Role of Pattern Recognition Receptors of the Neurovascular Unit in Inflamm-Aging

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Running head: PRRs in the aging brain

Abstract

Aging is associated with chronic inflammation (inflamm-aging) partly mediated by increased levels of damage-associated molecular patterns (DAMPs) which activate pattern recognition receptors (PRRs) of the innate immune system. Furthermore, many aging-related disorders are associated with inflammation. PRRs, like Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are not only expressed in cells of the innate immune system, but other cells as well, including cells of the neurovascular unit (NVU) and cerebral vasculature forming the blood-brain barrier (BBB). In this review we summarize our current knowledge about the relationship among activation of PRRs expressed by cells of the NVU/BBB, chronic inflammation and aging-related pathologies of the brain. The most important DAMP-sensing PRRs in the brain are TLR2, TLR4, NLRP1 and NLRP3, which are activated during physiological and pathological aging in microglia, neurons, astrocytes and possibly endothelial cells and pericytes.

Keywords

aging, astrocytes, blood-brain barrier (BBB), cerebral endothelial cells, damage-associated molecular patterns, inflamm-aging, inflammasome, neurovascular unit (NVU), pattern recognition receptors

Introduction

Due to the continuously growing life expectancy, aging and related morbidities are rapidly increasing unresolved health and socio-economic problems. Cerebrovascular dysfunctions are common among elderly persons and their incidence increases exponentially with age. The human brain has a very intense metabolism compared to other organs, by using about 20% of the body's resting oxygen consumption and accounting for only 2% of the body weight. Energy supply of the central nervous system (CNS) is provided by a very dense capillary system with an average distance of 40-50 µm between neighboring capillaries in the human brain. This implies that almost all neurons are in the close vicinity of a capillary, so that the concept of neurovascular unit (NVU) was coined, emphasizing the inseparable character of neural and vascular functions. Thus, the functional state of the CNS is greatly dependent on the quality of the microvasculature and the term "you are as old as your arteries" can be redefined in the brain to "you are as old as your microvessels".

The neurovascular unit (NVU) in aging

The NVU represents a close structural and functional relationship (i.e. coordinated action) of microvascular endothelial cells, pericytes, glial cells and neurons (Figure 1). Main functions of the NVU are formation of the blood-brain barrier (BBB) and neurovascular coupling (i.e. changes in cerebral blood flow in response to local neural activity). The BBB is a highly selective permeability barrier that separates the circulating blood from the extracellular fluid in the CNS. By strictly regulating the molecular and cellular traffic between the blood and the brain, it substantially contributes to the homeostasis of the CNS (1). The barrier itself is formed by endothelial cells of the cerebral microvasculature, which acquire special barrier characteristics in the brain microenvironment. Continuous tight junctions (TJs) interconnecting cerebral microvascular endothelial cells seal the paracellular cleft, forcing most molecular and even cellular traffic to use the highly regulated transcellular way of

transport or transmigration. Low level of endocytosis, intracellular enzymes and efflux transporters of the ATP-binding cassette family also contribute to the barrier (149). By sharing important regulatory functions, pericytes and astrocytes are also integral parts of the BBB (3, 137). In the present review we will particularly focus on these three cell types.

There is increasing evidence that dysfunction and senescence of the cerebral microvasculature play critical roles in age-related brain pathologies. The brain capillary endothelium suffers region- and species-specific morphological and functional changes during aging, including elongation, decrease in the number of mitochondria and decrease in choline and glucose transport (98). In addition, age-related morphological and functional microvascular changes include fibrosis and degeneration, basement membrane thickening, microhemorrhages, vessel rarefaction, impaired angiogenesis, dysregulation of cerebral blood flow, lower metabolic rates of glucose and oxygen and neurovascular uncoupling (17, 27, 45, 143, 144). Reduced blood flow in aging reflects an impaired vasodilatation and enhanced vasoconstriction (46), most probably mediated by imbalance in the production of and response to vasoconstrictor and vasodilator signals (129). After ischemia, inverse neurovascular coupling (i.e. vasoconstriction instead of vasodilatation) with spreading depolarizations may occur in the old brain (92). Diminished cerebral blood flow and reduction in functional hyperemia are largely dependent on loss of pericytes (9). Cerebral endothelial barrier functions can also be impaired in aging through changes in TJ structure (153). Loss of pericytes (133) might also contribute to this process. Aging-related BBB breakdown is most evident in the hippocampus, and is worsening with the appearance of cognitive impairment, that correlates with pericyte injury and increased levels of soluble PDGFRβ in the cerebrospinal fluid (97). (9). In the cortex and hippocampus of Alzheimer's disease (AD) subjects, pericyte number and coverage are reduced, correlating with BBB breakdown (127). Chronic BBB breakdown leads to

accumulation of neurotoxic serum proteins in the brain tissue contributing to neurodegeneration.

Altogether, changes in functions of the microvasculature during aging (i.e. neurovascular uncoupling and alterations in BBB integrity) lead to irreversible neuronal injury. Reductions in brain microcirculation and BBB breakdown may occur prior to neurodegeneration and neuroinflammation, as shown in pericyte-deficient mice However, the exact contribution of microvascular changes to neurodegeneration in aging is not well understood. Nevertheless, endothelial and glial, but not neuronal-specific genes are the best predictors of biological age (132). Therefore, the “you are as old as your microvessels” statement refers to the direct link between vascular and neuronal injury in aging.

Besides endothelial cells and pericytes, astrocytes are also changing during physiological and pathological aging. The total number of astrocytes in the human brain does not change with age (121); however, morphological and metabolic remodeling occurs. These changes might be region specific and might reflect astroglial adaptive plasticity (121). In astrocytes of aged animals, reduction of gap junction plaques (23), decrease in morphological complexity (32) and reduction in the ability to support survival of motor neurons (28) were described. Moreover, astrocytes having a senescence-associated secretory phenotype (SASP) accumulate with age, showing increased expression of glial fibrillary acidic protein (GFAP) and other intermediate filaments, secretion of inflammatory cytokines, chemokines and proteinases (124).

Other cell types of the NVU (neurons and microglia) also present morphological and functional changes in the elderly (reviewed in: (39)). Moreover, alteration of the structure and function of the NVU is even more accentuated in pathological aging (39). Unfortunately, hallmarks of aging are only referring to changes in the phenotypes of cells (86, 99), the relevance of these changes (i.e. causative or reactive role to aging) is largely uncharacterized.

Inflammation and aging-related functional changes of the NVU

In parallel with these mechanisms, inflammation is a central element affecting cells of the NVU during aging. Inflammation in the brain has mainly been linked to microglia and to a lower extent to astrocytes. However, other cells of the NVU are also participating in inflammatory responses; therefore, might also be key players in aging processes of the CNS.

Vascular inflammation is associated with BBB opening and neurovascular uncoupling, the main aging-related neurovascular dysfunctions. Inflammation is a well-characterized cause of BBB disruption (2, 145). Mechanisms of BBB opening may include cytokine-induced actin remodeling and modulation of TJ protein levels or subcellular relocalization (19). In mice, during normal aging, reduced amount of TJ proteins and elevated expression of TNF-α has been observed in CECs, without changes in adhesion molecules and with no leukocyte recruitment (37), suggesting a direct effect of the cytokine on the amount of TJ proteins. In aging-related brain pathologies, like AD or ischemia, increased secretion of inflammatory cytokines in the cerebral endothelium may enhance expression of adhesion molecules as well (57, 123). Consequent migration of circulating leukocytes through the activated brain endothelium may also contribute to deterioration in barrier properties of the BBB, which is also part of the pathogenesis of these diseases (114, 154). Besides increased BBB permeability, changes in cerebral blood flow and impaired hemodynamic coupling also occur in response to inflammatory cytokines, like IL-1β (10, 13). Moreover, reactive oxygen species (ROS) – which are key mediators of both neurovascular uncoupling (141) and of alteration of the brain endothelial junctional complex in aging (38) – can also trigger secretion of inflammatory cytokines (102). CECs are rich in mitochondria; therefore, may be important sources of ROS. Inflammatory cytokines can upregulate ROS generation in CECs leading to decreased expression of junctional proteins and consequent BBB disruption (120).

Inflammatory vascular dysfunctions not only depend on endothelial but on pericyte- and astrocyte-linked mechanisms as well. Pericytes respond to inflammatory cytokines through enhanced expression of adhesion molecules and secretion of inflammatory mediators (106, 111). In addition, astrocytes are a common source of inflammatory mediators in brain pathologies (43).

Therefore, cells of the NVU can both respond to and release inflammatory mediators, and vascular inflammation seems to be an important step in aging-related functional changes of the NVU. In the next chapters, we describe inflammatory aspects of the aging NVU.

Inflammation, damage-associated molecular patterns (DAMPs) and pattern recognition receptors (PRRs) in aging

Aging is the greatest risk factor for developing chronic diseases, many of which are directly linked to a persistent low grade inflammation, called inflamm-aging (49). Inflamm-aging is characterized by a pro-inflammatory environment in several tissues, consisting of activation of resident macrophages, leukocyte infiltration and increased production of inflammatory cytokines and ROS. Aging is also associated with the senescence of the immune system, characterized by loss of naïve T cells, accumulation of memory T cells, thymic involution, decline in the total number of phagocytes, impairment of dendritic cells and natural killers, delayed cytokine release, etc. (93, 130), leading to increased frequency of infections and chronic diseases.

Inflammation is highly regulated by the immune system, which has two main branches, the innate and the adaptive (acquired) immune system. Initialization of inflammatory processes is largely dependent on the innate immune system. Sensing of potentially dangerous molecules – like pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) – by the innate immune system depends on pattern recognition receptors

(PRRs) consisting of at least four major families (Table 1). Members of the Toll-like receptor (TLR) and the C-type lectin receptor (CLR) families are membrane-bound PRRs, while retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) detect intracellular patterns (91, 138).

Indeed, PAMPs are the most potent activators of these receptors. However, endogenous molecules released upon tissue damage (DAMPs) can activate the same receptors and signaling pathways driving a sterile inflammatory reaction. The most widely studied DAMPs are heat shock proteins, the chromatin protein high mobility group box-1 (HMGB1), extracellular matrix fragments and purine metabolites, such as ATP and uric acid. DAMP-dependent sterile inflammation is key player in aging-related pathologies (47). Moreover, DAMPs were proposed to be biomarkers and interventional targets in aging-associated diseases (70). In elderly, DAMP-induced chronic inflammation is more prevalent than in young individuals. Chronic oxidative stress is a hallmark of aging and reactive oxygen species induce oxidative damage leading to formation of DAMPs (38, 125). Accumulation of crystalline DAMPs, like urate crystals, cholesterol or amyloid deposits may also increase with age. The most important DAMP-identifying PRRs are TLRs and NLRP3 (47).

Inflammasome activation in aging

Activation of some NLRs leads to the assembly of inflammasomes, which are large multiprotein complexes mediating activation of inflammatory caspases. The best known inflammasome-forming NLRs are NLRP1, NLRP3 and NLRC4. Besides, other NLRs might also form inflammasomes (e.g. NLRP2, NLRP6, NLRP7 or NLRP12). In addition, non-NLR family members (e.g. AIM2 – absent in melanoma 2, IFI16 – γ-interferon-inducible protein 16 or pyrin) are also inflammasome-forming receptors (15).

Upon recognition of inflammatory signals, inflammasome initiators oligomerize, recruit the adaptor protein ASC (apoptosis-associated speck-like protein containing CARD) which interacts with and initiates autoactivation of an inflammatory caspase (caspase-1 or caspase-11/4/5). The active caspase processes precursors of inflammatory cytokines, mainly IL-1β or IL-18 (7) and can also initiate pyroptosis (15).

Inflammasomes can be activated in response to different PAMPs and DAMPs, the classical activators being diverse microbial components. NLRP1 inflammasomes assemble upon stimulation with anthrax lethal toxin or muramyl dipeptide (MDP; a constituent of both Gram-positive and Gram-negative bacteria, sensed by NOD2); the NLRC4 inflammasome is activated in response to bacterial flagellin sensed by NAIP (NLR family apoptosis inhibitory protein); pyrin detects RhoA protein inactivated by bacterial toxins, while AIM2 detects cytosolic microbial or host DNA. Activation of the NLRP3 inflammasome is dependent on potassium efflux associated with various stimuli including diverse pathogens and several DAMPs (extracellular ATP, crystalline material, amyloid-β, etc.). Therefore, the NLRP3 inflammasome pathway is one of the most important drivers of sterile inflammatory processes.

Inflammasomes can play a pivotal role in low-grade chronic inflammation associated with metabolic abnormalities and aging (i.e. inflamm-aging) as well. Moreover, inflammasome activation has been linked to diverse brain diseases (including ischemic and traumatic brain injury and neurodegenerative diseases) and to comorbidities and risk factors of CNS pathologies (e.g. diabetes, atherosclerosis, obesity, hypertension, etc.) (reviewed in: (74)).

It is well accepted that NLRP3 inflammasome assembly requires two signals: a priming signal inducing upregulation of the expression of inflammasome components and an activation signal required for the assembly of the inflammasome (58). It has been suggested that, although NLRP3 can be activated by a wide variety of pathogens, its primary role is sensing

metabolic disturbance and restoring homeostasis. However, chronic metabolic dysfunction in aging might result in aberrant NLRP3 response, leading to aging-associated inflammatory disorders (21). In the absence of PAMPs, elevated levels of TNF-α (110) seem to be one of the major inducers of NLRP3 expression in macrophages of the liver and in adipose tissue in aged mice (8). Nevertheless, NLRP3 inflammasome is involved in the induction of obesity and insulin resistance (135), conditions linked to aging and risk factors for brain disease.

PRRs and inflammasome activation in the aging brain

Inflamm-aging and immune senescence substantially affect the CNS (31). The low-grade inflammatory status of the aged CNS is associated with recruitment of leukocytes, i.e. dendritic cells and T cells, including memory CD8 T cells (119, 134). The majority of CNS diseases, including age-related pathological conditions, are characterized by neuroinflammatory processes (117). These pathological conditions include neurodegenerative disorders (50), like AD (113), Huntington's disease (96), Parkinson's disease (PD) (68), amyotrophic lateral sclerosis (ALS) (151) or multiple sclerosis (MS) (52). In addition, inflammation has been identified as an important player in cognitive dysfunctions (109), memory loss (64) and cerebral ischemia as well (147). A significant part of these disorders is age-related.

Activation of PRRs and of inflammasomes is an important event in these pathologies (Table 2) as a non-specific neuroinflammatory event (82). Aging was shown to induce upregulation of TLR1, TLR2, TLR4, TLR5 and TLR7 and downregulation of TLR9 expression in the mouse brain, while TLR3, TLR6 and TLR8 remain unchanged (81). Interestingly, upregulation of innate immune system-specific genes (complement genes; TLRs: TLR2, TLR4, TLR5; inflammasome-associated genes: caspase-1, IL-18 and IL-1β) in the human brain is more robust during normal aging than in AD (25).

Among cells of the CNS, **microglia**, the resident innate immune cells of the CNS, express the most PRRs, including a complex set of TLRs (TLR1-9) (75). Activation of TLR2, TLR4 and TLR9 in microglia can lead to an inflammatory response resulting in neuronal damage (122). Activation of microglial TLR2 and TLR4 receptors enhances microglial phagocytosis of neurons contributing substantially to neuronal loss during brain inflammation (105). TLR2 is involved in microglial activation in chronic neurodegenerative diseases such as AD and PD (63). In AD, TLR2, TLR4 and other TLRs expressed in microglial cells are involved in phagocytosis of amyloid-β in the early stages and contribute to neuroinflammatory responses in the late stages (54). Physiological aging is associated with an increased microglial response to LPS. However, in AD, TLR4 signaling is diminished contributing to the accumulation of amyloid-β in the brain (55). Similarly, cultured senescent microglial cells show decreased expression of TLR2 and TLR4 and reduced capacity to migrate and phagocytize (18). In contrast, upregulation of TLR4 was observed in microglial cells in the forebrain of postmenopausal women (126). In addition, TLR4 localized to microglia plays a role in ischemic brain injury (71).

Microglia express several inflammasome components as well, including inflammasome-forming NLRs, AIM2, the adaptor protein ASC and inflammatory caspases and can secrete active IL-1β through inflammasome-dependent and -independent mechanisms (16). Activation of NLRP3 inflammasome in microglial cells has been shown to be involved in the pathogenesis of AD (65). A proposed mechanism is that microglia phagocytize fibrillar amyloid-β, leading to lysosomal damage and release of cathepsin B from damaged lysosomes into the cytoplasm. Cathepsin B activates the NLRP3 inflammasome resulting in IL-1β release (62). Interestingly, only microglia isolated from aged mouse brains secrete IL-1β in response to fibrillar amyloid-β, while microglia isolated from young adult mouse brains do not, indicating the primed state of microglial inflammasomes in aged animals (152). Age-

associated priming of microglia can be induced by activation of the peripheral innate immune system (e.g. as a result of systemic infections) and plays a central role in exaggerated neuroinflammation (67).

In contrast to the well-accepted role of microglia in inflammatory processes of the aging brain, a recent study suggests that aging-induced upregulation of PRRs in cerebellar and hypothalamic brain regions does not primarily localize to microglia (11).

Neurons also express TLRs which may be involved in age-related pathologies (77, 108). TLR2 expression is significantly increased in PD brain neurons and is localized to α-synuclein positive Lewy bodies (36). Moreover, TLR signaling in sensory neurons contributes to persistent pain and neuroinflammation (85). TLR2 and TLR4 expression increases in cerebral cortical neurons in response to ischemia/reperfusion contributing to neurological deficits (140). TLR3, on the other hand, impairs working memory and inhibits hippocampal neurogenesis (107).

Neurons express diverse NLRs as well and are able to form functional NLRP1, NLRP3, NLRC4 and AIM2 inflammasomes (4, 74, 148). Aging-induced NLRP1 inflammasome activation in hippocampal neurons was shown to be involved in cognitive impairment (90). In AD, NLRP1 is upregulated in neurons (74) resulting in activation of the pyroptotic pathway, contributing to cognitive decline (139). In addition, NLRP1 and NLRP3 inflammasomes play a major role in neuronal cell death in stroke (42).

Besides microglia and neurons, other cell types of the NVU can also take part in aging-related neuroinflammation. Cells of the BBB (endothelial cells, pericytes and astrocytes) express different PRRs and inflammasome components (Figure 1) which may have an important role in inflammatory processes. Inflammation and related oxidative stress, developing naturally in aging, are important mechanisms of cerebrovascular malfunction. Moreover, inflammatory

mechanisms of the vasculature seem to be common and increasingly important in neurological disorders (80).

Role of PRR and inflammasome activation in cells of the BBB

Brain endothelial cells are critical in regulating the communication between the immune and central nervous systems (6), equipped with a whole set of signaling molecules (40, 44, 150). They are the first cells of the NVU coming in contact with circulating pathogens, activated immune cells and cytokines. Moreover, brain endothelial cells are essential in activating the hypothalamic-pituitary-adrenal inhibitory feedback in systemic inflammation (56). The key role of the BBB as a link between neuroinflammation and neurodegeneration has been increasingly recognized (59, 80).

Cerebral endothelial cells have been shown to express a whole set of TLRs including TLR2, TLR3, TLR4, TLR6 and TLR9 (22, 101), and these receptors have been shown to participate in important signaling processes. Besides TLR4, TLR2 is the main sensor of bacterial infections in brain endothelial cells (22, 76), while TLR3 responds to double stranded RNA with cytokine release (48, 83). Activation of TLR4 or TLR2/6 leads to an increased BBB permeability (101, 146).

Although brain endothelial TLRs have not been directly linked to physiological or pathological aging so far, TLR4/MyD88/NF-κB signaling in endothelial cells of the BBB is central in the regulation of both pro- and anti-inflammatory mechanisms, which have a key role in aging. Moreover, oxidative stress upregulates expression of TLR2, TLR3, TLR4 and TLR6 in vitro (101). In addition, ischemic stroke-induced fibrin deposition triggers TLR2 and TLR4 expression and activation in the cerebral vasculature of aged rats (155). In this process, both endothelial cells and pericytes are probably involved. Besides fibrin and fibrinogen, Hsp60 is another potential endogenous ligand for TLR2 and TLR4 in ischemia (14).

Nevertheless, using preconditioning with TLR2 or TLR4 ligands, tolerance to cerebral ischemia, maintenance of microvascular patency and attenuation of BBB disruption can be achieved (29, 69). In addition, increased TLR4 expression in brain endothelial cells can contribute to astrocyte swelling and brain edema formation (73).

TLR4 can be primarily activated by PAMPs, e.g. LPS and other pathogenic components. The envelope protein of MSRV (multiple sclerosis-associated retrovirus), a virus found in most patients with MS, is recognized by cerebral endothelial TLR4 and induces ICAM-1 overexpression, production of IL-6 and IL-8 and immune cell transmigration (35). Therefore, MSRV can maintain chronic inflammation through TLR4 activation. TLR4-dependent activation of ICAM-1 and of the inflammatory phenotype of brain endothelial cells has been proved in other studies as well (20, 78, 128). As a consequence, endothelial TLR4 has a decisive role in neuroinflammation through leukocyte recruitment into CNS (156). On the other hand, cerebral endothelial cells, and not perivascular microglia, are the main targets of circulating inflammatory mediators to activate brain circuits regulating release of anti-inflammatory glucocorticoids (56).

Regarding NLR expression and activation, experimental studies were mainly performed in non-cerebral endothelial cells. In a recent study (100), we detected expression of several NLRs – including NOD1, NOD2, NLRC4, NLRC5, NLRP1, NLRP3, NLRP5, NLRP9, NLRP10, NLRP12, NLRA and NLRX – in human brain endothelial cells. We have also shown that NLRP3 expression can be significantly induced by inflammatory stimuli. Expression of key inflammasome components (NOD2, NLRP3 and caspase-1) along with caspase-cleaved interleukins IL-1β and IL-33 can be induced by priming with LPS and activation with MDP. In addition, combining priming and activation of brain endothelial inflammasomes results in active IL-1β secretion. Since this is a recently described mechanism, further studies are needed to understand the role of brain endothelial

inflammasome activation in pathological processes of the CNS, including aging-related diseases. Nevertheless, NLRP3 activation has been shown to mediate endothelial senescence in non-cerebral endothelial cells (136), indicating that inflammasome activation might have an important role in aging and aging-related disorders, possibly both inside and outside the CNS.

Even much less is known about the expression and role of PRRs in cerebral **pericytes**. Besides TLR4, NOD1 and NOD2 (60, 104), we have recently shown the expression of TLR2, TLR5, TLR6, TLR10, NLRC5, NLRP1-3, NLRP5, NLRP9, NLRP10 and NLRX mRNA in cultured brain pericytes (106). TLR4 expressed in brain pericytes can not only respond to LPS, but to HMGB1 as well (60), suggesting the role of this receptor in sterile inflammation. In addition, TLR2 expression in the post-ischemic vasculature of aged rats was shown to partly co-localize with the pericyte marker PDGFRβ (155). Further investigations are needed to understand the role of PRRs expressed in pericytes in aging-related pathologies of the brain. Nevertheless, pericytes have a complex immunological role by secreting diverse chemokines and cytokines, expression of adhesion molecules and controlling immune cell trafficking (103). Moreover, inflammatory stimuli and oxidative stress – which can be aging-associated alterations – upregulate several PRRs in pericytes, although cannot activate inflammasomes (106).

Among cells of the BBB, **astrocytes** may have the most important role in sterile inflammatory reactions of the brain. Besides microgliosis, chronic neurodegeneration is characterized by astrogliosis as well, and both microglia and astrocytes can extensively respond to and release cytokines on this background (66). Moreover, these two cell types extensively cross-talk with cells of the adaptive and innate immune system infiltrating the CNS (116).

Astrocytes express several TLRs on the mRNA and protein level; however, their TLR expression profile is more limited in vivo than in vitro (77). Expression level of TLRs (TLR2,

TLR3 and TLR4) is lower in astrocytes than in microglia, and astrocytes may respond more robustly to TLR2/3/4 agonists in the presence of microglia (89). Moreover, TLR2/3/4 agonists are able to prime microglia but not astrocytes for ATP-dependent IL-1β release (41). In line with this observation, in animals with chronic neurodegenerative prion disease, IL-1β synthesis in response to IL-1β or TNF-α occurs exclusively in microglia and not in astrocytes (66). On the other hand, α-synuclein can activate proinflammatory TLR4 pathways in primary astrocytes (115).

Astrocyte-expressed TLRs, together with RLRs, can be involved in the antiviral response and type I IFN secretion. Viruses infecting and replicating in neurons (e.g. rabies virus or vesicular stomatitis virus) can abortively infect astrocytes, which have a decisive role in antiviral protection of the CNS (53, 112). Not only RNA viruses, but DNA viruses (e.g. herpes simplex virus-1) are also sensed in a RIG-I-dependent manner by astrocytes (26). Astrocytic RLRs (RIG-I, MDA5) are not only involved in anti-viral immune responses, but in type I IFN release after spinal cord injury and cerebral ischemia (12, 30), supporting the idea that astrocytes and RLRs contribute to several inflammatory processes in different CNS diseases.

In addition, astrocytes express several NLRs and are able to activate inflammasomes. Cultured cortical astrocytes express NLRP1, NLRP3, NLRC4 and AIM2, among which NLRP3 mRNA is the most abundant (5). Induction of NLRP1, NLRP3 and IL-1β in both neurons and astrocytes was shown to contribute to ethanol-dependent impairment in neurogenesis (157). In addition, NLRP3 inflammasome activation in astrocytes might be involved in the pathogenesis of PD (87). Aberrant NLRP3 activation was described in glioblastoma cells (142). However, no IL-1β or IL-18 secretion could be detected in microglia-free astrocyte cultures in response to cytokine priming and the NLRP3 activators ATP, nigericin, amyloid-β or α-synuclein (61). Others observed inflammasome-dependent

production of IL-1β in response to LPS/amyloid-β; however, in this case microglial contamination was not unambiguously excluded (24). On the other hand, inflammatory activation of brain endothelial cells in neurobrucellosis was shown to partly depend on IL-1β secreted by both microglia and astrocytes in a TLR2-, NLRP3- and AIM2-dependent manner (95).

Astrocytes can activate NLRC4 inflammasomes as well, and resulting IL-1β is involved in enhancing amyloid-β levels in neurons. This suggests an involvement of NLRC4 inflammasome in astrocytes in inflammatory responses associated with AD (84). Moreover, the functional NLRP2 inflammasome – consisting of NLRP2, ASC and caspase-1 – was first described in astrocytes. NLRP2 inflammasome in astrocytes is preassembled into a multiprotein complex with the pannexin 1 channel and the P2X7 receptor, does not require priming, is activated by extracellular ATP and contributes to the maturation of IL-1β and IL-18 (94). This suggests that NLRP2 – similarly to NLRP3 – can detect DAMPs released during injury. However, the role of NLRP2 inflammasome in pathological processes has not been evaluated so far.

Conclusions and possible future directions

Aging and aging-related CNS pathologies are accompanied by chronic sterile inflammation (inflamm-aging) which is largely determined by activation of pattern recognition receptors (PRRs), like TLR2, TLR4 or NLRP3 (Figure 2). These react to DAMPs (damage-associated molecular patterns) – i.e. self-molecules released upon cellular stress, tissue injury and necrosis – which accumulate during life. Due to age-dependent increase in cytokine production of senescent cells, PRRs and inflammasome components can be in a primed state in elderly. Therefore, inflammasome-activating signals (e.g. amyloid-β fibrils in AD or ROS released upon ischemia-reoxygenation) can directly lead to cytokine (IL-1β or IL-18) release and pyroptotic cell death.

Inflammatory reactions in the aging brain have mainly been linked to microglia and to a lower extent to astrocytes and neurons. Recently, CECs and pericytes have also been shown to express PRRs and to release inflammatory cytokines. Since the vasculature is largely involved in aging-related disorders, inflammatory reactions of the BBB might be involved in the pathomechanism of these conditions. It is our hope that further studies will elucidate the exact role of cells of the cerebral vasculature in inflammatory reactions of the aging brain. In addition, possible existence of any links between activation of PRRs and age-related BBB disruption or uncoupling of functional hyperemia also need to be clarified. Moreover, it would be important to understand whether changes in the functions of cells of the NVU are causes or compensatory consequences of aging.

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Legend to figures and tables

Figure 1. Expression of TLRs and inflammasome-forming NLRs in cells of the neurovascular unit (NVU).

Figure 2. Central role of sterile chronic inflammation and PRRs in aging and aging-related CNS disorders.

Table 1. Classification of pattern recognition receptors (PRRs) and their most important microbial and endogenous activators.

Table 2. Regulation and function of PRRs and inflammasomes in the aging brain and aging-related CNS disorders. ↑=upregulation/increase, ↓=downregulation/decrease.

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913 **Tables**

914 **Table 1.**

Pattern recognition receptors (PRRs)			Best characterized members	Best known activators		References
				PAMPs	DAMPs	
Membrane-bound PRRs	**Toll-like recepors (TLRs)**	TLR1-10 (human)				(47, 79, 138)
			TLR2	bacterial lipoproteins	Hsp60, Hsp70, HMGB1, fibrin	
			TLR3	double-stranded RNA	endogenous nucleic acids	
			TLR4	LPS	Hsp60, Hsp70, HMGB1, fibrin	
			TLR5	flagellin		
			TLR9	unmethylated CpG	endogenous nucleic acids	
	C-type lectin receptors (CLRs)					(138)
			mannose receptor 1	repeated mannose units	endogenous glycoproteins	
			mincle	bacterial glycolipids	spliceosome-associated protein 130	
			dectin-1	glucans from fungi	endogenous glycoproteins	
Cytoplasmic PRRs	**NOD-like receptors (NLRs)**	NLRA (CIITA), NLRB (NAIP), NOD1, NOD2, NLRC3-5, NLRP1-14, NLRX				(100, 138)
			NOD2	MDP		(15)
			NLRC4	flagellin		(15)
			NLRP1	MDP, anthrax lethal toxin		(15, 47, 61, 100)
			NLRP3	MDP, nigericin	ROS, amyloid-β fibrils, ATP, uric acid	

	RIG-I-like receptors (RLRs)				
		RIG-I	viral double stranded RNA		(138)
		MDA-5	viral double stranded RNA		

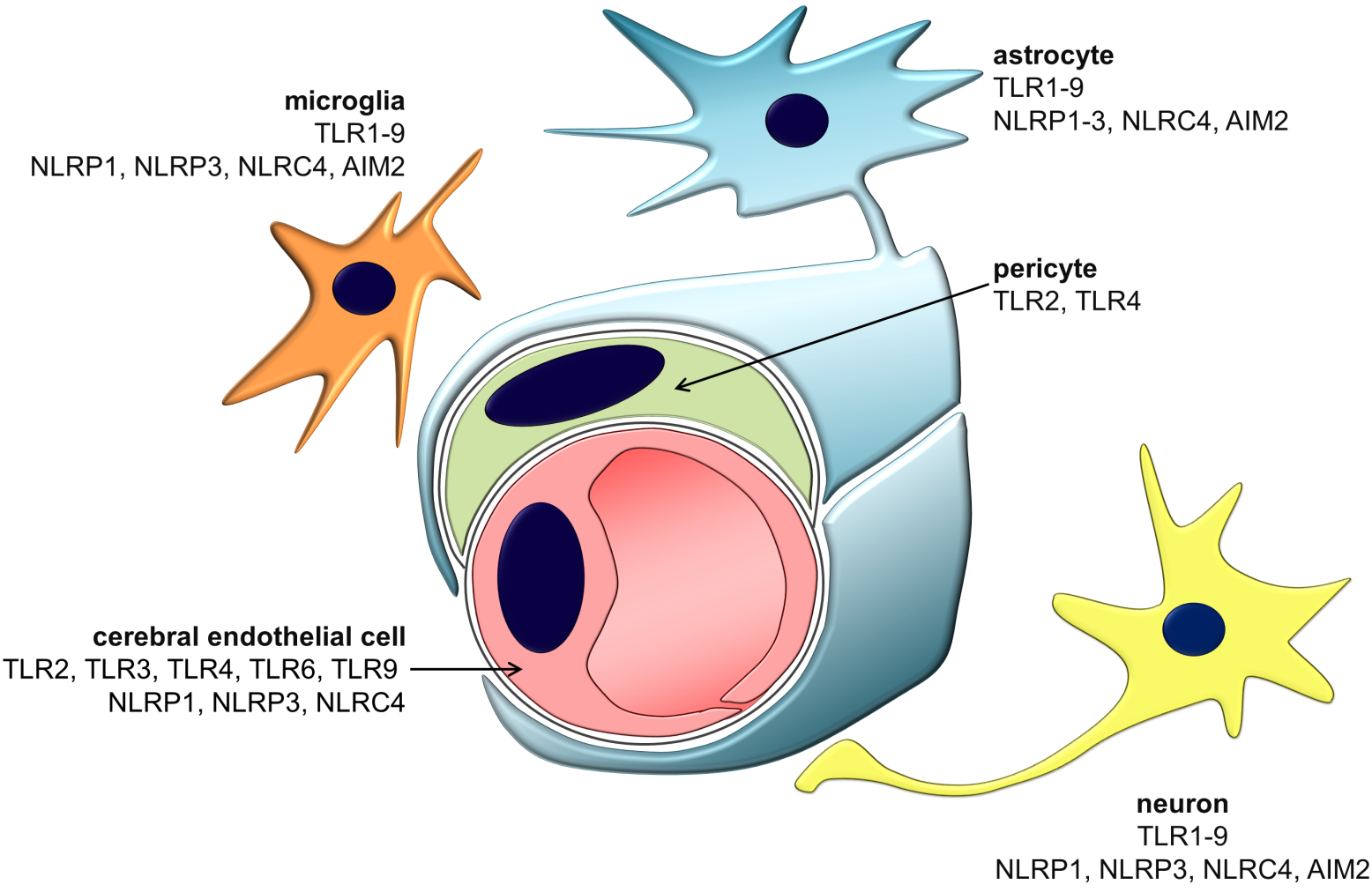
915

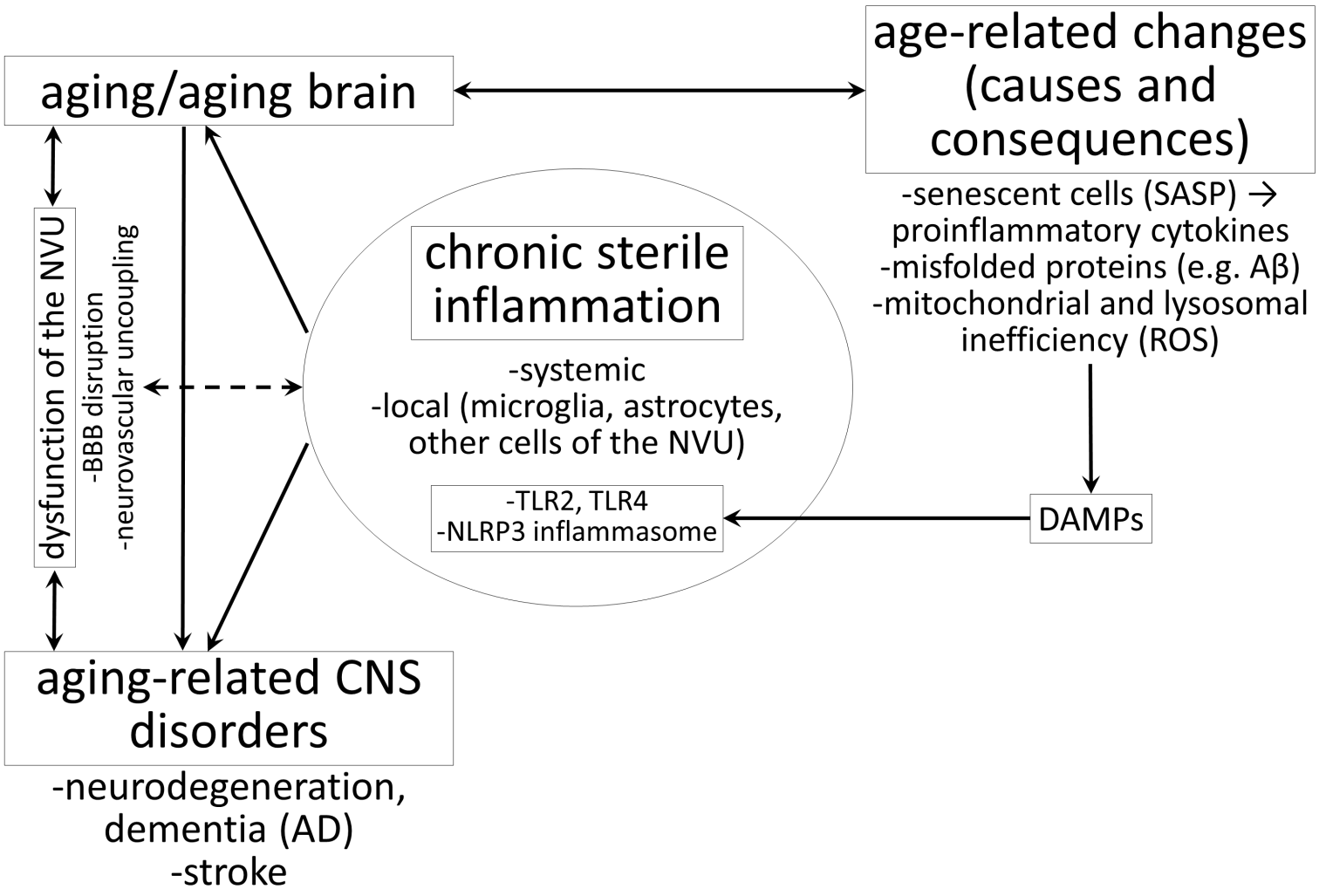
916 **Table 2.**

Pattern recognition receptor	Cell or tissue	Regulation or function	Remarks	Reference
TLR2	**mouse** brain (mononuclear phagocytes)	mRNA ↑ in aging		(82)
	mouse brain	mRNA ↑ in plaque-associated brain tissue of AD model mice		(51)
	human brain (hippocampus, entorhinal cortex, superior frontal gyrus, post-central gyrus)	mRNA ↑ in aging and AD	modest ↑ in AD relative to the aged brain which shows robust ↑ compared to young	(25)
	rat hippocampus (microglia)	mRNA ↑ in postoperative cognitive dysfunction in senile animals		(88)
	mouse microglia	involved in both amyloid-β uptake and inflammatory cytokine production		(72)
	human brain (neurons)	protein ↑ in PD		(36)
	neurons (**mouse** cortex)	protein ↑, proapoptotic in ischemia		(140)
	rat brain (vasculature)	protein ↑, activation in ischemic stroke		(155)
TLR4	**mouse** brain	mRNA ↑ in aging		(82)
	mouse brain	mRNA ↑ in plaque-associated brain tissue of AD model mice		(51)
	human brain (hippocampus, superior frontal gyrus, post-central gyrus)	mRNA ↑ in aging and AD	modest ↑ in AD relative to the aged brain which shows robust ↑ compared to young	(25)
	mouse microglia	involved in both amyloid-β uptake and inflammatory cytokine production		(118, 131)

	microglia (**mouse** striatum)	TLR4 knockout: neuroprotective in ischemia		(71)
	neurons (**mouse** cortex)	protein ↑, proapoptotic in ischemia		(140)
	rat brain (vasculature)	protein ↑, activation in ischemic stroke		(155)
	primary **mouse** astrocytes	activation in response to α-synuclein		(115)
other TLRs	**mouse** brain	TLR1,TLR5, TLR7 mRNA ↑ in aging		(82)
	mouse brain	TLR9 mRNA ↓ in aging		(82)
	mouse brain	TLR5, TLR7, TLR9 mRNA ↑ in plaque-associated brain tissue of AD model mice		(51)
	mouse hippocampus	TLR3: suppression of neural plasticity and inhibition of memory retention		(107)
	mouse microglia	TLR9: involved in amyloid-β uptake		(33, 34)
NLRP1	**rat** hippocampus	NLRP1 inflammasome activation in aging		(90)
	human hippocampus (neurons); **mouse** models of AD	protein ↑ in AD, role in neuronal pyroptosis and cognitive impairment		(74, 139)
	mouse cortical neurons (cell culture, stroke model), **human** brain	protein ↑ in ischemia/reperfusion, role in neuronal cell death and behavioral deficits		(42)
NLRP3	**mouse** microglia	NLRP3 inflammasome activation in AD models	only microglia isolated from aged mouse brains secrete IL-1β in response to fibrillar amyloid-β	(62, 65, 152)
	mouse cortical neurons (cell culture, stroke model), **human** brain	protein ↑ in ischemia/reperfusion, role in neuronal cell death and behavioral deficits		(42)
	rat primary astrocytes, **mouse** substantia nigra	protein ↑, activation of NLRP3 inflammasome in PD models		(87)
other inflammasome components	**human** brain (hippocampus)	caspase-1, IL-1β, IL-18 mRNA ↑ in aging and AD	modest ↑ in AD relative to the aged brain which shows robust ↑ compared to young	(25)

	rat hippocampus (neurons)	caspase-1, P2X7 receptor, pannexin-1 protein (involved in NLRP1 and NLRP3 inflammasome activation) ↑ in aging	(90)
	mouse cortical neurons (cell culture, stroke model), **human** brain	ASC, caspase-1 (pro- and active form), IL-1β (pro- and active form), IL-18 (pro- and active form) protein ↑ in ischemia/reperfusion	(42)
	rat primary astrocytes, **mouse** substantia nigra	caspase-1, IL-1β (pro- and active form) protein ↑ in PD models	(87)
	primary **rat** astrocytes, **human** neocortex	NLRC4 inflammasome activation in astrocytes: ↑ amyloid-β levels in neurons; NLRC4, ASC protein ↑ in sporadic AD	(84)


astrocyte
TLR1-9
NLRP1-3, NLRC4, AIM2
microglia
TLR1-9
NLRP1, NLRP3, NLRC4, AIM2
pericyte
TLR2, TLR4
cerebral endothelial cell
TLR2, TLR3, TLR4, TLR6, TLR9
NLRP1, NLRP3, NLRC4
neuron
TLR1-9
NLRP1, NLRP3, NLRC4, AIM2


aging/aging brain
age-related changes (causes and consequences)
-senescent cells (SASP) → proinflammatory cytokines
-misfolded proteins (e.g. Aβ)
-mitochondrial and lysosomal inefficiency (ROS)
dysfunction of the NVU
-BBB disruption
-neurovascular uncoupling
chronic sterile inflammation
-systemic
-local (microglia, astrocytes, other cells of the NVU)
-TLR2, TLR4
-NLRP3 inflammasome
DAMPs
aging-related CNS disorders
-neurodegeneration, dementia (AD)
-stroke