

# Mitochondria Dysfunction and Microglia Activation in Aging: A Mini Review

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## Abstract

Microglia are the resident immune cells in the central nervous system and critical in maintaining the homeostatic microenvironment to support neuronal survival. The microglial activation in the CNS can be divided into two opposite types: M1 pro-inflammation and M2 anti-inflammation phenotypes. Animal study suggests that during aging M1 microglia increase, accompanied by a decrease in M2 microglia. Intracellular Reactive Oxygen Species (ROS), produced by mitochondria, are known as mediators of M1 microglial activation. This review firstly discusses the changes in microglia phenotype and mitochondria function during aging. Secondly, it summarizes the current researches that report the association between the dysfunction of microglial mitochondria and alteration of microglial immune responses.

## Introduction

Microglia are the resident mononuclear phagocytes in the Central Nervous System (CNS), accounting for 5~20% of the total glial cells [1]. Microglia were firstly observed by Rio-Hortega early in the last century, who described microglia as a non-neuronal and non-astrocytic cells in the CNS with an elongated soma bearing two or more branched processes [2]. Upon immune stimuli or CNS injury, microglia rapidly change their morphology and migrate to the inflamed site to proliferate. Microglia can phagocytose dead, dying or viable neurons and release cytokines and neurotrophic factors to maintain the homeostasis of the CNS microenvironment and support neuronal survival [3]. However, as animals age, microglia appear to respond to environmental stimuli by increasing their propensity to pro-inflammatory responding [4]. Such change contributes to the increased production of pro-inflammatory cytokines and the Reactive Oxygen Species (ROS), generating a chronic mild inflammatory microenvironment in CNS [4].

The free radical theory of aging proposed that aging is a result of the accumulation of defects in macromolecules caused by free radicals. During aging, the functionality of mitochondria is decline, resulting in high ROS generation [5,6]. As a second messenger, intracellular ROS can modify gene expression by regulating the inflammatory-related kinase cascades and transcription factors [7]. In general, the triggering of inflammation response is directly related to intracellular ROS generation, until cell death occurs [7]. Considering the roles of ROS in aging and immune regulation, the review discusses the relationship between microglial mitochondria dysfunction and immune property alteration, which implicates the mechanism of aging-induced alteration of microglial phenotypes.

## Physiology of Microglia

In healthy CNS, microglia exist in a ramified morphology. These “resting” microglia act as surveying cells that continuously and actively scan the microenvironment by moving their thin and branched process, making these cells the first line of defense in response to an exogenous threat [8]. Maintaining microglia in a relatively quiescent state is, at least in part, due to the low expression of activation-associated molecules (CD46, major histocompatibility complex-II (MHC-II) and CD11b) [9,10] and specific inhibition signals derived from neurons (CD200 and CX3CL1) [11-13]. CD200 is an anti-inflammatory glycoprotein expressed on neuronal surface and functions by activating CD200R receptor on microglia [14]. Ablation CD200 increases the expression of microglial activation molecules, MHCII, CD11b and CD45 [15, 16]. *CX3CL1* is a neuron-associated chemokine which acts on a specific receptor CX3CR1 on microglia. CX3CL1-CX3CR1 signaling plays an important role in the regulation of microglial basal motility and maintenance of microglia in a non-neurotoxic quiescent [17]. Other factors, such as GABA neurotransmitter, CD22 and microRNA-124, have also been known to decrease microglia activity. GABA expressed by neurons and astrocytes suppresses the inflammatory pathways mediated by NF $\kappa$ B and P38 MAPK in microglia [18]. CD22 released by neurons inhibits the P38 and P44/42 MAPK signal pathways through binding with CD45 on microglia [19,20]. The microRNA-124 is largely expressed in microglia, which reduces the microglial activity and the ensuing inflammatory molecular cascades through restricting SPI1 (PU.1) and C/EBP- $\alpha$  transcription factors [21].

In the injured CNS, microglia are transformed from a resting to an activated state and recruited to the injured site. Activated microglia exhibit amoeboid morphology and can perform various functions such as cytotoxic response, immune regulation, tissue repair, and neuron regeneration.

Activated microglia can be classified into M1 phenotype (pro-inflammatory) or M2 phenotype (anti-inflammatory) depending on the activation pathway and their functions. M1 microglia is classically activated by toll-like receptors ligand or INF- $\gamma$ . Its activation is associated with the production of various pro-inflammatory cytokines, including tumor necrosis factor-alpha, interleukin-(IL-) $1\beta$ , NO, ROS and proteases [7,22]. These pro-inflammatory mediators are essential to enhancing host defense. However, due to the release of pro-inflammatory cytokines and various neurotoxic mediators, the activation of M1 microglia causes collateral damage to healthy cells and tissues [23,24]. Moreover, activated M1 microglia express CD80, CD86, and MHC class II on the cell surface [1], enabling M1 microglia to present antigens to T cells. The “reprime” or reactivated T cells in injured areas exacerbate the lesion by epitope spreading [25].

Compared to M1 microglia, M2 microglia are typically considered to be healing cells. M2 microglia exhibit beneficial immunological effector functions through antagonizing M1 microglia pro-inflammatory responses and releasing the neurotrophic factors, i.e., insulin-like growth factor 1 (IGF-1) and glial cell line-derived neurotrophic factor, to promote neuronal survival and growth [26]. M2 microglia can be activated via alternative activation and acquired deactivation.

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Alternative activation is limited to the activation state induced by IL-4 or IL-13 and is closely associated with M2 genes that promote immune resolution, tissue recovery, and extracellular matrix reconstruction [26]. Acquired deactivation is another state to mitigate acute inflammation and is induced by up taking apoptotic cells or exposure to the anti-inflammatory cytokines such as IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ) [26].

The molecular factors present within the microenvironment play a crucial role in mediating microglial activation phenotype. It remains unclear whether the two types of microglia can coexist. Nevertheless, the two types of microglia can transit into each other in different contexts [27,28]. A well-regulated M1/M2 microglia transition is critically associated with the immune homeostasis in CNS.

### Changes in immune properties in aged microglia

During aging, microglia show morphological changes and an increased inflammatory activity. Three dimensional morphological analysis of individual microglia in the cortex shows that microglia in old mice have fewer ramification and possess fewer branches compared to young mice [29]. The live imaging also exhibits that the phenotype of aged microglia involves alterations in their morphology and dynamic behavior [30]. For example, young microglia responds to extracellular ATP, an injury-associated signal, or to a focal tissue injury, through increasing their motility and extending their ramifications [30]. In contrast, aged microglia responds to ATP application through the retraction of their processes and reduction in their motility [30]. Additionally, the disaggregation of aged microglia from the injury site is significantly slower than young microglia, indicating that aged microglia respond to injury with a higher and more sustained level of activation [30].

The increased activation of microglia in the healthy aged brain has been reported in studies with different species [31-34]. For example, during normal aging of the monkey brain, MHC II expression are increased in an age-dependent fashion [31]. The study on 3- to 4- and 18- to 20-month-old mice suggests that less than 3% of the microglia isolated from young brains exhibit MHC II expression, whereas, approximately 25% of microglia isolated from aged brain have MHC II expression on the cell surface [34]. In addition to *MHC II* expression, olders exhibit higher expression of scavenger receptor CD68, CD11b and CD11c integrins, Toll-like receptors and co-stimulatory molecule CD86 [35]. This indicates that the induction of inflammation-response genes (i.e., MHC II components, toll-like receptors, complement, and downstream signaling factors) is upregulated in aged microglia [36]. On the other side, attenuation of neuronal control of microglial activation is recognized to enhance the activity of aged microglia [11,37]. It has been reported that the expression of CD200 mRNA and protein are reduced in the hippocampus and substantia nigra of older rats [16,37-39]. Moreover, the lower level of CX3CL1 protein is detected in the brain of aged (18-22 mo) BALB/c mice compared to adult (3-6 mo) controls [40]. While the lipopolysaccharide (LPS)-induced reduction of CX3CR1 is recovered in microglia of adult mice, the protracted down regulation of CX3CR1 is found in aged mice [40]. The decreased CD200 and CX3CL1 expression are coupled with the increased expression of IL-1 $\beta$ , IFN- $\gamma$ , CD86, MHCII and ICAM-1 (M1 microglial activation) [16,37-39] and decreased expression of TGF- $\beta$  (M2 microglia activation) [11,39-41].

Consistent with the increased M1 activation markers on aged microglia, there is also evidence of increased pro-inflammatory cytokine release by aged microglia. For example, elevated basal level of IL-6 has been found in the aged mouse cerebellum, cerebral cortex, and hippocampus [42] and in mixed primary glial cultures from aged rats [43]. This elevation of IL-6 with age corresponds to a decrease in IL-10 secretion [44]. Besides, studies have shown significant alterations in the responses of microglia to stimulatory events in aged rodents

when compared to their young and middle-aged counterparts. For instance, upon LPS stimulus, the microglia from aged mice produces greater amounts of ROS, whereas the microglia from young mice predominately secrete NO [45]. The aged microglia secrete significantly higher levels of IL-6, IL-1 $\beta$  and TNF- $\alpha$  [45-47] but the lower level of TGF- $\beta$  [40] than young microglia with LPS treatment. The change in cytokine production in aged microglia is thought to occur through activating pro-inflammatory kinase cascades [48] and increasing promoter methylation of cytokine DNA [49]. The evidence proves that decreased methylation of the IL-1 $\beta$  gene promoter basally or following systemic LPS is associated with increased production of IL-1 $\beta$  mRNA and intracellular IL-1 $\beta$  [49].

### Dysfunction of mitochondria in aging

Aging is considered to be a biological process caused by accumulated damage that leads to cellular dysfunction and death. In the 1950s, Denham Harman first proposed the Mitochondrial Free Radical Theory of Aging, which is one of the widely accepted theories in the current aging research [5,6]. According to this theory, the production of ROS during normal metabolic activity damages cellular macromolecules such as DNA, proteins, and lipids. The electron transport chain, or respiratory chain, in the inner mitochondrial membrane is the main location of ROS production. The electron transport chain consists of five complexes. Electrons move across the complexes generating electrochemical proton gradient to drive the synthesis of ATP. Some electrons prematurely leak from the electron transport chain, producing ROS. Specifically, complexes I and III are considered the major sources of ROS [50]. mtDNA is located in the matrix of the mitochondrion, adjacent to the electron transport chain. Lack of histone-like protective proteins makes mtDNA extremely vulnerable to the ROS. Intracellular ROS progressively damage mtDNA, causing mutation. The accumulation of mtDNA mutation leads to abnormalities of mitochondrial respiratory chain proteins which results in more ROS production [51,52], ultimately culminates in cellular dysfunction and death.

The increased levels of mtDNA damage during aging, including deletions, duplications and point mutations, have been found in various tissues of human, monkeys and rodents [53-56]. Many studies have showed the linkage between mtDNA mutation and aging. For example, the homozygous knock-in mice that express a proofreading-deficient catalytic subunit of mtDNA polymerase  $\gamma$  (PolgAmut) exhibit the accumulation of mtDNA mutations through lifespan and premature onset of the aging phenotypes including kyphosis, weight loss, reduced alopecia, heart disease, progressive hearing loss, and decreased spontaneous activity [58-60]. Oppositely, treatment of mitochondria-targeted antioxidant, SkQ1, in PolgAmut mutate mice can delay the appearance of aging phenotype and significantly increase their lifespan [61]. Besides, a mouse model with inducible mitochondrial-targeted *PstI* endonuclease expression (mito-PstI), an restriction enzyme to cleave mtDNA, shows an accelerated thymic involution and an increased kyphosis and fur graying expression after induction of *PstI* expression [57].

It is known that increased ROS triggers the replication of mtDNA [62]. Mitochondrial transcription factor A (TFAM) can regulate mtDNA replication and maintenance [63]. In addition to the control of mtDNA copy number, TFAM is also involved in mtDNA repair. TFAM can bind to damaged mtDNA and inhibits the in vitro incision activity of 8-oxoguanine DNA glycosylase, uracil-DNA glycosylase, apurinic endonuclease 1, and nucleotide incorporation by polymerase- $\gamma$  [64]. The increased expression of TFAM has been reported in skeletal muscle from aged healthy subjects [65,66] and in cerebellum, liver, and kidney from aged rats. Although the TFAM over expression increases in mtDNA copy number, the capacity of respiratory or mitochondrial mass are not enhanced [67]. Thus, it can be speculated that increased

TFAM in aged tissue is used to repair the defects in mtDNA, which is necessary for maintaining energy metabolism. .

### Microglial mitochondria dysfunction and microglial immune response

The intracellular ROS has been known to act as second messengers to amplify pro-inflammatory response [7]. Intracellular ROS in microglia triggers a set of signaling cascades that includes mitogen-activated protein kinase (MAPK), Nuclear Factor (NF)  $\kappa$ B, and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways. This results in increased proinflammatory cytokine release and neurotoxic microglial activity [68-70]. Studies have suggested that the responses of microglia to external immune stimuli are affected by intracellular ROS in microglia. For example, the application of Mito-TEMPO, a mitochondria-targeted superoxide dismutases, to reduce LPS-induced intracellular ROS in BV-2 murine microglia significantly inhibits the LPS-stimulated activation of MAPK and NF- $\kappa$ B pathways and downregulates the mRNA levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and iNOS [70]. On the contrary, siRNA knockdown superoxide dismutase in rat primary microglia increases the LPS-induced NF- $\kappa$ B transcriptional activity and mRNA levels of TNF $\alpha$  and IL-1 $\beta$  [71]. Except for the initiation of inflammatory cascades, ROS also drive inflammasome activation in microglia. A recent study indicated that the induction of intracellular ROS in primary microglia by mitochondrial complex-I inhibitor, rotenone or tebufenpyrad, triggers NLRP3 inflammasome signaling, which promotes the proteolytic processing of pro-caspase-1 to caspase-1 p20 and pro- IL-1 $\beta$  to IL-1 $\beta$  [72]. The mito-apocynin, an inhibitor of NADPH oxidase, attenuates inflammasome activation in microglia by decreasing superoxide generation [72].

Mitochondria dysfunction in microglia not only enhances the microglial classical pro-inflammatory activation but also inhibits the alternative anti-inflammatory activation. The study in primary microglia proves that IL-4-induced arginase activity, and expressions are inhibited by 3-NP and rotenone treatment. The 3-NP and rotenone not only inhibits IL-4-induced antagonization of IL-6 and TNF- $\alpha$  production but also disrupt IL-4-induced IGF-1 production [73]. Although it is still unknown whether the changes of immune responses in aged microglia is caused by mitochondria damage and ROS accumulation during aging, a previous study has proved that the prevention of mitochondria dysfunction during the aging process ameliorates the microglial pro-inflammatory responses in aged brain. The study shows that aged transgenic mice with human TFAM over expression exhibit better activity of mitochondrial complex I-V enzymes and lower mtDNA oxidation in the hippocampus as compared with wild-type animals [74]. After intraperitoneal LPS injection, the level of IL-1 $\beta$  in the whole brain of aged TFAM transgenic mice is significantly lower than aged wild-type mice [74]. The results suggest the relationship among mitochondrial dysfunction, ROS and microglia response during the process of aging.

### Conclusion

Aging is a complex and multi factorial process involving diverse molecular and cellular mechanisms. It is clear that a wide change in both adaptive and innate immune systems occurs during aging, including the microglia. Since microglia play a critical role in several aging-related neurodegenerative diseases, it is important to gain a better understanding of how the microglia immune properties change during aging in the field of immunology and neuroscience. The review summarizes the current studies that suggest the dysfunction of mitochondria leads to alterations in the microglia immune functions. During aging, mitochondria dysfunction causes microglia prone to be activated through classical pathway, which will increase the detrimental effects in CNS and probably induce the neuropathology of neurodegeneration. Molecular strategies for targeting antioxidants to mitochondria may increase the tendency of the activation of the

alternative pathway in microglia and thus, produce the neuro protective effects in the aged brain.

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