NEUROSCIENCE

The link between neuroinflammation and the neurovascular unit in synucleinopathies

Qing Wang[1]†*, Jialing Zheng[1]†, Sven Pettersson[2,3,4,5], Richard Reynolds[6,7]*, Eng-King Tan[8]*

The neurovascular unit (NVU) is composed of vascular cells, glial cells, and neurons. As a fundamental functional module in the central nervous system, the NVU maintains homeostasis in the microenvironment and the integrity of the blood-brain barrier. Disruption of the NVU and interactions among its components are involved in the pathophysiology of synucleinopathies, which are characterized by the pathological accumulation of α-synuclein. Neuroinflammation contributes to the pathophysiology of synucleinopathies, including Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies. This review aims to summarize the neuroinflammatory response of glial cells and vascular cells in the NVU. We also review neuroinflammation in the context of the cross-talk between glial cells and vascular cells, between glial cells and pericytes, and between microglia and astroglia. Last, we discuss how α-synuclein affects neuroinflammation and how neuroinflammation influences the aggregation and spread of α-synuclein and analyze different properties of α-synuclein in synucleinopathies.

INTRODUCTION

The neurovascular unit (NVU) is a structural and functional multicellular module composed of vascular cells, glial cells, neurons, and extracellular matrix (ECM). Endothelial cells, pericytes, and vascular smooth muscle cells make up the vascular layer. Glial cells in the NVU include microglia, oligodendrocytes, and astrocytes. Disruption of the NVU and the interactions among its components are involved in the pathogenetic mechanisms of central nervous system (CNS) disorders (*1*).

Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) are all characterized by abnormal and misfolded α-synuclein aggregates and can be collectively grouped together as synucleinopathies. Recent studies have shown that the form and location of α-synuclein misfolding and aggregation differ between synucleinopathies and may be responsible for the clinical distinctions (*2*). PD and DLB both exhibit pathological α-synuclein inclusions in neurons, which are called Lewy bodies and Lewy neurites, while a hallmark feature of MSA pathology is oligodendroglial cytoplasmic inclusions.

Neuroinflammation is an important contributor to the pathogenesis and progression of the majority of neurodegenerative disorders. Our previous studies discussed the relationship of immune-inflammatory axes with α-synuclein and the gut microbiota in neurodegenerative diseases (*3*). However, very few articles have comprehensively summarized the effects of neuroinflammatory factors on single-cell types in the NVU and how they interact.

In this review, we discuss how the components of the NVU are disrupted as a result of neuroinflammation. We focus on the changes and mechanisms of each element of the NVU and provide details on how neuroinflammation alters cell-to-cell communication. Last, we review the interactions between α-synuclein and neuroinflammatory mechanisms. Understanding how different α-synuclein pathologies in synucleinopathies work in the NVU-related microenvironment could clarify their neuropathogenesis and clinical manifestations.

DISRUPTION OF THE NVU AND ITS ASSOCIATION WITH SYNUCLEINOPATHIES

The effect of neuroinflammation on vascular cells in the NVU

In the brain, cerebrovascular endothelial cells form tightly sealed monolayers linked by tight junctions and adherens junctions. Under normal conditions, the endothelial barrier can regulate the infiltration of immune cells into the brain and control macromolecule movement through specific transport processes. Under inflammatory conditions and infection, the endothelial barrier can be penetrated by activated mononuclear cells through diapedesis without disrupting tight junctions (*4*). This response of microvascular endothelial cells is therefore critical in promoting neuroinflammation. For example, the up-regulation of epithelial cell adhesion molecules that promote neutrophil and lymphocyte adhesion to the endothelium is a well-known feature of the inflammatory process (*5*). Moreover, alterations in blood-brain barrier (BBB) permeability caused by proinflammatory cytokine release directly and indirectly result in glutamate excitotoxicity, astrocyte and microglial activation, and free radical production (Fig. 1) (*6*).

The identity and specific molecular mechanisms by which proinflammatory factors affect endothelial cells are unclear. Wang *et al.* (*7*) found that the immune cytokine interleukin-1β (IL-1β) could easily diffuse through the BBB by decreasing the expression of tight junction proteins in endothelial cells and suppressing the

[1]Department of Neurology, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong 510282, China. [2]ASEAN Microbiome Nutrition Centre, National Neuroscience Institute, Singapore 308433, Singapore. [3]Karolinska Institutet, Department of Odontology, 171 77 Solna, Sweden. [4]Faculty of Medical Sciences, Sunway University, Subang Jaya, 47500 Selangor, Malaysia. [5]Department of Microbiology and Immunology, National University Singapore, Singapore 117545, Singapore. [6]Department of Brain Sciences, Imperial College London, Hammersmith Hospital Campus, Burlington Danes Building, Du Cane Road, London W12 0NN, UK. [7]Centre for Molecular Neuropathology, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 308232, Singapore. [8]Department of Neurology, National Neuroscience Institute, Singapore General Hospital, Duke-NUS Medical School, Singapore, Singapore.
†These authors contributed equally to this work.
*Corresponding author. Email: tan.eng.king@sgh.com.sg (E.-K.T.); richard.reynolds@ntu.edu.sg (R.R.); wqdennis@hotmail.com (Q.W.)

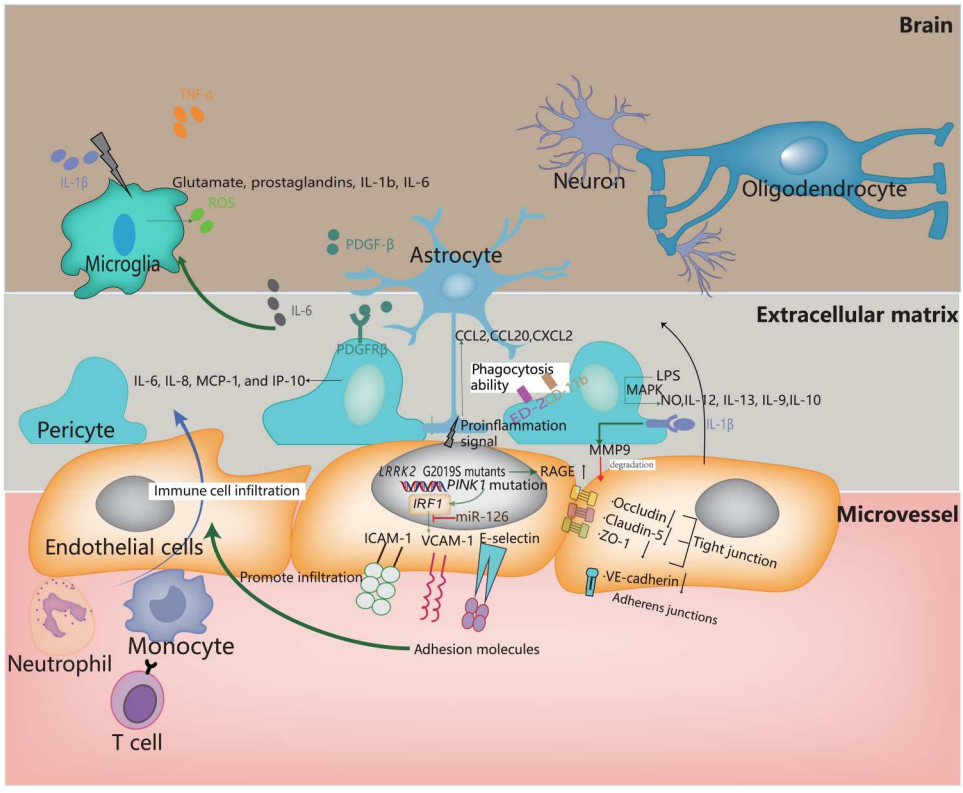

Fig. 1. Neuroinflammation in the NVU. In the NVU, endothelial cells form the BBB, the integrity of which is maintained by the vascular basement membrane. This vascular basement membrane constitutes a three-dimensional protein network consisting of ECM proteins. Under inflammatory conditions, the disruption of endothelial cells and degradation of tight junctions and adherens junctions promote the infiltration of immune cells into the BBB. Microglia release proinflammatory cytokines in response to the BBB disruption and the presence of interleukin-6 (IL-6) and IL-1β. Astrocytes can communicate with endothelial cells via CCL2 and CXCL2. After LPS stimulation, pericytes produce proinflammatory mediators such as NO and IL-10. ECM provides a stable structure in which the neuroinflammation signals in the NVU can be transmitted and spread quickly.

expression of sonic hedgehog in astrocytes to increase BBB permeability. In addition, after stimulation with IL-1β, astrocytes up-regulated the expression of proinflammatory chemokines, including CCL2, CCL10, CCL20, and CXCL2, which attracted immune cells from the peripheral circulation to the CNS.

Extensive evidence has shown that endothelial dysfunction is involved in synucleinopathies such as PD. In the PD brain, endothelial cells form clusters with degenerative morphology and contribute to the fragmentation of capillaries (*8*). Reduced expression of tight junction proteins, including occludin and zonula occludin-1, was observed in the brains of PD patients and in brain endothelial cells that were continuously stimulated with α-synuclein in vitro (*9*). Moreover, the interruption of tight junctions and messengers located in endothelial cells results in increased leukocyte infiltration, which occurs during the early stage of PD. Moreover, capillary structural damage and loss in the brains of PD patients result in the generation of abnormal capillary fragments and endothelial cell thickening and atrophy, which can be detected in the substantia nigra, cerebral cortex, and brainstem (*8*). The mechanisms underlying the initiation of endothelial cell impairment are currently unclear. However, there are some factors that influence immune activity in endothelial cells. For example, the growth factor neuregulin-1 has an anti-inflammatory effect on endothelial cells (*10*). In vitro, when endothelial cells were activated by IL-1β, neuregulin-1β down-regulated the expression of the adhesion molecules vascular cell adhesion molecule–1 and E-selectin, which reduced the attachment of immune cells such as neutrophils.

Pericytes show an elongated, stellate-shaped morphology with highly differentiated branches and tightly surround the endothelium, forming part of the barrier of the vascular structure. Here, we will focus on the roles of pericytes in neuroinflammation, which mainly include BBB disruption, leukocyte recruitment, and the secretion of neuroinflammatory mediators. Under physiological conditions, pericytes maintain BBB integrity. Pericyte impairment markedly disrupts vascular permeability in the diseased brain, which promotes leukocyte adhesion, transmission, and trafficking into the NVU (*11*). Because of contractile and cytoskeletal proteins, including α-smooth muscle actin, vimentin, desmin, myosin, and nestin, pericytes have contractile functions that affect leukocyte trafficking across the BBB and thereby promote neuroinflammation in the NVU (*12*). It has been reported that pericytes play important roles in other features of neuroinflammation. Four pathways involved in pericyte-mediated regulation of neuroinflammation have been identified. (i) After lipopolysaccharide (LPS) treatment, mouse brain pericytes released a series of immune mediators, including cytotoxic factors such as nitric oxide (NO), cytokines, and chemokines, among which IL-12, IL-13, and IL-9 were markedly increased. These immune mediators were dose-dependently

produced by pericytes in response to LPS, and this effect was generally modulated by mitogen-activated protein kinase signaling (*13*). (ii) Human brain pericytes produce matrix metalloproteinase 9 (MMP9) in response to IL-1β. MMP9 was shown to be partly responsible for increasing BBB permeability to sodium fluorescein following the disruption of adhesion molecules and tight junction molecule binding, such as that between vascular endothelial cadherin, occludin, claudin-5, and zonula occludin-1 (*14*). (iii) Rat brain pericytes respond to inflammatory mediators [e.g., tumor necrosis factor (TNF)], which promote cytokine (e.g., IL-6) release and thus play a role in both innate and adaptive immune response (*15*). (iv) Rat pericytes can increase expression of macrophage markers, such as ED-2 and CD11b, and have phagocytic abilities (*16*). In α-synuclein–overexpressing transgenic PD mouse models, pathological pericyte activation was shown to be accompanied by neuroinflammation (*17*). In conclusion, pericytes mediate neuroinflammation by releasing proinflammatory factors in response to inflammatory stimuli.

Pericytes are widely classified by their expression of platelet-derived growth factor receptor β (PDGFRβ), and up-regulated expression of soluble PDGFRβ has been found in clinical samples from patients with neurodegenerative diseases, suggesting that PDGFRβ is a biomarker of induced pericyte injury (*18*). When PDGFRβ on pericytes binds to PDGF-β released by endothelial cells, pericytes are recruited to blood vessels and initiate the dimerization, autophosphorylation, and activation of their receptors (*19*). This PDGFRβ signal transduction further shifts the proinflammatory reaction from gene to protein expression in the BBB. For example, the activation of immune response genes, which then stimulate the recruitment of immune cells, could be directly promoted in the brain. Using a human cytokine array, Gaceb *et al.* (*20*) demonstrated that PDGF-BB, an isoform of PDGF, increased the expression of cytokines and chemokines in pericytes, although these levels were significantly lower than the cytokine expression induced by LPS. PDGF-BB also reversed the activation of pericytes in 6-hydroxydopamine–induced mouse models of PD, thus emphasizing its role in regenerative processes (*21*). Overall, the main functions of pericytes in the CNS involve regulating microcirculation and vessel diameter, manipulating the integrity of the endothelial cell lamina, participating in the regeneration of vascular cells, and modulating neuroinflammation. In addition, pericytes can mediate endothelial cell proliferation, apoptosis, and degeneration, and the loss of pericytes leads to leukocytes entering the brain, which may modulate the inflammatory response (*22*). Overall, as a part of the microvasculature, pericytes are pivotal cellular mediators of NVU function in neuroinflammation.

The ECM also plays a critical role in mediating neuroinflammation and is composed of proteoglycans, glycoproteins, and glycosaminoglycans. Heparan sulfate, which is the covalent attachment of proteoglycans, may be associated with misfolded protein aggregation by changing their interactions with signaling proteins (*23*). The ECM of the NVU is a dynamic structure that is remodeled during neurodegenerative disorders, and microglia are activated by changes in the ECM properties and structure, leading to neuroinflammation in 6-hydroxydopamine–induced mice (*24*). MMPs are also the major components of the ECM, and these factors can up-regulate neuroprotective α6β4 integrin levels and alter the balance of degradation and deposition of proteins (*25*). The decrease of MMP1, which is one of the major enzymes that participate in ECM degradation, was observed in PD patients, indicating the disruption of ECM remodeling in PD (*26*). Disruption of endothelial cells, pericytes, and the ECM leads to the infiltration of immune cells and the subsequent generation of proinflammatory molecules in the NVU, which activates innate immune responses in the brain via astrocytes and microglia (*27*). For example, $CD3^+$ $CD8^+$ T lymphocytes and immunoglobulin deposition were found in the substantia nigra in PD patients (*28*). Therefore, once disruption of vascular cells in the NVU occurs, the homeostatic balance of the NVU is lost, which then leads to the glial activation and a proinflammatory state.

The role of glial cells in neuroinflammation in the NVU

The main cells responsible for neuroinflammation in the NVU are microglia and astrocytes. Microglia are classically divided into two major phenotypes: M1 and M2. The M1-like phenotype, which is defined as proinflammatory and damage inducing, is associated with the release of cytotoxic and proinflammatory factors, such as inducible NO synthase (iNOS), TNF, IL-1β, and interferon-γ (IFN-γ). In contrast, the M2-like phenotype is characterized by the production of neuroprotective and anti-inflammatory factors, including brain-derived neurotrophic factor, insulin-like growth factor-1, IL-4, and IL-10 (*29*, *30*). Under physiological conditions, astrocytes maintain the peripheral immune privilege of the BBB by restricting the infiltration of peripheral immune cells through their close interaction with blood vessels (*31*). In particular, when these cells sense neuroinflammation, reactive astrocytes can change into the A1 and A2 subtypes. A1 astrocytes have highly up-regulated expression of many classic complement cascade genes, including the inflammatory cytokines IL-1α, TNF, and IL-6, the complement component C1q, and cytotoxic effect molecules such as NO, all of which are generally destructive to neurons and other cells (*32*). Therefore, it is thought that A1 astrocytes might exert harmful effects. In contrast, A2 astrocytes have up-regulated expression of many immunomodulatory and neurotrophic factors, such as IL-10, transforming growth factor-β, and IL-4, and thus, it is thought that the A2 phenotype is protective (*33*). The role of oligodendrocytes in neuroinflammation has received less attention, but studies using human tissues have suggested that these cells may play an important role in regulating inflammatory processes by up-regulating the expression of anti-inflammatory genes through signal transducer and activator of transcription 6 (STAT6) signaling (*34*).

The cells of the NVU are interconnected and may produce a cascade of effects that result in further neuroinflammation and more serious impairment. Once an insult occurs in the NVU, microglia can quickly switch from the resting state to an activated state to maintain brain homeostasis. Various stimuli, such as pathological insults, injury, and peripheral inflammation, can activate resting microglia, which release cytotoxins and inflammatory factors (*35*). First, the interaction between microglia and environmental cues is essential for stimulating repair processes that are initiated by inflammation, but over time, the persistent microglial activation undoubtedly leads to further persistent inflammation. Evidence is emerging that this sustained microglial activation occurs in the NVU and cross-talk between neurons, astrocytes, and microglia has been shown to play important roles in neuroinflammatory responses.

NEUROINFLAMMATORY INTERACTIONS IN THE NVU AND THEIR ASSOCIATION WITH SYNUCLEINOPATHIES

Cross-talk between glial cells and vascular cells

Previous studies have indicated that microglial activation can occur in response to vascular damage. Microglial activation is likely to be mediated not only by the release of inflammatory factors but also by notable enlargement of both soma size and proximal processes that are juxtaposed with the vasculature. In addition, several studies supported a neuroprotective role for Wnt signaling in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–induced models by activating microglia and repairing midbrain dopaminergic neurons (*36*). Astrocytes produce Wnt growth factors, which are crucial for maintaining Wnt/Frizzled-1/β-catenin activity in endothelial cells, thereby protecting the BBB and NVU integrity. In PD, Wnt signaling in astrocytes is suppressed by neurodegenerative mechanisms in the brains, which is coupled with proinflammatory microglia, thereby resulting in dysfunctional neuron-astrocyte and astrocyte-endothelial cross-talk (*37*). In contrast, in an astrocyte/endothelial cell coculture model treated with proinflammatory molecules, adhesion molecules and inflammatory chemokines, especially CCL3, were increased on the luminal side, suggesting that extracellular inflammatory factors are crucial mediators that control immune cell infiltration into the NVU during neuroinflammation (*38*).

Cross-talk between glial cells and pericytes

One of the most important interactions between pericytes and glial cells is the up-regulation of neuroinflammatory cytokines. IL-6, which is a multifunctional cytokine, plays a vital role in BBB impairment and neuroinflammation by mediating the activation of microglia and astrocytes and subsequent neuronal damage. The cells in the NVU all express IL-6, and brain pericytes have been reported to express more IL-6 than brain endothelial cells, microglia, and astrocytes after being stimulated with TNF (*39*). Furthermore, IL-6 released from pericytes stimulated iNOS mRNA expression in microglia via the induction of TNF. In turn, activated microglia may promote pericyte apoptosis through reactive oxygen species production *(40)*. Moreover, analysis of cytokines/chemokines levels provided further evidence to elucidate the inflammatory interactions between activated microglia and astrocytes, pericytes, and endothelial cells (*41*). Coculturing microglia and astrocytes induced marked increases in the levels of cytokines/chemokines, emphasizing the importance of the coordinated secretion of chemokines and cytokines by microglia, astrocytes, and pericytes in neuroinflammatory reactions.

Astrocytes can secrete a variety of molecules that transduce different signals to brain microvessels and target pericytes. One study examined the relationship between astrocytes and pericytes and suggested that glutamic acid could influence brain pericyte contractility, survival, reproduction, and movement. Soluble factors released by astrocytes independently mediate pericyte contractility in vitro (*42*). MMPs are known to promote pericyte migration by degrading several components of the ECM, including vascular endothelial growth factor-A, intercellular adhesion molecule–1 (ICAM-1), cytokine-induced neutrophil chemoattractant–1, LPS-induced CXC chemokines, monocyte chemoattractant protein–1, macrophage inflammatory protein–3, and L-selectin, all of which have fundamental effects on vascular permeability and the inflammatory state (*43*). A recent study showed that connexin 30, which was previously thought to be predominantly expressed by astrocytes, was also expressed on pericytes, suggesting that connexin 30 may be a shared protein that forms gap junctions (*44*). Astrocytes are anatomically adjacent to pericytes, and these cells are tightly connected in neuroinflammatory conditions (*11*). Several studies have demonstrated BBB disruption in PD, which is characterized by increases in BBB leakage and peripheral inflammatory markers, prompting astrocyte and pericyte dysfunction that may contribute to PD pathology (*45*).

Cross-talk between microglia and astrocytes

Microglia and astrocytes both play important roles in neuroinflammation. Microglia transmit danger signals to other neighboring cells, especially astrocytes. For example, after in vitro LPS stimulation, activated microglia secrete multiple soluble mediators, such as IL-1, TNF, and complement component C1q, all of which induce A1-type astrocytes (*31*). A1-type astrocytes can then induce the death of neurons and oligodendrocytes by up-regulating the expression of a range of classic complement cascade genes. After being exposed to both LPS and α-synuclein oligomers, microglia in PD mice were activated and became proinflammatory, while astrocytes atrophied (*46*). However, gene expression profiling of a brain injury model revealed that early up-regulation of glial fibrillary acidic protein, which is a well-known marker of astrocytes, preceded the increase in allograft inflammatory factor 1, which is a marker of microglial activation. This finding suggested that the activation of astrocytes may occur before that of microglia and that astrocytes may play an important role in triggering microglial activation. Cells expressing both astrocyte and microglial markers were detected in DLB brains, indicating their shared characteristics in neurodegeneration (*47*). This interaction is maintained by neurotransmitters, the binding of ligands and receptors, soluble neuromodulators, transcriptional regulatory factors, and extracellular vesicles (EVs) shared between microglia and astrocytes (Fig. 2).

Neurotransmitters and purinergic receptors

Numerous neurotransmitter receptors are expressed by astrocytes and microglia, and it is widely accepted that these factors are an important communication route between glial cells and neurons, particularly with respect to the NVU. Among these signaling factors, extracellular adenosine 5′-triphosphate (ATP) released by glial cells and neurons plays a complex role in the CNS. ATP secreted by astrocytes causes microglial activation by binding to P2X7 receptor, which is a ligand-gated cation channel that drives IL-1β and TNF secretion by microglia (*48*). The nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome complex can respond to ATP-induced activation of the purinergic P2X7 receptor (P2X7R), which then triggers the cleavage of IL-1β, thereby activating IL-1. It is thought that astrocytes predominantly initialize such signals from the NVU and then trigger the microglial activation. Unexpectedly, ATP also alters microglial protrusions and promotes the release of adenosine, which then mediates neuronal activity and changes in animal behavior via the adenosine A_1 receptor (*49*). To demonstrate the specific role of P2X7R in neurodegenerative diseases, an Alzheimer′s disease transgenic mouse model was examined, and the results showed that ATP released from microglia and astrocytes

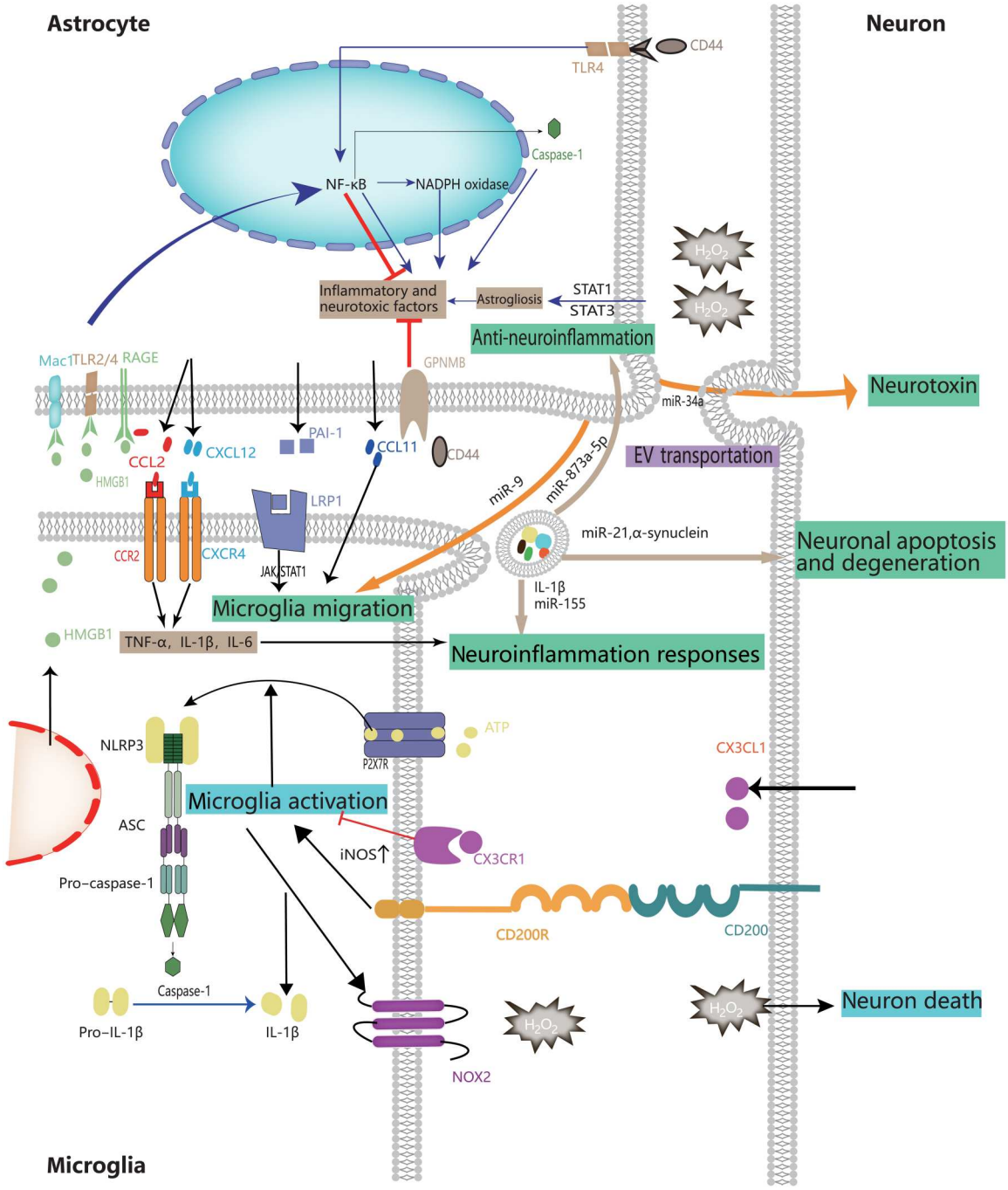

Fig. 2. The cross-talk among microglia, astrocytes, and neurons. The cross-talk between microglia and astrocytes includes neurotransmitters, ligand-receptor binding, soluble mediators, transcriptional regulatory factors, and EVs. EVs carrying miRNAs and α-synuclein can be transported among microglia, astrocytes, and neurons. The neuroinflammatory signals and corresponding mechanisms that lead to neurotoxicity have been shown.

acted on P2X7R, elevated the levels of chemokines, particularly CCL3, and promoted pathogenic T cell recruitment (*50*).

In addition, the excitatory neurotransmitter glutamate is released from astrocytes and has been reported to cause microglial activation in a TNF-α–dependent manner, resulting in neuroinflammation and unbalanced neurotoxicity (*51*). Histamine, a monoamine neurotransmitter, is known to regulate microglial histamine responses mediated by histamine receptor isoform 1, which is expressed mostly on astrocytes (*52*). These observations suggest that an unbalanced neurotransmitter system affects glial cross-talk (Fig. 3).

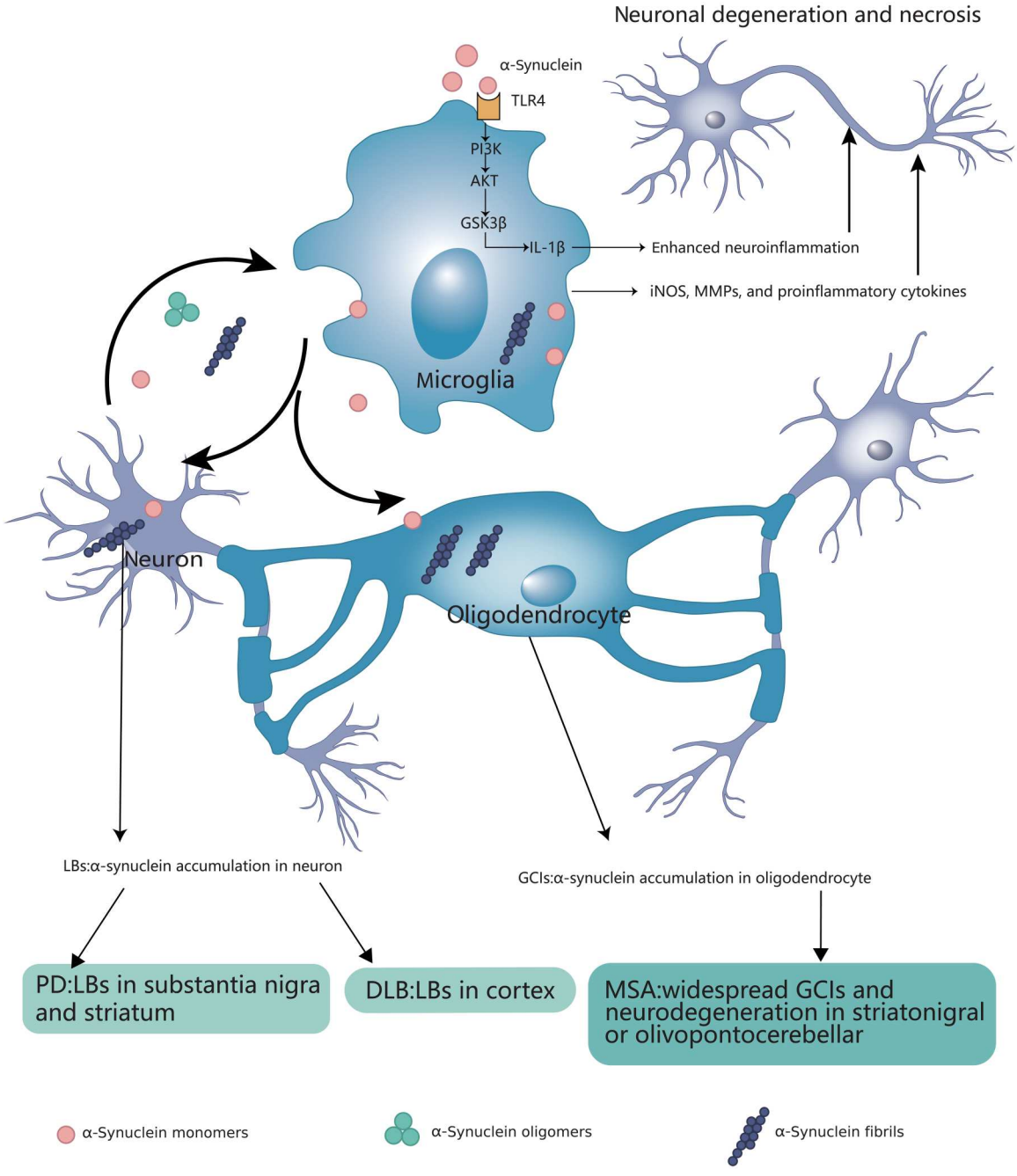

Fig. 3. The mutual effects of neuroinflammation and α-synuclein. Different sites of α-synuclein accumulation result in different synucleinopathies. Microglia can engulf fibrillar α-synuclein released from neurons and oligodendrocytes, and inflammation is activated, which further causes neuronal degeneration and necrosis.

Ligand-receptor binding

Ligand-receptor binding is important for the interaction of microglia and astrocytes, and this triggers neuroinflammatory signal transductions in NVU. Receptor for advanced glycation end products (RAGE) is a cell surface transmembrane receptor that can bind numerous ligands, many of which are pathogen-associated molecular patterns or damage-associated molecular patterns (*53*). RAGE is expressed mainly by neurons, microglia, and vascular endothelial cells in the context of neuroinflammation (*54*). Increased levels of RAGE have been observed in patients with PD, which suggests that RAGE plays a role in the mechanism of neurodegeneration in PD (*55*). In the MPTP model of PD, the combination of RAGE and its ligand, the S100 protein, activates nuclear factor κB (NF-κB) to upregulate the expression of proinflammatory mediators (*56*). In addition, eliminating RAGE on neuron can inhibit the phosphorylation of p38 in the substantia nigra and protect dopaminergic neurons from dying, suggesting that RAGE participates in the neuropathogenesis of PD by stimulating neuroinflammation (*57*).

RAGE activates astrocytes and microglia and increases the levels of the cytokines IL-1β and TNF (*58*). In essence, RAGE can induce neuronal death and promote neurodegenerative processes by activating neuroinflammation in NVU.

High-mobility group box 1 (HMGB1) is one of the most important damage-associated molecular patterns produced by microglia and astrocytes (*59*). Once microglia sense pathogens or tissue injury in the microenvironment, they rapidly release HMGB1 into the extracellular milieu. In the NVU, neurons, microglia, and astrocytes that express pattern recognition receptors, including Toll-like receptor 2 (TLR2), TLR4, RAGE, and macrodomain 1, are activated when they come into contact with HMGB1 (*60*). These cells subsequently induce the NF-κB pathway and produce NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase to activate the transcription of multiple inflammatory and neurotoxic mediators. In addition, HMGB1 can effectively promote the formation of the NLRP3 inflammasomes by activating NF-κB in astrocytes (*61*). In contrast, some receptors that bind to HMGB1, including CXCR7 and regulatory ribonuclease 1, are involved in the negative regulation of neuroinflammatory signaling by stimulating the release of anti-inflammatory mediators (*62*). Binding of HMGB1-CXCR7 inhibits neuroinflammation by modifying BBB integrity and preventing peripheral immune cell infiltration into the CNS (*62*). Overall, extracellular HMGB1 released from activated microglia and damaged neurons binds to the receptors on other cells in the NVU and triggers them to produce more HMGB1, forming a positive feedback loop that is associated with different neuroinflammatory pathways.

Ligand-receptor binding can also transmit signals inhibiting neuroinflammation. Glycoprotein nonmetastatic melanoma protein B, a transmembrane glycoprotein specifically expressed by astrocytes, directly binds to CD44 and plays a critical role in attenuating astrocyte-induced neuroinflammatory responses (*63*). In addition, CD44 is able to interact with TLR2 and inhibit the downstream NF-κB signaling, reducing neuroinflammatory responses (*64*). These two studies provide evidence that the ligand-receptor binding is also able to reduce neuroinflammation, thus highlighting the complexity of endogenous signaling pathways in the NVU.

Many studies have suggested that the nature of the communication between astrocytes and microglia is highly dependent on the exact combination of chemokines and ligands. Astrocytes can rapidly receive signals from vascular endothelial cells via cytokines (e.g., CCL2, CXCL1, CXCL2, and IL-6) through chemokine receptors on astrocytic end feet after peripheral LPS injection (*65*). In addition, astrocyte-derived CCL2 can induce microglial activation by binding to CCR2 on microglia, contributing to neuroinflammation in vitro (*66*). Emerging studies demonstrated the mutual activation of microglia and astrocytes through chemokines and their receptors in a neuroinflammatory environment. Subsequently, in response to the appropriate signal, CCL11 is secreted by astrocytes, which has been shown to promote microglial migration and induce microglia to produce reactive oxygen species (*67*). An in vitro study showed that the migration and proinflammatory state of microglia depended on the response to CXCL10, which is mainly expressed by astrocytes (*68*). These studies suggest that astrocytes transmit information to microglia after sensing signals from the peripheral immune system and the NVU. However, which cells are the initial triggers and the precise molecular mechanisms of this interaction are not yet clear. In conclusion, the cross-talk between astrocytes and microglia via ligand-receptor biding also plays a critical role in inflammation in the NVU.

Soluble protein mediators

Glia maturation factor (GMF) is a recently discovered proinflammatory protein that is associated with neuroinflammation and contributes to dopaminergic neurodegeneration. There is evidence that GMF, which is mainly secreted by astrocytes, microglia, and neurons, can activate mast cells to produce inflammatory cytokines and chemokines. GMF overexpression in glial cells leads to neuroinflammation and neurodegeneration. GMF deficiency regulates microglial polarization (*69*). In one study, it was shown that overexpression of GMF and 1-methyl-4-phenylpyridinium ion (+), the active metabolite of the neurotoxin MPTP, drove the release of MMP3, a multifunctional proinflammatory mediator from glial cells or neurons (*70*). The release of GMF via mast cell activation mediated the microglial activation and the expression of ICAM-1, which played an important role in immune-induced cell-to-cell adhesion. Moreover, GMF-dependent macrophage activation after MPTP treatment markedly up-regulated calpain expression in neurons, resulting in dopaminergic neuron damage and motor deficits in mice (*71*). However, genetic knockout of GMF by using CRISPR-Cas9 inhibited 1-methyl-4-phenylpyridinium–induced oxidative stress and suppressed the production of inflammatory mediators in BV2 microglia (*72*). GMF knockout mice had reduced pathological changes and improved motor behavioral performance (*71*). However, the secretory protein lipocalin-2 was reported to be expressed mainly by astrocytes and mediated microglial activation, neuronal cell death, and cognitive deficits in a glia-neuron coculture model. Therefore, astrocytes can also be a major source of neuroinflammatory mediators in the NVU. These studies suggest that the cells in the NVU can cooperate with each other by expressing brain-specific proinflammatory proteins and that these interactions can modulate pathological features.

Reactive oxygen species

A recent study suggested that hydrogen peroxide (H_2O_2) served as a direct signal connecting microglial activation with astrogliosis by regulating transcription factors. In LPS- and MPTP-induced mouse PD models, superoxide produced by NADPH oxidase 2 was the first cytotoxic factor released by activated microglia in response to neuroinflammation (*73*). Then, the H_2O_2 generated by microglial NADPH oxidase 2 activation spreads quickly into the cytoplasm of astrocytes, stimulated STAT1 and STAT3, and subsequently activated the immunological functions of astrocytes, ultimately leading to the expression of proinflammatory and neurotropic factors in astrocytes (*74*). Moreover, a microglia/neuron coculture model showed that H_2O_2 activation in microglia was a source of neuroinflammatory cytokines or chemokines that resulted in neuronal death (*75*). These observations suggest that microglial activation can regulate astrogliosis through reactive oxygen species (such as H_2O_2). Hence, factors that influence microglial activation can be used as potential useful targets for inhibiting astrogliosis and neuroinflammation.

Extracellular vesicles

Recently, EVs, which are carriers of many kinds of factors, including lipids, proteins, DNAs, mRNAs, microRNAs (miRNAs),

noncoding RNAs, and organelles, that shuttle from donors to recipient cells have attracted much attention in the context of the regulation and amplification of neuroinflammation (*76*). Most cells in the NVU have the ability to release EVs. Under neuroinflammatory conditions, EV production is distinctly increased because of the increased expression of proteins related to translation and transcription in EVs (*77*). Thus, the ability of EVs to facilitate intercellular neuroinflammatory communication in the NVU deserves further attention. EVs released from astrocytes carry molecules that are necessary and sufficient to trigger neuroinflammation. After inflammatory stimulation, astrocytes release vesicles containing miR-34a, which strengthens the sensitivity and vulnerability of dopaminergic neurons to neurotoxins by precisely targeting the antiapoptotic protein Bcl-2 (*78*). Exosomes containing miR-873a-5p that originate from activated astrocytes induced the microglial transformation of M1 microglia to the M2 phenotype and exert anti-inflammatory effects by decreasing the phosphorylation of extracellular signal–regulated kinase and NF-κB transcription factor p65 (*79*). These EVs and their contents are of particular interest because they can affect gene expression in recipient cells. EVs secreted from microglia can form a positive feedback loop because they can stimulate other microglia in an autocrine manner. Moreover, microglia release microparticles, which are a type of the EV family, that contain the proinflammatory molecules IL-1β and miRNA-155, resulting in the initiation of systemic immune responses. In addition, neurons treated with EVs from microglia undergo apoptosis and degeneration (*80*). Previous studies have reported that cytotoxic viral proteins such as HIV transactivating regulatory protein can stimulate astrocytes to produce EVs carrying miR-9, which are then taken up by microglia, resulting in enhanced microglial migration. The expression of miR-21 in EVs was detected, and miR-21 was located in damaged neurons in the injured brain regions where neighboring microglia were activated, revealing that miR-21 is a neuroinflammation mediator of cell-to-cell connections (*81*). Overall, microglia-astrocyte cross-talk forms a feed-forward loop, and factors that modulate the neuroinflammatory response of either cell type can easily magnify neuroinflammation.

Cross-talk between neurons and glial cells

Both CD200 and fractalkine (CX3CL1) are mainly expressed on neurons, and their receptors are specifically found on microglia; these factors are the two main inactivation signals and regulators of immune homeostasis in neurons and microglia (*82*). Through this fractalkine and its receptor, CX3CR1 interaction, neurons can communicate with microglia to mediate inflammatory responses and control cell survival in neurodegenerative diseases. Furthermore, the CX3CL1/CX3CR1 axis may protect against neuroinflammation by improving the transmission exosomal miR-124 to microglia and reducing the expression of proinflammatory genes in activated microglia to limit neuroinflammation and organize a neuroprotective response (*83*). Moreover, inhibition of this axis impairs the autophagy-lysosome degradation pathway by inactivating the NF-κB pathway (*84*). Treatment of $CX3CL1^{-/-}$ mice with the soluble cleaved form of fractalkine partially reversed cognitive and motor damage and restored neurogenesis, suggesting that the CX3CL1/CX3CR1 axis may mediate these protective effects. Increasing the production of the soluble cleaved form of CX3CL1 may be a possible therapeutic strategy for treating neurodegeneration. MiRNA-195 may act as an upstream regulator of the CX3CL1/CX3CR1 axis to mediate neuroinflammation in neurons and microglia (*85*). CD200 can mediate microglial activation by binding with microglial CD200R. Blocking the CD200-CD200R axis could promote microglial activation of microglia and increase the levels of iNOS and dopaminergic neurodegeneration in an α-synuclein–overexpressing PD model, showing that the midbrain is a distinctly sensitive region that responds to changes in CD200. There is evidence that CD200 knockdown can enhance peripheral inflammation and the lymphocyte response following microglial activation (*86*). However, in another LPS-induced animal model, peripheral inflammation occurred first (*87*). CD200-CD200R signaling transmitted the inflammatory signals from the peripheral nervous system to the CNS. Overall, the interaction between these ligands and receptors maintains the microglial resting state. Once these factors are inhibited, neuroinflammation becomes overactive, ultimately leading to neuronal damage. Excessive levels of these proinflammatory interactions in the NVU lead to neuronal injury. Dysfunctional and dying neurons also activate microglia, which, once activated, can produce additional mediators that affect neighboring neurons and astrocytes.

THE RELATIONSHIP BETWEEN NEUROINFLAMMATION AND α-SYNUCLEIN IN SYNUCLEINOPATHIES

Clinical evidence of neuroinflammation in synucleinopathies

PD patients have high levels of inflammatory indicators, including genetic biomarkers such as miRNAs and cell-free DNA, inflammasomes, and various inflammatory proteins such as IL-6, C-reactive protein, IFN-γ, and IL-1, in their plasma, blood, cerebrospinal fluid (CSF), and brain tissue compared with healthy controls (Table 1) (*88*). The presence of α-synuclein oligomers in $CD11b^{+}$ exosomes derived from microglia in the CSF of PD patients suggests that neuroinflammation regulates the translation and spread of α-synuclein (*89*). However, increased levels of many inflammatory factors, including inflammatory markers and autoantibodies, have been identified in the brain, CSF, and blood samples of DLB patients (*90*). The levels of inflammatory markers during different stages of the disease process were compared, and a more notable increase was reported in the early stage of DLB, suggesting that inflammation occurs early as a triggering event (*91*). Moreover, increased microglial activation was observed in DLB brains with mild disease status compared with those with moderate/severe disease status (*92*). In contrast, one study appeared to contradict the previous study, showing the inhibition of microglial activation in the postmortem brain tissue of 50 donors (*93*) despite increased DLB neuropathology. Regarding adaptive neuroinflammation, increased adaptive immune responses in DLB were shown to promote cerebral attachment of T lymphocytes. However, the systemic inflammatory profile and specific cell signaling mechanisms of inflammation in DLB deserve further investigation.

Recent work has provided evidence of the neuroinflammatory response in human disease and animal models of MSA. For example, proinflammatory cytokine levels were increased in the CSF, blood, and brain tissue samples of MSA patients compared to healthy controls (*94*). In addition, widespread microglial activation was shown in patients with the Parkinsonian phenotype of MSA by [^{11}C](*R*)-PK11195 positron emission tomography (PET) examination. Moreover, there was an active correlation between

Table 1. Different inflammatory biomarkers for distinguishing synucleinopathy disorders. ↑, increased expression; ↓, decreased expression; –, not significant; /, not measured.

Methods	Samples	Markers	PD	MSA	DLB	Reference
PEA	CSF	DNER	–	↓*	/	(*102*)
		β-NGF	–	↓*	/	
ELISA		NFL	–	↑*†	/	(*94*)
		CRP	–	↑*	/	
		SAA	–	↑*†	/	
		YKL-40	↓†	↑*	/	
Bead-based Luminex assays		C3	↑†	↓*†	/	(*88*)
Bead-based Luminex assays		CRP	–	↑*	/	(*90*)
		TNF-α	–	↑*	/	
		IL-1β	–	↑*	/	
		IL-6	–	↑*	/	
ELISA	Brain in temporal cortex	ICAM-1	–	/	↑†	(*93*)
	Brain in cingulate cortex	ICAM-1	–	/	↑†	
		CD-200	–	/	↓†	
Multiplex analysis	Serum	TNF-α	↑†	–	/	(*90*)
		CCL2	↑†	–	/	
		CXCL10	↑†	–	/	
		CCL7	–	↓†	/	
		IL-17	–	↓†	/	
PCR		miR-30-5p	↑†	↑*†	/	(*94*)
PCR	Blood	P2Y6R	↑†	↓*	/	(*96*)

*$P < 0.05$ versus PD. †$P < 0.05$ versus controls.

locally restricted neuroinflammation and increased numbers of α-synuclein inclusions (*95*). These studies of pathological samples have implicated circulating factors such as P2Y6R, miR-30-5p, and ICAM-1 in synucleinopathies, highlighting the pathogenic mechanisms initiated by different neuroinflammatory elements in the NVU (*96*).

Similarities and differences of neuroinflammation in synucleinopathies

Neuroinflammation has been observed in patients with PD, DLB, and MSA. Clinical and experimental studies suggest some similarities and also differences. Evidence of microglia activation has generally been demonstrated in these conditions. In MSA, there is proliferation and activation of microglia in the brain, causing degeneration and apoptosis of oligodendrocytes (*97*). Microglia activation has been shown in the brains of PD and DLB; however, the level of activation was different and occurred in varied brain regions. Microglia were recruited to nearby neurons in PD and DLB, while they were more aligned to oligodendrocytes in MSA (*98*, *99*). A number of astrocytes have been observed in close proximity to the oligodendrocytes containing glial cytoplasmic inclusion in MSA (*100*). In PD, reactive astrocytes act by increasing the release of proinflammatory cytokines such as TNF-α and IL-1 (*101*). Upregulation of NLRP3 has been commonly found in synucleinopathies. NLRP3 on microglia is co-located with CD68 in MSA brains (*102*). In PD, when NLRP3 on microglia was activated, it released proinflammatory cytokines and led to mitochondrial damage, which, in turn, promoted dopaminergic neuronal damage (*103*, *104*). The increased infiltration of peripheral immune cells suggests an additional role in the neuroinflammation of synucleinopathies (*105*). In the brains of MSA patients, $CD3^+$, $CD4^+$, and $CD8^+$ T cells were increased. $CD4^+$ T cells tended to differentiate into proinflammatory T helper cells, which secreted IFN-γ and TNF-α to enhance neuroinflammation (*106*).

Clinical studies suggest evidence of neuroinflammation in patients with PD, DLB, and MSA. Neuroinflammatory cytokines in blood, serum, CSF, and brain tissue have been shown to differentiate different types of synucleinopathies (Table 1). These observations suggest that different neuroinflammatory responses predominate depending on the specific disease (*102*). In PD mice model overexpressing human α-synuclein using recombinant adeno-associated virus vector, microglial activation could be observed in the early stage of pathology, indicating that inflammatory responses happened in the early stage in synucleinopathies (*98*). Animal models overexpressing α-synuclein preformed fibrils demonstrated that α-synuclein activated neuroinflammation by inducing the expression of TLR2 on microglia. In addition, TLR2 was identified to interact with myeloid differentiation primary response. This enhanced interaction further increased the release of NF-κB (*107*). It is important to remember that the α-synuclein overexpression models

unfortunately do not fully recapitulate human PD; thus, current observed differences in responses and mechanisms underlying the neuroinflammation among these diseases need to be interpreted with caution.

How α-synuclein affects neuroinflammation

The abnormal aggregation of α-synuclein and neuroinflammation are shared characteristics of synucleinopathies, as verified by in vivo and in vitro studies; however, how these factors interact with each other and generate combined effects has not been systematically demonstrated. Here, we will discuss how α-synuclein aggregation contributes to the activation of microglia, astrocytes, and pericytes and the effects of different forms of α-synuclein on neuroinflammation.

α-Synuclein can trigger microglia activation via three different ways: the pattern recognition pathway activation, protease secretion, and iron deposits. First, the aggregation of extracellular α-synuclein stimulates microglial activation and up-regulates the expression of proinflammatory factors such as TNF-α and IL-1β via the TLR4/phosphatidylinositol 3-kinase (PI3K)/AKT/glycogen synthase kinase 3β (GSK3β) signaling pathway (Fig. 3) (*108*). The miR-155 and Janus kinase (JAK)/STAT pathways in microglia and macrophages showed direct effects on proinflammatory responses to α-synuclein and α-synuclein–induced neurodegeneration in vitro (*109*). In addition, microglia exposed to α-synuclein fibrils exhibited increased IL-1β expression, which required inflammasome activation and participated in the recruitment of caspase-1 (*110*). On the other hand, the increases in iNOS, MMPs, proinflammatory cytokines such as ionized calcium binding adaptor molecule 1, CD68, and chemotaxis were observed in α-synuclein–treated primary rat microglia (*111*). MMP inhibition suppressed α-synuclein–induced microglial activation, revealing the important regulatory role of MMPs, especially MMP3, MMP8, and MMP9. In addition, these results showed that protease-activated receptor−1, a protease receptor located on the surface of microglia, was responsible for this activation (*112*). Iron deposits are specifically localized to microglia, while transferrin (TF), TF receptor 1, TF receptor 2, and ferroportin are increasingly expressed in dopaminergic neurons. Iron deposition in microglia could play a role in α-synuclein preformed fibril treatment-induced neuroinflammation as an early cellular response before neuronal death (*113*). In addition, the prolonged process of α-synuclein formation is associated with neuroinflammation.

α-Synuclein aggregates not only activate microglia releasing proinflammatory factors but also affect microglial motility. A recent study showed that neuron-derived α-synuclein can accelerate the migration of microglia toward neuron. It also found that α-synuclein can directly bind to integrin CD11b on microglia to trigger the microglial migration, and this promoted the release of extracellular H_2O_2 (*114*). Then, H_2O_2 diffuses rapidly into the cytoplasm in microglia, and the F-actin–associated protein cortactin is phosphorylated by the tyrosine protein kinase Lynin in response to the increased concentration of H_2O_2. In response to phosphorylated cortactin, the actin skeleton is rearranged and promotes microglia-directed migration toward neurons, which is strictly controlled by the Rho family of small guanosine triphosphate hydrolyzing enzymes (*114*).

However, whether α-synuclein induces astrocyte activation, which is an important component of neuroinflammation, and the detailed mechanism have not been elucidated. Because imidazoline 2 is mainly expressed on astrocytes, a highly specific and selective detection method targeting the imidazoline 2 binding site in PD demonstrated a neuroprotective effect of reactive astroglia in response to α-synuclein accumulation in the early stage (*115*). However, because the disease progresses with constant stimulation from α-synuclein inclusions and the microenvironment, astrocytes may become overactive and respond accordingly. The release of neuroinflammatory factors, including NF-κB, TNF-α, and IL-1β, by astrocytes can be amplified by IFN-γ (*116*). In addition, after CX3CR1 deletion, the A53T mutant of α-synuclein exacerbated neuroinflammatory markers and microgliosis, which were accompanied by neurodegeneration, indicating the modulatory effect of CX3CR1 on pathological proteins (*117*).

A recent study showed that when rat brain endothelial cells (RBECs) were cocultured with rat brain pericytes, RBEC permeability was increased after α-synuclein treatment. However, in the absence of pericytes, RBECs were not affected (*118*). These results showed that pericytes reacting to monomeric α-synuclein were more sensitive than RBECs to the release of inflammatory cytokines and chemokines, such as MMP9. Therefore, monomeric α-synuclein–induced pericytes may play roles in BBB breakdown and inflammatory infiltration, contributing to neuroinflammation in patients with PD. Whether pericytes play a role in α-synuclein–associated PD pathology needs further study.

It has been reported that posttranslational modifications of α-synuclein, including truncation, phosphorylation, and ubiquitination, can also affect neuroinflammation. Among these modifications, truncation is widespread in Lewy bodies in PD brains, suggesting that truncation may be involved in the formation of pathological structures (*119*). Exogenous C-terminal truncation of α-synuclein activates neuroinflammation by increasing the release of reactive oxygen species. Some in vitro studies demonstrated that phosphorylation at serine-129 (pS129) was associated with the production of fibrillar α-synuclein by disrupting the endocytic vesicle or slowing down the rate of proteolytic pathways (*120*). Furthermore, the CSF levels of pS129 of patients with MSA were significantly higher than those of patients with PD and DLB. It has been suggested that pS129 could prevent initial α-synuclein fibril from further aggregation in PD (*121*), and hence, this effect should be further investigated. One study indicated that α-synuclein was phosphorylated at S87, and this may increase α-synuclein conformational flexibility, inhibit α-synuclein oligomerization, influence α-synuclein–membrane interactions, and block its fibrillization (*122*). Furthermore, pS87 may cause reduction of α-synuclein binding to membranes. pS87 was identified in the brains of different synucleinopathies, and this phosphorylation affected the helical conformation of membrane binding to α-synuclein, with lower lipid binding affinity. Several studies indicated that with tyrosine residue, nitration may lead to α-synuclein aggregation due to the damage of α-helix conformation and the disruption of binding to lipid vesicles (*123*). Under oxidative stress condition, excess NO induced α-synuclein nitration, which resulted in α-synuclein aggregation. Despite these interesting observations, more in vivo studies are needed to investigate the pathological roles of posttranslational modifications in α-synuclein aggregation.

Furthermore, neuronal debris induces further inflammatory reactions through a cycle of neuronal injury, α-synuclein aggregation, and sustained inflammation. The α-synuclein monomer has been shown to maintain the physiological and anti-inflammatory

microglial phenotype by attenuating the activation of NF-κB, increasing peroxisome proliferator–activated receptor γ expression, and promoting neuroprotective functions (*124*). In addition, fibrillar α-synuclein exhibited the highest selectivity for triggering microglial activation and induced a faster persistent major histocompatibility complex class II (MHC II) response in the brain than α-synuclein monomers and oligomers (*125*).

The effect of neuroinflammation on the aggregation and spread of α-synuclein

While neuroinflammation is a contributory factor in neuronal death and neurodegeneration in synucleinopathies, how this contributes to the accumulation of intracellular α-synuclein is still being investigated. In several LPS-induced animal models, increased inflammatory mediators may induce the loss of dopaminergic neurons and defects in the molecular networks that clear misfolded proteins (*126*). Increasing evidence has indicated that the overproduction of proinflammatory cytokines and oxidative stress contribute to neuronal degeneration. Experimental animal models have shown that the overactivation of cells in the NVU, including glia and vascular cells, causes continuous and excessive production of neuroinflammatory mediators that are cytotoxic to neurons and subsequently results in neuronal degeneration and necrosis, which releases neuronal debris (*127*). For example, the proinflammatory mediator S100A9 has recently been shown to colocalize with α-synuclein within Lewy bodies in PD patients (*128*). The cytotoxicity of α-synuclein was maximized when S100A9 was added in vitro, emphasizing the important role of S100A9 in neurodegeneration. TNF-α was also shown to modulate α-synuclein levels in inducing pluripotent stem cell–derived neurons (*129*). Moreover, the lack of triggering receptor expressed on myeloid cells 2, a transmembrane protein that is specifically expressed on microglia, may promote α-synuclein aggregation by modulating the proinflammatory state of microglia (*130*). TLR4 deficiency impairs microglial endocytosis, leads to poor α-synuclein clearance, and promotes neurodegeneration (*131*). These data suggest the involvement of specific inflammatory pathways in α-synuclein aggregate formation.

To clarify the temporal relationship between neuroinflammation and synucleinopathy, mice were injected with preformed α-synuclein fibrils, and during the proinflammatory stage, a significant increase in ionized calcium binding adaptor molecule 1^+ cells, glial fibrillary acidic protein–positive cells, and IL-1β expression was shown to precede α-synuclein aggregation in the substantia nigra. In contrast, there was no change in inflammation in mice that received α-synuclein monomers or the vehicle (*132*). Thus, these findings suggest that neuroinflammation is an early event that precedes α-synuclein inclusion formation. However, an animal model of late-onset Parkinsonism showed that a mild inflammatory state occurred in the late stage of PD pathology, which was even later than the progressive accumulation of pathological α-synuclein in the substantia nigra (*133*). Together, these results suggest that a vicious cycle exists between astrocytic and microglial activation and neuroinflammation and the aggregation of α-synuclein.

In parallel with a major glial immune response, other cells in the NVU also contribute to the dynamic accumulation of pathological α-synuclein by secreting factors such as proteoglycan neuron glia antigen 2 (NG2). NG2 may generally mediate the proliferation, movement, and differentiation of pericytes in the brain. Inhibiting NG2 in pericytes reduces the levels of the vessel basal lamina molecules laminin, collagen VI, and collagen IV (*134*). It was shown that the levels of soluble NG2 in the CSF of patients with PD were associated with the expression of α-synuclein, indicating that NG2 expression was linked to synucleinopathies.

In addition, immune cells and the regulation of neuroinflammatory factors are affected by the degradation of α-synuclein. Many studies have shown that microglia, which are prime scavenger cells in the brain, are in charge of phagocytosis to degrade α-synuclein (*135*). Microglia can engulf fibrillar α-synuclein by up-regulating the autophagy receptor p62/SQSTM1, which is mediated by TLR4–NF-κB signaling (*136*). Meanwhile, the fibrillar form of α-synuclein, but not monomeric α-synuclein, was found to induce lysosomal damage and active autophagy of microglia. In addition, α-synuclein can impair microglial autophagy flux through TLR4-dependent p38 and AKT-mammalian target of rapamycin (mTOR) signaling. Exosomes are crucial mediators in the transmission and propagation of α-synuclein between microglia and neurons. It has been reported that exosomes derived from the plasma and CSF of PD patients containing higher level of total α-synuclein can preferentially activate microglia in vivo and in vitro (*137*). Neurons can release exosomes containing α-synuclein. These exosomes can be phagocytosed by nearby healthy neurons or microglia (*138*). Microglia degrade extracellular α-synuclein via autophagy-lysosomal pathway and secrete exosomes (*89*). A recent study described that microglia-derived exosomes carrying α-synuclein were able to trigger protein aggregation in recipient neurons, and inhibition of exosomes in microglia inhibited α-synuclein transmission (*138*). Studies in in vitro and animal models and humans suggest that these exosomal α-synuclein can be absorbed by nearby neurons, promoting cell-to-cell transmission, and this can lead to accumulation of pathogenic α-synuclein (*139*). Together, microglia can directly take up α-synuclein or exosomal α-synuclein and can release microglial exosomes with α-synuclein to contribute the α-synuclein transmission and propagation. It has been suggested that monomeric α-synuclein can be internalized into microglia by binding to ganglioside GM1 (*136*). Moreover, the internalization can be disrupted by DJ-1 protein deficiency (*135*). Xia *et al.* (*139*) found that TLR2 in microglia could be activated by exosomal α-synuclein, which leads to the further propagation and spread of α-synuclein pathology, thereby highlighting the pivotal roles of reactive microglia in α-synuclein transmission. These studies provide notable insights into the role of microglia in degrading α-synuclein and its transmission. Thus, because of the defects in autophagy regulation, microglia cannot take up α-synuclein. A lack of neuronal IFN-β and interferon-α/β receptor (IFNAR) signaling, which is a classic anti-inflammatory pathway, causes dysfunction in α-synuclein degradation in neurons and ultimately results in Lewy body formation (*132*). In conclusion, whether inflammation precedes the pathological formation of α-synuclein or is a result of that loss remains to be further investigated.

Different properties of α-synuclein in synucleinopathies

The aggregation of α-synuclein is thought to be deleterious and a notable step that leads to neuronal dysfunction and death. Although three synucleinopathies share the α-synuclein burden, how α-synuclein develops and aggregates distinctly in specific brain regions and then leads to distinctive clinical symptoms remains unclear. In PD and DLB, pathological α-synuclein inclusions in neurons in the form of Lewy bodies are the major causes of neurotoxicity and

progressive neurodegeneration, while pathological α-synuclein in MSA is characterized by glial cytoplasmic inclusions, especially oligodendroglial cytoplasmic inclusions (*140*). The cryo-electron microscopy showed that there were two filament types in patients with MSA, which showed a shortened average twisting distance and greater toxicity than the filaments in patients with PD (*141*). Because neuroinflammation mainly affects the aggregation and spread of α-synuclein, we will discuss how these differences in α-synuclein differ among the three synucleinopathies.

Emerging PD studies in cells, animal models, and humans suggest that misfolded α-synuclein species is released by neurons and can be absorbed by nearby neurons, inducing cell-to-cell transmission and the accumulation of pathogenic α-synuclein (*142*). A human single-nucleus transcriptomic analysis was performed on the human substantia nigra and cortex regions and identified a noticeable cell type connection between an increased risk of PD and oligodendrocyte-specific gene expression, but how oligodendrocyte dysfunction occurs in PD has not been well demonstrated (*132*).

Pathogenic mutations in human α-synuclein play important roles in its pathological transmission, which may explain the different clinical manifestations and pathological phenotypes of synucleinopathies at the gene level. Among these phenotypes, the mutations H50Q and A53T not only greatly increased α-synuclein secretion and seeding activity in vivo but also rapidly induced neuroinflammation and microglial activation in vitro and in the rat substantia nigra pars compacta (*143*). For example, the mutant A53T α-synuclein activated robust microglial reactivity through phosphorylation mechanisms that were modulated by mitogen-activated protein kinases and subsequent NF-κB/activator protein–1/nuclear factor erythroid 2 p45–related factor 2 pathway activation (*144*). A recent study suggested that chemically synthesized α-synuclein peptides containing specific amino acid sequences could directly increase the release of superoxide by microglia and facilitate dopaminergic neuronal damage (*145*). Inspired by this study, we suggest that the constitution of different individual peptides generated from α-synuclein mediates the different pathological natures of synucleinopathies and intermediate metabolites during neurodegeneration.

Previous studies have demonstrated that reducing α-synuclein levels has beneficial effects on neurotoxin-induced models of PD (*146*). In contrast, a recent study yielded discrepant findings and showed that inhibiting nigrostriatal α-synuclein initiated a neuroinflammatory cascade, as indicated by rapidly increased expression of MHC I, the transformation of reactive microglia, and the loss of nigrostriatal neurons (*147*). These results challenged the therapeutic efficacy of simply decreasing α-synuclein in PD patients, which may lead to severe neuronal loss.

As a rare and rapidly progressive synucleinopathy, MSA is characterized by a combination of clinical Parkinsonism symptoms, cerebellar impairment, and autonomic and motor dysfunctions (*148*). Oligodendrocytes are not traditionally involved in inflammation, but these cells play a major role in MSA. The accumulation of misfolded α-synuclein within oligodendrocytes is also involved in both oligodendroglial dysfunction and neurodegeneration, which can initiate the onset of clinical symptoms in MSA.

DLB is a disease with brain atrophy caused by abnormal aggregation in neuronal synapses. In DLB, Lewy bodies are predominantly located in the cortex. It was found that two mutants of the α-synuclein isoform β-synuclein in DLB, V70M and P123H, had higher membrane binding affinity and stronger ability to aggregate than wild-type β-synuclein (*149*). However, it is still unclear how these two mutants of β-synuclein contribute to the abnormal aggregation of synuclein and lead to neurodegeneration in DLB.

The relevance of anatomical variations in these three synucleinopathies to the functions of α-synuclein in different diseases will be discussed below. In vitro and in vivo studies provide strong evidence that the aggregation of α-synuclein in mature amyloid fibrils in Lewy bodies is preceded by oligomerization. Extracts from the brains of MSA patients showed a unique strain of α-synuclein prion, which had variable conformations and the ability to self-propagate (*150*). The morphological and biochemical features of α-synuclein in synucleinopathies have drawn great attention, and the different properties and seeding activities may contribute to the classification and specific pathogenesis of synucleinopathies. To determine the structures of α-synuclein filaments in the human brain, Schweighauser *et al.* (*141*) used cryo–electron microscopy to observe the structures of α-synuclein filaments, which consisted of two types of filaments in the brains of patients with MSA. Studies have reported that brain extracts from patients had much higher seeding activity than those extracted from patients with DLB and PD both in vivo and in vitro. Moreover, the α-synuclein filaments in MSA are distinctly different from the α-synuclein filaments in DLB by two-dimensional class averaging, which provides a clue to the precise pathogenic mechanism and prospective therapeutic targets of the different conformations. On the basis of these structural analyses, many notable methods have been established to distinguish the structures of α-synuclein in MSA and PD, such as silver staining, luminescent conjugated oligothiophenes, fluorescence lifetime imaging, nuclear magnetic resonance spectroscopy, and electron paramagnetic resonance. Further studies are needed to determine how pathological α-synuclein accumulates, aggregates, and spreads in different synucleinopathies.

DISCUSSION

Disruption of the NVU has been shown to play a role in the initiation and progression of synucleinopathies. Both neuroinflammation and abnormal misfolding of α-synuclein are important contributors to the underling pathophysiologic processes, although this may not be specific to synucleinopathies. There is evidence of intercellular cross-talk among different cell types including glia cells, vascular cells, pericytes, and neurons during neuroinflammation. Although how the neuroinflammatory networks lead to neurodegeneration is unclear, current data suggest that they may contribute to the aggregation and transmission of α-synuclein.

FUTURE PROSPECTS

Studies to elucidate the exact interplay between microglia and astrocytes and its effects on immune cell activation and release of neuroinflammatory cytokines are needed. The cause-and-effect relationship between neuroinflammation and α-synuclein is unclear. It is useful to investigate their temporal relationship in experimental models and to evaluate whether adjustments of the microenvironment can influence the outcome. Identification of the specific mechanisms that contribute to the initiation and aggregation of α-synuclein will provide prospective insights on the pathophysiologic role of neuroinflammation. Because most published

reports have focused on experiments using coculture of limited cell types, investigations involving additional cell types such as pericytes in NVU and to evaluate their relationship with glial cells and oligodendrocytes will be beneficial.

Optimization and developing physiologic NVU models that can imitate neuroinflammatory response will provide an ideal platform to interrogate the relationship between neuroinflammation and α-synuclein. Because the predominant cell involvement differs in different synucleinopathies (e.g., neurons in PD and DLB and oligodendrocytes in MSA), studies to identify the cell types most at risk to neuroinflammation and to determine how the predominant cell type interacts with the other elements in NVU will further our understanding on the role of inflammatory processes in the disease pathophysiology. Potential therapeutic approaches in the treatment of synucleinopathies can target inhibitions of these neuroinflammatory cross-talks.

REFERENCES AND NOTES

1. B. V. Zlokovic, The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* **57**, 178–201 (2008).
2. E. A. Coon, W. Singer, Synucleinopathies. *Continuum (Minneap Minn)* **26**, 72–92 (2020).
3. E.-K. Tan, Y.-X. Chao, A. West, L.-L. Chan, W. Poewe, J. Jankovic, Parkinson disease and the immune system—Associations, mechanisms and therapeutics. *Nat. Rev. Neurol.* **16**, 303–318 (2020).
4. M. D. Sweeney, A. P. Sagare, B. V. Zlokovic, Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat. Rev. Neurol.* **14**, 133–150 (2018).
5. T. M. Bui, H. L. Wiesolek, R. Sumagin, ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J. Leukoc. Biol.* **108**, 787–799 (2020).
6. S. Cheng, W. Gao, X. Xu, H. Fan, Y. Wu, F. Li, J. Zhang, X. Zhu, Y. Zhang, Methylprednisolone sodium succinate reduces BBB disruption and inflammation in a model mouse of intracranial haemorrhage. *Brain Res. Bull.* **127**, 226–233 (2016).
7. Y. Wang, S. Jin, Y. Sonobe, Y. Cheng, H. Horiuchi, B. Parajuli, J. Kawanokuchi, T. Mizuno, H. Takeuchi, A. Suzumura, Interleukin-1β induces blood-brain barrier disruption by downregulating Sonic hedgehog in astrocytes. *PLOS ONE* **9**, e110024 (2014).
8. J. Guan, D. Pavlovic, N. Dalkie, H. J. Waldvogel, S. J. O'Carroll, C. R. Green, L. F. B. Nicholson, Vascular degeneration in Parkinson's disease. *Brain Pathol.* **23**, 154–164 (2013).
9. W. L. Kuan, N. Bennett, X. He, J. N. Skepper, N. Martynyuk, R. Wijeyekoon, P. V. Moghe, C. H. Williams-Gray, R. A. Barker, α-Synuclein pre-formed fibrils impair tight junction protein expression without affecting cerebral endothelial cell function. *Exp. Neurol.* **285**, 72–81 (2016).
10. L. Wu, S. Walas, W. Leung, D. B. Sykes, J. Wu, E. H. Lo, J. Lok, Neuregulin1-β decreases IL-1β-induced neutrophil adhesion to human brain microvascular endothelial cells. *Transl. Stroke Res.* **6**, 116–124 (2015).
11. M. D. Sweeney, S. Ayyadurai, B. V. Zlokovic, Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nat. Neurosci.* **19**, 771–783 (2016).
12. D. Attwell, A. Mishra, C. N. Hall, F. M. O'Farrell, T. Dalkara, What is a pericyte? *J. Cereb. Blood Flow Metab.* **36**, 451–455 (2016).
13. A. Kovac, M. A. Erickson, W. A. Banks, Brain microvascular pericytes are immunoactive in culture: Cytokine, chemokine, nitric oxide, and LRP-1 expression in response to lipopolysaccharide. *J. Neuroinflammation* **8**, 139 (2011).
14. W. Qin, J. Li, R. Zhu, S. Gao, J. Fan, M. Xia, R. C. Zhao, J. Zhang, Melatonin protects blood-brain barrier integrity and permeability by inhibiting matrix metalloproteinase-9 via the NOTCH3/NF-κB pathway. *Aging (Albany NY)* **11**, 11391–11415 (2019).
15. A. Bhattacharya, D. K. Kaushik, B. M. Lozinski, V. W. Yong, Beyond barrier functions: Roles of pericytes in homeostasis and regulation of neuroinflammation. *J. Neurosci. Res.* **98**, 2390–2405 (2020).
16. R. Balabanov, R. Washington, J. Wagnerova, P. Dore-Duffy, CNS microvascular pericytes express macrophage-like function, cell surface integrin αM, and macrophage marker ED-2. *Microvasc. Res.* **52**, 127–142 (1996).
17. O. Elabi, A. Gaceb, R. Carlsson, T. Padel, R. Soylu-Kucharz, I. Cortijo, W. Li, J.-Y. Li, G. Paul, Human α-synuclein overexpression in a mouse model of Parkinson's disease leads to vascular pathology, blood brain barrier leakage and pericyte activation. *Sci. Rep.* **11**, 1120 (2021).
18. Q. Chen, H. Song, C. Liu, J. Xu, C. Wei, W. Wang, F. Han, The interaction of EphA4 with PDGFRβ regulates proliferation and neuronal differentiation of neural progenitor cells *in vitro* and promotes neurogenesis *in vivo*. *Front. Aging Neurosci.* **12**, 7 (2020).
19. M Hellström, H Gerhardt, M Kalén, X Li, U Eriksson, H Wolburg, C Betsholtz, Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *The Journal of cell biology* **153**, 543–596 (2001).
20. A Gaceb, I Özen, T Padel, M Barbariga, G Paul, Pericytes secrete pro-regenerative molecules in response to platelet-derived growth factor-BB. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* **38**, 45–102 (2018).
21. T. Padel, I. Özen, J. Boix, M. Barbariga, A. Gaceb, M. Roth, G. Paul, Platelet-derived growth factor-BB has neurorestorative effects and modulates the pericyte response in a partial 6-hydroxydopamine lesion mouse model of Parkinson's disease. *Neurobiol. Dis.* **94**, 95–105 (2016).
22. J. Cheng, N. Korte, R. Nortley, H. Sethi, Y. Tang, D. Attwell, Targeting pericytes for therapeutic approaches to neurological disorders. *Acta Neuropathol.* **136**, 507–523 (2018).
23. A. Freitas, M. Aroso, S. Rocha, R. Ferreira, R. Vitorino, M. Gomez-Lazaro, Bioinformatic analysis of the human brain extracellular matrix proteome in neurodegenerative disorders. *Eur. J. Neurosci.* **53**, 4016–4033 (2021).
24. A. Freitas, M. Aroso, A. Barros, M. Fernández, E. Conde-Sousa, M. Leite, E. D. Carvalho, C. C. Ribeiro, R. Ferreira, A. P. Pêgo, R. Vitorino, M. Gomez-Lazaro, Characterization of the striatal extracellular matrix in a mouse model of Parkinson's disease. *Antioxidants (Basel)* **10**, 1095 (2021).
25. J. V. Welser, S. K. Halder, R. Kant, A. Boroujerdi, R. Milner, Endothelial α6β4 integrin protects during experimental autoimmune encephalomyelitis-induced neuroinflammation by maintaining vascular integrity and tight junction protein expression. *J. Neuroinflammation* **14**, 217 (2017).
26. V. Gupta, M. K. Singh, R. K. Garg, K. K. Pant, S. Khattri, Evaluation of peripheral matrix metalloproteinase-1 in Parkinson's disease: A case-control study. *Int. J. Neurosci.* **124**, 88–92 (2014).
27. V. Kumar, Toll-like receptors in the pathogenesis of neuroinflammation. *J. Neuroimmunol.* **332**, 16–30 (2019).
28. C. F. Orr, D. B. Rowe, Y. Mizuno, H. Mori, G. M. Halliday, A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain* **128**, 2665–2674 (2005).
29. M. Wu, L. Xu, Y. Wang, N. Zhou, F. Zhen, Y. Zhang, X. Qu, H. Fan, S. Liu, Y. Chen, R. Yao, S100A8/A9 induces microglia activation and promotes the apoptosis of oligodendrocyte precursor cells by activating the NF-κB signaling pathway. *Brain Res. Bull.* **143**, 234–245 (2018).
30. C. L. Myhre, C. Thygesen, B. Villadsen, J. Vollerup, L. Ilkjær, K. T. Krohn, M. Grebing, S. Zhao, A. M. Khan, L. Dissing-Olesen, M. S. Jensen, A. A. Babcock, B. Finsen, Microglia express insulin-like growth factor-1 in the hippocampus of aged $APP_{swe}/PS1_{\Delta E9}$ transgenic mice. *Front. Cell. Neurosci.* **13**, 308 (2019).
31. S. Guérit, E. Fidan, J. Macas, C. J. Czupalla, R. Figueiredo, A. Vijikumar, B. H. Yalcin, S. Thom, P. Winter, H. Gerhardt, K. Devraj, S. Liebner, Astrocyte-derived Wnt growth factors are required for endothelial blood-brain barrier maintenance. *Prog. Neurobiol.* **199**, 101937 (2021).
32. E. J. Goetzl, J. B. Schwartz, E. L. Abner, G. A. Jicha, D. Kapogiannis, High complement levels in astrocyte-derived exosomes of Alzheimer disease. *Ann. Neurol.* **83**, 544–552 (2018).
33. X. Zong, Y. Li, C. Liu, W. Qi, D. Han, L. Tucker, Y. Dong, S. Hu, X. Yan, Q. Zhang, Theta-burst transcranial magnetic stimulation promotes stroke recovery by vascular protection and neovascularization. *Theranostics* **10**, 12090–12110 (2020).
34. T. Zeis, U. Graumann, R. Reynolds, N. Schaeren-Wiemers, Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. *Brain* **131**, 288–303 (2008).
35. X. J. Zhang, M. M. Lv, X. Q. Zhu, L. Y. Tian, J. J. Li, Y. P. Shao, C. J. Gao, X. D. Sun, Microglia M1/M2 polarization contributes to electromagnetic pulse-induced brain injury. *J. Biol. Regul. Homeost. Agents* **33**, 1051–1062 (2019).
36. K. Maiese, Moving to the rhythm with clock (Circadian) genes, autophagy, mTOR, and SIRT1 in degenerative disease and cancer. *Curr. Neurovasc. Res.* **14**, 299–304 (2017).
37. F. L'Episcopo, C. Tirolo, M. F. Serapide, S. Caniglia, N. Testa, L. Leggio, S. Vivarelli, N. Iraci, S. Pluchino, B. Marchetti, Microglia polarization, gene-environment interactions and Wnt/β-catenin signaling: Emerging roles of glia-neuron and glia-stem/neuroprogenitor crosstalk for dopaminergic neurorestoration in aged Parkinsonian brain. *Front Aging Neurosci.* **10**, 12 (2018).
38. M. De Laere, C. Sousa, M. Meena, R. Buckinx, J. P. Timmermans, Z. Berneman, N. Cools, Increased transendothelial transport of CCL3 Is insufficient to drive immune cell transmigration through the blood-brain barrier under inflammatory conditions in vitro. *Mediators Inflamm.* **2017**, **2017**, 6752756

39. J. Matsumoto, F. Takata, T. Machida, H. Takahashi, Y. Soejima, M. Funakoshi, K. Futagami, A. Yamauchi, S. Dohgu, Y. Kataoka, Tumor necrosis factor-α-stimulated brain pericytes possess a unique cytokine and chemokine release profile and enhance microglial activation. *Neurosci. Lett.* **578**, 133–138 (2014).
40. X. Ding, M. Zhang, R. Gu, G. Xu, H. Wu, Activated microglia induce the production of reactive oxygen species and promote apoptosis of co-cultured retinal microvascular pericytes. *Graefes Arch. Clin. Exp. Ophthalmol.* **255**, 777–788 (2017).
41. Y. Shigemoto-Mogami, K. Hoshikawa, K. Sato, Activated microglia disrupt the blood-brain barrier and induce chemokines and cytokines in a rat *in vitro* model. *Front. Cell. Neurosci.* **12**, 494 (2018).
42. E. Isasi, N. Korte, V. Abudara, D. Attwell, S. Olivera-Bravo, Glutaric acid affects pericyte contractility and migration: Possible implications for GA-I pathogenesis. *Mol. Neurobiol.* **56**, 7694–7707 (2019).
43. Y. Shan, S. Tan, Y. Lin, S. Liao, B. Zhang, X. Chen, J. Wang, Z. Deng, Q. Zeng, L. Zhang, Y. Wang, X. Hu, W. Qiu, L. Peng, Z. Lu, The glucagon-like peptide-1 receptor agonist reduces inflammation and blood-brain barrier breakdown in an astrocyte-dependent manner in experimental stroke. *J. Neuroinflammation* **16**, 242 (2019).
44. N. Mazaré, A. Gilbert, A. C. Boulay, N. Rouach, M. Cohen-Salmon, Connexin 30 is expressed in a subtype of mouse brain pericytes. *Brain Struct. Funct.* **223**, 1017–1024 (2018).
45. S. Al-Bachari, J. H. Naish, G. J. M. Parker, H. C. A. Emsley, L. M. Parkes, Blood-brain barrier leakage is increased in Parkinson's disease. *Front. Physiol.* **11**, 593026 (2020).
46. T. I. Kam, J. T. Hinkle, T. M. Dawson, V. L. Dawson, Microglia and astrocyte dysfunction in Parkinson's disease. *Neurobiol. Dis.* **144**, 105028 (2020).
47. U. Wilhelmsson, D. Andersson, Y. de Pablo, R. Pekny, A. Ståhlberg, J. Mulder, N. Mitsios, T. Hortobágyi, M. Pekny, M. Pekna, Injury leads to the appearance of cells with characteristics of both microglia and astrocytes in mouse and human brain. *Cereb. Cortex* **27**, 3360–3377 (2017).
48. H. M. Nguyen, J. di Lucente, Y.-J. Chen, Y. Cui, R. H. Ibrahim, M. W. Pennington, L.-W. Jin, I. Maezawa, H. Wulff, Biophysical basis for Kv1.3 regulation of membrane potential changes induced by P2X4-mediated calcium entry in microglia. *Glia* **68**, 2377–2394 (2020).
49. A. Badimon, H. J. Strasburger, P. Ayata, X. Chen, A. Nair, A. Ikegami, P. Hwang, A. T. Chan, S. M. Graves, J. O. Uweru, C. Ledderose, M. G. Kutlu, M. A. Wheeler, A. Kahan, M. Ishikawa, Y.-C. Wang, Y.-H. E. Loh, J. X. Jiang, D. J. Surmeier, S. C. Robson, W. G. Junger, R. Sebra, E. S. Calipari, P. J. Kenny, U. B. Eyo, M. Colonna, F. J. Quintana, H. Wake, V. Gradinaru, A. Schaefer, Negative feedback control of neuronal activity by microglia. *Nature* **586**, 417–423 (2020).
50. E. Martin, M. Amar, C. Dalle, I. Youssef, C. Boucher, C. Le Duigou, M. Brückner, A. Prigent, V. Sazdovitch, A. Halle, J. M. Kanellopoulos, B. Fontaine, B. Delatour, C. Delarasse, New role of P2X7 receptor in an Alzheimer's disease mouse model. *Mol. Psychiatry* **24**, 108–125 (2019).
51. T. Canedo, C. C. Portugal, R. Socodato, T. O. Almeida, A. F. Terceiro, J. Bravo, A. I. Silva, J. D. Magalhães, S. Guerra-Gomes, J. F. Oliveira, N. Sousa, A. Magalhães, J. B. Relvas, T. Summavielle, Astrocyte-derived TNF and glutamate critically modulate microglia activation by methamphetamine. *Neuropsychopharmacology* **46**, 2358–2370 (2021).
52. P. Xia, F. Logiacco, Y. Huang, H. Kettenmann, M. Semtner, Histamine triggers microglial responses indirectly via astrocytes and purinergic signaling. *Glia* **69**, 2291–2304 (2021).
53. N. Jangde, R. Ray, V. Rai, RAGE and its ligands: From pathogenesis to therapeutics. *Crit. Rev. Biochem. Mol. Biol.* **55**, 555–575 (2020).
54. P. Alexiou, M. Chatzopoulou, K. Pegklidou, V. J. Demopoulos, RAGE: A multi-ligand receptor unveiling novel insights in health and disease. *Curr. Med. Chem.* **17**, 2232–2252 (2010).
55. M. Santoro, W. Maetzler, P. Stathakos, H. L. Martin, M. A. Hobert, T. W. Rattay, T. Gasser, J. V. Forrester, D. Berg, K. J. Tracey, G. Riedel, P. Teismann, In-vivo evidence that high mobility group box 1 exerts deleterious effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model and Parkinson's disease which can be attenuated by glycyrrhizin. *Neurobiol. Dis.* **91**, 59–68 (2016).
56. K. Sathe, W. Maetzler, J. D. Lang, R. B. Mounsey, C. Fleckenstein, H. L. Martin, C. Schulte, S. Mustafa, M. Synofzik, Z. Vukovic, S. Itohara, D. Berg, P. Teismann, S100B is increased in Parkinson's disease and ablation protects against MPTP-induced toxicity through the RAGE and TNF-α pathway. *Brain* **135**, 3336–3347 (2012).
57. X. Wang, X. Sun, M. Niu, X. Zhang, J. Wang, C. Zhou, A. Xie, RAGE silencing ameliorates neuroinflammation by inhibition of p38-NF-κB signaling pathway in mouse model of Parkinson's disease. *Front. Neurosci.* **14**, 353 (2020).
58. R. Bianchi, I. Giambanco, R. Donato, S100B/RAGE-dependent activation of microglia via NF-κB and AP-1 co-regulation of COX-2 expression by S100B, IL-1β and TNF-α. *Neurobiol. Aging* **31**, 665–677 (2010).
59. A. Klegeris, Regulation of neuroimmune processes by damage- and resolution-associated molecular patterns. *Neural Regen. Res.* **16**, 423–429 (2021).
60. Z. Zhou, J. Hou, Y. Mo, M. Ren, G. Yang, Z. Qu, Y. Hu, Geniposidic acid ameliorates spatial learning and memory deficits and alleviates neuroinflammation via inhibiting HMGB-1 and downregulating TLR4/2 signaling pathway in APP/PS1 mice. *Eur. J. Pharmacol.* **869**, 172857 (2020).
61. X. Yao, Q. Jiang, W. Ding, P. Yue, J. Wang, K. Zhao, H. Zhang, Interleukin 4 inhibits high mobility group box-1 protein-mediated NLRP3 inflammasome formation by activating peroxisome proliferator-activated receptor-γ in astrocytes. *Biochem. Biophys. Res. Commun.* **509**, 624–631 (2019).
62. S. Das, K. P. Mishra, S. Chanda, L. Ganju, S. B. Singh, CXCR7: A key neuroprotective molecule against alarmin HMGB1 mediated CNS pathophysiology and subsequent memory impairment. *Brain Behav. Immun.* **82**, 319–337 (2019).
63. M. L. Neal, A. M. Boyle, K. M. Budge, F. F. Safadi, J. R. Richardson, The glycoprotein GPNMB attenuates astrocyte inflammatory responses through the CD44 receptor. *J. Neuroinflammation* **15**, 73 (2018).
64. G. Vinukonda, P. Dohare, A. Arshad, M. T. Zia, S. Panda, R. Korumilli, R. Kayton, V. C. Hascall, M. E. Lauer, P. Ballabh, Hyaluronidase and hyaluronan oligosaccharides promote neurological recovery after intraventricular hemorrhage. *J. Neurosci.* **36**, 872–889 (2016).
65. S. Hasegawa-Ishii, M. Inaba, H. Umegaki, K. Unno, K. Wakabayashi, A. Shimada, Endotoxemia-induced cytokine-mediated responses of hippocampal astrocytes transmitted by cells of the brain-immune interface. *Sci. Rep.* **6**, 25457 (2016).
66. M. He, H. Dong, Y. Huang, S. Lu, S. Zhang, Y. Qian, W. Jin, Astrocyte-derived CCL2 is associated with M1 activation and recruitment of cultured microglial cells. *Cell. Physiol. Biochem.* **38**, 859–870 (2016).
67. B. Parajuli, H. Horiuchi, T. Mizuno, H. Takeuchi, A. Suzumura, CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. *Glia* **63**, 2274–2284 (2015).
68. T. Clarner, K. Janssen, L. Nellessen, M. Stangel, T. Skripuletz, B. Krauspe, F. M. Hess, B. Denecke, C. Beutner, B. Linnartz-Gerlach, H. Neumann, L. Vallières, S. Amor, K. Ohl, K. Tenbrock, C. Beyer, M. Kipp, CXCL10 triggers early microglial activation in the cuprizone model. *J. Immunol.* **194**, 3400–3413 (2015).
69. M. E. Ahmed, G. P. Selvakumar, D. Kempuraj, S. P. Raikwar, R. Thangavel, K. Bazley, K. Wu, O. Khan, A. Khan, S. Zaheer, S. Iyer, C. Burton, D. James, A. Zaheer, Glia maturation factor (GMF) regulates microglial expression phenotypes and the associated neurological deficits in a mouse model of traumatic brain injury. *Mol. Neurobiol.* **57**, 4438–4450 (2020).
70. D. Kempuraj, G. P. Selvakumar, S. Zaheer, R. Thangavel, M. E. Ahmed, S. Raikwar, R. Govindarajan, S. Iyer, A. Zaheer, Cross-talk between glia, neurons and mast cells in neuroinflammation associated with Parkinson's disease. *J. Neuroimmune Pharmacol.* **13**, 100–112 (2018).
71. G. P. Selvakumar, M. E. Ahmed, R. Thangavel, D. Kempuraj, I. Dubova, S. P. Raikwar, S. Zaheer, S. S. Iyer, A. Zaheer, A role for glia maturation factor dependent activation of mast cells and microglia in MPTP induced dopamine loss and behavioural deficits in mice. *Brain Behav. Immun.* **87**, 429–443 (2020).
72. G. P. Selvakumar, M. E. Ahmed, S. P. Raikwar, R. Thangavel, D. Kempuraj, I. Dubova, D. Saeed, H. Zahoor, K. Premkumar, S. Zaheer, S. Iyer, A. Zaheer, CRISPR/Cas9 editing of glia maturation factor regulates mitochondrial dynamics by attenuation of the NRF2/HO-1 dependent ferritin activation in glial cells. *J. Neuroimmune Pharmacol.* **14**, 537–550 (2019).
73. B. Liu, H.-M. Gao, J.-S. Hong, Parkinson's disease and exposure to infectious agents and pesticides and the occurrence of brain injuries: Role of neuroinflammation. *Environ. Health Perspect.* **111**, 1065–1073 (2003).
74. L. Hou, X. Zhou, C. Zhang, K. Wang, X. Liu, Y. Che, F. Sun, H. Li, Q. Wang, D. Zhang, J. S. Hong, NADPH oxidase-derived H2O2 mediates the regulatory effects of microglia on astrogliosis in experimental models of Parkinson's disease. *Redox Biol.* **12**, 162–170 (2017).
75. E. Hansen, M. Krautwald, A. E. Maczurek, G. Stuchbury, P. Fromm, M. Steele, O. Schulz, O. B. Garcia, J. Castillo, H. Körner, G. Münch, A versatile high throughput screening system for the simultaneous identification of anti-inflammatory and neuroprotective compounds. *J. Alzheimers Dis.* **19**, 451–464 (2010).
76. A. Kumar, B. A. Stoica, D. J. Loane, M. Yang, G. Abulwerdi, N. Khan, A. Kumar, S. R. Thom, A. I. Faden, Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. *J. Neuroinflammation* **14**, 47 (2017).
77. Y. Yang, A. Boza-Serrano, C. Dunning, B. H. Clausen, K. L. Lambertsen, T. Deierborg, Inflammation leads to distinct populations of extracellular vesicles from microglia. *J. Neuroinflammation* **15**, 168 (2018).
78. S. Mao, Q. Sun, H. Xiao, C. Zhang, L. Li, Secreted miR-34a in astrocytic shedding vesicles enhanced the vulnerability of dopaminergic neurons to neurotoxins by targeting Bcl-2. *Protein Cell* **6**, 529–540 (2015).
79. X. Long, X. Yao, Q. Jiang, Y. Yang, X. He, W. Tian, K. Zhao, H. Zhang, Astrocyte-derived exosomes enriched with miR-873a-5p inhibit neuroinflammation via microglia phenotype modulation after traumatic brain injury. *J. Neuroinflammation* **17**, 89 (2020).

80. X. Chen, B. Qian, X. Kong, J. Hao, Y. Ye, K. Yang, T. Xu, F. Zhang, A20 protects neuronal apoptosis stimulated by lipopolysaccharide-induced microglial exosomes. *Neurosci. Lett.* **712**, 134480 (2019).
81. E. B. Harrison, C. G. Hochfelder, B. G. Lamberty, B. M. Meays, B. M. Morsey, M. L. Kelso, H. S. Fox, S. V. Yelamanchili, Traumatic brain injury increases levels of miR-21 in extracellular vesicles: Implications for neuroinflammation. *FEBS Open Bio* **6**, 835–846 (2016).
82. L. Wang, Y. Liu, S. Yan, T. Du, X. Fu, X. Gong, X. Zhou, T. Zhang, X. Wang, Disease progression-dependent expression of CD200R1 and CX3CR1 in mouse models of Parkinson's disease. *Aging Dis.* **11**, 254–268 (2020).
83. X. Chen, M. Jiang, H. Li, Y. Wang, H. Shen, X. Li, Y. Zhang, J. Wu, Z. Yu, G. Chen, CX3CL1/CX3CR1 axis attenuates early brain injury via promoting the delivery of exosomal microRNA-124 from neuron to microglia after subarachnoid hemorrhage. *J. Neuroinflammation* **17**, 209 (2020).
84. C. Liu, K. Hong, H. Chen, Y. Niu, W. Duan, Y. Liu, Y. Ji, B. Deng, Y. Li, Z. Li, D. Wen, C. Li, Evidence for a protective role of the CX3CL1/CX3CR1 axis in a model of amyotrophic lateral sclerosis. *Biol. Chem.* **400**, 651–661 (2019).
85. M. Mao, Y. Xu, X.-Y. Zhang, L. Yang, X. B. An, Y. Qu, Y.-N. Chai, Y. R. Wang, T.-T. Li, J. Ai, MicroRNA-195 prevents hippocampal microglial/macrophage polarization towards the M1 phenotype induced by chronic brain hypoperfusion through regulating CX3CL1/CX3CR1 signaling. *J. Neuroinflammation* **17**, 244 (2020).
86. R. M. Ritzel, M. A. Al, J. Crapser, R. Verma, A. R. Patel, B. E. Knight, N. Harris, N. Mancini, M. Roy-O'Reilly, B. P. Ganesh, F. Liu, L. D. McCullough, CD200-CD200R1 inhibitory signaling prevents spontaneous bacterial infection and promotes resolution of neuroinflammation and recovery after stroke. *J. Neuroinflammation* **16**, 40 (2019).
87. J. J. Li, B. Wang, M. C. Kodali, C. Chen, E. Kim, B. J. Patters, L. Lan, S. Kumar, X. Wang, J. Yue, F.-F. Liao, In vivo evidence for the contribution of peripheral circulating inflammatory exosomes to neuroinflammation. *J. Neuroinflammation* **15**, 8 (2018).
88. Y. Wang, A. M. Hancock, J. Bradner, K. A. Chung, J. F. Quinn, E. R. Peskind, D. Galasko, J. Jankovic, C. P. Zabetian, H. M. Kim, J. B. Leverenz, T. J. Montine, C. Ginghina, K. L. Edwards, K. W. Snapinn, D. S. Goldstein, M. Shi, J. Zhang, Complement 3 and factor h in human cerebrospinal fluid in Parkinson's disease, Alzheimer's disease, and multiple-system atrophy. *Am. J. Pathol.* **178**, 1509–1516 (2011).
89. M. Guo, J. Wang, Y. Zhao, Y. Feng, S. Han, Q. Dong, M. Cui, K. Tieu, Microglial exosomes facilitate α-synuclein transmission in Parkinson's disease. *Brain* **143**, 1476–1497 (2020).
90. W. Maetzler, D. Berg, M. Synofzik, K. Brockmann, J. Godau, A. Melms, T. Gasser, S. Hörnig, M. Langkamp, Autoantibodies against amyloid and glial-derived antigens are increased in serum and cerebrospinal fluid of Lewy body-associated dementias. *J. Alzheimers Dis.* **26**, 171–179 (2011).
91. E. King, J. T. O'Brien, P. Donaghy, C. Morris, N. Barnett, K. Olsen, C. Martin-Ruiz, J.-P. Taylor, A. J. Thomas, Peripheral inflammation in prodromal Alzheimer's and Lewy body dementias. *J. Neurol. Neurosurg. Psychiatry* **89**, 339–345 (2018).
92. A. Surendranathan, L. Su, E. Mak, L. Passamonti, Y. T. Hong, R. Arnold, P. V. Rodríguez, W. R. Bevan-Jones, S. A. E. Brain, T. D. Fryer, F. I. Aigbirhio, J. B. Rowe, J. T. O'Brien, Early microglial activation and peripheral inflammation in dementia with Lewy bodies. *Brain* **141**, 3415–3427 (2018).
93. J. Amin, C. Holmes, R. B. Dorey, E. Tommasino, Y. R. Casal, D. M. Williams, C. Dupuy, J. Nicoll, D. Boche, Neuroinflammation in dementia with Lewy bodies: A human post-mortem study. *Transl. Psychiatry* **10**, 267 (2020).
94. T. Kim, E. Valera, P. Desplats, Alterations in striatal microRNA-mRNA networks contribute to neuroinflammation in multiple system atrophy. *Mol. Neurobiol.* **56**, 7003–7021 (2019).
95. A. Hoffmann, B. Ettle, K. Battis, S. Reiprich, J. Schlachetzki, E. Masliah, M. Wegner, T. Kuhlmann, M. J. Riemenschneider, J. Winkler, Oligodendroglial α-synucleinopathy-driven neuroinflammation in multiple system atrophy. *Brain Pathol.* **29**, 380–396 (2019).
96. X. Yang, Y. Lou, G. Liu, X. Wang, Y. Qian, J. Ding, S. Chen, Q. Xiao, Microglia P2Y6 receptor is related to Parkinson's disease through neuroinflammatory process. *J. Neuroinflammation* **14**, 38 (2017).
97. D. K. Stone, T. Kiyota, R. L. Mosley, H. E. Gendelman, A model of nitric oxide induced α-synuclein misfolding in Parkinson's disease. *Neurosci. Lett.* **523**, 167–173 (2012).
98. R. Rydbirk, B. Elfving, M. D. Andersen, M. A. Langbøl, J. Folke, K. Winge, B. Pakkenberg, T. Brudek, S. Aznar, Cytokine profiling in the prefrontal cortex of Parkinson's disease and multiple system atrophy patients. *Neurobiol. Dis.* **106**, 269–278 (2017).
99. B. D. Vieira, R. A. Radford, R. S. Chung, G. J. Guillemin, D. L. Pountney, Neuroinflammation in multiple system atrophy: Response to and cause of α-synuclein aggregation. *Front. Cell. Neurosci.* **9**, 437 (2015).
100. R. Radford, A. Rcom-H'Cheo-Gauthier, M. B. Wong, E. D. Eaton, M. Quilty, C. Blizzard, A. Norazit, A. Meedeniya, J. C. Vickers, W. P. Gai, G. J. Guillemin, A. K. West, T. C. Dickson, R. Chung, D. L. Pountney, The degree of astrocyte activation in multiple system atrophy is inversely proportional to the distance to α-synuclein inclusions. *Mol. Cell. Neurosci.* **65**, 68–81 (2015).
101. J. Tong, L.-C. Ang, B. Williams, Y. Furukawa, P. Fitzmaurice, M. Guttman, I. Boileau, O. Hornykiewicz, S. J. Kish, Low levels of astroglial markers in Parkinson's disease: Relationship to α-synuclein accumulation. *Neurobiol. Dis.* **82**, 243–253 (2015).
102. A. Santaella, H. B. Kuiperij, A. van Rumund, R. Esselink, A. J. van Gool, B. R. Bloem, M. M. Verbeek, Inflammation biomarker discovery in Parkinson's disease and atypical parkinsonisms. *BMC Neurol.* **20**, 26 (2020).
103. Y. Li, Y. Xia, S. Yin, F. Wan, J. Hu, L. Kou, Y. Sun, J. Wu, Q. Zhou, J. Huang, N. Xiong, T. Wang, Targeting microglial α-synuclein/TLRs/NF-kappaB/NLRP3 inflammasome axis in Parkinson's disease. *Front. Immunol.* **12**, 719807 (2021).
104. R. Que, J. Zheng, Z. Chang, W. Zhang, H. Li, Z. Xie, Z. Huang, H. T. Wang, J. Xu, D. Jin, W. Yang, E. K. Tan, Q. Wang, Dl-3-*n*-butylphthalide rescues dopaminergic neurons in Parkinson's disease models by inhibiting the NLRP3 inflammasome and ameliorating mitochondrial impairment. *Front. Immunol.* **12**, 794770 (2021).
105. G. P. Williams, D. J. Marmion, A. M. Schonhoff, A. Jurkuvenaite, W.-J. Won, D. G. Standaert, J. H. Kordower, A. S. Harms, T cell infiltration in both human multiple system atrophy and a novel mouse model of the disease. *Acta Neuropathol.* **139**, 855–874 (2020).
106. H. González, R. Pacheco, T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J. Neuroinflammation* **11**, 201 (2014).
107. D. Dutta, M. Jana, M. Majumder, S. Mondal, A. Roy, K. Pahan, Selective targeting of the TLR2/MyD88/NF-κB pathway reduces α-synuclein spreading in vitro and in vivo. *Nat. Commun.* **12**, 5382 (2021).
108. Q.-H. Shao, W.-F. Yan, Z. Zhang, K.-L. Ma, S.-Y. Peng, Y.-L. Cao, Y.-H. Yuan, N.-H. Chen, Nurr1: A vital participant in the TLR4-NF-κB signal pathway stimulated by α-synuclein in BV-2 cells. *Neuropharmacology* **144**, 388–399 (2019).
109. H. Qin, J. A. Buckley, X. Li, Y. Liu, T. R. Fox, G. P. Meares, H. Yu, Z. Yan, A. S. Harms, Y. Li, D. G. Standaert, E. N. Benveniste, Inhibition of the JAK/STAT pathway protects against α-synuclein-induced neuroinflammation and dopaminergic neurodegeneration. *J. Neurosci.* **36**, 5144–5159 (2016).
110. A. F. Pike, T. Varanita, M. Herrebout, B. C. Plug, J. Kole, R. Musters, C. E. Teunissen, J. Hoozemans, L. Bubacco, R. Veerhuis, α-Synuclein evokes NLRP3 inflammasome-mediated IL-1β secretion from primary human microglia. *Glia* **69**, 1413–1428 (2021).
111. S. E. Navone, M. Peroglio, L. Guarnaccia, M. Beretta, S. Grad, M. Paroni, C. Cordiglieri, M. Locatelli, M. Pluderi, P. Rampini, R. Campanella, M. Alini, G. Marfia, Mechanical loading of intervertebral disc modulates microglia proliferation, activation, and chemotaxis. *Osteoarthr. Cartil.* **26**, 978–987 (2018).
112. E.-J. Lee, M.-S. Woo, P.-G. Moon, M.-C. Baek, I. Choi, W.-K. Kim, E. Junn, H.-S. Kim, α-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. *J. Immunol.* **185**, 615–623 (2010).
113. J.-J. Guo, F. Yue, D.-Y. Song, L. Bousset, X. Liang, J. Tang, L. Yuan, W. Li, R. Melki, Y. Tang, P. Chan, C. Guo, J.-Y. Li, Intranasal administration of α-synuclein preformed fibrils triggers microglial iron deposition in the substantia nigra of *Macaca fascicularis*. *Cell Death Dis.* **12**, 81 (2021).
114. S. Wang, C. H. Chu, T. Stewart, C. Ginghina, Y. Wang, H. Nie, M. Guo, B. Wilson, J. S. Hong, J. Zhang, α-Synuclein, a chemoattractant, directs microglial migration via H2O2-dependent Lyn phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* **112**, –E1926, E1935 (2015).
115. H. Wilson, G. Dervenoulas, G. Pagano, R. J. Tyacke, S. Polychronis, J. Myers, R. N. Gunn, E. A. Rabiner, D. Nutt, M. Politis, Imidazoline 2 binding sites reflecting astroglia pathology in Parkinson's disease: An in vivo11C-BU99008 PET study. *Brain* **142**, 3116–3128 (2019).
116. J. Wang, Z. Chen, J. D. Walston, P. Gao, M. Gao, S. X. Leng, α-Synuclein activates innate immunity but suppresses interferon-γ expression in murine astrocytes. *Eur. J. Neurosci.* **48**, 1583–1599 (2018).
117. S. Castro-Sánchez, Á. J. García-Yagüe, T. López-Royo, M. Casarejos, J. L. Lanciego, I. Lastres-Becker, Cx3cr1-deficiency exacerbates alpha-synuclein-A53T induced neuroinflammation and neurodegeneration in a mouse model of Parkinson's disease. *Glia* **66**, 1752–1762 (2018).
118. S. Dohgu, F. Takata, J. Matsumoto, I. Kimura, A. Yamauchi, Y. Kataoka, Monomeric α-synuclein induces blood-brain barrier dysfunction through activated brain pericytes releasing inflammatory mediators in vitro. *Microvasc. Res.* **124**, 61–66 (2019).
119. Z. A. Sorrentino, B. I. Giasson, The emerging role of α-synuclein truncation in aggregation and disease. *J. Biol. Chem.* **295**, 10224–10244 (2020).
120. V. Gupta, S. Salim, I. Hmila, N. N. Vaikath, I. P. Sudhakaran, S. S. Ghanem, N. K. Majbour, S. A. Abdulla, M. M. Emara, H. B. Abdesselem, T. Lukacsovich, D. Erskine, O. M. A. El-Agnaf, Fibrillar form of α-synuclein-specific scFv antibody inhibits α-synuclein seeds induced aggregation and toxicity. *Sci. Rep.* **10**, 8137 (2020).
121. K. E. Paleologou, A. W. Schmid, C. C. Rospigliosi, H. Y. Kim, G. R. Lamberto, R. A. Fredenburg, P. J. Lansbury, C. O. Fernandez, D. Eliezer, M. Zweckstetter, H. A. Lashuel, Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of α-synuclein. *J. Biol. Chem.* **283**, 16895–16905 (2008).

122. K. E. Paleologou, A. Oueslati, G. Shakked, C. C. Rospigliosi, H.-Y. Kim, G. R. Lamberto, C. O. Fernandez, A. Schmid, F. Chegini, W. P. Gai, D. Chiappe, M. Moniatte, B. L. Schneider, P. Aebischer, D. Eliezer, M. Zweckstetter, E. Masliah, H. A. Lashuel, Phosphorylation at S87 is enhanced in synucleinopathies, inhibits α-synuclein oligomerization, and influences synuclein-membrane interactions. *J. Neurosci.* **30**, 3184–3198 (2010).
123. R. Hodara, E. H. Norris, B. I. Giasson, A. J. Mishizen-Eberz, D. R. Lynch, V. M.-Y. Lee, H. Ischiropoulos, Functional consequences of α-synuclein tyrosine nitration: Diminished binding to lipid vesicles and increased fibril formation. *J. Biol. Chem.* **279**, 47746–47753 (2004).
124. N. Li, T. Stewart, L. Sheng, M. Shi, E. M. Cilento, Y. Wu, J. S. Hong, J. Zhang, Immunoregulation of microglial polarization: An unrecognized physiological function of α-synuclein. *J. Neuroinflammation* **17**, 272 (2020).
125. A. Hoffmann, B. Ettle, A. Bruno, A. Kulinich, A.-C. Hoffmann, J. von Wittgenstein, J. Winkler, W. Xiang, J. Schlachetzki, Alpha-synuclein activates BV2 microglia dependent on its aggregation state. *Biochem. Biophys. Res. Commun.* **479**, 881–886 (2016).
126. H.-F. Zheng, Y.-P. Yang, L.-F. Hu, M.-X. Wang, F. Wang, L.-D. Cao, D. Li, C.-J. Mao, K.-P. Xiong, J.-D. Wang, C.-F. Liu, Autophagic impairment contributes to systemic inflammation-induced dopaminergic neuron loss in the midbrain. *PLOS ONE* **8**, e70472 (2013).
127. M. G. Tansey, T. C. Frank-Cannon, M. K. McCoy, J. K. Lee, T. N. Martinez, F. E. McAlpine, K. A. Ruhn, T. A. Tran, Neuroinflammation in Parkinson's disease: Is there sufficient evidence for mechanism-based interventional therapy? *Front. Biosci.* **13**, 709–717 (2008).
128. I. Horvath, I. A. Iashchishyn, R. A. Moskalenko, C. Wang, S. Wärmländer, C. Wallin, A. Gräslund, G. G. Kovacs, L. A. Morozova-Roche, Co-aggregation of pro-inflammatory S100A9 with α-synuclein in Parkinson's disease: Ex vivo and in vitro studies. *J. Neuroinflammation* **15**, 172 (2018).
129. D. M. R. López, V. Lang, A. Zubiarrain, A. Sousa, N. Vázquez, A. Gorostidi, J. Águila, D. M. A. López, M. Rodríguez, R. Sánchez-Pernaute, Mutations in LRRK2 impair NF-κB pathway in iPSC-derived neurons. *J. Neuroinflammation* **13**, 295 (2016).
130. Y. Guo, X. Wei, H. Yan, Y. Qin, S. Yan, J. Liu, Y. Zhao, F. Jiang, H. Lou, TREM2 deficiency aggravates α-synuclein-induced neurodegeneration and neuroinflammation in Parkinson's disease models. *FASEB J.* **33**, 12164–12174 (2019).
131. I. Choi, Y. Zhang, S. P. Seegobin, M. Pruvost, Q. Wang, K. Purtell, B. Zhang, Z. Yue, Microglia clear neuron-released α-synuclein via selective autophagy and prevent neurodegeneration. *Nat. Commun.* **11**, 1386 (2020).
132. P. Ejlerskov, J. G. Hultberg, J. Wang, R. Carlsson, M. Ambjørn, M. Kuss, Y. Liu, G. Porcu, K. Kolkova, R. C. Friis, K. Ruscher, B. Pakkenberg, T. Goldmann, D. Loreth, M. Prinz, D. C. Rubinsztein, S. Issazadeh-Navikas, Lack of neuronal IFN-β-IFNAR causes Lewy body- and Parkinson's disease-like dementia. *Cell* **163**, 324–339 (2015).
133. C. Baiguera, M. Alghisi, A. Pinna, A. Bellucci, M. A. De Luca, L. Frau, M. Morelli, R. Ingrassia, M. Benarese, V. Porrini, M. Pellitteri, G. Bertini, P. F. Fabene, S. Sigala, M. G. Spillantini, H.-C. Liou, P. F. Spano, M. Pizzi, Late-onset Parkinsonism in NFκB/c-Rel-deficient mice. *Brain* **135**, 2750–2765 (2012).
134. F. Girolamo, M. Errede, G. Longo, T. Annese, C. Alias, G. Ferrara, S. Morando, M. Trojano, D. R. N. Kerlero, A. Uccelli, D. Virgintino, Defining the role of NG2-expressing cells in experimental models of multiple sclerosis. A biofunctional analysis of the neurovascular unit in wild type and NG2 null mice. *PLOS ONE* **14**, e213508 (2019).
135. Y. Nash, E. Schmukler, D. Trudler, R. Pinkas-Kramarski, D. Frenkel, DJ-1 deficiency impairs autophagy and reduces alpha-synuclein phagocytosis by microglia. *J. Neurochem.* **143**, 584–594 (2017).
136. J.-Y. Park, K. S. Kim, S.-B. Lee, J.-S. Ryu, K. C. Chung, Y.-K. Choo, I. Jou, J. Kim, S. M. Park, On the mechanism of internalization of alpha-synuclein into microglia: Roles of ganglioside GM1 and lipid raft. *J. Neurochem.* **110**, 400–411 (2009).
137. Y. Xia, G. Zhang, C. Han, K. Ma, X. Guo, F. Wan, L. Kou, S. Yin, L. Liu, J. Huang, N. Xiong, T. Wang, Microglia as modulators of exosomal alpha-synuclein transmission. *Cell Death Dis.* **10**, 174 (2019).
138. C. Porro, M. A. Panaro, D. D. Lofrumento, E. Hasalla, T. Trotta, The multiple roles of exosomes in Parkinson's disease: An overview. *Immunopharmacol. Immunotoxicol.* **41**, 469–476 (2019).
139. Y. Xia, G. Zhang, L. Kou, S. Yin, C. Han, J. Hu, F. Wan, Y. Sun, J. Wu, Y. Li, J. Huang, N. Xiong, Z. Zhang, T. Wang, Reactive microglia enhance the transmission of exosomal α-synuclein via toll-like receptor 2. *Brain* **144**, 2024–2037 (2021).
140. R. M. Meade, D. P. Fairlie, J. M. Mason, Alpha-synuclein structure and Parkinson's disease–Lessons and emerging principles. *Mol. Neurodegener.* **14**, 29 (2019).
141. M. Schweighauser, Y. Shi, A. Tarutani, F. Kametani, A. G. Murzin, B. Ghetti, T. Matsubara, T. Tomita, T. Ando, K. Hasegawa, S. Murayama, M. Yoshida, M. Hasegawa, S. Scheres, M. Goedert, Structures of α-synuclein filaments from multiple system atrophy. *Nature* **585**, 464–469 (2020).
142. J. A. Steiner, E. Quansah, P. Brundin, The concept of alpha-synuclein as a prion-like protein: Ten years after. *Cell Tissue Res.* **373**, 161–173 (2018).
143. Y. Guan, X. Zhao, F. Liu, S. Yan, Y. Wang, C. Du, X. Cui, R. Li, C. X. Zhang, Pathogenic mutations differentially regulate cell-to-cell transmission of α-synuclein. *Front. Cell. Neurosci.* **14**, 159 (2020).
144. C. Hoenen, A. Gustin, C. Birck, M. Kirchmeyer, N. Beaume, P. Felten, L. Grandbarbe, P. Heuschling, T. Heurtaux, Alpha-synuclein proteins promote pro-inflammatory cascades in microglia: Stronger effects of the A53T mutant. *PLOS ONE* **11**, e162717 (2016).
145. S. Wang, C.-H. Chu, M. Guo, L. Jiang, H. Nie, W. Zhang, B. Wilson, L. Yang, T. Stewart, J. S. Hong, J. Zhang, Identification of a specific α-synuclein peptide (α-Syn 29-40) capable of eliciting microglial superoxide production to damage dopaminergic neurons. *J. Neuroinflammation* **13**, 158 (2016).
146. A. D. Zharikov, J. R. Cannon, V. Tapias, Q. Bai, M. P. Horowitz, V. Shah, A. A. El, T. G. Hastings, J. T. Greenamyre, E. A. Burton, shRNA targeting α-synuclein prevents neurodegeneration in a Parkinson's disease model. *J. Clin. Invest.* **125**, 2721–2735 (2015).
147. M. J. Benskey, R. C. Sellnow, I. M. Sandoval, C. E. Sortwell, J. W. Lipton, F. P. Manfredsson, Silencing alpha synuclein in mature nigral neurons results in rapid neuroinflammation and subsequent toxicity. *Front. Mol. Neurosci.* **11**, 36 (2018).
148. C. Bettencourt, Y. Miki, I. S. Piras, R. de Silva, S. C. Foti, J. S. Talboom, T. Revesz, T. Lashley, R. Balazs, E. Viré, T. T. Warner, M. J. Huentelman, J. L. Holton, MOBP and HIP1 in multiple system atrophy: New α-synuclein partners in glial cytoplasmic inclusions implicated in the disease pathogenesis. *Neuropathol. Appl. Neurobiol.* **47**, 640–652 (2021).
149. K. Sharma, S. Mehra, A. S. Sawner, P. S. Markam, R. Panigrahi, A. Navalkar, D. Chatterjee, R. Kumar, P. Kadu, K. Patel, S. Ray, A. Kumar, S. K. Maji, Effect of disease-associated P123H and V70M mutations on β-synuclein fibrillation. *ACS Chem. Neurosci.* **11**, 2836–2848 (2020).
150. S. B. Prusiner, A. L. Woerman, D. A. Mordes, J. C. Watts, R. Rampersaud, D. B. Berry, S. Patel, A. Oehler, J. K. Lowe, S. N. Kravitz, D. H. Geschwind, D. V. Glidden, G. M. Halliday, L. T. Middleton, S. M. Gentleman, L. T. Grinberg, K. Giles, Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E5308–E5317 (2015).

Acknowledgments

Funding: This work was supported by the National Natural Science Foundation of China (no. 82071414), Leading Talent in Talents Project Guangdong High-level Personnel of Special Support Program, Scientific Research Foundation of Guangzhou (no. 202206010005) to Q.W., ASEAN microbiome nutrition centre to SP, CIFAR to SP, and National Medical Research Council Singapore to E.-K.T. **Author contributions:** Conceived and designed the study: Q.W. and J.Z. Performed the study: Q.W. and J.Z. Revised the paper for intellectual content: E.-K.T., S.P., and R.R. Wrote the paper: Q.W., J.Z., R.R., and E.-K.T. All authors read and approved the final manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper.

Submitted 26 March 2022
Accepted 19 January 2023
Published 15 February 2023
10.1126/sciadv.abq1141