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Age-related changes in microglial physiology: the role for healthy brain ageing and neurodegenerative disorders

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Abstract: Microglia are the main immune cells of the brain contributing, however, not only to brain's immune defense but also to many basic housekeeping functions such as development and maintenance of functional neural networks, provision of trophic support for surrounding neurons, monitoring and modulating the levels of synaptic activity, cleaning of accumulating extracellular debris and repairing microdamages of the brain parenchyma. As a consequence, age-related alterations in microglial function likely have a manifold impact on brain's physiology. In this review, I discuss the recent data about physiological properties of microglia in the adult mammalian brain; changes observed in the brain innate immune system during healthy aging and the probable biological mechanisms responsible for them as well as changes occurring in humans and mice during age-related neurodegenerative disorders along with underlying cellular/molecular mechanisms. Together these data provide a new conceptual framework for thinking about the role of microglia in the context of age-mediated brain dysfunction.

Keywords: Alzheimer's disease; inflammaging; macrophage; microglial senescence; neuroglia.

Introduction

Microglia, the resident innate immune cells of the central nervous system (CNS), are derived from primitive yolk sac macrophages, which colonize the brain before the blood-brain-barrier is formed (Ginhoux et al., 2010; Zhao et al., 2015). Microglial cells were classically seen as immune sentinels within the brain. In the healthy adult brain these cells are highly ramified with each cell covering its own territory (so called spatial domain). The processes of microglia are spread relatively uniformly throughout this territory and move continuously, thereby sampling the surface of all the surrounding cellular elements (Davalos et al., 2005; Nimmerjahn et al., 2005). Under healthy conditions immune responses of microglia are under the tight control of so-called OFF signals, which are constitutively present in the intact brain microenvironment (Biber et al., 2007). The molecular substrates of the OFF signals comprise membrane-bound immunoglobulins (e. g. CD200, TREM-2, Siglecs), conventional G-protein-coupled seven-transmembrane receptors (e. g. CX3CR1), receptors for neurotransmitters, neurotrophins, and TGF-β (transforming growth factor-β) interacting with the respective ligand, etc. Some of them represent receptor-ligand pairs with one of the two being selectively expressed on neurons and the other on microglia, whereas the others are microglia-located receptors for molecules released during physiological neuronal activity. Collectively OFF signals convey the "everything is good" message to microglia by creating an immunosuppressive environment.

The appearance of either pathogen- (PAMPs) or damage-associated molecular patterns (DAMPs) in microglial microenvironment causes activation of microglia and triggers microglia's immune response. DAMPs also comprise neuronal damage-associated ON signals (e. g. ATP and its derivatives, RNA, DNA, high concentrations of neurotransmitter glutamate, etc.). In addition, microglia's immune response can also be triggered by disruption of the OFF signaling. Activated microglia change their morphology towards an hypertrophic or amoeboid phenotype, lose their territoriality, proliferate, migrate to the site of injury, upregulate the expression of surface molecules (e. g. major histocompatibility complex type II glycoprotein (MHCII)) and start releasing immune mediators such as pro- or anti-inflammatory cytokines as well as reactive oxygen species (ROS). Moreover, microglial cells may transform into highly phagocytic cells removing cell debris, pathological protein aggregates or bacterial/viral pathogens. In analogy to activation of T lymphocytes, microglia were proposed to adopt two different activation phenotypes with M1 phenotype referring to potentially neurotoxic, "classically activated" microglia releasing pro-inflammatory mediators

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(e. g. tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), ROS, nitric oxide, etc.) and M2 phenotype referring to "alternatively activated", neuroprotective microglia releasing anti-inflammatory mediators (e. g. interleukin-4 (IL-4) and interleukin-10 (IL-10), TGF-β). However, many recent experiments have refuted this hypothesis by showing that microglia can adopt many different activation states combining classical features of M1 as well as M2 phenotypes (Hanisch and Kettenmann, 2007).

In addition to their classical role as the first and main form of brain's active immune defense, microglia were recently shown to play a key role in many "homeostatic" processes impacting the development and maintenance of functional neural networks. Prenatally, microglia are responsible for controlling the number of neural precursor cells in the cerebral cortex as well as their disappearance during late stages of neurogenesis (Cunningham et al., 2013) and contribute to the embryonic wiring of the forebrain (Squarzoni et al., 2014). During early postnatal development microglia are involved both in formation (Miyamoto et al., 2016) and elimination (Paolicelli et al., 2011; Schafer et al., 2012) of synaptic contacts and dendritic spines, whereas in the intact mature brain microglia monitor the level of synaptic activity, dynamically interact with synapses and contribute to ongoing structural plasticity of synaptic contacts (for a review see (Wake et al., 2013; Wu et al., 2015)). Moreover, recent data suggest that microglia can influence the efficacy of synaptic connections between neurons, under some circumstances even via physical separation of the pre- and postsynaptic elements, and that interaction between neurons and microglia modify synaptic plasticity (i.e. activity-dependent modulation of synaptic strength) (Parkhurst et al., 2013; Chen et al., 2014; Wu et al., 2015).

Given the fundamental role of microglia within the framework of both immune and neural systems, it is becoming increasingly clear that age-related impairment of microglial physiology is very likely to impact not only brain's immune defense but also its key cognitive functions related to motivation, vigilance as well as learning and memory formation. This review aims to summarize recent findings about functional properties of microglia during normal aging as well as in conditions of age-related neurodegenerative diseases and to discuss potential consequences of age-dependent microglial dysfunction.

Normal aging of microglia: reactivity or senescence?

As already outlined above, in the healthy young CNS microglia have a typical ramified morphology and microglia's spatial domains are evenly distributed throughout the entire brain parenchyma, thus providing efficient spatial coverage of the brain tissue. With aging, microglial cells undergo morphological, physiological, and molecular changes defining the senescent microglial phenotype (Fig. 1).

The length of microglial processes, for example, is reduced with ageing, resulting in a smaller tissue volume covered by a single cell (Baron et al., 2014; Hefendehl et al., 2014). Moreover, the order and regularity of microglial domains seems to deteriorate, with large areas devoid of microglial processes on the one hand side and cells sticking to each other on the other (Tremblay et al., 2012; Askew et al., 2017). In addition, aged microglial cells show an increase in soma volume, a decrease in the complexity/circularity of the branching pattern of their processes (Fig. 1) and an increase in cell number in some but not all brain regions/mouse strains tested (Sierra et al., 2007; Tremblay et al., 2012; Baron et al., 2014; Hefendehl et al., 2014). These morphological changes are accompanied by a decrease in the baseline motility of microglial processes and a slower directed process movement towards a lesion site (Hefendehl et al., 2014). In aged human brains, some microglial cells exhibit dystrophic morphologies in which dendritic arbors appear deramified, with residual processes showing increased tortuosity and cytoplasmic fragmentation (Streit et al., 2004).

Another typical feature of the aged microglia is the accumulation of large inclusions (Fig. 1), so called lipofuscin granules, containing "wear-and-tear" pigment lipofuscin (Sierra et al., 2007; Eichhoff et al., 2008). Aside from a large lipid content, sugars and metals, microglial lipofuscin granules were recently shown to contain myelin fragments (Safaiyan et al., 2016). Moreover, genetic manipulations interfering with lysosomal degradation or long-term stability of myelin potentiated the accumulation of these undegradable lysosomal aggregates in adult, not aged, microglia, paralleled by accumulation of morphological and molecular signs of microglial senescence. These and other (Tremblay et al., 2012) data suggest that microglial senescence is largely caused by saturation of its degradative pathways.

From the molecular point of view, aging has been associated with a profound change in the expression pattern of microglial genes (Wong, 2013). Thus, aged microglia

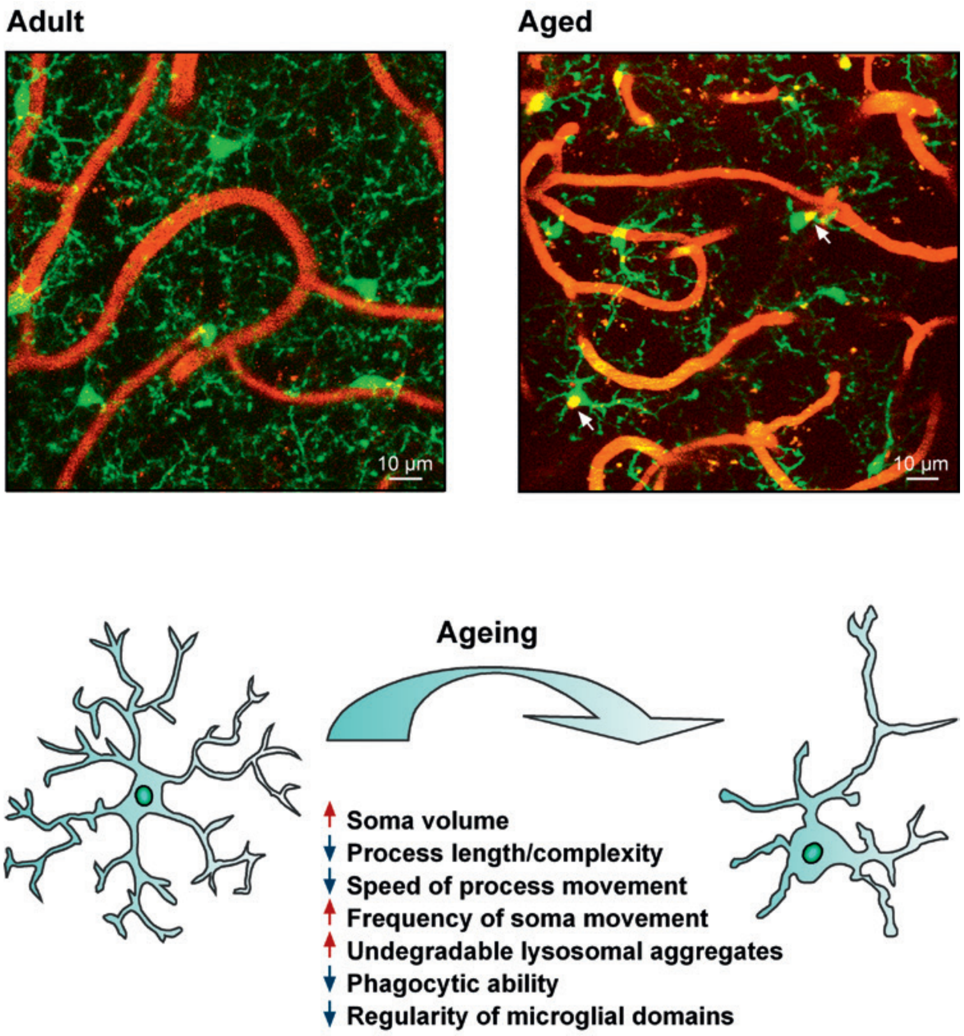

Fig. 1: Differences in morphological and functional properties between adult and aged microglia. Upper row: maximum intensity projection images of layer 2/3 imaged *in vivo* in the frontal/motor cortex of 5-month-old (left image; 30-68 µm depth, step 1 µm) and 18-month-old (right image; 9-47 µm depth, step 1 µm) CX3CR1$^+$/GFP mice. All images were taken at similar illumination intensities. Blood vessels were labeled in red by an i.p. injection of the red fluorescent dye sulforhodamine B. Arrows point towards large lipofuscin granule inside the microglia. Lower row: a schematic drawing of an adult (left) and aged (right) microglial cells along with a list of morphological and functional features changing in the course of ageing (see text for further details). Colored arrows show the direction of change.

showed an increased mRNA expression of pro- (TNF-α, IL-1β, IL-6) and anti-inflammatory (IL-10, TGF-β) cytokines (Sierra et al., 2007), in line with the notion that low grade immune activation is a highly prominent feature of the cognitively normal aging brain (von Bernhardi et al., 2010; Cribbs et al., 2012; Baron et al., 2014).

Indeed, both in rodents and humans aging is accompanied by a widespread upregulation of immune/ inflammation-related genes (von Bernhardi et al., 2010; Cribbs et al., 2012), allowing Franceschi and colleagues to coin a new term describing the chronic pro-inflammatory status of the aged organism as “inflammaging” (Franceschi et al., 2007). In the brain inflammaging is mainly defined by chronically increased expression levels of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-18 and a modulatory cytokine TGF-β, upregulation of the expression levels of caspase-1 (an enzyme responsible for L-1β and IL-18 production), MHCII, complement receptor 3 (CD11b), as well as a downregulation of anti-inflammatory genes such as IL-10, IL-4 or brain-derived nerve growth factor (BDNF) (von Bernhardi et al., 2010; Cribbs et al., 2012; Norden and Godbout, 2013). As the brain’s immune system is mainly represented by microglia, microglia was thought to mediate the observed pro-inflammatory status of the aging brain. However, a more recent study specifically addressing the age-dependent changes in the gene expression pattern of microglia by means of direct mRNA sequencing (without amplification or cDNA synthesis), challenged this established view by showing that several pro-inflammatory or neurotoxic pathways appeared to be downregulated in aged microglia while pathways involved in neuroprotection appeared to be upregulated (Hickman et al., 2013). Specifically, 24 out of 37 markers of "alternatively activated" M2 phenotype were significantly upregulated in aged microglia in contrast to 5 out of 12 markers of potentially neurotoxic M1 phenotype. In addition, the authors discovered a significant alteration in the expression pattern of genes required for sensing the microglia's microenvironment. The transcripts associated with endogenous ligand recognition appeared to be downregulated, whereas those involved in pathogen recognition and neuroprotection were upregulated (Hickman et al., 2013). According to the authors, there was little change in the expression pattern of genes involved in phagocytosis, such as CD11b, CD14, CD68 and ICAM genes, suggesting that aged microglial cells might preserve their phagocytic ability. The authors concluded that aged "microglia retain their ability to defend against infectious pathogens and clear debris, but attempt to 'tone down' the stimulatory effects of endogenous debris, as if to keep from becoming constantly activated".

It has to be mentioned, however, that the observed differential up- or downregulation of endogenous ligand recognition genes caused an alteration in a delicate balance between the ON and OFF signals. The transcripts encoding classical microglia-located OFF molecules such as TREM-2, DAP12, Siglecs or CD200R decreased with advancing age (Fig. 2), in parallel to a decrease in transcripts encoding the counterpart OFF molecules located on or released from neurons (e. g. CD200, CX3CL1(Fractalkine)) (Hickman et al., 2013; Wong, 2013; Grabert et al., 2016). At the same time, the expression of purinergic receptors (ATP is a classical ON signal), is differentially regulated by aging with an increase in the number of transcripts for P2X4 and a decrease in the number of transcripts for P2X7, P2Y12 and P2Y13 receptors (Hickman et al., 2013).

In summary, microglia in the aged brain face a different environment compared to their counterparts in the young adult brain, with a different relationship between ON and OFF signals and a mild chronic pro-inflammatory state. Therefore it is not surprising that many morphological and biochemical characteristics of senescent microglia point towards an activated phenotype. While the classical literature emphasizes the neurotoxic potential of senescent microglia, some recent studies suggest that microglia try to adapt to the changing environment, upregulate their neuroprotective potential, keep calm and remain functional.

How does ageing of microglia influence its housekeeping functions?

The housekeeping functions of microglia include (i) provision of trophic support for surrounding neurons, (ii) monitoring and modulating the levels of synaptic activity, (iii) phagocytosis of accumulating extracellular debris and (iv) repairing microdamages of brain parenchyma. The trophic factors released by microglia include BDNF, supporting neuronal growth, differentiation, survival and learning-dependent synapse formation (Parkhurst et al., 2013), and TNF-α, required for neuroprotection during cerebral ischemia (Lambertsen et al., 2009). In addition, microglia-derived TNF-α plays a key role in controlling the amount of glutamate release from astrocytes, thus directly controlling neuronal firing as well as glutamate-induced neurotoxicity (Santello and Volterra, 2012). Furthermore, the contacts between microglial processes and neuronal somata were shown to decrease both spontaneous and sensory-driven neuronal activity (Li et al., 2012). As already mentioned above, ageing of microglia is accompanied by a decrease in the expression, and probably also release of BDNF, increased levels of TNF-α in the brain tissue and a decreased motility of microglial processes (von Bernhardi et al., 2010; Cribbs et al., 2012, Hefendehl, 2014 #443; Wong, 2013), all leading to decreased neuroprotection and increased extracellular levels of glutamate in the brain parenchyma. This potentiates activity of ageing neurons (Maier et al., 2014) and promotes glutamate-induced neurotoxicity.

The slower, less efficient movement of microglial process towards a lesion site (Hefendehl et al., 2014) decreases the ability of microglia to limit minute brain damages, caused, for example, by small ischemic events or ruptures of tiny blood vessels, increasing the size of the damaged brain tissue. In addition, these microdamages are the likely cause of the enhanced "spontaneous" calcium signaling in ageing microglia (Brawek et al., 2014). The increased intracellular calcium concentration, in turn, may activate many calcium dependent processes, such as processing and release of pro-inflammatory cytokines IL-1β and IL-18 (Murakami et al., 2012) as well as nitric oxide (Hoffmann et al., 2003), leading to alterations in synaptic transmission and plasticity (Sama and Norris, 2013). A decreased

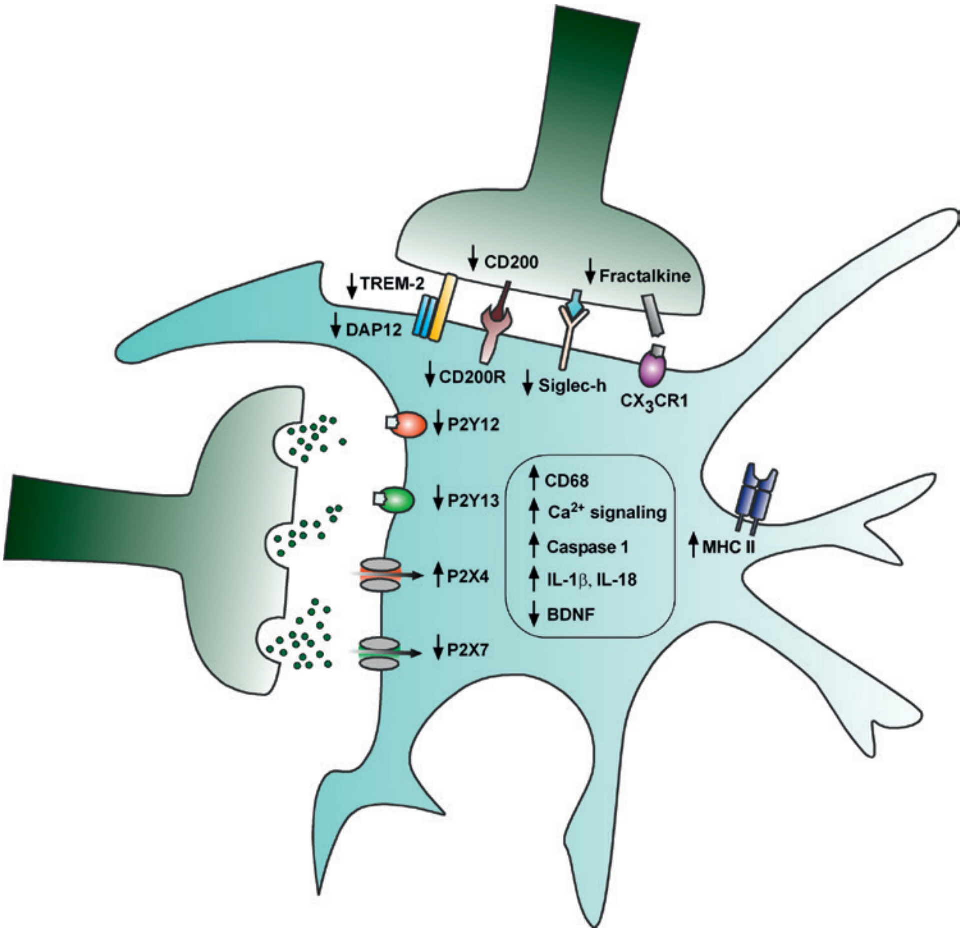

Fig. 2: Molecular make-up of the aged microglia. Microglial ageing is accompanied by changes in the expression patterns of OFF signals (e. g. TREM-2/DAP12, CD200R/CD200, Siglecs, CX3CR1/fractalkine), purinergic receptors (P2Y12, P2Y13, P2X4, P2X7), MHC II as well as an increase in the intracellular calcium signaling, causing an activation of calcium-sensitive signaling cascades.

motility of microglial processes might also decrease the efficiency of inflammation-induced displacement of axosomatic synapses by microglial processes, which was recently shown to be neuroprotective (Chen et al., 2014).

As specialized brain macrophages, aged microglia accumulate large amounts of lipofuscin granules (Fig. 1) as well as other types of noncellular inclusions and appear to be almost completely filled by cellular debris (Tremblay et al., 2012). This state is likely a byproduct of life-long phagocytic activities of microglia (in Greek “macrophage” literally means ‘‘big eater’’) and points out to a possible exhaustion of its phagocytic capacity (Streit, 2006). Moreover, the expression of TREM-2, a microglial receptor promoting phagocytosis, is known to be downregulated with the advanced age (Fig. 2), in line with the assumed impairment of the phagocytic ability of microglia.

Thus, the mounting evidence suggests that all housekeeping properties of microglia are changing with advancing age and these changes often result in the deterioration of the microglial function.

How does ageing of microglia influence brain’s immune defense?

As the main immune cells of the brain, microglia react to acute injury, infections, or disease with a biphasic response starting with a rapid, pro-inflammatory activation phase, followed by a slower anti-inflammatory, neuroprotective tissue repair phase during the resolution of inflammation. The correct timing of both phases is necessary for efficient responses to injury and infections on the one hand and protection of the brain parenchyma from microglia-induced damage on the other (Wong, 2013). Accumulating evidence suggest that aged microglia generate exaggerated pro-inflammatory responses to both peripheral and central immune challenges (reviewed in (Norden and Godbout, 2013; Wong, 2013)). The activation of the immune system by a peripheral injection of lipopolysaccharide (LPS), for example, causes a prolonged and exaggerated brain immune response accompanied with elevated expression levels of mRNA encoding IL-1β, IL-6 and TNF-α and increased levels of respective proinflammatory cytokines. In addition, in the aged brain the mRNA expression levels of IL-1β and TNF-α remained elevated for up to 72 hours after LPS injection and thus much longer than in the adult brain (up to 24 hours) (Richwine et al., 2008). The prolonged inflammatory response within the brain correlated well with the prolonged LPS-induced behavioral impairment of ageing experimental animals, characterized by a reduction of appetite and depressive withdrawal from normal social activities (Godbout et al., 2005; Norden and Godbout, 2013). Aged animals also show exaggerated immune responses to other stressors, such as hemorrhagic stroke, injury, neurotoxins or trauma (Wong, 2013). This partial loss of control over the brain's innate immune system might lead to a prolonged impairment of brain function, memory and cognition and, in the extreme case, also to neurodegeneration.

Microglia and age-related neurodegenerative disorders

Neurodegenerative disorders including, among others, Alzheimer's, Parkinson's, and Huntington's disease, are incurable diseases resulting in progressive functional impairment, degeneration and death of neural cells. Although each of the neurodegenerative disorders has its own etiology, many of them are associated with advancing age and with the deposition of abnormal proteins within the brain. Because of the worldwide ageing of the human population (the number of people over the age of 65 is expected to double over the next 15-20 years, with the fraction of oldest old (85+) increasing from 7% to 12% (Qiu et al., 2009)), these diseases will pose huge challenges to public healthcare systems across the world. Even nowadays dementias, among which Alzheimer's disease (AD) is the prevailing form, affect more than 25 million people worldwide. The hallmarks of AD include intracellular accumulations of hyperphosphorylated microtubule-associated protein tau, parenchymal accumulation of amyloid β deposits, synaptic loss and a progressive impairment of neuronal function, finally leading to a pronounced neuronal death.

Recent Genome Wide Association Studies have identified many genes related to microglia and/or immune response, mutations in which are associated with an increased risk of late-onset AD, the most prevalent form of the disease (Villegas-Llerena et al., 2016). Moreover, the risk associated with a specific mutation in the gene encoding the TREM2 protein is as high as the one associated with the ε4 allele of the apolipoprotein E, until recently the only known genetic risk factor associated with late-onset AD. However, the difference in the expression of the immune-related genes between young and old healthy subjects seems to be much larger than the one between healthy subjects and AD patients of the same age (Cribbs et al., 2012). These results support the notion that inflammaging in general and the increased reactivity of the aged microglia in particular might promote the development of AD. One of the possible mechanisms connecting inflammaging and AD was recently discovered by Beth Stevens's group. They showed that the classical complement cascade-mediated pathways, which normally contribute to synaptic pruning of excess synapses during early postnatal development, cause engulfment and elimination of synapses by microglia in the amyloid-depositing brain (Hong et al., 2016).

Both in humans and in mouse models of AD microglial cells, especially those located in the immediate vicinity of the amyloid β accumulations (so-called senile plaques), have an activated morphology (see, for example, Fig. 1 in (Brawek et al., 2014)) and an increased expression of pro-inflammatory markers such as MHC II and CD68 (Norden et al., 2015). Moreover, plaque-associated microglia undergo a dramatic functional impairment with more that 80% of cells becoming "hyperactive" in respect to their ongoing calcium signaling (Brawek et al., 2014). In parallel, microglia in the amyloid-depositing brain show a reduced phagocytic activity and impaired responses to cell/tissue damage in their vicinity (Krabbe et al., 2013; Brawek et al., 2014), thus failing to sufficiently protect the surrounding tissue. Of note, TREM2 and CD33, recently identified as AD risk genes, are implicated in microglial phagocytosis (Norden et al., 2015). While the latter findings point towards amyloid-induced microglial senescence, an exaggerated microglial calcium signaling alludes towards the increased reactivity of plaque-associated microglia. As already mentioned above for normally aged microglia, the increased intracellular calcium concentration may cause processing and release of proinflammatory cytokines IL-1β and IL-18 via the caspase-1-mediated pathway, release of nitric oxide and activation of the calcium-dependent protein phosphatase calcineurin, upregulating cytokine expression via activation of the nuclear factor of activated

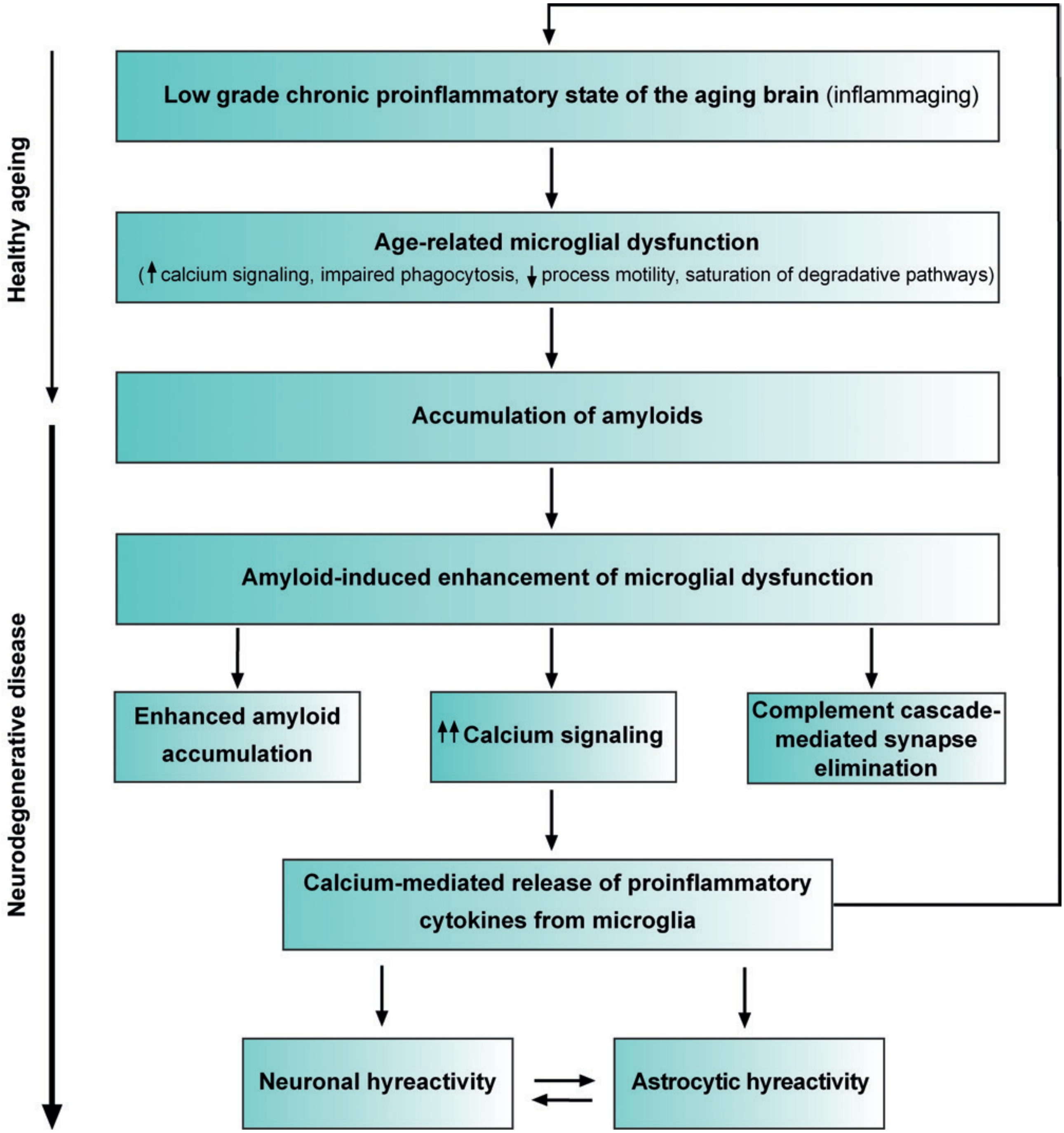

Fig. 3: A flow chart illustrating an inflammaging-based hypothesis of the development of neurodegenerative diseases (see text for details).

T-cells (Felderhoff-Mueser et al., 2005; Sama and Norris, 2013). Interestingly, an NLRP3 inflammasome, a multiprotein complex activating caspase-1, itself requires increased intracellular calcium levels for activation and this activation is enhanced in both human and mouse AD brains (Murakami et al., 2012; Norden et al., 2015). Moreover, genetic deletion of NLPR3 inflammasome reduces plaque burden in AD mice and protects them from loss of spatial memory (Heneka et al., 2013).

Although the fact that amyloid deposition inside the AD brain causes activation of glial cells is known since the pioneering work of Alois Alzheimer, till recently the activated microglia was seen as a late consequence of amyloidosis, as a bystander either potentiating or failing to cope with the ongoing pathology. Only recently it became apparent that age-related microglial dysfunction, as for example, a decrease in its phagocytic ability (see above), might be crucial for age-dependent accumulation of amyloids (i.e. abnormal protein aggregates) in the first place (Fig. 3).

Thus, low grade inflammation within the ageing brain and inflammaging-associated microglial dysfunction result in the reduced efficiency of microglial surveillance because of the reduced process motility paralleled by the impaired phagocytosis due to saturation of microglia's degradative pathways. Under these conditions naturally produced amyloids cannot be efficiently found and eliminated by microglia and therefore accumulate in the brain parenchyma, causing further microglial dysfunction. This amyloid-induced dysfunction potentiates microglial senescence, triggers complement cascade-dependent synapse elimination and further exaggerates intracellular calcium dyshomeostasis in microglia. The latter boosts the production of pro-inflammatory cytokines (e. g. TNF-α and IL-β) via different calcium-dependent pathways (e. g. activation of caspase-1, calcineurin or NFκB (Sama and Norris, 2013)). The pro-inflammatory cytokines (i) potentiate the inflammation within the ageing brain thus closing the vicious cycle and (ii) cause the hyperactivity of calcium signaling in neurons and astrocytes (Santello and Volterra, 2012; Brawek and Garaschuk, 2014), exacerbating amyloid-induced dysfunction of neural networks (Fig. 3). Although this hypothesis still needs a rigorous experimental validation, its plausibility moves microglia and inflammaging in the very center of AD-related pathological events and allows to view AD, but likely also some other neurodegenerative disorders, as a direct consequence of the age-related dysfunction of the brain's immune system.

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Glossary

TREM-2 (triggering receptor expressed on myeloid cells 2), an immunoglobulin inhibiting inflammatory responses in macrophages. In the brain TREM-2 is exclusively expressed by microglia.

DAP12 (DNAX-activating protein 12 kDa), an adaptor molecule used by TREM-2 for intracellular signaling.

Siglecs (sialic acid-binding immunoglobulin-like lectins), immunoglobulins binding to sialic acid. Under physiological conditions the recognition of sialic acids on the neuronal glycocalyx seems to signal cellular integrity inhibiting microglia activation.

CX3CL1 (CX3C ligand, fractalkine), a cytokine involved in the adhesion and migration of leukocytes.

CX3CR1 (CX3C chemokine receptor 1), receptor for the chemokine fractalkine.

MHCII (major histocompatibility complex II), a surface protein normally found only on antigen-presenting cells.

ROS (reactive oxygen species), reactive chemical species containing oxygen. Under physiological conditions ROS are involved in cell signaling and homeostasis, at high concentrations they cause cell damage via oxidative stress.

BDNF (brain-derived neurotrophic factor), a growth factor critically important for neuronal differentiation and survival.

TNF-α (tumor necrosis factor α), one of the key pro-inflammatory cytokines produced by activated macrophages.

TGF-β, (transforming growth factor β), an immunosuppressive cytokine secreted by many cell types, including macrophages.

IL-1β (interleukin-1β), one of the key pro-inflammatory cytokines. IL-1β is involved in many processes including cell proliferation, differentiation, apoptosis, inflammatory pain hypersensitivity, etc.

IL-6 (interleukin-6), a pro-inflammatory cytokine secreted by macrophages and T cells. IL-6 is an important mediator of fever and of the acute phase response.

IL-4 (interleukin-4), an anti-inflammatory cytokine playing an important role in wound repair.

IL-10 (interleukin-10), an anti-inflammatory cytokine inhibiting LPS-mediated induction of pro-inflammatory cytokines.

CD11b (cluster of differentiation molecule 11b), a surface molecule expressed by many leukocytes, including monocytes, granulocytes, macrophages, and natural killer cells. Under inflammatory conditions it regulates chemotaxis, leukocyte adhesion, cell-mediated cytotoxicity and phagocytosis.

CD14 (cluster of differentiation molecule 14), a surface molecule expressed mainly by macrophages acting as a co-receptor for the detection of LPS.

CD68 (cluster of differentiation molecule 68), a glycoprotein found in lysosomal and surface membranes of myeloid cells. CD68 is indicative of phagocytic capability.

CD33 or **Siglec-3** (sialic acid binding Ig-like lectin 3), a sialic acid receptor expressed on myeloid cells.

ICAMs (intercellular adhesion molecules), immunoglobulins, continuously present in low concentrations in the membranes of leukocytes.

CD200 (cluster of differentiation molecule 200), an immunoglobulin regulating myeloid cell activity and delivering an inhibitory signal to macrophages.

CD200R, receptor for cluster of differentiation molecule 200. In the CNS, microglial cells express CD200R, while CD200 is expressed on the membrane of neurons and astrocytes. Together this ligand-receptor pair inhibits inflammatory responses of microglia.

LPS (Lipopolysaccharide), component of bacterial cell wall of Gram-negative bacteria provoking innate immune response.

ApoE (Apolipoprotein E), a class of apolipoproteins transporting lipoproteins, fat-soluble vitamins, and cholesterol.

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