Microglia Modulate Neurodegeneration in Alzheimer's and Parkinson's Diseases

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Dementia is a rapidly rising global health crisis that silently disables families and kills lives and livelihoods around the world. However, to date, no early biomarkers or effective therapy exist. It is now clear that brain microglia are more than bystanders or amyloid phagocytes; they can act as governors of neuronal function and homeostasis in the adult brain. Here, we highlight the fundamental role for microglia as tissue-resident macrophages in neuronal health. Then we suggest how chronic impairment in microglia-neuron crosstalk may secure the permanence of failure of synaptic and neuronal function and health in Alzheimer's and Parkinson's diseases. Understanding how to assess and modulate microglia-neuron interaction critical for brain health will be key to developing effective therapies.

It is becoming increasingly clear that amyloids are not necessarily the smoking gun of neuronal dysfunction and cognitive decline in neurodegenerative diseases. This is displayed in centenarians with apparent good cognitive health but whose brains are populated with amyloids (*1*). Epidemiological data also point to the concept of cognitive reserve where certain individuals appear more resilient to pathological changes in their brains (*2*). Hence, a challenge in neurodegeneration is to understand how certain aging brains successfully maintain proper neuronal function despite chronic amyloid build-up, whereas others do not. Several genetic studies suggest that microglia, the major tissue-resident macrophages of the brain, may be key in determining this success (*3*, *4*). Emerging data in developing, adult and diseased brains collectively suggest that microglia are critical to neuronal homeostasis and health. These altogether raise a major question of whether, and which, microglia-neuron interactions may be impaired in Alzheimer's disease (AD) and Parkinson's disease (PD) to confer neurodegeneration. Insight into this will enable novel methods to assess and modulate microglia-neuron interaction in the aging brain and allow for a desperately needed focus expansion from clearing amyloids alone in biomarker and target engagement efforts.

Here, we propose several pathways by which microglia may contribute to neuronal dysfunction in AD and PD. In AD, we focus specifically on complement-mediated synaptic loss and suggest lipid-centric mechanisms in microglia-neuron crosstalk at the synapse. In PD, we discuss the potential roles of tissue-resident macrophages in the brain and gut in modulating amyloid spreading and toxicity, including lysosomal degradation pathways.

Microglia are Central to Neuronal Function and Health

Genomic and proteomic tools indicate that microglia, akin to other tissue-resident macrophages, are functionally diverse depending on context, i.e., brain region, age, health and metabolic needs (region-specific microglial heterogeneity and their interdependence on neuronal microenvironment were recently reviewed in (5)). Microglia constantly survey their local milieu for signals of danger and injury, including pathogens, disease stimuli and apoptotic neurons. In addition to their immune functions, microglia crucially support brain development, for example, by sculpting neuronal synapses in the developing brain. In the adult brain, microglia perform multiple functions, including monitoring changes in neuronal activity, modulating learning and memory, and acting as local phagocytes and damage sensors in the brain parenchyma (*6-12*).

Many of these microglia-neuron interactions are mediated by cell-cell signaling pathways including purinergic signaling, cytokines, neurotransmitters and neuropeptides (5). These functions often require high energy expenditure and mitochondrial metabolism, for which microglia display metabolic flexibility in acute hypoglycemic states (9). An intriguing question is whether chronic mitochondrial dysfunction observed in AD and PD (13, 14) impairs microglia's ability to be metabolically flexible, and thus properly monitor and govern neuronal function and health. Interestingly, in models of neuronal mitochondrial defect and neurodegeneration (15), glia accumulate lipid droplets, which can modulate macrophage function. Alternatively, lipid droplets accumulate with aging in microglia (16), raising the hypothesis of whether improper lipid metabolism in aged microglia underlie susceptibility to neurodegeneration in AD and PD. In support of this, various AD and PD risk factors, including Triggering receptor expressed on myeloid cells 2 (TREM2), Apolipoprotein E (ApoE), GBA1 and Steroyl-CoA-Desaturase (SCD), have been found to modulate lipid metabolism, lysosomal pathways, and microglial metabolic fitness (3, 17-23).

How Microglia May Mediate Synaptic Loss in AD

In AD, synaptic loss and dysfunction are region-specific, early and strongly correlate with cognitive impairment (24). Pre-fibrillar oligomeric β -amyloid (A β) and/or tau accumulate on synapses and induce pathological synaptic dysfunction and loss (25-30). More than half of the identified genetic risk factors in AD are expressed by myeloid cells (31). These altogether raise the need to understand how mutations in risk genes and alleles impair the crosstalk between microglia, the major myeloid cell population in the brain, and neurons at the synapse.

Multiple studies in animal models of AD suggest dysregulation of neuroimmune signaling pathways on synapses involving classical complement cascade (C1q, C3), TREM2, phosphatidylserine (PS) and ApoE (Fig. 1). These raise the intriguing question of whether accumulation of local pathological proteins on synapses dysregulates neuron-glia interactions critical for synaptic health. For example, pathological Aß or tau accumulated on synapses upregulate complement (C1q or C3) in surrounding microglia and astrocytes, and promote microglial engulfment of synapses (26, 29, 32, 33). Blocking activation of the classical complement cascade in AD mouse models using genetic or antibody-based means protected synapses from loss and dysfunction and downstream memory loss (26, 29, 32-34), suggesting microglia-synapse pruning pathway as a potential therapeutic target. What remains unclear is whether this pruning mechanism is, at least in the beginning, a beneficial process that then becomes dysregulated in a chronic manner to impair the very neurons they were trying to save. Microglial engulfment of synapses likely involves a fine balance of 'eat me' and 'don't-eat me' signals (35). Because many of the microglial functions including synaptic pruning appear activity-dependent (7, 8, 12, 36), it will be important to determine whether neuronal hyperactivity observed in early AD mouse models (30, 37, 38) instructs microglia to aberrantly engulf synapses (36). Insight into the pathways that regulate pruning, as well as the specific signals that guide microglia to engulf synapses, will be crucial for identifying potential therapeutic targets against cognitive decline and for developing biomarker candidates to quantify microglial dysfunction in relation to synaptic loss.

Another biologically and therapeutically important question is to understand whether particular synapses are targeted for elimination by microglia. Proteomic studies in synaptosomes from human and mouse AD brains highlight synaptic mitochondrial dysfunction (14, 39). However, whether complement factors including C1q and C3b target specific, i.e., dysfunctional and/or damaged, synapses is not known. Lipid signaling in neuron-glia interplay may be a crucial determinant. For instance, TREM2, a key damage sensor on microglia (40), was shown to mediate synaptic refinement in the developing mouse hippocampus (41). A proposed ligand for TREM2 is exposed PS on the outer leaflet of neuronal membranes (19). Exposed PS on synapses may thus be

an 'eat-me' signal for microglial TREM2 in AD (42, 43). Furthermore, recent elegant work in a model of tauopathy suggested a potential link between TREM2 and microglia-mediated synaptic elimination in AD (44). The AD risk variant of TREM2 was associated with less synaptic localization of C1q and less engulfed synaptic elements by microglia, as opposed to the common variant of TREM2. These data altogether suggest a potential role for microglial TREM2 in sensing damaged synaptic membranes in AD, perhaps through PS signaling. Gangliosides, a family of sialic acid-containing lipids enriched in the brain, have also been postulated to be crucial for A β -induced synaptic dysfunction in mice (45). GM1 ganglioside-bound A β is enriched on membranes in early AD brains (46). Moreover, anti-GM1 ganglioside antibodies have been shown to fix complement on neuronal membranes, and the same antibody targeting C1q that was used in AD models (26, 29) ameliorated anti-ganglioside antibody-mediated neuronal injury in a mouse model of acute motor axonal neuropathy (47). These studies altogether raise the question of whether brain gangliosides contribute to synaptic loss in AD and complement-mediated synaptic engulfment by microglia.

An additional neuroimmune and lipid-related pathway to consider is ApoE. Previous research suggests a possible link between astrocytic ApoE and microglial synaptic pruning: ApoE allele-dependent rate of synaptic engulfment by astrocytes appears important for normal synapse plasticity (48). This rate appears to slow down during aging, thus potentially increasing vulnerability of synapses to complement-mediated pruning by microglia. Interestingly, ApoE ε 4 has been associated with enhanced synaptic localization of pathological A β in human AD brains (25). Furthermore, ApoE has been recently shown to bind C1q and to regulate activation of the classical complement cascade (49). Altogether, these data suggest a role for ApoE at the synapse in astrocyte-neuron-microglia crosstalk. This is of high interest, especially in light of cell-type specific dysregulation of ApoE in AD and critically linked cholesterol and other lipid metabolic pathways.

Studies involving TREM2 and ApoE, two of the major risk factors in late-onset AD, altogether suggest that lipid metabolism in microglia is a determinant to how well our brain's immune system can respond to the

chronic build-up of amyloids. For example, TREM2-deficient microglia fail to properly metabolize lipids in a chronic demyelination paradigm (21). TREM2 also appears to be a key regulator of ApoE, a major lipid transporter (18). Interestingly, ApoE has been shown to transport excess lipids from hyperactive neurons to lipid droplets in astrocytes where they are metabolized, suggesting a key role for ApoE in ameliorating neuronal hyperactivity-induced lipid toxicity (20). It will thus be interesting to elucidate the relationship between amyloid-related neuronal hyperactivity and lipid metabolism in astrocytes and microglia, and how this relationship falters in the aged brain or in brains with mutations in AD risk genes.

Glial Cells as Modulators of aSyn Toxicity in PD

PD pathology is often accompanied by distinct accumulation of alpha-Synuclein (α Syn) in astrocytes and microglia (50), which also has recently been described as a prominent feature in PD mouse models (51). Furthermore, manipulating microglia-astrocyte crosstalk alleviates PD-like pathology in α Syn-aggregation models (52). These studies suggest a direct role for glia in mediating neurotoxicity of α Syn. Notably, neuronophagia—microglial phagocytosis of neurons—is evidenced in PD by accumulation of neuromelanin within microglia (50). This could point to a synaptic engulfment mechanism analogous to what is observed in AD, given that synapses in PD tissue are enriched for pathological α Syn aggregates (53). However, whether complement and microglia mediate synaptic loss in PD is not known.

From a genetics perspective, the link between PD and microglia is, at first sight, not as apparent. Familial synucleinopathies can be tied to the expression levels of total neuronal α Syn (54). However, in sporadic PD, neurodegeneration strongly correlates with certain bioactive forms of α Syn rather than total levels of α Syn (55). Furthermore, three "synucleinopathies"—PD, Dementia with Lewy Bodies (DLB) and Multiple System Atrophy (MSA)—are all characterized by amyloid α Syn burden but interestingly show distinct brain region-specific patterns of amyloid accumulation and neuronal dysfunction (56). This remarkable region-specific pattern of α Syn spreading is thought to be caused by a "prion-like" spread of specific extracellular α Syn

aggregates or "disease strains", analogous to prion disease (57). These findings collectively raise the critical need to understand what governs the brain region-specific formation and local abundance of these "disease strains". Recent genetic studies in PD suggest enrichment of genetic risk factors in sphingolipid metabolism (58). The risk genes *GBA1*, *SMPD1*, *GALC*, *ASAH1*, *CTSD*, *SPTLC1* and *SLC17A5* altogether point to dysfunctional lysosomal degradation of aggregates as crucial determinants in disease manifestation. *GBA1*, in particular, has received spotlight as one of the biggest risk factors for PD (17), notably for its potential role in creating toxic variants of α Syn aggregates through defective lysosomal function (59). It is important to note that past studies have investigated these genes in neuronal context, but recent mouse brain single-cell atlases indicate that most of these genes, including *Gba1*, are expressed by microglia rather than neurons (60).

Taken together with the "prion-like" spread of α Syn aggregates, an interesting question is whether, and how, glia are involved in blocking or promoting the transmission of extracellular α Syn aggregates throughout the different brain regions, thus contributing to region-specific vulnerability in synucleinopathies. In support of this, the only known uptake receptor for extracellular α Syn aggregates, LAG3 (*61*), is mainly expressed by microglia (*60*). Furthermore, in a recent synucleinopathy model, disruption in microglial clearance of extracellular α Syn via autophagy led to dopaminergic neuron degeneration (*62*), whereas in another study, oligodendrocytes were shown to selectively enhance the toxicity of exogenous α Syn aggregates after uptake (*57*). These altogether demonstrate that glial uptake and processing is critical in modulating the activity of α Syn. Thus, while glia can act in a physiological context as the "waste disposal system" of expelled misfolded aggregates by neurons, this is potentially a double-edged sword in disease: the uptake and processing of non-toxic α Syn by glia could actually be the process that generates the disease-specific toxic "strains" through autophagy and defective lysosomal degradation (Fig. 2). Pathological modification of extracellular α Syn by microglia mediated by imbalances in sphingolipid metabolism could be a crucial determinant for chronic α Syn dysfunction leading to PD, DLB or MSA.

Looking Beyond the Brain in PD: Macrophage-Neuron Signaling in the Gut

Emerging preclinical and genetic data suggest that the enteric nervous system (ENS) or 'little brain of the gut' may be implicated in PD pathology. αSyn aggregation has been observed in the ENS, from where it potentially spreads to the brain in a cell-to-cell transsynaptic manner (63). In support of this, truncal vagotomy in mice prevented transmission of pathological α Syn into the brain and related motor deficits, suggesting the vagus nerve as a potential conduit of α Syn (64). Notably, gut-injected α Syn not only induced phosphorylation of aSyn in enteric neurons, but also stimulated the production of CX3CL1 and CSF1, ligands that bind to CX3CR1 and CSF1R on gut macrophages (65). A recent study highlighted a unique type of tissue-resident macrophages in the ENS that are, analogous to microglia in the brain, long-lived and crucial for neuronal survival and function of the gut (66) (Fig. 3). These ENS-resident gut macrophages express high levels of transcripts involving vesicular trafficking and endolysosomal pathways including GBA1 and LRRK2. Mutations in LRRK2 are a common cause of autosomal dominant PD; however, how LRRK2 contributes to αSyn pathology and PD-like symptoms is unclear (67). Of note, macrophages deficient for Lrrk2 show higher proteolytic activity and contain higher levels of lysozyme (68), suggesting that LRRK2 regulates lysosomal function and phagosome maturation. Furthermore, LRRK2 interacts with the actin-remodeling factor WAVE2 to regulate phagocytosis specifically in macrophages (69). Thus, it will be interesting to assess how gut macrophages become affected in PD and regulate clearance of pathological α Syn along the gut-brain axis.

Conclusion

In AD and PD, failure of the tissue-resident macrophages in our brain and gut to sense dysfunctional neurons may lead to the pathological dismantling of neuronal homeostasis and function. Studies from both patient tissues and animal models pinpoint glia as more than cleaners and phagocytes of amyloids; they govern and modulate neuronal health. Therefore, it will be critical to develop methods to monitor glia-neuron crosstalk in living brains, and assess which ones are relevant to disease. Employing amyloids, which are pathological hallmarks of neurodegeneration, as biomarkers and diagnostics is undoubtedly critical. However, adding specific neuroimmune modulators has the potential to change how we diagnose and treat, particularly during early stages of disease when relatively few neurons may be affected but certain neuroimmune markers or relevant microglial cell states may be detectable, for e.g., in cerebrospinal fluid (70) or by brain imaging. Furthermore, macrophage-neuron crosstalk in the ENS may present an early opportunity to intervene in PD. In therapy, early screening for risk factors and preventive application of drugs (like in cardiovascular disease with statins) that stabilize amyloidogenic proteins (gamma-secretase modulators, aggregation inhibitors, anti-sense oligonucleotides or antibodies) with modulators of neuroglial pathways (potentially through targeted modulation of lipid metabolism and enhancing the autophagosome-lysosome system) could be employed.

Finally, whether neuroinflammation is beneficial or detrimental depends on the context. For e.g., classical complement cascade helps to reduce amyloids but also mediates synapse loss (*26*, *34*), and enhancing TREM2 activity may prove detrimental in tangle-bearing brains but not in amyloid-burdened ones (*44*). Therefore, in chronic, multifaceted and multifactorial diseases such as AD and PD, we need to carefully consider the local milieu when assessing function and impact. It will be important to employ distinct strategies at various disease stages to target appropriate biological processes for effective treatment.

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FIGURE LEGENDS

Fig. 1. Complement-Mediated Synapse Loss by Microglia in Alzheimer's Disease

Potential mechanisms leading to complement-mediated synapse elimination by microglia. A: Whether microglia target specific synapses is not known. Neuronal hyperactivity and/or mitochondrial dysfunction observed in AD mouse models and patients may lead to upregulation of complement factors (C1q, CR3) in microglia to target the dysfunctional synapses. B: In AD mouse models, pathological A β or tau accumulated on gangliosides may upregulate complement signaling pathways through membrane damage sensors like TREM2, resulting in synaptic elimination by microglia. C: Recognition of exposed phosphatidylserine (PS) on synapses by myeloid TREM2 may lead to synaptic engulfment by microglia. Alternately, lipid transporter Apolipoprotein E (ApoE) potentially ameliorates hyperactivity- or membrane damage-induced lipid toxicity by transporting excess lipids to lipid droplets in astrocytes and microglia.

Fig. 2. Glial Cells as Modulators of Alpha-synuclein Toxicity in Parkinson's Disease

Schematic representation of glia in the central nervous system (CNS) contributing to spreading of toxic alphasynuclein. Extracellular alpha-synuclein is internalized by microglia, potentially via LAG3 receptor-mediated uptake, and processed via endo-lysosomal machinery. Defective autophagy and impairment in lysosomal degradation could potentially modulate the internalized alpha-synuclein aggregates and expel them after failed degradation. These newly modified "disease strains" then may contribute to differential region-specific pathology observed in DLB, MSA and PD.

Fig. 3. Macrophage-Neuron Crosstalk Along the Gut-Brain Axis in Parkinson's

Schematic of the enteric nervous system (ENS) harboring a unique type of tissue-resident macrophages that are long-lived and provide critical neurotrophic support. These macrophages express transcripts involving vesicular trafficking and endolysosomal pathways, including *Lrrk2* and *Gba1*, suggesting a potential role for uptake, processing and clearance of alpha-synuclein aggregates in PD. Pathological modification of

extracellular α Syn by gut macrophages could be a potential modifier of alpha-synuclein spreading between enteric ganglia in the ENS or from ENS to CNS via the vagus nerve.





