., 2007) led to the speculation that complement 3 serves to tag the extranumerary synaptic inputs for CR3-mediated phagocytosis by microglia (Schafer and Stevens, 2010).

Microglia are not only associated with synapse elimination but influence synaptic strength through the release of TNFα (Zhong et al., 2010) and by promoting surface expression of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA)-type glutamate receptors on hippocampal neurons (Beattie et al., 2002) via β3 integrin expression (Cingolani et al., 2008). Neuronal activity can directly activate microglia (Hung et al., 2010). For example, prolonged inhibition of spontaneous neuronal activity in hippocampal cells stimulates TNFα release, while activity-regulated signals decrease TNFα release (Stellwagen and Malenka, 2006). The authors proposed that this was related to synaptic scaling, where the strength of synapses is modulated in response to changes in network activity. These data suggest s that microglia can detect the functional state of neurons and initiate an appropriate response targeted to the specific neuronal activation pattern. Thus, one can propose that alterations in microglia functioning during synapse formation and maturation of the brain can have significant long-term effects on the final established neural circuitry.

3. Microglia Morphology and Distribution in the Adult Brain

Once established, resident microglia comprise approximately 15–20% of the total number of cells in the brain (Carson et al., 2006b). Slight differences in morphology are seen across brain regions (Harry and Kraft, 2012); however, there is a relative uniform distribution with the exception of a higher relative number of in the olfactory telencephalon, dentate gyrus of the hippocampus, the substantia nigra, and portions of the basal ganglia (Lawson et al., 1990; Mittelbronn et al., 2001; Sharaf et al., 2010). In general, ramified microglia in gray matter show a small amount of perinuclear cytoplasm and a small, dense, and heterochromatic nucleus with processes extending in multiple directions. In white matter, microglial cells align their cytoplasmic extensions in parallel or at right angles to nerve processes. Differences in localization of microglia throughout the brain are suggested to be associated with the microenvironment as well as functional differences and phenotypic heterogeneity, including receptor expression patterns (Olah et al., 2011) and expression of cell surface antigens (Kaur & Ling, 1991). For example, in the corpus callosum, a white matter tract, microglia express a constitutively higher level of the co-stimulatory molecule, CD86 (B7.2) as compared to cells within the cortex (Carson et al., 2007). This expression within the white matter has been proposed to be associated with mechanisms related to

nondestructive immunity (Bechmann et al., 2001). Microglia within unique brain regions maintaining a neurogenic capability such as the subgranular zone of the dentate gyrus in the hippocampus, also show a phenotype that allows for phagocytosis at the tips of process extension (Sierra et al., 2010) and exists under higher basal expression levels of CD45 and CD11b (Marshall et al., 2008). This may be linked to the activity of microglia within this region to regulate the cell-death, clearance, and survival of the newly generated neurons in order to ensure an appropriate number of mature dentate granule neurons (McPherson et al., 2011a;b: Sierra et al., 2010). Within brain regions lacking a blood-brain-barrier (BBB) such as the circumventricular organs, microglia display shorter thicker processes. Given their location, this morphology is likely influenced by the presence of serum proteins (Giulian et al., 1995; Fujita et al., 1996; Chamak and Mallat, 1991). Perivascular macrophages represent a separate population of cells located in the basal lamina of brain capillaries and the choroid plexus (Streit et al., 1989; Graeber & Streit, 1990; Guillemin and Brew, 2004; Sedgwick et al., 1991).

4. Phenotype Changes in Microglia

Microglia actively survey surrounding parenchyma via dynamic movement of processes allowing each cell to sample its environment over the course of a few hours (Davalos et al., 2005; Nimmerjahn et al., 2005). Maintaining microglia in such a relatively quiescent state is in part due to specific signals derived from neuronal and astrocyte-derived factors (Cardona et al., 2006; Neumann, 2001; Simard et al., 2006). Healthy neurons accomplish this via secreted and membrane bound signals including, CX3CL1 (Barclay et al., 2002; Hoek et al., 2000; Lyons et al., 2007; Sunnemark et al., 2005; Wright et al., 2003), neurotransmitters (Pocock and Kettenmann, 2007), and neurotrophins. During development, in the normal adult brain, and in the brain following injury, microglia display varied morphologies. The morphological differences in microglia are suggested to represent different functional states. Functional changes in microglia are often accompanied by a morphological transformation to cells with larger somata and shorter, coarser cytoplasmic processes displaying a bushy appearance possibly progressing to a full amoeboid morphology (Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009). *In vivo*, the transformation of resident microglia into those with a phagocytic phenotype is strictly regulated and occurs in response to stimuli such as cell death (apoptotic or necrotic), accumulated debris, excess aberrant protein, or the presence of viral or bacterial pathogens. Alternatively, a microglia response can be induced by a variety of input signals and may not necessarily result in a shift to an amoeboid phenotype as has been seen in the morphologies associated with synaptic or neural progenitor cell clearance. Upon the removal and thus absence of the stimulating stimuli or in the presence of the down-regulating signal, activated microglia commonly down-regulate to a ramified phenotype.

Brain macrophages exist in various states of activation within injured tissue and retain the capability to shift their functional phenotype within specific stages of the inflammatory response (Stout et al., 2005; Graeber 2010). In efforts to characterize functional changes and thus activation states, stages of peripheral macrophages have been recently adapted and proposed to describe the various stages that brain macrophages undergo during injury or neurodegeneration (Colton and Wilcock, 2010). Compared to peripheral macrophages or immature dendritic cells, CNS resident microglia do not represent a cellular source to present tissue antigens to T cells located within the draining lymph nodes and thus are relatively ineffective at initiating antigen-driven CD4+ T cell responses (Carson et al., 1998; 2006b). However, expression of MHC class II may serve to re-stimulate T cells in the target organ (Carson et al., 2006b; Siffrin et al., 2007). With presentation, CD4+ T-cells are stimulated toward one of a broad array of phenotypes (Lo et al., 1999; Siffrin et al., 2007). For example, the pro-inflammatory (Th1, Th17) $CD4^+$ T cells promote myeloid cells

towards a classical activation state (M1 state) characterized by high expression levels of pro-inflammatory cytokines (IL-1β, IL-12, IL-23, TNFα) as well as inducible nitric oxide synthase (iNOS), CD40, MHC I and MHC II (Goerdt et al., 1999; Colton and Wilcock, 2010; Gordon and Martinez, 2010). In contrast, IL-4- and IL-13+ Th2 CD4+T-cells are essential to promote myeloid cells toward alternative activation states (M2a and M2b) as characterized by expression of macrophage mannose receptor (CD206), Chitinase-3-like-1 (YM1 in rodents), stabilin-1, and arginase I. Under this alternative activation, the cells are functionally associated with enhanced phagocytosis, tissue repair, and parasite elimination. A regulatory activation state (M2c) is promoted by IL-10 and TGFβ-producing Th3 cells and regulatory T cells and characterized by expression of molecules related to tissue repair, reconstruction, and immunosuppression (Colton and Wilcock, 2010; Gordon and Martinez, 2010). Each state may have its own particular phenotype and there are likely transitional phenotypes between wound healing classical and regulatory macrophages (Mosser and Edwards, 2008) and microglia (Colton and Wilcock, 2010; Durafourt et al., 2012). This hypothesis that microglia can display an M1/M2 stage distinction as in peripheral macrophages would account for the heterogeneity of function of microglia (Ajami et al., 2007; Kigerl et al., 2009; Michelucci et al., 2009; Colton and Wilcock, 2010). Microglia are capable of exhibiting a M1 pro-inflammatory phenotype as occurs following activation with lipopolysaccharide (LPS). An M2 anti-inflammatory phenotype can be generated with IL-4, characterized by the release of neurotrophic factors such as insulin like growth factor -1 and anti-inflammatory cytokines such as, IL-10 and expression of YM1 (Perego et al., 2011). Microglia isolated from MS patients undergoing glatiramer acetate therapy displayed type 2 phenotypes, suggestive of a shift from a pro-inflammatory phenotype (Kim et al., 2004). Polarization of microglia has been demonstrated in human adult microglia (Durafourt et al., 2012) following focal cerebral ischemia (Hu et al., 2012) and in a severe relapsing experimental rat model of MS (Mikita we al., 2011). While data are still limited, there are a few studies reporting that aging shifts the sensitivity of activated microglia with diminished anti-inflammatory and M2-promoting effects of IL-4 (Fenn et al., 2012). In the microglia response to the Aβ protein, the brain oligomeric form of Aβ is a stronger inducer of the M1-phenotype as compared to the fibrillar form of the protein Michelucci et al (2009). When the cells are maintained in a cytokine-induced anti-inflammatory environment the level of reactivity is reduced (Michelucci et al., 2009). While the M1/M2 distinction has been considered under injury or disease states, expression of M1 and M2 related factors have been demonstrated in the developing rodent brain (Lenz et al., 2013). Thus, with additional experimental studies, examination of microglia responses and regulatory factors within the framework of an M1/M2 response may lead to a better understanding of the diverse functions of these cells and how they shift during various stages of life. For example, the inability of microglia to follow a normal healthy pattern could likely contribute to neurodegeneration, neuroinflammation, and absence of repair and injury resolution, which could occur under disease conditions or with aging.

5. Receptors for Microglia Activation

The normal functional capacity of microglia to rapidly return an injured tissue back to normal homeostasis is a critical component for maintaining a healthy tissue environment. This response requires migration of microglia to the damaged area and phagocytosis of aberrant material. To accomplish this, microglia express many surface receptors allowing for a direct interaction with the target. A multitude of signals posing potential threat to brain homeostasis are sensed by microglial receptors (Hanisch & Kettenmann, 2007) including the presence of inwardly-rectifying potassium (K^+) channels (Kettenmann et al., 1993) other ion channels (Table 2), receptors for adenosine triphosphate (ATP; Langosch et al, 1994; Walz et al., 1993), calcitonin gene-related peptide (Priller et al., 1995), pattern recognition receptors (PRR), and multiple neurotransmitter (Table 3), neurohormone receptors (Table 4)

and chemokines (Table 5). Initiation of a microglia response can involve purinergic receptors (P2X, P2Y; Langosch et al., 1994), the scavenger receptor CD36 (Stolzing and Grune, 2004), toll-like receptors (TLRs) (Landreth and Reed-Geaghan, 2009), and chemokine receptors (Cartier et al., 2005). These include the CX3C chemokine fractalkine receptor (CX3CR1) (Cardona et al., 2006; Lee et al., 2010) and CXCR3 as triggered by the neuronal chemokine CCL21 elevated with cell death (de Jong et al., 2005) and regulated by CXCL10 (Dijkstra et al., 2004). Signaling through these receptors can induce changes in membrane potential, intracellular calcium, cellular motility, and cytokine release (for review see, Kettenmann et al. 2011; Pocock and Kettenmann, 2007).

5.1. Distinguishing what to destroy and what to protect

One of the necessary functions of microglia in performing their tasks appropriately and efficiently is the ability to distinguish “friend from foe” (Carson et al., 2006a; 2006b). This function can serve to influence the purpose and outcome of a microglia response and direct outcome effects on other neural cells. In many cases, the ability of the cell to discriminate between self and invader is dependent on the presence or absence of antigen presentation (Medzhitov and Janeway, 2000). In the periphery, the rapid innate and slow adaptive immune response systems are responsible for the “self”-identifier function and the integrated system allows for a specific response based upon the nature of the threat (Lo et al., 1999). In contrast to the periphery, there is limited infiltration of T-cells into the brain and microglia are relatively ineffective at initiating antigen-driven CD4+ T-cell responses (Carson et al., 2006b). There however, is evidence suggesting that microglia are capable of contributing to the antigen driven response in autoimmune diseases such as, multiple sclerosis (Coyle, 2011).

Even in the absence of a strong APC, a productive innate immune response to successfully drive a rapid response to insult is dependent upon a number of factors including expression of pattern recognition receptors (PRRs) (for review, Krishnaswamy et al., 2013). PRRs expressed on microglia include Toll-like receptors (TLRs; TLR 1–9 (Farina et al., 2005); TLR2 (Babcock et al., 2006; Lehnardt et al., 2007), and TLR4 (Lehnardt et al., 2003; Visintin et al., 2001)), receptor for advanced glycation end products (RAGE), and specific scavenger receptors targeted to detect the aberrant expression of phosphatidylserine (PS) on the extracellular surface of dying cells (Ravichandran, 2003). Ligation of PRRs leads to the activation of signal transduction pathways and transcriptional and post-transcriptional molecules of the interferon regulator factor families. These molecules modulate pro-inflammatory target genes encoding cytokines, chemokines, enzymes, and other molecules essential for pathogen elimination (Akira et al., 2006). In comparison, signaling of the triggering receptor expressed on myeloid cells-2 (TREM-2) was found to facilitate debris clearance by microglia *in vitro* in the absence of an inflammatory response (Takahashi et al., 2005). Based upon studies examining the various TLRs, it was suggested that innate immune response activation in the brain is tailored according to the cell type and environmental signal. For example, TLR2 and TLR4 are capable of sensing damage induced by ischemia and, as such, boost the pro-inflammatory response leading to an increase in infarct size (Caso et al., 2008; Kilic et al., 2008; Lehnardt et al., 2007). TLR2-mediated responses are primarily associated with release of IL-6 and IL-1β (Jack et al., 2005) and thus the outcome would be related to those cytokine-signaling pathways. The presence of activated microglia is required for a TLR4 response in astrocytes and to optimize the TLR2 and TLR3 response (Holm et al., 2012). TLR4 is used by microglia and astrocytes to detect Aβ (Landreth and Reed-Geaghan, 2009) and the release of heat shock protein 60 from dying CNS cells (Lehnardt et al., 2008). In a pro-oxidant and inflammatory environment, RAGE (Neeper et al., 1992; Schmidt et al., 1992) is activated on microglia and responds to the increased production of Aβ, serum amyloid A (SAA), S100 protein, and high-mobility

group protein B1 (HMGB1). Increased production of these ligands is observed with cellular dysfunction and inflammation (Schmidt et al., 2009; Srikanth et al., 2011). Upon detection, signal-dependent transcription factors are activated to up-regulate inflammatory response genes and initiate the clearance of the aberrant protein (Reed-Geaghan et al., 2009; Walter et al., 2007). RAGE increases M-CSF release from neurons, activating the cognate receptor, c-fms, on microglia resulting in a prolonged survival of microglia within the injured tissue (Yan et al., 1997). However, RAGE also recognizes PS and induces apoptotic cell clearance (Friggeri et al., 2011). HMGB1 is an intracellular protein that activated p53 and NF-κB transcription factors and through ERK-mediated signaling pathway, inhibits the phagocytosis of apoptotic cells (Friggeri et al., 2010).

Degenerated neurons also release several signaling molecules, including nucleotides, cytokines, and chemokines, to recruit microglia (Biber et al., 2007). Signaling between microglia and degenerating neurons is conducted by a number of different mechanisms with one of the strongest signals being adenosine triphosphate (ATP). ATP release can mediate purinergic signaling to promote microglia process extension and phagocytosis. Microglia recognize the release of purine from dying cells by purinergic receptors. The physiological effects of purines and pyrimidines are mediated through purinoceptors classified as metabotropic P1 adenosine receptors, metabotropic P2Y purinoceptors, and ionotropic P2X purinoceptors (Fredholm et al., 2007; North, 2002; Surprenant and North, 2009). Receptor expression is abundant in glia with microglia expressing $P2X_4$, $P2X_7$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, and $P2Y_{14}$ receptors (Verkhratsky et al., 2009) and purine metabolism-related enzymes are expressed by microglia in the developing brain (Dalmau et al., 1998). In the case of neuropathic pain, one such signaling molecule is the chemokine, CCL2 (monocyte chemoattractant protein 1, MCP-1). Its cognate receptor, CCR2, is expressed on microglia (Zhang et al., 2007) and upon activation, promotes cell surface expression of $P2X_4R$ (Toyomitsu et al., 2012) as a critical modulator for the pain response (Trang et al., 2009; Tsuda et al., 2003; 2009; Ulmann et al., 2008). Also critical for the up-regulation of $P2X_4R$ are chemokines released from neurons e.g., CCL21 (Biber et al., 2011), interferon gamma (Tsuda et al., 2009), fibronectin (Tsuda et al., 2009), as well as protease tryptase activating proteinase-activated receptor 2 (PAR2) from mast cells (Yuan et al., 2010), and brain derived neurotrophic factor (BDNF) released from microglia (Trang et al., 2009). P2XRs are expressed on rat microglia during early development (Xiang and Burnstock, 2005). $P2X_7R$ expressing microglia are diffusely scattered throughout the brain (Yu et al., 2008) and are induced with brain injury and in neurodegenerative disorders including amyotrophic lateral sclerosis (Yiangou et al., 2006), Alzheimer's disease (Takenouchi et al., 2010), ischemia (Melani et al., 2006) localized injection of $A\beta_{1–42}$ (McLarnon et al., 2006) or LPS (Choi et al., 2007), and kainite-induced seizures (Rappold et al., 2006). As with other microglia receptors, the outcome of $P2X_7R$ activation can be cytotoxic or trophic. Activation of $P2X_7$ receptors promotes release of IL-1α and IL-1β (Bernardino et al., 2008; Ferrari et al., 1997; Mingam et al., 2008), TNFα (Hide et al., 2000), superoxide (Parvathenani et al., 2003), and plasminogen (Inoue et al., 1998). However, a dual role is proposed given that $P2X_7R$ activation can protect neurons against glutamate-induced neurotoxicity (Suzuki et al., 2004).

In comparison to the P2X receptors, P2Y receptors can promote down-regulation of a pro-inflammatory response. Activation of P2YR inhibits LPS-induced release of TNFα, IL-1β, IL-6, and IL-12 (Boucsein et al., 2003; Ogata et al, 2003) and activation of $P2Y_1/P2Y_{11}R$ increases release of the anti-inflammatory cytokine, IL-10 (Seo et al., 2008). $P2Y_{12}Rs$ are localized on the cell processes and somatic membrane of quiescent microglia. The expression of this receptor contributes to the cell's ability to migrate, proliferate, and extend processes toward a lesion (Haynes et al., 2006). As with other stimuli of chemotaxis, this is associated with engagement of integrin-β1 signaling (Ohsawa et al., 2010), K^+ channel activation (Wu et al., 2007) and ATP signaling (Ohsawa et al., 2007). Migration of

microglia mediated by the $P2Y_{12}$ receptor is dependent upon activation of phosphoinositide 3-kinase (PI3K) and Akt pathways (Irino et al., 2008). Microglia express subclasses of the P1 adenosine receptors (A1, A2A, A2B, and A3) (Dare et al., 2007; Fredholm et al., 2001; Hammarberg et al., 2003). It has been suggested that a co-stimulation of purinergic and adenosine receptors is required for cell migration with the ATP/adenosine balance regulated by expression of the prototype nucleoside triphosphate diphosphohydrolase, CD39 (Draheim et al., 1999).

While ATP serves as the more potent signal, microglia chemotaxis can be mediated by other receptors including α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA), serotonin (Krabbe et al., 2012), histamine (Ferreira et al., 2012), dopamine and epinephrine (Farber et al., 2005), bradykinin (Ifuku et al., 2007), metabotropic glutamate receptors (mGluR) (Liu et al., 2009; McMullan et al., 2012), cannabinoid receptors (McHugh, 2012), platelet-activating factor receptors (Aihara et al., 2000), as well as, expression of CD39 ectonucleotidase (Draheim et al., 1999). Noda et al (2000) reported the presence of AMPA/kainite (KA) type receptors on microglia but on only a subpopulation of the cells. While KA-induced inward currents could be induced in only 20% of quiescent cells, this increased to 80% when the cells were in a proliferative state (Yamada et al., 2006). This suggests that the influence of KA type receptor activation is directed more toward immature or proliferating microglia rather than process bearing cells. While increased excitatory neurotransmission and purinergic signaling can increase microglial process motility, inhibitory neurotransmissions have been implicated in diminished motility. The inhibitory neurotransmitter, γ–aminobutyric acid (GABA), has been implicated in the decrease of microglia process motility in the brain (Fontainhas et al., 2011; Nimmerjahn et al., 2005) and retinal explants (Fontainhas et al., 2011).

6. Microglia in the Aged Brain

It has been proposed that microglia change as a function of aging and that such change may reflect a diminished capability for the cells to perform their normal function (Streit, 2006; Streit and Xue, 2012). To maintain homeostasis cells are required to rapidly respond in a manner that will allow for clearance of aberrant proteins (ApoE, α-synuclein, Aβ42), invading pathogens, damaged tissue debris, synaptic stripping and remodeling. The activation of microglia cells and pro-inflammatory cytokine signaling representing an activation of the innate immune system can serve in a neuroprotective manner with responding and clearing invading pathogens and local debris or protein (for reviews: Aravalli et al., 2007; Glezer et al., 2006; 2007; Lehnardt, 2010; Olson and Miller, 2004; Rodgers and Miller, 2012). Such actions ensure appropriate signaling to extend these clearance processes and transition the response to one of resolution and repair, providing trophic support with the release of various growth factors. Any deficit in the ability of microglia to perform these functions, whether due to a decrease in cell number or diminished function, would have significant impact on the state of health of the brain.

When the overall distribution of microglia was examined in the mouse brain, cells were found less evenly distributed in the aged cortex as compared to the young adult (Tremblay et al., 2012). Dendritic arbors are smaller, less symmetrical and more elongated rather than circular in shape (Sierra et al., 2007; Tremblay et al., 2012). In examining the evolution of structural differences in cortical microglia from rats aged 3 to 30 months, Vaughan and Peters (1974) observed changes in cell shape and an increase in cell number Similar to the morphology in the brain, live cell imaging of retinal microglia *in situ* demonstrated that aged microglia are smaller with less ramifications, have slower process motility, and as a response to ATP they become less dynamic and ramified. Upon a laser injury, aged microglia show slower migration response and remain at the injury site longer, as compared

to young microglia suggesting not only a deficit in the initial response but also in the down-regulatory signaling for resolution (Damani et al., 2011). With aging, microglia are less restricted from the outer retina and accumulate in the subretinal space (Xu et al., 2008). This shift in position brings microglia into contact with photoreceptors and retinal pigmented epithelial cells (Ma et al., 2012). In the retina, a para-inflammatory state exists with aging (Xu et al., 2009). This is a tissue adaptive response to noxious stress or system malfunction and exists under conditions that are intermediate between basal and inflammatory states. A dysregulation of this intermediate state has been linked to the immune dysfunction associated with age-related macular degeneration (Chen et al., 2011). With normal aging, microglia aggregate in the outer retina and acquire intracellular autofluorescent lipofuscin deposits (Xu et al., 2008). Examination of A2E accumulation as representative of ocular lipofuscin, increased microglia activation, lowered expression of chemokine receptors, suppressed chemotaxis and increased complement activation (Ma et al., 2013). The accumulation of lipofuscin in microglia has also been described in the brains of rats aged between 3 and 30 months (Vaughan and Peters, 1974).

In both rat and human brains observations of altered microglia morphology have been observed and associated a more reactive/activated phenotype as a function of aging (Vaughan and Peters, 1974; Samorajski, 1976; Schuitemaker et al., 2010). Markers normally present in activated microglia are also elevated in aged microglia including MHC II antigens, CD11b, CD14, and pattern recognition receptors (Finch et al., 2002; Frank et al., 2006; Letiembre et al., 2007; Ogura et al., 1994; Perry et al., 1993). Basal pro-inflammation cytokine levels are elevated with aging (Sheng et al., 1998; Ye and Johnson, 1999); yet, data on anti-inflammatory cytokines such as IL-10 are less than clear (Ye and Johnson, 2001; Sierra et al., 2007). In addition to a possible shift in pro-inflammatory proteins with aging, changes in ligands and their receptors important for ensuring normal microglia function are changed. Signaling between CX3CL1 and its receptor CX3CR1 is critical for microglia migration in the adult brain yet in the aged brain expression levels of each are diminished in the aged brain (Bachstetter et al., 2011; Wynne et al., 2010). This shift is evident under basal conditions and upon induction by LPS (Wynne et al., 2010). Not only are these receptors which contribute to an activation of microglia diminished but levels of CD200 are also decreased (Frank et al., 2006) suggesting of loss of the regulatory ability of neurons to maintain microglia in a quiescent state.

While it was initially thought the changes in cell morphology observed in the aged brain represented cells in a more activated state, additional research has found that upon morphological changes in cytoplasmic structure with aging were not characteristic of activation (Sheng et al., 1998; Miller and Streit, 2007) but rather reflective of dystrophy and senescence (Streit et al., 2008). Microglial cells showed cytoplasmic inclusions, de-ramification of processes, and membrane blebbing with aging. The dysmorphic characteristics of aged microglia suggested that, rather than the cells being maintained in an over-activated state, they displayed decreased ability to mount a normal response to injury (Sheng et al., 1998). Age-related changes in cytokine production and dysmorphic microglia morphology (Perry et al., 1993; Sheffield and Berman, 1998) have been proposed to underlie microglia contributions to neurodegenerative diseases. Recent work demonstrated that a systemic challenge with LPS to aged mice elevated the level of cytokine synthesis as compared to young animals (Dilger and Johnson, 2008; Henry et al., 2009). It was considered that such an exaggerated response could represent either a “primed” state with over-production of pro-inflammatory cytokines or a diminished induction of anti-inflammatory factors. The phenomenon “priming” represents a phenotypic shift of microglial cells toward a more sensitized state. This primed microglia will then respond to a secondary “triggering” stimulus more rapidly and to a greater degree than would be expected if non-primed.

Further examination by Streit and colleagues (2008) suggested that microglial senescence is triggered by an intracellular oxidative stress response to enhanced intracellular accumulation of iron. Alternatively, the senescence may be related to a decrease in replicative ability as a result of a lifetime of activation/replication resulting in telomere attrition, DNA damage, and chromatin perturbation (Flanary and Streit, 2004). Microglia exhibit telomere shortening and decreased telomerase activity with aging (Flanary and Streit, 2004) and in Alzheimer s disease brains (Flanary et al., 2007) supporting the hypothesis of microglial replicative senescence in normal and pathological aging. Thus, age-related changes observed in receptor expression such as a downregulation of CX3CR1 (Wynne et al., 2010), diminished motility, and speed of an acute response to injury (Damani et al., 2011) may be the result of cell senescence. While a classic outcome of cell senescence is a diminished proliferative ability, it is likely that a more general blunting of functional activities occurs. This could involve a decrease in other properties of microglia such as production of neurotrophic factors, phagocytosis, and protein clearance. How senescence is induced is still uncertain. It may be the result of intracellular oxidative stress as suggested by Streit (2008). Or related to the increased demand for clearance of such proteins as Aβ (Streit et al., 2004), or existing in a prolonged activated state.

It has been hypothesized that changes in the aging microglia drive pathogenic progression of diseases or injury through a diminution of neuroprotective functions, increase in neurotoxicity, and dysregulation of responses to signals and perturbations (Flanary et al., 2007; Lopes et al., 2008; Luo et al., 2010). Evidence of microglia dysregulation in response to perturbation is accumulating from studies models of infection/inflammatory challenge (Norden and Godbout, 2012; Sierra et al., 2007; Sparkman et al., 2005), stroke (Buchanan et al., 2008; Rosczyk et al., 2008), and trauma (Sandhir et al., 2008). In each case the response tended to be larger and sustained for a longer period of time. However, age-related neurodegeneration might not only be due to a loss of neuroprotective properties, but also to the actual loss of microglia (Streit et al., 2008). Work by Baker et al. (2011) demonstrated that lifetime removal of cyclin-dependent kinase inhibitor 2A ($p16^{Ink4a}$)-positive senescent cells in the adipose tissue, skeletal muscle, and eye resulted in a delayed onset of age-related pathologies. Furthermore, they demonstrated that late-life clearance of senescent cells attenuated the progression of already established age-related disorders. While this work was not conducted in the nervous system, one could envision that a deficit for any organ system to clear dysfunctional cells would result in an adverse and potentially “disease-related” environment.

One of the more prominent features of microglia is their normal phagocytic capability to clear invading pathogens, aberrant proteins, and debris from the CNS. Thus, the loss of efficiency in this function would likely result in a more toxic environment for the neural cells. Cytoplasmic projections from microglia surround Aβ fibrillary aggregates and microglia cultured from aged mice showed a diminished ability to internalize Aβ peptide which would translate to a lack of amyloid elimination by parenchymal microglia. Recent work demonstrated an age related shift in CD47 (Rh-related antigen, integrin-associated signal transducer)-dependent ability of isolated microglia to phagocytize Aβ fibrils. In this case, microglia isolated from brain at birth or at 2 months of age could effectively phagocytize Aβ fibrils; however, this ability was lost when cells were isolated from 6-month-old animals (Floden and Combs, 2011). When cells from each age group, including 12–17 month old animals, were examined for expression of putative Aβ interacting proteins, only CD36 was shown to decrease as a function of age suggesting that adult cells remained able to interact with Aβ fibrils, just not to phagocytize it. This effect was not due to a generalized loss of phagocytic capability as no age related difference was observed in clearance of bacteria, suggesting a specific alteration with regards to Aβ and possibly other aberrant protein. Whether this loss of function reflects actions *in vivo* or occurs as the result

of culturing techniques is not clear, however, when isolated microglia were allowed to adhere to AD or control brain sections the cells obtained at birth showed a greater ability to decrease plaque load as compared to microglia obtained from 6–8 month old brains. While interesting and supportive of a more effective clearance process with the younger microglia, the impact of a loss of cell viability in the older cultures could not allow for a direct comparison of function. In the older cultures, more microglia adhered to Aβ immunoreactive plaques in the AD brain as compared to that seen in with younger microglia. This data suggests concern for other specific loss of functions of microglia that may occur not only with maturation of the brain but also with increased aging. One hypothesis is that interactions with Aβ peptide or other inflammatory stimuli in the diseased brain drives the cells to an early and maintained alternative activation state that shifts the ability of the cells to respond in a normal manner (Floden and Combs, 2011). In the apolipoprotein E (ApoE) transgenic mice as a model of risk factor for AD, an age-related and genotype-related deficit was observed in the ability of the animals to mount a host-resistance response to injury (Harry et al., 2000). Until the age of 8 months, constitutive mRNA levels of pro-inflammatory cytokines were not altered between mice expressing various allelic forms of the human ApoE gene (ApoE2, ApoE3, ApoE4, or ApoE null). With hippocampal injury and activation of resident microglia, no differences were observed at 2 months of age; however, by 8 months of age the ApoE4 allele mice showed a reduced ability to mount a host-response and pro-inflammatory cytokine response. In this model, microglia did not yet display morphological dystrophic phenotypes and microglia activation upon injury was diminished.

7. Conclusions

While characteristic features of microglia structure and function have been identified as they relate to brain development and as they change with aging, there are limited experimental data to fully demonstrate the functions or alterations thereof in microglia cells across the lifespan. However, based upon what is known about the general signaling of the cells and their different functions, it could be assumed that microglia cells change with regard to how they detect changes in the environment, how they respond to those changes, their capability for motility and phagocytosis, the severity and duration of the a response, and the nature of the response. Modifications to resident immune cells of the brain can manifest as alterations in the intrinsic function of the cells or in interactions with ongoing cellular processes. The nature of these alterations can be dependent upon life-stage events (e.g., development, senescence), presence/absence of antigen, vascular cell contribution, leakage of the blood-brain-barrier to serum proteins and other factors. The outcome is also dependent upon the contribution of T-cells and the cellular source of the brain macrophages, i.e., whether the response is driven by resident microglia or infiltrating macrophages. Our knowledge of the function of microglia is expanding; yet, we are still limited with regards to biochemical and molecular changes that occur during development and in the aged brain (for review, Kettenmann et al., 2011). While only critical concepts and limited specific features associated with microglia are presented in the current review, we believe that they set the framework for readers to begin to integrate new information as it becomes available. After having been categorized as brain macrophages, mounting data suggests that not only are microglia heterogeneous with regards to normal morphology and reaction to injury, but that this heterogeneity likely crosses over to the multiple roles that microglia play throughout the lifespan. Gaining a better understanding of their capability and regulatory processes will allow for a more accurate picture of their functions and thus, allow for targeted intervention strategies for approaching brain injury and neurodevelopmental and neurodegenerative disorders.

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Abbreviations

ATP	Adenosine triphosphate
AD	Alzheimer's disease
Aβ	amyloid beta
BBB	blood brain barrier
Cl-	chloride
CD	cluster of differentiation
CR3	complement receptor 3
C1q	complement 1q
CNS	central nervous system
CX3CL1	fractalkine or neurotactin
CX3CR1	fractalkine receptor
GD	gestational day
IFN	interferon
MHC	major histocompatibility complex
MAPK	mitogen-activated protein kinase
PND	postnatal day
IL	Interleukin
LPS	lipopolysaccharide
NO	nitric oxide
PRR	pattern recognition receptors
PI3K	phosphoinositide 3-kinase
K^+	potassium
P2	purinergic receptors
TREM-2	triggering receptor expressed on myeloid cells-2
TLR	toll-like receptors
TGFβ	transforming growth factor beta
TNF	tumor necrosis factor

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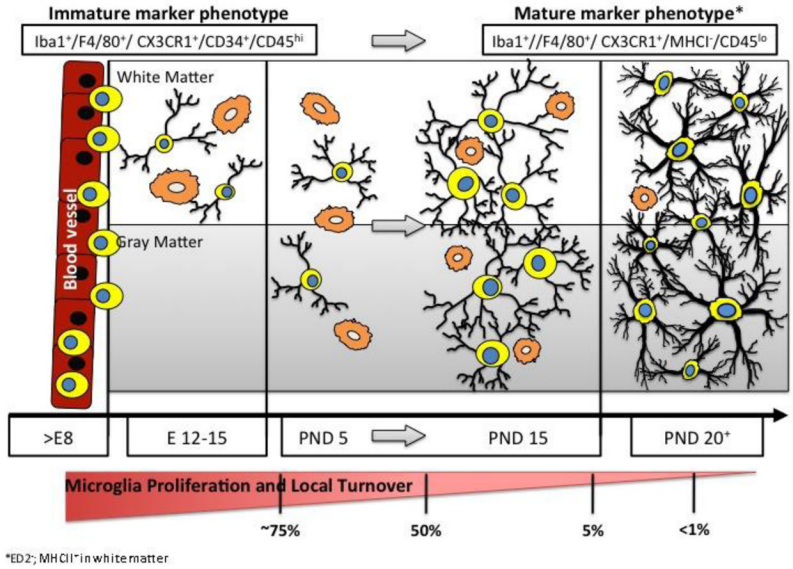

Figure 1.

Developmental Stages Leading to Establishment of a Mature Population of Brain Microglia. During gestation (E9 – mice; E11- rat) microglial precursors cross the blood vessel wall and begin to take up residence in the brain parenchyma. Microglia progenitor cells (small round cells) line up along the blood vessels. These cells typically possess little cytoplasm and have small or nonexistent appendages (indicated as process-free round cells with distinct nuclei). At early stages of colonization (approx. E12–15), these cells are identifiable in white matter regions (or along vascular/ ventricular margins) possessing an amoeboid/ phagocytic morphology (larger ruffled round cells [orange]). In the gray matter, the cells are somewhat smaller than in the white matter and begin to show processes. During early postnatal stages (~PND5), these highly proliferative mononuclear cells are observed in both white matter and gray matter regions of the brain with showing both amoeboid and process-bearing phenotypes. During a critical period of postnatal microglia development (PND2- PND14), the number of microglia increases dramatically and has been associated with expression of Runx1. During this time, there is an increased ratio of ramified versus amoeboid microglia, with the cells having noticeably more complex process arbors and cytoplasmic material. By ~PND15 are well distributed throughout the brain and can be seen in close proximity to apoptotic neurons (swollen cells [light brown]), facilitating surveillance of the majority of the parenchyma; they exhibit a significantly reduced proliferative capacity; they begin to exhibit more heterogeneity (both within and across brain regions) in terms of their morphology, orientation, and process field organization; and they begin to express a somewhat modified constellation of cell surface markers. These maturation features continue such that by PND20 the adult population of microglia is fairly well established. In the absence of stimulation, these cells are highly ramified with complex process arbors encompassing the entire brain parenchyma integrated around healthy neurons and they possess little to no evidence of proliferation or from the systemic population (Lawson et al., 1992).

Table 1

Cytokine Receptors on Microglia

Receptor	Ligand	Expression
TNFR1	TNFα	cell-death receptor
TNFR2	TNFα	trophic receptor/2nd cell-death receptor
IFNγR	IFNγ	M1 phenotype
IFNAR type I IFN	IFNβ	suppression of glutamate and super oxide
IL-1R1/IL-1R2	IL-1α; IL-1β	induction of inflammatory mediators (IL-6)
IL-2Rα/β/γ(γ_c)	IL-2	augment NO production, M2-like phenotype
IL-10R	IL-10	induction of M2-like deactivation phenotype
IL-13R	IL-13	induction of M2-like phenotype
IL-15R	IL-15	reduced NO production, microglia survival
IL-18R (IL-1 related)	IL-18	attenuation of induced IL-12

Table 2

Ion Channels in Microglia

Channel Type/Subunit	*In vivo/In vitro*	Expression
ORAI1/CRAC	rat primary	Activation with ER Ca2+ depletion
TRPM7; TRPC6, TRPM2,	primary	Activation for Ca^{2+} release/IL-6
TRPV1; TRPM4	primary	Activation for Ca^{2+} release
IK1R, Kir 2.1	primary/brain slice	Activated microglia
IK_{DR}; Kv1.1; Kv1.2; Kv1.3; Kv1.5	primary/brain slice	Activated microglia
Ca2+-dependent K	primary/brain slice	Increased in activated microglia
G protein-activated K+	mouse primary	metabotropic receptor activation
TTX-sensitive I_{NA}	primary	20% rat; 95% brain tumors
Nav1.1, Nav1.5, Nav1.6	primary	TTX blocked LPS activation
Nav1.6	EAE mice/MS human	optic nerve/spinal cord
Volume-regulated Cl-	mouse primary/cell line	proliferation, phagocytosis
CLIC-1 chloride	rat primary/cell line	proliferation and ROS
Proton channels	primary/cell lines	respiratory burst, cell volume
Aquaporins	rat	LPS activated microglia
Connexins	rat, human, mouse	activated microglia TBI

Source indicates species and type of cell culture or brain slice preparation

Table 3

Neurotransmitter Receptors in Microglia

Receptor	Source	Expression
A1 A2A; A2B; A3	rat/mouse primary	proliferation, survival, cytokine, trophic effect
$P2X_1$, $P2X_4$, $P2X_7$	rat brain sections	Embryonic P2x1, P2X4; Adult P2X4; P2X7
$P2X_4$	rat spinal cord	Activated microglia/neuropathic pain
$P2X_7$	primary/line/slice	Activated microglia, cytokine release
$P2Y_2$; $P2Y_6$; $P2Y_{12}$; $P2Y_{13}$	Mouse primary	Microglia activation, migration, pain
GluR1, GluR2, GluR3, GluR4	primary/cell line	TNFα, cytoskeleton remodeling, chemotaxis
mGluR5a	rat primary	Ca^{2+} transient
mGluR2, mGluR4	rat primary	TNFα, microglia activation
mGluR6, mGluR8	rat primary	reduced neurotoxicity
$GABAB_{(1a);\ b(1b);\ B(1c)}$	rat/mouse primary/slice	increased facial nerve axotomy microglia
α7 nACh	rat/mouse primary	nicotine triggered Ca^{2+} transients
α1A, α2A, β1, β2	rat primary	cytokine release
Dopamine (D1, D2)	rat/mouse primary/slice	activated outward K^+ currents
AMPA/KA	primary	only on proliferating microglia

Source indicates species and type of cell culture

Table 4

Neurohormone and Neuromodulator Receptors on Microglia

Receptor	Source	Expression
PAF	rat/mouse primary	Ca^{2+} entry, IL-6 expression
Bradykinin (B_1; B_2)	rat primary	B1 LPS induced; B_2 resting
Histamine	rat primary	Ca^{2+} release
Endothelin ET_B	mouse, human/primary	Ca^{2+} release
Cannabinoid (CB_1, CB_2)	rat, mouse, human/primary	Activated microglia, cytokine
Angiotensin II (AT_1, AT_2)	rat embryo/primary	AT_1 LPS induced; AT_2 resting
Somatostatin (sst2, sst3, sst4)	rat primary	inhibited proliferation
Glucocorticoid	rat primary	inhibited proliferation
Mineralcorticoid	rat primary	microglia
Opioid (MOR)	rat primary	upregulates $P2X_4$ expression
Neuokinin (NK-1)	human embryo/mouse	activation of NF-κB
VIP ($VPAC_1$)	rat primary	inhibits LPS-induced activation
Trk-B1	rat primary	BDNF triggered Ca^{2+} release

Source indicates species and type of cell culture

Table 5

Chemokine Receptors on Microglia

Receptor	Agonists	Expression
CCR1, CCR2	CCR2-MCP-1	Ca^{2+} transients
CCR5	MIP-1α, RANTES	Ca^{2+} transients
CXCR1, CXCR3, CCR3		expression increased by TNFα and IFNγ
CCR5		increase with hypoxia/ischemia in rat forebrain
CCR5		MIP-1α-induced K^+ current human brain
CXCR3	CCL21T	Activated vol-regulated Cl^- channels