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Microglia and Neurodegenerative Diseases

Microglia are the primary immune cells, the so-called professional phagocytes, of the mammalian brain. Microglia continually scan the entire brain in their resting state and when neurotoxic pathogens and damaged cellular machinery are detected, they are activated to eliminate them[1,2] (Fig. 1). In healthy neurons, microglia also contribute to essential cellular functions such as neurogenesis, neurodevelopment, and neural plasticity[3,4]. Of similar importance are the roles microglia have in neurodegenerative diseases characterized by aggregates of pathogenic, misfolded proteins such as Parkinson's disease (PD; Lewy bodies) and Alzheimer's disease (AD; Aβ plaques). Microglia-mediated neuroinflammation is a common pathophysiology in PD and AD human brains, as well as in in vivo animal model brains[1,2,4-9]. Moreover, recent genetic and transcriptomic studies revealed microglia-associated signaling cascades as critical players in AD pathogenesis[1,7]. This newsletter discusses microglia activation and neuroinflammation in PD and AD.

PD is characterized by a loss of dopamine (DA) neurons in the substantia nigra pars compacta, a corresponding loss of dopaminergic (DAergic) terminals throughout the basal ganglia, and Lewy bodies which are composed primarily of intracellular α-synuclein aggregates. Lewy bodies are a pathophysiological hallmark of PD and trigger microglia-mediated neuroinflammation, which itself is another neuropathological correlate of PD[1,2,8,10] (Fig. 1). In the brains of PD patients, Lewy body neurites are associated with activated microglia and α-synuclein deposits are correlated with inflammatory markers. *In vitro* cell culture and *in vivo* animal models also demonstrate a relationship between α-synuclein aggregates and microglial activation[2,8,10]. *In vitro*, α-synuclein concentration-dependently activates microglia and microglia can also remove α-synuclein aggregates through phagocytosis, though the exact relationship between the two processes remains unclarified[10]. Over-expressing wild-type or mutant α-synuclein in mice produces early microglial activation. *In vivo* over-expression of wild-type human α-synuclein in mouse PD models display early and sustained microglial activation and increased release of multiple pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha [TNF-α], interleukins [IL] such as IL-1b, IL-6, and IL-18) and chemokines[2,6]. This inflammatory response is restricted to midbrain DA neurons that comprise the nigrostriatal pathway, even though α-synuclein expression encompasses the entire brain[2,6,8]. In the MPTP mouse model of PD, the MPTP toxin provokes microglial-mediated inflammation with the expected increase in pro-inflammatory cytokines prior to MPTP-induced DAergic neuron neurodegeneration[8].

The classic histopathological hallmarks of AD are extracellular Aβ plaque deposits and intracellular hyperphosphorylated tau tangles. Microglia activation and release of pro-inflammatory cytokines, and the subsequent neuroinflammation are also pathological hallmarks of AD[1,2,7] (Fig. 1). Studies support neuroprotective and neurotoxic roles for microglia and associated proteins in AD. On the one hand, pathogenic Aβ soluble species and insoluble plaques undergo phagocytosis, and at later stages of AD, microglia encircle and compact plaques (Fig. 1). Compaction may prevent further deposition onto the existing plaque[1,2,7]. Additionally, recent genetic studies reveal multiple AD risk genes, many of which are related to microglia either directly or indirectly (e.g., triggering receptor expressed on myeloid cells 2 [TREM2] and complement receptor 1 [CR1] genes)[1,7]. TREM2 is necessary for phagocytic signaling and microglia-mediated neuroprotection, and its dysfunction exacerbates AD pathophysiology[1,2,7]. In healthy neurons, CR1 and other members of the innate immune system's complement signaling cascade assists microglia in phagocytosis, removal of immune complexes, and negative regulation of the complement system[11,12], as well as the removal of excess synapses (i.e., synaptic pruning) during development, maturation, and refinement of neuronal circuitry[13-15]. Neurotoxicity occurs when the complement proteins are up-regulated and activated in human AD brains and in mouse AD models[13-16]. Interestingly, the up-regulation occurs prior to Aβ plaque deposition and when microglial dysfunction is observed[13-16]. These observations suggest that dysfunctional microglia and complement proteins contribute to early synapse loss due to a malfunctioning of the physiological synaptic pruning process, resulting in microglia engulfing mature, functional synapses[1,2,16] (Fig. 1). AD-associated synapse loss and synaptic dysfunction are rescued following inhibition of complement signaling[1,16]. These data are noteworthy because synapse loss is an early pathological event in AD (and similar diseases) and robustly correlates with cognitive decline[17,18]. Microglia dysfunction is exacerbated with continuing plaque formation

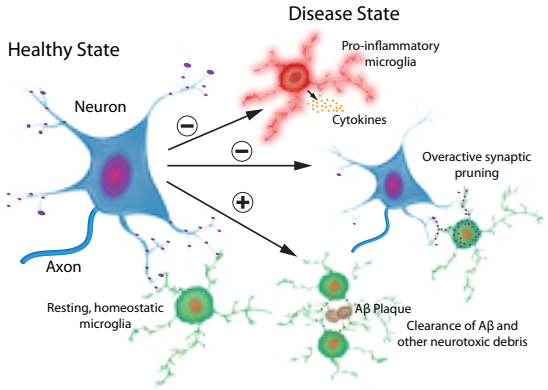

Fig. 1. Physiological and pathological microglial processes. Microglia-mediated signaling cascades trigger neuroprotective/neurotrophic and neurotoxic effects on healthy and diseased neurons.

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as *in vivo* two-photon microscopy in different AD mouse models shows that as Aβ plaque numbers increase, microglia function decreases[19].

Microglia and microglia-associated proteins are implicated in tau pathology. Through an unknown pathway, activation of complement proteins appears to worsen tau pathology in *in vivo* mouse models[20,21]. Moreover, over-activation of microglia in an *in vivo* transgenic tau mouse model worsened the onset and progression of tau pathology[22,23]. An *in vivo* model of mutant tau pathology suggests that microglial uptake of toxic tau species and exosomal release of tau contributes to cell-to-cell transmission of the pathogenic tau[7,24].

Summary

Microglia display a diverse set of physiological and pathological functions in the brain which presents multiple challenges. In neurodegenerative diseases, an open question is when and how the switch from anti- to pro-inflammatory signaling occurs. Understanding the sequence and timing of alterations in the two opposing microglial phenotypes is a complicated undertaking which requires new research tools[25]. Indeed, a recent advance in live cell imaging with calcium indicators offers an opportunity to better understand the in vivo role of microglia in neuron function and dysfunction[26]. Cytoskeleton, Inc. offers a variety of tools, including live cell imaging reagents, purified cytoskeletal proteins, functional assay kits, and Signal-Seeker™ kits for quantifying post-translational modifications of target proteins to decipher microglia-regulated signaling cascades in the mammalian brain.

Actin Biochem Kits

Actin Biochem Kit	Reactions	Cat. #
Actin Binding Protein Spin-Down Assay Biochem Kit rabbit skeletal muscle actin	30-100 assays	BK001
Actin Binding Protein Spin-Down Assay Biochem Kit human platelet actin	30-100 assays	BK013
Actin Polymerization Biochem Kit (fluorescence format) rabbit skeletal muscle actin	30-100 assays	BK003
G-Actin/F-actin In Vivo Assay Biochem Kit	30-100 assays	BK037

Tubulin Kits and Reagents

Product	Reactions	Cat. #
Tubulin Polymerization Assay Biochem Kit (absorbance format), porcine tubulin	24-30	BK006P
Tubulin Polymerization Assay Biochem Kit (fluorescence format): 99% pure porcine tubulin	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038

Product	Amount	Cat. #
Anti-Tubulin Polyclonal Ab (sheep host)	2 x 100 µl reconstituted	ATN02
Paclitaxel (Taxol)	10x100 µl	TXD01
Tau protein: bovine brain	1x50 ug	TA01
Tubulin protein (fluorescent HiLyte 488): porcine brain	5x20 ug	TL488M

G-LISA Activation Assay Kits

Product	Reactions	Cat. #
G-LISA RhoA Activation Assay Biochem Kit (colorimetric format)	96	BK124
G-LISA RhoA Activation Assay Biochem Kit (luminescence format)	96	BK121

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Live Cell Imaging Products

Description	Ex / Em	Amount	Cat. #
SiR-Actin Kit Includes SiR-Actin and Verapamil	630 / 680 nm	50 nmol	CY-SC001
SiR-Tubulin Kit Includes SiR-Tubulin and Verapamil	630 / 680 nm	50 nmol	CY-SC002
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol each	CY-SC006
SiR-DNA Kit Includes SiR-DNA and Verapamil	630 / 680 nm	50 nmol	CY-SC007
SiR-Lysosome Kit Includes SiR-Lysosome and Verapamil	630 / 680 nm	50 nmol	CY-SC012