Chapter 13
Aging

Rommy von Bernhardi, Betsi Flores, and Hiroshi Nakanishi

Abstract Microglial cells undergo multiple morphological and immunophenotypic changes during normal aging. Abnormal morphology, which includes fewer and shorter ramifications, beading and spheroid swellings, has been observed particularly in the cerebral cortex, as well as in and around the white matter. In aged animals, microglia express some surface antigens which are not normally present in their young counterparts, in addition to presenting altered motility and phagocytosis. Aged microglia exhibit an aberrant production of pro- and anti-inflammatory mediators, accompanied by an exacerbated inflammatory response to pathological changes, a phenomenon known as microglial "priming." Lysosomal dysfunction and mitochondrial DNA oxidative damage further accumulate in aged microglia, resulting in an increased production of reactive oxygen species. These changes could contribute to mediating the neuronal dysfunction observed during normal aging and facilitate the onset of age-associated cognitive decline, as well as neurodegenerative diseases. In this chapter, we describe microglial aging at the cellular and molecular levels, the implications for diseases, and potential strategies to slow down aging based on preserving lysosomal and mitochondrial function.

Keywords Microglia • Neuroinflammation • Cytokines • Priming • Reactive oxygen species • Oxidative stress • lysosome • Mitochondria • DNA damage • NFκB • Cathepsin B

Bullet Points

- Microglial cells undergo various morphologic and immunophenotipic changes during normal aging.

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- Aged microglia show an impaired motility towards damage signals, probably reflecting a decreased surveillance capacity.
- Microglial proliferation and phagocytosis of different types of cargo are affected with aging.
- Aged microglia exhibit an altered expression of cytokines and exacerbated inflammatory response, a phenomenon known as microglial "priming."
- Lysosomal dysfunction and mitochondrial DNA oxidative damage also accumulate in microglia during aging, resulting in the increased production of reactive oxygen species (ROS) and activation of the microglial inflammasome.
- These changes are probably both cell autonomous and the result of an altered aging brain milieu.
- The overall contribution of aging microglia to the onset of cognitive dysfunction and neurodegenerative diseases remains to be experimentally tested.

13.1 Introduction

Aging (or senescence) is a complex process of cumulative changes affecting an organism with the passage of time. Aging increases the vulnerability to death and is a primary risk factor for major human pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. It is characterized by a progressive loss of physiological integrity, leading to impaired function in various levels and systems of the organism, such as skeletal muscles (Klein et al. 2001; Thompson 2009), cardiovascular, endocrine, respiratory processes (Fadel et al. 2004; Lipsitz 2002; Smith et al. 2005), and central nervous system (CNS) activity (Smith et al. 2005). These changes are generally accompanied by alterations in behavior, associated with increased tremor, loss of balance control, and decreased walking proficiency (Glenn et al. 2004; Lipsitz 2002; Lipsitz and Goldberger 1992). The ability to perform complex dual learning tasks, such as memorizing word lists while walking, also significantly decreases during aging (Lindenberger et al. 2000; Salat et al. 2005), even though many elderly people preserve to a certain degree their cognitive abilities (Shock et al. 1984).

At the cellular level, senescence is characterized by the accumulation of DNA damage, oxidative stress, chronic inflammatory activity, and an imbalance between the levels of pro- and anti-inflammatory cytokines in various tissues, including the brain. Cellular aging is also associated with the shortening of telomeres and the activation of tumor suppressor genes (reviewed in Lopez-Otin et al. 2013). The potentially damaging elements may be produced by the organism itself (e.g., cytokines, radical species, eicosanoids, among other mediators) or derived from the prolonged exposure to physical, chemical, or biological agents (e.g., ionic radiation, pollutants, pathogens; see Chap. 6 for further reading) (Droge and Schipper 2007; Vijg and Campisi 2008). In addition, some responses of the immune system particularly decline with age, increasing the susceptibility to infections and cancer, whereas other responses are exacerbated, facilitating the onset of autoimmune diseases (Yung and Julius 2008). As the blood-brain barrier (BBB) undergoes several changes during aging (Marques

et al. 2013), it is possible that aging leads to an increased surveillance of the brain parenchyma by peripheral monocytes and lymphocytes, which could further contribute to the aging process. In fact, the expression levels of chemotactic molecules such as interferon-inducible protein 10 (IP-10) and monocyte chemotactic protein-1 (MCP-1), but also the infiltration of $CD11b^{+}$ $CD45^{high}$ cells identified as monocytes, were shown to be increased ex vivo in hippocampal tissue prepared from aged rats (Blau et al. 2012; Enciu et al. 2013).

The CNS also undergo pronounced structural and functional alteration during normal aging, even in clinically healthy middle-age individuals, i.e., 40–50 years old. In particular, brain weight decreases in the order of 2–3 % per decade after the age of 50 and accelerates in later years to reach 10 % at the age of 80 (Drachman 2006). Using magnetic resonance imaging (MRI) and voxel-based morphometry (VBM), it has been shown that the gray matter volume of prefrontal, parietal, and temporal cortices of the human brain progressively decreases during aging (Courchesne et al. 2000; Ge et al. 2002; Good et al. 2001; Jernigan et al. 2001; Salat et al. 2004; Sowell et al. 2003). The temporal cortex is more affected in the left hemisphere than in the right hemisphere, in agreement with an age-related decline in language functions (Sowell et al. 2003). White matter volume also decreases with age; the process begins later but progresses at a more accelerated rate than in the gray matter (Courchesne et al. 2000; Ge et al. 2002; Jernigan et al. 2001).

Some of these changes are undoubtedly related to cell autonomous alterations in the aging neurons (Jurk et al. 2012) and astrocytes (Sheng et al. 2013), but aging microglia may further contribute to the aging pathology, notably by their production of reactive oxygen species (ROS) and pro-inflammatory cytokines, which could together increase neuronal vulnerability to oxidative stress. An accepted view is that neuroinflammation and oxidative stress collectively induce neuronal dysfunction and degeneration, thus resulting in the decline of motor and cognitive functions during aging (Forster et al. 1996; Navarro et al. 2002). In addition, compromised microglial properties have been proposed to cause impaired reaction to neuronal abnormalities during aging (Streit 2006; Aguzzi et al. 2013; Kettenmann et al. 2013; Conde and Streit 2006b; Siskova and Tremblay 2013). In this chapter, we will discuss the cellular and molecular changes observed in senescent microglia, and their implication for our understanding of the normal aging process, related cognitive dysfunction, and brain diseases.

13.2 Changes in Microglial Morphology, Dynamics, Phagocytosis, and Proliferation During Aging

Several authors have shown that microglial cells display various changes in morphology and functional behavior over the course of normal aging. Morphological characteristics associated with normal aging include fewer and shorter ramifications, excessive beading, and formation of spheroid swellings (Conde and Streit 2006b; Flanary 2005; Streit 2006; Streit et al. 2004). These changes are commonly referred to as microglial cell "dystrophy" (Streit 2006; Streit et al. 2004). Moreover,

it was shown that microglia often colocalize with neurodegenerating neurons in the aging brain and display additional deterioration such as higher incidence of clumping, irregular distribution, and accumulation of phagocytic debris (lysosomal lipopigments, cellular elements, vacuoles, and large vesicles), particularly observed in cortical areas, as well as in and near the white matter (Hart et al. 2012; Perry et al. 1993; Tremblay et al. 2012; Hefendehl et al. 2013). Microglial accumulation of phagocytic debris might contribute to reducing their dynamism and impairing their phagocytic capacity as discussed below. However, no systematic quantification of microglial interactions with neurons has been performed to determine the extent of their changes during aging. Furthermore, no study has yet clearly determined the functional consequences of their abnormal morphology.

In terms of functional behavior, live imaging of microglial cells in retinal explants has demonstrated ex vivo that the dynamic responses of senescent microglia to injury also show age-dependent variations (Damani et al. 2011). In particular, young microglia were shown to rapidly increase their motility and number of ramifications when exposed to the nucleotide ATP, an injury-associated signal, or to a laser-induced focal tissue injury. In contrast, aged microglia were less dynamic and ramified, as compared to younger counterparts, and became even less dynamic and ramified in the presence of ATP, resulting in slower responses to a laser-induced injury. Moreover, their migration away from the site of injury was retarded in senescent versus young microglia (Damani et al. 2011). A recent characterization of the changes in microglial morphology and dynamic behavior in vivo using two photon imaging also showed similar age-related processes, such as a shortening of processes, increased soma volume, and loss of homogeneous tissue distribution and surveillance rate, in the cerebral cortex of aged mice. In addition, aged microglia examined in vivo also presented a diminished dynamic response to a laser-induced tissue injury, as in the retina, but their migration was however found to be accelerated (Hefendehl et al. 2013). Together, these findings suggest that the microglial capacity to detect and respond to pathological signs might be compromised in the aging brain, probably resulting both from altered microglial properties, especially their motility, and from the altered brain microenvironment.

This altered motility of microglia also seems to be closely related to additional alterations in their phagocytic capacity. As the brain professional phagocytes, microglia have the capacity to engulf apoptotic cells, myelin and axonal debris, deposits of extracellular proteins including beta amyloid (Aβ), and neurites (reviewed in Sierra et al. 2013). During aging, microglial cells over-express ED1, the rodent equivalent of CD68, a lysosomal protein upregulated during inflammation, which has been associated with phagocytosis (Perry et al. 1993). However, the function of ED1 is unknown since reducing ED1 expression with anti-ED1 monoclonal antibodies in cultured macrophages did not impair phagocytosis (reviewed in Sierra et al. 2013). Acutely isolated microglial cells from aged mice also show a decreased ability to phagocytose Aβ, contrarily to microglia derived from young mice (Floden and Combs 2011). In vitro, the phagocytosis of Aβ was also comparable between microglia derived from young and old mice, but only enhanced by a bacterial lipopolysaccharide (LPS) challenge in microglia from young mice

(Tichauer et al. 2014). In addition, once internalized, the proteolytic degradation of Aβ was shown to be impaired in aged mice, due to deficits in lysosome acidification, as required for proper functioning of the lysosome degradation enzymes (Majumdar et al. 2007). Nevertheless, microglial phagocytosis of newborn apoptotic cells in the adult hippocampus neurogenic niche remained functional at least until 12 months of age (Sierra et al. 2010) (see Chap. 10 for further reading), although no systematic observations were carried out in older ages. Thus, the extent to which microglial phagocytosis is impaired during aging remains to be fully determined. A related process, protein homeostasis or proteostasis, which involves chaperone-mediated protein folding and stability, protein trafficking, protein degradation, and autophagy pathways, is also impaired in aged microglia in vitro, possibly explaining the microglial accumulation of phagocytic debris observed at the ultrastructural level (Tremblay et al. 2012). A major consequence of this declining proteostasis is the aggregation of abnormal proteins, which has been linked to the pathogenesis of neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) (Taylor and Dillin 2011).

The proliferative capacity of microglia could also become altered during aging. Microglial cells exhibit an increased density in the facial nucleus upon nerve injury in aged versus young rats, and a similar finding was observed in mouse cerebral cortex during age-related impairment of audition and vision, possibly suggesting a less regulated proliferative response (Conde and Streit 2006a; Tremblay et al. 2012). However, microglial replication is generally considered as being reduced during aging, and there is no significant evidence for an overall increase in microglial density in the aging postmortem human brain (VanGuilder et al. 2011). It has been speculated that a reduced microglial replication could induce the depletion of healthy microglia in the aged brain, shifting the balance towards a more senescent and dysfunctional population (Mosher and Wyss-Coray 2014). Clumped microglia have been observed in the aged cerebral cortex, but their possible colocalization with markers of proliferation was not examined, and alternative interpretations were also proposed, such as a breakdown of the mechanisms which regulate their territorial organization (Tremblay et al. 2012). Additionally, interpreting the increased microglial density observed in some studies is limited by the impossibility to distinguish microglia from monocytes/macrophages derived from the periphery using CX3CR1, ionized calcium-binding adapter molecule 1, and other commonly used immunocytochemical markers. Thus, it remains unclear whether there are regional or species-specific changes in microglial density in the aging brain, and if so, whether they result from the proliferation of resident microglial cells, or the infiltration of peripheral inflammatory cells.

Together, these observations suggest the appearance of dysfunctional microglial phenotypes in the aging brain, which combined with the immunophenotypic changes described below, might contribute to the age-associated neuronal dysfunction and cognitive decline.

13.3 "Priming" of Microglia During Aging

During normal aging, a decreased secretion of the anti-inflammatory cytokine interleukin (IL)-10 has been observed (Ye and Johnson 2001), found to be accompanied by increased levels of pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) and IL-1β in the CNS (Lukiw 2004; Streit et al. 2004), and IL-6 in plasma (Godbout and Johnson 2004; Ye and Johnson 1999, 2001), using gene expression profiling, flow cytometry, and ELISA in mice and human. Aged microglia studied in situ (Sheng et al. 1998) and isolated ex vivo (Njie et al. 2012; Sierra et al. 2007) also showed increased expression of several pro-inflammatory mediators, but reports regarding the levels of anti-inflammatory cytokines, such as IL-10, are less uniform (Sierra et al. 2007; Ye and Johnson 2001). Furthermore, microglia display a significant upregulation of several Toll-like receptors (TLRs), such as TLR1, TLR2, TLR4, TLR5, and TLR7, as well as an increased expression of the TLR4 co-receptor, CD14, during aging (Letiembre et al. 2007). An age-related alteration in the signal transduction of TLR4 and conspicuous changes in the expression profile of scavenger receptors (SRs) have also been reported (Hickman et al. 2008; Yamamoto et al. 2002). TLRs, CD14, and SRs are pattern recognition receptors (PRRs) that participate in host defense response and phagocytosis of pathogen-associated molecules pattern (PAMPs), as well as damage-associated molecules pattern (DAMPs), which are crucial for the innate immune response. Signaling through these receptors is accompanied by microglial cell activation, including their production of pro-inflammatory mediators, and uptake of pathogens and macromolecules, such as the neurotoxic peptide Aβ. Therefore, changes in the expression profile of these receptors might account for the alterations observed in microglial inflammatory profile during normal aging.

Additionally, increased mRNA levels of transforming growth factor β isoform 1 (TGFβ1) have been measured in microglia from aged mice and rats (Bye et al. 2001; Sierra et al. 2007). TGFβ1 is a potent regulator of cytotoxicity and inflammatory response in the CNS. Its downstream signaling involves members of the Smad family, i.e., intracellular proteins that transduce extracellular signals from TGFβ ligands to the nucleus, thus acting as transcription factors. These proteins are homologs of the *Drosophila* mothers against decapentaplegic (MAD) protein and *Caenorhabditis elegans* SMA protein, named after the gene *Sma* for small body size, acting as mitogen-activated protein kinases (MAPKs), although their activation is highly variable and dependent on the cell type (Schmierer and Hill 2007). TGFβ1 modulates the activation of microglial cells induced by a LPS challenge, by decreasing their production of pro-inflammatory molecules, with the consequence of protecting cultured neurons from neurotoxicity and oxidative stress (Herrera-Molina and von Bernhardi 2005; Hu et al. 1995; Lieb et al. 2003). It has also been demonstrated in culture that this influence of TGFβ1 on microglial activation is regulated in a Smad3-dependent manner (Le et al. 2004; Werner et al. 2000). The TGFβ1 and Smad3 pathways were further linked to the reduction of radical species production induced by inflammatory stimuli and to the induction of Aβ phagocytosis in vitro

(Tichauer and von Bernhardi 2012). Additionally, it has recently been shown that the induction of the Smad3 pathway is decreased in normal aging under inflammatory conditions (Tichauer et al. 2014), which could explain, at least partially, that microglial activation is overall increased in the aging brain, even though microglial expression of TGFβ1 is concomitantly increased ex vivo (Sierra et al. 2007).

The over-production of pro-inflammatory cytokines is associated with a repertoire of symptoms commonly known as sickness behavior, an adaptive response that occurs following exposure to infectious microorganisms, and that is exacerbated during aging (Hart 1988). Upon systemic inflammatory stimulation, aged microglia display an exacerbated inflammatory phenotype, compared with young ones, possibly resulting in enhanced sickness behavior (Combrinck et al. 2002; Cunningham et al. 2005; Godbout et al. 2005; Sierra et al. 2007). In particular, systemic inflammation resulted in an exacerbated production of the pro-inflammatory cytokines IL1-β, IL-6, TNFα ex vivo in aged versus young microglia (Sierra et al. 2007). This exacerbated response to inflammatory challenges is also referred to as microglial "priming," a concept first introduced by Perry and colleagues (Perry 2004; Perry et al. 1993). By definition, primed microglia undergo a phenotypic shift towards a more sensitized state, in which they respond more rapidly and to a greater extent to a secondary "triggering" stimulus than non-primed microglia (Perry 2004; Perry et al. 2003, 2007; Harry 2013). An important question is to what extent aging microglial cells do become intrinsically dysfunctional, versus simply react to the changes in their local aging brain environment. Importantly, aging microglial properties were replicated in a mouse model where only neurons are made senescent. In these mice considered as a model of accelerated senescence, deleting the expression of a nucleotide repair protein (Ercc1) exclusively in forebrain neurons results in decreased neuronal plasticity, progressive neuronal pathology, and learning impairment (Borgesius et al. 2011). Importantly, in spite of not carrying the mutation, microglia also displayed hallmark features of "priming" such as an exaggerated response to peripheral LPS exposure in terms of pro-inflammatory cytokines expression, ROS production, and phagocytosis (Raj et al. 2014). Therefore, the exacerbated response of "primed" microglia to inflammatory stimuli could result, at least partially, from an accumulation of neuronal genotoxic stress, in addition to changes in the expression of TLRs (Letiembre et al. 2007) and other alterations including shortening of telomeres (Flanary and Streit 2004; Flanary et al. 2007). In turn, the exaggerated inflammatory response of microglia could further enhance the neuronal dysfunction and sickness behavior associated with normal aging.

13.4 Aged Microglia and Their Relationship with Neurodegeneration

While it remains unclear whether microglial "priming" could directly result from microglial senescence, aging microglial cells have been linked to several age-related neurodegenerative diseases, including PD, amyotrophic lateral sclerosis (ALS),

and AD (von Bernhardi 2007) (for further reading, refer to Chap. 18). Microglial "priming" could not only result in an increased inflammatory response and cytotoxicity, but also in the impairment of microglial neuroprotective functions (see Chap. 5 for further reading). Indeed, aged microglia appear to actively participate to the neuronal damage observed in neurodegenerative diseases, especially through their production of ROS (Block et al. 2007). Thus, inflammation, possibly related to the activity of microglia, has been suggested to contribute to the death of dopaminergic neurons in PD, forebrain neurons in AD, and motor neurons in ALS (Boillee et al. 2006; Mount et al. 2007). In particular, it has been shown that TNFα promotes PD progression (McCoy et al. 2006), whereas the absence of TNF receptor 1 protects against AD- and PD-like disease in mice (He et al. 2007; Sriram et al. 2002). Moreover, administration of the anti-inflammatory derivative of thalidomide, lenalidomide, which was accompanied by a reduced expression of TNFα and IL-1β, was shown to improve motor behavior even after the onset of symptoms and to extend the survival in mouse models of ALS (Neymotin et al. 2009). Nonetheless, microglia are not likely the sole producers of TNFα and IL-1β in these diseases, and more specific experimental approaches are necessary to dissect out the effects of other inflammatory cells, including infiltrating macrophages, at different stages of their time course.

Altered responses of the aging microglia have also been linked to AD. In rhesus monkeys, a microinjection of fibrillar Aβ in the cortex was found to trigger neuronal loss, tau phosphorylation, and microglial cell proliferation in aged but not young adult monkeys. This in vivo observation suggests that Aβ neurotoxicity is a pathological response specific to the aging brain (Geula et al. 1998). Moreover, aged microglia are less capable of phagocytosing (Floden and Combs 2011) and degrading Aβ (Majumdar et al. 2007) than young microglia, as discussed above. Microglial cell reactivity to Aβ and phagocytic activity is further modulated by astrocytes, at least in vitro, whose presence attenuates the cytotoxic response of cultured microglia (DeWitt et al. 1998; von Bernhardi and Ramirez 2001). However, this modulation was not observed in "primed" microglia exposed to Aβ (von Bernhardi and Eugenin 2004), which showed increased cytotoxicity, Aβ precursor protein (APP) synthesis, Aβ aggregation, and impaired uptake and degradation of Aβ as compared with non-activated microglia (Rogers et al. 2002; Ramirez et al. 2008; von Bernhardi et al. 2007). TGFβ1 secreted by hippocampal neurons and astrocytes has been identified as an important modulatory cytokine of microglial activation, attenuating the release of pro-inflammatory mediators (Chen et al. 2002; Herrera-Molina and von Bernhardi 2005; Mittaud et al. 2002; Herrera-Molina et al. 2012) and promoting microglia-mediated Aβ phagocytosis and degradation (Wyss-Coray et al. 2001). It has been recently shown that these effects are mediated by Smad3-dependent mechanisms as described above (Flores and von Bernhardi 2012; Tichauer and von Bernhardi 2012). Interestingly, this signaling pathway is impaired in the brains of AD patients and mouse models, resulting in Aβ accumulation, Aβ-induced neurodegeneration, and neurofibrillary tangle formation (Tesseur et al. 2006; Ueberham et al. 2006), even though TGFβ1 levels are elevated in the cerebrospinal fluid of these patients (Blobe et al. 2000). Therefore, the Smad3 pathway could be considered as a target for therapeutic approaches against AD.

13.5 Increased Mitochondrial DNA Damage and Resultant Over-production of Reactive Oxygen Species and Inflammatory Cytokines by Microglia During Aging

In the aging brain, microglia constitute a primary cellular source of inflammatory molecules and oxidative products (Hayashi et al. 2008; Pawate et al. 2004; Qin et al. 2005). In the hippocampus of aged mice, immunoreactivity for 8-oxo-deoxyguanosine (8-oxo-dG), a major DNA peroxidation product, has been mainly observed in microglial cells, and partially in neurons, but not in astrocytes (Hayashi et al. 2008). Furthermore, this immunoreactivity for 8-oxo-dG mainly colocalized with the cytochrome *b*, a marker of mitochondria, thus suggesting that microglia could accumulate oxidative damage to their mitochondrial DNA (mtDNA) over the course of aging. Mitochondrial DNA (mtDNA) serves to encode components of the mitochondria electron transfer complexes and is highly susceptible to oxidative damage due to its close proximity to ROS generated through the respiratory chain, and its paucity of protective histones and DNA-binding proteins. Accumulation of mtDNA damages during aging results in a reduced expression of the mitochondria electron transfer complexes, especially the complexes I and IV, which contain a relatively large number of mtDNA-encoded subunits. Reduced activity of the complex I further increases the generation of ROS (Corral-Debrinski et al. 1992; Lin et al. 2002), thus forming a vicious cycle in the mitochondria (Kang et al. 2007) (Fig. 13.1).

In parallel, several changes induced by the aging environment, such as an increase in systemic inflammation and BBB permeability, as well as dysfunction, oxidative stress and degeneration of the other resident cells including neurons and astrocytes (Fig. 13.2), could further contribute to the production and release of ROS. In aged animals, several studies have proposed that BBB permeability could increase (Blau et al. 2012; Enciu et al. 2013), and therefore, a possible production of ROS by peripheral immune cells in the aged brain must be considered. Lastly, neuronal cells could be implicated as well, since DNA damage to cortical, hippocampal, and peripheral neurons (from the myenteric plexus) has been found to induce their production of ROS and release of the pro-inflammatory cytokine IL-6 (Jurk et al. 2012). A similar role of human astrocytes was also recently suggested from in vitro observations (Sheng et al. 2013).

The over-production of ROS, through a vicious cycle in the aging mitochondria, might also activate redox-sensitive transcription factor NFκB, implicated in the regulation of immunity, inflammation, and cell death (Adler et al. 2007, 2008), thereby provoking excessive inflammation in the aged brain (Hayashi et al. 2008; Nakanishi and Wu 2009) (Fig. 13.1). Increased NFκB signaling during aging further potentiates the expression of NLRP3, a member of the NLR family of cytosolic pattern recognition receptors that control the activity of caspase-1 by forming multiprotein complexes which are termed inflammasomes. After being activated, the pyrin domain containing-3 protein (NLRP3) recruits the adaptor protein ASC, which in turn binds to pro-caspase-1, leading to its autocatalytic processing and activation. Active caspase-1 cleaves the inactive precursors of two inflammatory

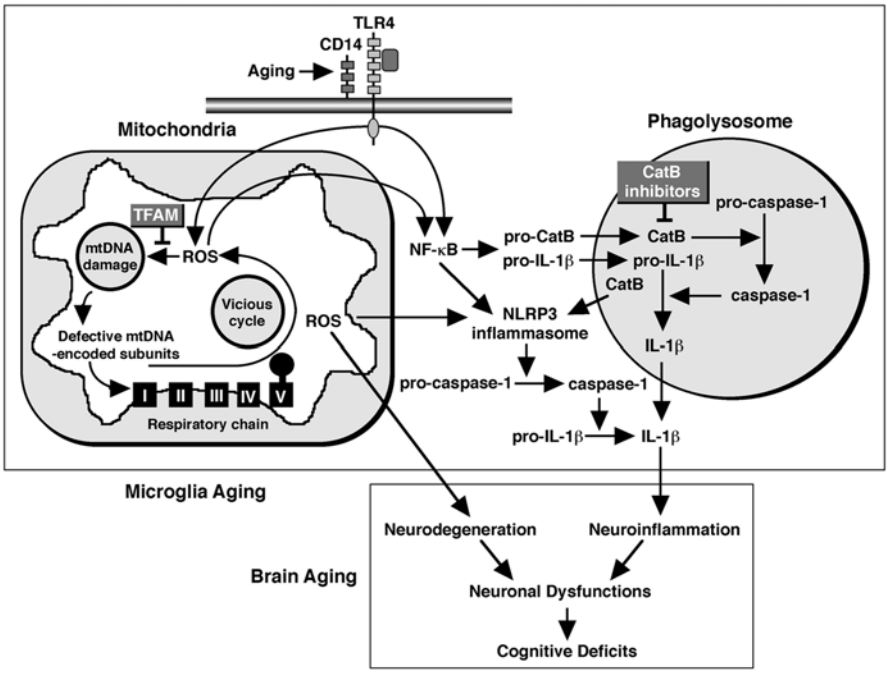

Fig. 13.1 Schematic representation of the "Microglia-Aging" hypothesis. Increased intracellular ROS activate the redox-sensitive NFκB to provoke excessive inflammation in the aged brain. During aging, the NFκB pathway is activated through two different pathways, the mitochondria-derived ROS-dependent pathway and the direct ROS-independent pathway. The activation of the NFκB pathway induces the expression of pro-CatB, pro-IL-1β and NLRP3. CatB and the NLRP3 inflammasome are involved in the activation of pro-caspase-1, an essential enzyme for the proteolytic processing of pro-IL-1β. Therefore, TFAM and CatB inhibitors inhibit the IL-1β-producing pathways in microglia, thereby improving the age-dependent deficits in memory

cytokines, IL-1β and IL-18, into their mature forms (Tschopp and Schroder 2010). NLPR3 has been proposed to be activated by several danger signals, including PAMPs and DAMPs, via three different models: the "ROS model," the "lysosomal rupture model," and the "channel model" (Tschopp and Schroder 2010). In the "channel model," NLRP3 is activated by extracellular ATP released from damaged cells, which binds to the nucleotide receptor P2X7, and triggers a rapid efflux of K^+ and the formation of a pore in the cell membrane, leading to the entry of extracellular factors acting as NLRP3 ligands (including PAMPs and DAMPs). Herein, we will focus on the other two models, which have been associated with the altered function of microglia during aging.

According to the "ROS model" proposed by Tschopp's group, particulate activators of the NLRP3 inflammasome, including asbestos fibers and silica crystals, trigger the generation of short-lived ROS, whereas treatment with various ROS scavengers blocks NLRP3 activation in response to these particulate activators. Monosodium urate crystal and asbestos fiber, which are major causative factors of gout and asbestosis,

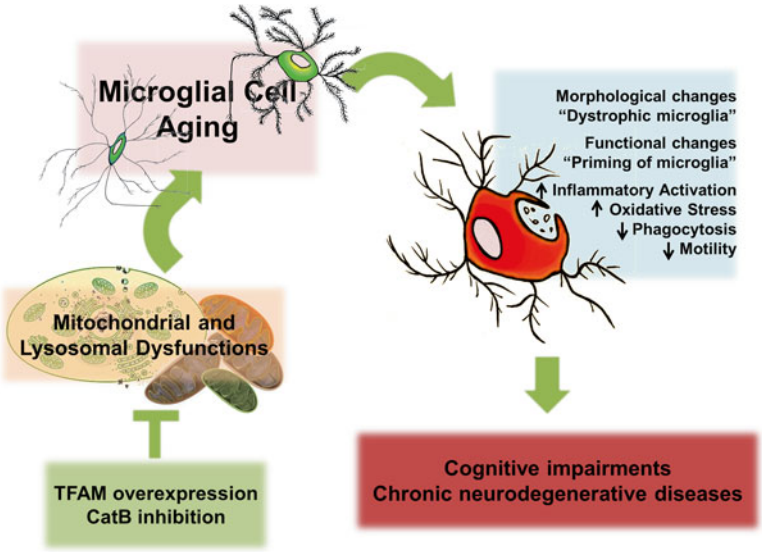

Fig. 13.2 Schematic representation of microglial cell changes during aging. Aged microglia show alterations in their morphology and function, which can lead to neuronal damage and cognitive impairments, facilitating the onset of chronic neurodegenerative diseases. Mitochondrial and lysosomal dysfunctions observed during aging, which are modulated by the over-expression of TFAM and the inhibition of CatB, could represent the cellular basis of these changes. Therefore, TFAM and CatB could be considered as potential targets for the development of pharmacological interventions against aging-associated impairments

respectively, activate the NLRP3 inflammasomes in a ROS-dependent manner (Dostert et al. 2008). Recent studies have demonstrated that autophagic uptake capacity can regulate mitochondrial integrity, ROS production, and subsequent NLRP3 activation (Nakahira et al. 2011; Salminen et al. 2012; Zhou et al. 2011). Furthermore, NLRP3 activation is negatively regulated by autophagy, which plays an important role in clearing the damaged ROS-hypergenerating mitochondria. During aging, the efficiency of autophagic uptake declines and waste materials accumulate within cells (Salminen et al. 2012). Furthermore, the inhibition of autophagy triggers the accumulation of damaged ROS-hypergenerating mitochondria, which augments the activation of the NLRP3 inflammasomes in human macrophages (Zhou et al. 2011). As discussed above, a dysfunction of autophagy has been reported in microglia during aging (reviewed in Wong 2013). Therefore, in addition to the activation of redox-sensitive NFκB, it is also hypothesized that the dominance of ROS-hypergenerating mitochondria, due to the dysfunction of autophagy, could contribute to activating the NLRP3 inflammasome in microglia during aging, leading to excessive production of IL-1β and IL-18 in the aged brain (Fig. 13.1).

On the other hand, according to the "lysosomal rupture model" proposed by Latz's group, the uptake of fibrillar $A\beta_{42}$ or silica crystals by LPS-primed microglia/macrophages causes phagosomal destabilization and lysosomal rupture. The subsequent secretion of cathepsin B (CatB), a typical lysosomal cysteine protease, into the

cytoplasm triggers the activation of the NLRP3 inflammasome directly or indirectly, leading to the production and secretion of IL-1β and IL-18 (Halle et al. 2008; Hornung et al. 2008). More recently, CatB was found to directly interact with the leucine-rich-repeat (LRR) domain of NLRP3 (Bruchard et al. 2013). This model is supported by the observation that a specific inhibitor of CatB, CA074Me, significantly inhibits IL-1β secretion from LPS-primed microglia and macrophages following the phagocytosis of fibrillar Aβ and silica crystals, respectively, (Halle et al. 2008; Hornung et al. 2008). Following the phagocytosis of fibrillar Aβ, the secretion of IL-1β from CatB-deficient macrophages is significantly reduced compared with wild-type macrophages (Hornung et al. 2008). Furthermore, NLRP3-deficient mice carrying mutations associated with familial AD demonstrate improvement in spatial memory, a reduced expression of caspase-1 and IL-1β in the brain, and enhanced Aβ clearance (Heneka et al. 2013). Besides fibrillar Aβ and silica crystals, cholesterol crystals and islet amyloid peptide, which are major causative factors of age-related diseases such as atherosclerosis and type 2 diabetes, respectively, also activate the NLRP3 inflammasome in a CatB-dependent manner (Duewell et al. 2010; Masters et al. 2010). More direct evidence on the importance of the "lysosomal rupture model" will require identification of the putative CatB substrates that activate the NLRP3 inflammasome. Phagocytosed particles that are too large to be efficiently cleared are likely to induce the production of ROS on their way to lysosomes. Therefore, the "lysosomal rupture model" could be viewed as part of a more general "ROS model." It is likely that the activation of the NLRP3 inflammasome is more complex, requiring a combination of factors, including enzymatic activity of CatB and ROS activity.

13.6 Preventing or Reversing Microglia Aging by Inhibition of Cathepsin B

In addition to mediating the maturation of pro-caspase-1 through activation of the NLRP3 inflammasome, CatB also directly contributes to the proteolytic maturation of pro-caspase-1. CatB can efficiently cleave pro-caspase-11 in a cell-free system at a neutral pH, but only cleaves pro-caspase-1 at an acidic pH (Vancompernolle et al. 1998). Further cleavage is necessary for the full maturation of pro-caspase-1 after its proteolytic cleavage by CatB, because the fragments generated by CatB cleavage are still larger than the mature caspase-1 (Hentze et al. 2003). This suggests that CatB is involved in the activation of pro-caspase-1 through its direct activation of pro-caspase-11, which in turn activates pro-caspase-1 (Kang et al. 2000). CatB deficiency and selective pharmacological inhibition with CA074Me prevent the activation of precursor forms of IL-1β and IL-18 in microglial cell cultures following treatment with chromogranin A (CGA), a potent activator of microglia, through inhibition of proteolytic maturation of pro-caspase-1 (Terada et al. 2010). CGA does not induce leakage of CatB in microglia (Sun et al. 2012; Wu et al. 2013), but it is known to activate microglia through scavenger receptors class-A (SRA) (Hooper et al. 2009).

CatB-containing enlarged lysosomes are considered to be phagolysosomes formed by the fusion of SRA-mediated phagosomes and primary lysosomes (Sun et al. 2012; Wu et al. 2013). Therefore, pro-caspase-1 and the inactive forms of IL-1β and IL-18 in the cytoplasm may be trapped in phagosomes, which are fused with CatB-containing primary lysosomes to form phagolysosomes and thus degraded rather than being released extracellularly. It is also noted that CatB is increased in the brain during aging (Nakanishi 2003). Therefore, a pharmacological inhibition of CatB could be a potent strategy for slowing brain aging through inhibition of the pro-caspase-1 activation in microglia, and resultant reduction of neuroinflammation (Fig. 13.1).

13.7 Preventing or Reversing Microglia Aging by Elevation of Mitochondrial Transcription Factor-A

Mitochondrial transcription factor-A (TFAM) is a nucleus-encoded protein that binds upstream of the light-strand and heat-strand promoters of mtDNA and induces the transcription of mtDNA (Parisi and Clayton 1991). Therefore, the level of TFAM is a major determinant of the amount of mtDNA (Kanki et al. 2004; Seidel-Rogol and Shadel 2002). In addition to maintaining mtDNA by acting as a transcription factor, TFAM stabilizes mtDNA by forming a nucleoid structure (Kanki et al. 2004). The amounts of both TFAM and mtDNA are significantly increased during aging in the brain and peripheral organs including the liver of rodents (Dinardo et al. 2003; Masuyama et al. 2005). There is growing evidence that mtDNA deficiency and mitochondrial dysfunction play a major role in the development and progression of cardiac failure (Ikeuchi et al. 2005), but whether these changes occur in aging microglia is not known. The over-expression of human TFAM has been shown to prevent the decrease in mtDNA copy number and mitochondrial electron transfer function in a partial myocardial infarction model of a mouse (Ikeuchi et al. 2005). The increased mtDNA copy number observed in hTFAM-transgenic mice could be due to nucleoid formation or the stabilization of mtDNA by hTFAM, because hTFAM is not expected to function as a transcription factor in murine cells (Kang et al. 2007). Therefore, oxidative stress may cause deficiencies of mtDNA, leading to cardiac failure through mitochondrial dysfunction and resultant over-production of ROS.

The increased expression level of hTFAM in HeLa cells effectively reduces ROS generation induced by rotenone, an inhibitor of mitochondrial complex I, and the subsequent nuclear translocation of NFκB, probably through stabilization of mtDNA, which could reduce mitochondrial dysfunction and resultant ROS generation (Corral-Debrinski et al. 1992). Furthermore, hTFAM-transgenic mice exhibit a significant improvement of age-dependent motor and memory impairments, associated with a marked reduction of mtDNA damage and IL-1β production in microglia (Hayashi et al. 2008; Nakanishi and Wu 2009). In addition to the motor and memory functions, sickness behaviors induced by LPS are also affected by aging

(Huang et al. 2008), as discussed above. Increasing evidence suggest that LPS-induced NFκB activation through TLR4-CD14 complex is dependent on the production of ROS (Baeuerle and Henkel 1994; Janssen-Heininger et al. 2000), and a broad range of antioxidants abolish NFκB activation (Blackwell et al. 1996; Zhang et al. 1994). These observations prompted further investigation of the effect of human TFAM over-expression on the age-dependent prolongation of LPS-induced sickness behaviors. In particular, human TFAM-transgenic mice were found to exhibit a significant improvement of age-dependent prolonged sickness behaviors following treatment with LPS, which is closely correlated with attenuation of mtDNA damages and IL-1β expression in microglia (Nakanishi et al. 2011) (Fig. 13.1). Therefore, over-expression of hTFAM could improve the age-dependent memory impairment and prolonged LPS-induced sickness behaviors by ameliorating the mtDNA damage and the resulting redox-regulated inflammatory response.

In the aging brain, there is an impairment of electron transfer in some mitochondrial complex, shifting the intracellular redox balance towards a more oxidized state (Navarro et al. 2002). Aged dysfunctional mitochondria may not respond to sudden increases in ATP demands, which has been speculated to lead to impaired performance in behavioral tests (Navarro et al. 2002). Whether this impairment also occurs in aged microglia is not known, but it is likely that alterations in mitochondrial function result in a decreased microglial motility and phagocytosis, as they are energy-requiring events (Fig. 13.2). However, this hypothesis needs to be experimentally determined. Microglia with highly branched fine processes are now being considered as active players in the normal healthy brain (see Chaps. 3, 4 and 9 for further information). Therefore, the accumulation of damaged ROS-hypergenerating mitochondria in microglia might limit microglial cell defensive behaviors, including motility and phagocytosis, during aging.

13.8 Alternative Strategies for Preventing or Reversing Microglial Cell Aging

There is accumulating evidence that exercise and caloric restriction can play a role in reducing microglial activation and "priming" during aging. In aged animals, small amounts of exercise were found to prevent the infection-induced exaggerated neuroinflammatory response, which is associated with increased cytokine production and increased cognitive deficits (Barrientos et al. 2011). Moreover, voluntary exercise was found to abrogate the age-related "priming" of microglia (Barrientos et al. 2011; Kohman et al. 2013), suggesting that exercise might be an effective intervention to prevent or reverse microglial cell aging. These beneficial effects of exercise may, in part, result from its induction of brain-derived neurotrophic factor (BDNF), which is a potent regulator of synaptic development and plasticity (Barrientos et al. 2011). On the other hand, caloric restriction could also attenuate the age-related activation of microglia, resulting in beneficial effects on neurodegeneration and cognitive decline (Morgan et al. 2007). Caloric restriction has

anti-inflammatory and anti-apoptotic properties (Loncarevic-Vasiljkovic et al. 2012). Interestingly, both exercise and caloric restriction were recently shown to promote mitochondrial biogenesis and expression of TFAM in the rat brain (Picca et al. 2012; Zhang et al. 2012). Collectively, both exercise and caloric restriction may effectively slow down the brain aging through preventing or reversing microglial aging. However, their exact underlying mechanisms remain unknown.

13.9 Conclusion

During normal aging, microglia undergo several morphological and functional changes, affecting their neuronal environment and facilitating the appearance of cognitive impairments. Among these changes, increased production of ROS and pro-inflammatory cytokines by microglia and other resident and infiltrating cells, including monocytes, could facilitate the onset of neurodegenerative diseases. Decline of both lysosomal function and mitochondrial DNA damage in these cells results in an exacerbated generation of ROS and pro-inflammatory mediators, which could represent the cellular basis of microglia aging. Therefore, molecules implicated in lysosomal and mitochondrial dysfunction, such as CatB and TFAM, may be considered as potential therapeutic targets. Further research would be necessary, however, to develop effective pharmacological interventions against brain aging. Within this perspective, pharmacological approaches aimed to rejuvenate old microglia in the elderly brain may constitute a promising future research avenue for slowing senescence. Furthermore, non-pharmacological strategies, like exercise and dietary restriction, could promote a healthy aging through their effects on promoting microglial surveillance and physiological functions, while reducing inflammation and ROS production.

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