Accepted Manuscript

Toll-like receptors and their therapeutic potential in Parkinson's disease and α-synucleinopathies

A. Kouli, C.B. Horne, C.H. Williams-Gray

PII: S0889-1591(19)30082-0
DOI: https://doi.org/10.1016/j.bbi.2019.06.042
Reference: YBRBI 3778

To appear in: *Brain, Behavior, and Immunity*

Received Date: 24 January 2019
Revised Date: 13 June 2019
Accepted Date: 29 June 2019

Please cite this article as: Kouli, A., Horne, C.B., Williams-Gray, C.H., Toll-like receptors and their therapeutic potential in Parkinson's disease and α-synucleinopathies, *Brain, Behavior, and Immunity* (2019), doi: https://doi.org/10.1016/j.bbi.2019.06.042

Toll-like receptors and their therapeutic potential in Parkinson's disease and α-synucleinopathies

Kouli A[1]*, Horne CB[1]*, Williams-Gray CH[1].

[1]John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom.

*These authors have contributed equally to this work.

Abstract

Toll-like receptors (TLRs) are pattern recognition receptors which mediate an inflammatory response upon the detection of specific molecular patterns found on foreign organisms and on endogenous damage-related molecules. These receptors play a major role in the activation of microglia, the innate immune cells of the CNS, and are also expressed in peripheral tissues, including blood mononuclear cells and the gut. It is well established that immune activation, in both the brain and periphery, is a feature of Parkinson's disease as well as other α-synucleinopathies. Aggregated forms of α-synuclein can act as ligands for TLRs (particularly TLR2 and TLR4), and hence these receptors may play a critical role in mediating a detrimental immune response to this protein, as well as other inflammatory signals in Parkinson's and related α-synucleinopathies. In this review, the potential role of TLRs in contributing to the progression of these disorders is discussed. Existing evidence comes predominantly from studies in *in vitro* and *in vivo* models, as well as analyses of postmortem human brain tissue

and pre-clinical studies of TLR inhibitors. This evidence is evaluated in detail, and the potential for therapeutic intervention in α-synucleinopathies through TLR inhibition is discussed.

Introduction

Toll-like receptors (TLRs) are a family of transmembrane pattern recognition receptor (PRR) proteins key to the activation of the innate immune response. Their primary role is in the detection of foreign microbial and viral molecules which they achieve through their recognition of pathogen-associated molecular patterns (PAMPs). TLRs can also recognize damage-associated molecular patterns (DAMPs) which are found on molecules endogenous to the host (Reviewed by Bianchi, 2007). On their recognition of either PAMPs or DAMPs, TLRs induce a signalling cascade resulting in the activation of an immune response to clear the pathogen from the host or to resolve the damage. TLRs are involved in the activation of both the innate immune system, consisting of non-specific responses such as initiation of pro-inflammatory cytokine production and phagocytosis, and the adaptive immune system consisting of a response specific to the pathogen mediated by T and B lymphocytes (Hoebe and Beutler, 2004).

Microglia, the resident innate immune cells of the CNS, express all human TLRs and can be activated by them, eliciting inflammatory responses (Bsibsi et al., 2002). This involvement of TLRs in the initiation of inflammation and microglial activation in the brain suggests that they may play a pivotal role in neuronal dysfunction and death. In fact, several recent studies have implicated TLRs in neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis and Parkinson's disease (PD) (Miranda-Hernandez and Baxter, 2013; Su et al., 2016). In PD, a key theory is that the pathological protein α-synuclein activates Toll-like receptors found on microglia, ultimately leading to chronic neuroinflammation which

exacerbates neuronal dysfunction and loss. Thus, TLRs may be an attractive therapeutic target for disease modification. In this review, we will discuss the evidence for a crucial role of Toll-like receptors in α-synuclein-mediated inflammation, how this affects α-synucleinopathy progression in various model systems and the emerging therapeutic strategies to modulate the activity of these receptors.

Function and localisation of TLRs

The TLR family is made up of 10 functional receptors in humans (TLR1-10), and 12 in mice (TLR1-13; murine TLR10 is a non-functional pseudogene due to gene insertions) (Akira et al., 2006; Hasan et al., 2005). Each of these receptors has the ability to recognise distinct molecular patterns, and so allowing an appropriate immune response to be initiated. TLRs are type I transmembrane proteins formed of an extracellular leucine-rich repeat (LRR) domain responsible for specific pattern recognition, a transmembrane region, and a cytoplasmic TIR (Toll/interleukin-1 receptor) domain which transduces the signal to activate the correct downstream signalling pathway (Reviewed by Kawai and Akira, 2011). The human TLR family can be split into two groups based on where the receptors are located in the cell: TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are found on the plasma membrane, while TLR3, TLR7, TLR8, and TLR9 are located in intracellular compartments **(Figure 1)**. This differential localisation occurs due to the specialisation of the different members of the TLR family to recognise specific pathogens. The TLRs on the cell surface membrane recognise components of the outer membrane of microbes, such as lipopolysaccharides which are found in the membrane of Gram-negative bacteria and are recognised by TLR4 (Poltorak et al., 1998). The intracellular TLRs primarily recognise foreign nucleic acids. These TLRs are mainly expressed in membranes of endosomal or lysosomal compartments (Latz et al., 2004; Nishiya et al.,

2005). This endolysosomal localisation allows for the discrimination between self and nonself molecules, such as viral single-stranded RNA (ssRNA) recognised by TLR7 & TLR8 (Heil et al., 2004), due to self nucleic acids not being present in such compartments under normal physiological conditions (Reviewed by Brencicova and Diebold, 2013). Similarly, TLR3 recognises and is activated by double-stranded RNA (dsRNA) which is mainly encountered in viruses (Alexopoulou et al., 2001), while TLR9 cascades are triggered by unmethylated cytosine/guanine (CpG)-rich DNA strands which are common in bacteria but less frequent in mammalian cells (Hemmi et al., 2000).

TLRs normally function in homodimers, with the exception of TLR2 which forms heterodimers with either TLR1 or TLR6, as well as homodimers (Jin et al., 2007; Kang et al., 2009). Some TLRs work in combination with additional molecules or co-receptors that enable more sensitive ligand recognition, notably TLR4 requires both MD2 (Shimazu et al., 1999) and CD14 (Wright et al., 1990) to recognize and be activated by bacterial lipopolysaccharides (LPS).

On PAMP or DAMP recognition by the LRR domain, the signal is transduced intracellularly, and the correct adapter proteins (each with a TIR domain) are recruited to and activated by the cytoplasmic TIR domain of the TLR to trigger the correct immune response (Akira et al., 2006). There are multiple adapter proteins which can be activated on pattern recognition by the TLR, and the combination of these proteins determines the specific downstream pathway. MyD88 (myeloid differentiation 88) is one such adapter protein. It can be activated by all TLRs with the exception of TLR3, and as such is known as the central adapter protein (Deguine and Barton, 2014). Recruitment of MyD88 triggers the downstream activation of mitogen-activated protein kinases (MAPKs) and the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), ultimately leading to the expression of multiple genes involved in the immune response. These include genes

responsible for the production of pro-inflammatory cytokines such as TNFα, IL-1β and IL6 (Kawai and Akira, 2011). NF-κB can also be activated in a MyD88-independent pathway involving the TLR3 and TLR4 adapter protein TRIF (TIR-domain-containing adapter-inducing interferon-β). This pathway results in the activation of the IRF (interferon regulatory factor) transcription factors in addition to NF-κB, which leads to the upregulation of cytokines and chemokines involved in the inflammatory response, including IP-10, MCP1, and RANTES (Yamamoto et al., 2003). Most signalling pathways resulting from TLR activation can be classified as either MyD88-dependent (the "canonical" pathway) or TRIF-dependent. Further adapter proteins include TRAM (TRIF-related adapter molecule) and TIRAP (TIR domain-containing adapter protein). TRAM acts to recruit TRIF to TLR4, and TIRAP to recruit MyD88 to TLR2 and TLR4 **(Figure 1)** (Akira et al., 2006; Fitzgerald et al., 2003; Horng et al., 2001, 2002; Yamamoto et al., 2002). A detailed overview of the molecules and adapter proteins involved in the TLR signalling pathways can be found in Kawai and Akira, 2011.

In the periphery, TLR expression is highest in tissues which are in close contact with the environment, namely the lungs and the gastrointestinal tract, as well as in peripheral blood mononuclear cells and the spleen (Zarember and Godowski, 2002). Several cell types, both immune and non-immune, have been found to express TLRs including dendritic cells, macrophages, B and T lymphocytes (both cytotoxic and helper T lymphocytes, although expression is lower in the latter), natural killer (NK) cells, as well as epithelial, endothelial and fibroblast cells (Yao et al., 2015; McClure and Massari, 2014; Fitzner et al., 2008; Muzio et al., 2000).

Within the central nervous system, TLRs are expressed in microglia, astrocytes, and neurons. Human and mouse microglia express all the members of the TLR family, and their expression varies depending on the stimulation state of the cell and the TLR type (Bsibsi et al., 2002; Olson and Miller, 2004). TLR expression is low in resting microglia but rises rapidly on

their activation. The most highly expressed microglial TLRs are TLR1-4, with TLR2 having the highest expression (Bsibsi et al., 2002). Mouse astrocytes also express TLRs but at a lower level than microglia and lack the expression of TLRs 7 and 8 (Carpentier et al., 2005). Human astrocytes express TLR1-5 and TLR9 to some extent, with TLR3 being the most highly expressed (Bsibsi et al., 2002; Jack et al., 2005). A healthy individual shows low TLR expression on astrocytes, with upregulation on activation, though at a slower rate than that seen in microglia (Trudler et al., 2010). Microglia and astrocytes have distinct functional responses on exposure to TLR ligands, such as differential pro-inflammatory cytokine induction (Jack et al., 2005). Mouse neurons have also been shown to express TLR2, TLR3, TLR4, TLR7 and TLR8 (Barajon et al., 2009; Lafon et al., 2006; Ma et al., 2006; Tang et al., 2007). Human neurons have been shown to express TLRs 1-10, however the expression levels show high variation dependent on the specific TLR (Zhou et al., 2009). In neurons, a different variant of MyD88 is used as an adapter protein; this neuronal variant acts independently of NF-κB resulting in responses involving the negative regulation of growth, development, and survival of the TLR-induced neurons (Kim et al., 2007a).

The activation of microglia through TLR engagement by PAMPs results in an inflammatory response induced to clear pathogens, but it can also be induced by endogenous signals (DAMPs) such as heat shock proteins, uric acid, HMGB-1 (high mobility group box chromosomal protein 1), and aggregated, modified, or misfolded proteins (Beraud et al., 2011; Bianchi, 2007). The function of the inflammatory response in the brain is to protect and restore neurons and glia within the CNS, particularly neurons as these cells lack the ability to regenerate (Ransohoff, 2016). However, in addition to neuroprotective functions such as the phagocytosis and subsequent destruction of misfolded proteins, neuroinflammation can also be detrimental. Chronic neuroinflammation has been shown to occur in many neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic

lateral sclerosis (ALS), and may accelerate disease development and progression through increased levels of proinflammatory cytokines, as well as increased oxidative and nitrosative stress, which all contribute to neuronal death (Frank-Cannon et al., 2009). This forms a self-perpetuating cycle of degeneration whereby neuronal death causes immune activation, resulting in increased production of pro-inflammatory cytokines, chemokines and increased oxidative stress, which in turn leads to further neuron loss (Gao and Hong, 2008).

Toll-like receptors in Parkinson's disease

PD is the second most common neurodegenerative disease after Alzheimer's disease. It is characterised by the loss of dopaminergic neurons within the substantia nigra and the presence of α-synuclein aggregates (Lewy bodies) within the brain. Activated microglia were first observed in the substantia nigra of patients with Parkinson's disease at postmortem by McGeer and colleagues in 1988 (McGeer et al., 1988). Subsequent studies reported an increase in the levels of proinflammatory cytokines in the striatum of PD brains compared to healthy controls, including TNFα, IL-1β, and IL-6, as well as other proteins related to microglial activation like cyclooxygenase and iNOS (inducible nitric oxide synthase) (Knott et al., 2000; Mogi et al., 1994a, 1994b). Complement activation and T lymphocyte infiltration in the substantia nigra of Parkinson's postmortem brains have also been observed (Brochard et al., 2008; Loeffler et al., 2006). In agreement with these postmortem studies, positron emission tomography (PET) neuroimaging using the tracer [^{11}C]-PK11195 has demonstrated increased microglial activation in the brains of PD patients compared to age-matched healthy individuals, and this appears to start early on in the disease course (Gerhard et al., 2006; Ouchi et al., 2005).

Microglial activation in α-synucleinopathies is thought to be triggered by α-synuclein aggregation. α-Synuclein was first linked to PD on the discovery of a familial form of the

disease where patients carry a mutation in the α-synuclein gene, *SNCA* (Polymeropoulos et al., 1997). Following on from this, misfolded fibrillar α-synuclein was shown to be the main component of Lewy bodies and Lewy neurites in both PD and dementia with Lewy bodies (DLB) brains; Lewy bodies and neurites are the defining pathological feature of PD and DLB (Spillantini et al., 1997). Further research (Zhang, 2005), showed that aggregated α-synuclein oligomers induced microglial activation *in vitro*, in a primary neuron-glia co-culture system and that this led to increased dopaminergic neurotoxicity. Su et al. (2008) also found evidence for this in a PD model where mice over-expressing wild-type (WT) α-synuclein show early microglial activation, and in primary microglial cultures where dose-dependent α-synuclein-mediated activation was observed. The mechanism by which aggregated α-synuclein activates microglia is still being established, but a key theory involves α-synuclein working as a DAMP and activating Toll-like receptors (Beraud et al., 2011). Initial experiments using the BV2 microglial cell line demonstrated that treatment with α-synuclein led to altered expression of several TLRs, including upregulation of TLR1, TLR2, TLR3, and the adapter MyD88, and an increase in expression and release of pro-inflammatory molecules (Beraud et al., 2011). It has also been reported that the priming of cells with α-synuclein results in differential responses of microglia to TLR ligands, including increased secretion of pro-inflammatory cytokines (Roodveldt et al., 2013). In transgenic animal models, such as Thy1-α-synuclein mice which overexpress wild-type human α-synuclein, there is an increase in TLR1, TLR2, TLR4, and TLR8 mRNA expression in brain regions showing microglial activation (Watson et al., 2012). This upregulation of TLRs is widely seen across a number of different PD models, but the level and time-course of upregulation depend on the specific model (McCabe et al., 2017). It is worth noting that TLRs, and particularly TLR2, TLR4 and TLR9 have also been shown to be activated in PD models which do not show α-synuclein pathology, such as the MPTP model (Drouin-Ouellet et al., 2011; Ros-Bernal et al., 2011; Panaro et al., 2008). Activation of TLRs

in this and similar toxin models may be induced by DAMPs related to oxidative stress, such as cell debris or molecules released by damaged or dying neurons including heat-shock proteins, the nuclear protein HMGB1 (High mobility group box 1), uric acid and others (Bianchi, 2007).

Of particular note, TLR2 and TLR4 have been consistently reported to be upregulated in many α-synuclein-overexpressing or toxin-induced animal models (Drouin-Ouellet et al., 2015; Kim et al., 2013; Noelker et al., 2013; Watson et al., 2012), and accumulating evidence from human studies further implicates these receptors in the pathogenesis of PD,. The majority of studies investigating the roles of TLRs in PD pathogenesis have focused on TLRs 2 and 4, with a small proportion also looking at TLR9. Though it is possible that further TLRs are involved in PD or other α-synucleinopathies, there is as yet no evidence to support this and as such, this review will specifically focus on TLR2, TLR4 and TLR9. The putative role of these receptors in PD is summarized in **Figure 2**.

Toll-like receptor 2

In human PD, upregulation of TLR2 has been observed in postmortem brains **(Table 1)**. Specifically, transcriptomic analysis revealed increased TLR2 expression in the substantia nigra, accompanied by an increase in the expression of genes involved in the TLR2 signalling pathway including CD14, IRAK2 and NF-κB (Kim et al., 2013). An increase in TLR2 protein levels was also observed in the caudate/putamen of postmortem PD brains (Drouin-Ouellet et al., 2015). Furthermore, TLR2 was found to co-localise with amoeboid microglia, suggesting upregulation of TLR2 expression by activated microglia in PD brains (Doorn et al., 2014; Kim et al., 2013; Ping et al., 2018). TLR2 has also been reported to be expressed on neurons in human PD brains, and the level of neuronal TLR2 expression was strongly correlated with disease stage and disease duration (Dzamko et al., 2017). However, analysis of publicly

available transcriptomic data from the cortex of control postmortem brains has indicated that TLR2 is almost exclusively expressed on microglia (Hughes et al., 2018), hence neuronal TLR2 expression may be disease-specific. TLR2 upregulation, as well as NF-κB activation, have also been identified in peripheral immune blood cells of PD patients (Ping et al., 2018; Drouin-Ouellet et al., 2015).

There is also a growing body of evidence from *in vitro* and *in vivo* studies in animal models implicating TLR2 in the pathogenesis and progression of PD (summarized in **Table 2**). Endogenous α-synuclein binds to TLR2 to trigger microglial activation, and it has been suggested that this occurs in a conformation-dependent manner, with the oligomeric, but not monomeric or dimeric forms, acting as a TLR2 agonist (Kim et al., 2013). This was further verified with the use of microglia from TLR2-deficient mice, where Kim et al. (2013) showed diminished microglial activation indicated by a complete loss of cytokine and chemokine upregulation on treatment with endogenous α-synuclein released by human α-synuclein overexpressing SHSY5Y cells, compared to wild-type microglia. The same group later demonstrated that endogenous α-synuclein released from neurons induced TLR2-mediated microglial activation and this appeared to be neurotoxic. This neurotoxicity was entirely abolished by TLR2 gene depletion (Kim et al., 2016). Daniele et al. (2015) similarly showed that the oligomeric form of α-synuclein activates microglia, and additionally reported that this is mediated through a TLR1/2 heterodimer, resulting in the nuclear translocation of the p65 NF-κB subunit. However, work by others has contradicted that it is only the oligomeric form of α-synuclein which can activate TLRs. Gustot et al. (2015) reported that α-synuclein fibrils, not oligomers or monomers, cause the activation of the NF-κB pathway through TLR2 activation *in vitro*. Another study in primary human monocytes suggested that both monomeric and fibrillar α-synuclein instigated a TLR2-mediated inflammatory response, though α-synuclein oligomers were not used in this work (Codolo et al., 2013). It is possible that this

discrepancy results partly from a lack of consistency in the definition and size of oligomers and fibrils across different studies. Although the precise aggregate size which is most pathologically relevant in this context remains uncertain, it seems clear that at least one, if not several species of the protein, can act as DAMPs (Roberts and Brown, 2015). Studies in models of other diseases have similarly suggested that other misfolded proteins can also act as DAMPS, for instance, Liu et al. (2012) identified TLR2 as the receptor to which amyloid-β binds and initiates microglial inflammatory responses.

Activation of neuronal TLR2 has been suggested to result in increased intracellular α-synuclein protein. This was seen in studies by Dzamko et al. (2017) on the SHSY5Y cell line and IPSC-derived human neural progenitor cells using the TLR2 agonist PAM3CSK4. It is interesting to note that in this study there was no increase in α-synuclein expression, which alongside the marked increase in the expression of the autophagy marker p62 suggests impairment in the autophagy/lysosome degradation pathway (Dzamko et al., 2017). The TLR4 agonist LPS did not show this effect on intracellular α-synuclein. Building on their earlier work, Kim et al. (2015) proposed that TLR2 activation can lead to an AKT/mTOR-mediated inhibition of autophagy resulting in accumulation of α-synuclein within neurons. In this study, $TLR2^{-/-}$ mice had reduced neuronal α-synuclein accumulation, reduced microgliosis and astrogliosis, and showed improved motor behavioural deficits compared to control mice (Kim et al., 2015).

TLR2 has also been implicated in the MPTP toxin model of PD, as shown by its upregulation on MPTP treatment, and subsequent upregulation of proinflammatory cytokines (Chao et al., 2016; Drouin-Ouellet et al., 2011; Jang et al., 2017; Koo et al., 2017). In α-synuclein-overexpressing mice, immunization with anti-TLR2 antibodies resulted in reduced α-synuclein accumulation, reduced microgliosis and proinflammatory cytokine secretion, and a reduced memory deficit as shown using behavioural tests (Kim et al., 2018; La Vitola et al.,

2018). Furthermore, the use of a TLR2 neutralizing antibody in an *in vitro* set of experiments revealed additional roles for TLR2 in the neuron-to-neuron and neuron-to-astrocyte transfer of α-synuclein. Specifically, after TLR2 inhibition the neuron to neuron transfer of α-synuclein was markedly reduced. A similar reduction in α-synuclein transfer was observed in a co-culture of SHSY5Y neurons with primary human astrocytes (Kim et al., 2018).

Toll-like receptor 4

In human studies, analysis of transcriptomic data from postmortem control brains revealed ubiquitous expression of TLR4 and the adapter MyD88 throughout the brain, with their expression being higher in the substantia nigra and the putamen (Hughes et al., 2018) **(Table 1)**. Additional studies comparing PD with control brains showed increased TLR4 protein levels in PD cases, specifically in the substantia nigra (Shin et al., 2015) and the caudate/putamen (Drouin-Ouellet et al., 2015). This increase was also observed in peripheral immune cells in PD patients (Ping et al., 2018; Drouin-Ouellet et al., 2015; Shin et al., 2015). Additional evidence supporting a role of TLR4 in human PD is provided from genetic studies and the discovery that a polymorphism in the TLR4 gene is linked with the risk of PD in a Chinese Han population (Zhao et al., 2015).

TLR4 has also been implicated through *in vitro* and *in vivo* work in animal models of PD, though some studies support a neuroprotective role while others argue that it acts in a detrimental manner (summarized in **Table 3**).

Stefanova et al. (2011) showed that TLR4 ablation leads to impaired microglial phagocytosis of α-synuclein in an *in vitro* model using BV2 microglia. In the same study, TLR4 deficiency was found to increase the dopaminergic neuron loss in the SN of an α-synuclein overexpressing mouse model, in addition to exacerbating the motor problems seen in this

model. This neuronal death was proposed to occur due to the lack of clearing of α-synuclein by microglial phagocytosis, implicating TLR4 in this process (Stefanova et al., 2011). Further implicating TLR4 in neuroprotection, work by Mariucci et al. (2018) demonstrated that overexpression of α-synuclein mRNA occurs in $TLR4^{-/-}$ mice in several brain areas, though the causes of this have not been elucidated.

Conversely, TLR4 has been implicated in the MPTP toxin model of PD, with MPTP treatment resulting in TLR4 upregulation, and inhibition of TLR4, either by gene knockout or with siRNA silencing, ameliorating MPTP induced deficits. For instance, $TLR4^{-/-}$ mice appeared to be less vulnerable to the toxin as they showed less dopaminergic cell death, in addition to fewer activated nigral microglia (Mariucci et al., 2018; Conte et al., 2017; Zhao et al., 2016; Noelker et al., 2013). Recently, Campolo et al. (2018) added to this data by showing that the absence of TLR4 also leads to inhibition of inflammasome activation and an improvement in MPTP-induced motor impairments. Zhou et al. (2016) using an *in vitro* TLR4 siRNA silencing approach found a reduction in microglial activation after MPTP treatment and decreased NF-κB activation. Upregulation of TLR4 was observed in an additional toxin model induced by 6-hydroxydopamine (6-OHDA); in this model TLR3 expression was also upregulated (McCabe et al., 2017). In a rotenone-induced model, motor deficits and dopaminergic cell loss were ameliorated in $TLR4^{-/-}$ animals (Perez-Pardo et al., 2018).

Recent work suggests that not only TLR2 but also TLR4 is necessary for the recognition of α-synuclein and initiation of inflammatory responses. Upon α-synuclein stimulation, TLR4-deficient microglia produced significantly less TNFα and reactive oxygen species, compared to wild-type microglia (Fellner et al., 2013). In agreement with this, Shao et al. (2018) recently showed that either pharmacological inhibition or gene depletion of TLR4 in microglia *in vitro* attenuates α-synuclein-induced TNFα secretion. Further work showed TLR4 to be involved in mediating the astrocyte response to α-synuclein as well, resulting in upregulation of

proinflammatory cytokines through activation of the NF-κB pathway (Rannikko et al., 2015). This data suggests that TLR4 is responsible for detecting α-synuclein as a DAMP and initiating an immune response. Work by Hughes et al., 2018 revealed that physiological levels of soluble oligomeric α-synuclein (but not monomers or fibrils) could induce TLR4-mediated cytokine production in microglia in vitro, with a 10- to 100-fold reduction in TNFα production induced by α-synuclein in TLR4$^{-/-}$ microglia compared to wild-type. The reduction in the inflammatory response to α-synuclein in TLR2$^{-/-}$ microglia compared to wild type was much more modest, thus suggesting that the TLR4 pathway is the primary mechanism underlying the microglial response to oligomeric α-synuclein at physiological concentrations.

Finally, recent evidence implicates TLR4 activation in the gastrointestinal tract with PD pathogenesis. It has been widely proposed that α-synuclein pathology in PD may be initiated in the periphery, specifically in the intestinal and/or olfactory mucosa (Hawkes et al., 2007), and α-synuclein aggregates are present in human colonic mucosa even in very early disease (Malek et al., 2014). In further support of early gut involvement in PD, patients have been found to have an altered microbiome in the intestine (Keshavarzian et al., 2015; Scheperjans et al., 2015). Furthermore, studies in germ-free α-synuclein-overexpressing mice have demonstrated that colonisation of the gut with microbiota from PD patients exacerbates disease severity, compared with colonisation with microbiota from healthy human donors (Sampson et al., 2016). As such, the microbiome itself may play a role in driving disease progression by triggering an abnormal inflammatory response. TLR4 is primarily activated by lipopolysaccharides found on the surface of Gram-negative bacteria (Poltorak et al., 1998) and is therefore uniquely poised to sense and be activated by dysbiotic gut microbiota. In line with this theory, Perez-Pardo et al. (2018) have demonstrated increased expression of TLR4 mRNA in human intestinal mucosal biopsy samples from PD patients compared to controls, with a concomitant increase in the expression of pro-inflammatory cytokines (IL-1β, IFNγ) and

chemokines (CCL2, CCL5) in the sigmoid mucosa. In a second set of experiments using the rotenone mouse model of PD, the same authors showed that TLR4$^{-/-}$ mice were protected to some extent from the toxic effects of rotenone both in the gut and the brain, and their motor deficit was significantly less severe compared to wild-type rotenone-treated mice. However, in a different study it was found that the knockout of MyD88 had no effect on the MPTP treatment-induced degeneration of the dopaminergic nigrostriatal pathway (Drouin-Ouellet et al., 2011) despite the increased survival of myenteric dopaminergic neurons seen in the same MPTP-treated MyD88$^{-/-}$ mice (Côté et al., 2011). In another animal study, transplantation of faecal microbiota from wild-type to MPTP mice led to reduced activation of the TLR4 pathway, dopaminergic neuron rescue and improved motor function (Sun et al., 2018).

In summary, there is evidence to suggest that TLR4 may have both neuroprotective and detrimental roles in PD, although this may depend on the model system, or on the timepoint in the disease process. Specifically, in an acute setting or in early disease, TLR4 activation by α-synuclein may promote microglial phagocytosis to facilitate intracellular degradation of aggregated α-synuclein. Conversely in the setting of chronic inflammation, in either the gut or brain, TLR4 may play a critical role in escalating the inflammatory response (Fellner et al., 2013; Lee et al., 2008).

Toll-like receptor 9

Evidence is emerging that there may also be a role for TLR9 in PD (Table 1). TLR9 was found to be upregulated in human striatal homogenates from PD patients, mirroring an MPTP-induced upregulation of TLR9 in mice (Ros-Bernal et al., 2011). Polymorphisms in the TLR9 gene (as well as TLR4) have also been reported to be associated with PD in a Chinese Han population; specifically, the rs352140 TLR9 single nucleotide polymorphism (SNP) was

linked with PD susceptibility, but only in females (Zhu et al., 2016). This SNP is relatively common (found in 44% of Caucasians, Japanese and Chinese, and in 67% of African Americans), though its functional significance has not been explored yet (Cheng et al., 2007). The role of TLR9 in PD was recently investigated in an MPTP mouse model where TLR9 ablation by gene knockout was found to be protective against dopaminergic cell death in the substantia nigra (Maatouk et al., 2018). The authors further proposed a regulatory role for glucocorticoid receptors (GR) in the TLR9 mediated response. Specifically, TLR9 stimulation via injection of its agonist CpG ODN in control and GR knockout mice led to dopaminergic cell loss which was more pronounced in the GR knockout group. This data suggests that GR may play a protective role in TLR9-mediated neurodegeneration (Maatouk et al., 2018), which if replicated in further studies, would raise interesting therapeutic implications.

Toll-like receptors in other synucleinopathies

If α-synuclein plays a key role in the activation of Toll-like receptors 2 & 4, as the literature suggests, one would expect TLR activation to be a common mechanism across other "α-synucleinopathies" including dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Although studies have been more limited in these conditions, evidence of TLR activation has been similarly reported, as reviewed below.

Dementia with Lewy Bodies

DLB is a common form of dementia. Pathologically and clinically there is overlap with Parkinson's disease dementia (PDD), although clinically the 2 conditions differ in terms of symptom evolution (cognitive deficits being the first presenting feature in DLB, versus motor

features in PDD) (McKeith et al., 2005), and recent evidence suggests that they have distinct genetic associations at the SNCA locus (Guella et al., 2016). However, the cortical α-synuclein aggregates (Lewy bodies) formed in DLB cannot be distinguished from those seen in PD dementia.

Similar to work previously discussed in human postmortem PD brains, increased numbers of activated microglia have been found in the trans-entorhinal cortex of DLB brains (Mackenzie, 2000), and subsequent PET neuroimaging studies using the TSPO ligand PK11195 have confirmed microglial activation in subcortical and cortical regions in early stage DLB (Iannaccone et al., 2013). In conjunction with this, TLR2 protein levels are increased in DLB post mortem brain. Specifically, western blotting in temporal cortex homogenates showed a significant increase in TLR2, while immunohistochemical analysis revealed increased co-localization of TLR2 with microglia but not neurons, as compared to control brains (Kim et al., 2013). Garcia-Esparcia et al. (2017) found no change in the mRNA expression level of TLR4 and TLR7 in postmortem frontal cortices of cases with DLB, or rapid DLB (2 years from symptom onset to death) compared to neurologically intact controls but did not evaluate TLR2.

Multiple system atrophy

Multiple system atrophy is an α-synucleinopathy which is more distinct in its pathology and clinical phenotype than DLB and PD. It is characterised by early autonomic disturbance as well as parkinsonism and cerebellar features, and a more rapidly progressive course than PD with minimal response to levodopa. Pathologically, the α-synuclein aggregates in MSA differ from Lewy bodies both in their glial location and their solubility – these aggregates are known as glial cytoplasmic inclusions (GCIs) (Wenning and Jellinger, 2005).

As in other α-synucleinopathies, human MSA postmortem brains exhibit increased region-specific microglial activation correlating with α-synuclein glial cytoplasmic inclusions as well as neuronal loss compared to controls (Stefanova et al., 2007; Ishizawa et al., 2004), with similar findings in an animal model of the disease (Stefanova et al., 2007). TLR4 expression was found to be increased both in an animal model of MSA and in human postmortem MSA brain (Stefanova et al., 2007). Other TLRs have also been reported to be upregulated in MSA in a brain region-specific manner: TLR3, TLR4, and TLR5 in the SN, striatum, cerebral cortex, and nucleus dentatus; TLR1 in the SN and striatum only, and TLR8 and TLR9 in the cerebellum (Brudek et al., 2013). Interestingly TLR4 expression was also increased in the colonic sigmoid mucosa of MSA patients raising the possibility that neuroinflammation in MSA may be sustained or driven by inflammation in the gut, as has also been suggested in PD (Engen et al., 2017). A change in TLR2 expression in MSA has not been reported. This raises the possibility that TLRs may play distinct roles according to the nature of the α-synuclein aggregates (GCIs versus Lewy bodies).

The potential neuroprotective role of TLR4 in MSA has also been investigated using the TLR4 agonist monophosphoryl lipid A (MPLA), a chemically detoxified form of the common TLR4 agonist lipopolysaccharide (LPS), which does not instigate neuroinflammatory responses (Venezia et al., 2017). Upon MPLA injection in the brain of α-synuclein-overexpressing mice, increased α-synuclein clearance, reduced nigral dopaminergic neuron death, and a region-specific decrease in intracellular α-synuclein aggregates was seen. The beneficial role of TLR4 stimulation seen in this work indicates that MPLA may warrant further consideration as a potential therapeutic compound (Venezia et al., 2017). Interestingly, in this same study, chronic TLR4 stimulation by LPS resulted in decreased monomeric α-synuclein, and a shift to (putatively) more toxic oligomeric α-synuclein species (Venezia et al., 2017).

Hence TLR4 may have dual roles to play in both neuroinflammation and α-synuclein aggregate clearance in MSA.

Potential therapeutic strategies

There is an increasing interest in the possible use of therapeutics aimed at preventing or stopping neuroinflammation in PD and other α-synucleinopathies. Dying or injured neurons can release noxious molecules in the extracellular milieu such as misfolded or aggregated proteins (e.g., α-synuclein), cytosolic compounds like neuromelanin, or other altered molecules (e.g. the matrix metalloproteinase-3) (Kim et al., 2007b; Zhang, 2005; Zecca et al., 2003). These self-molecules can serve as stimuli (DAMPs) to initiate an inflammatory response. This initial acute response can be beneficial for the injured tissue by promoting clearance of cell debris and by releasing neurotrophic factors to enhance neuronal survival (Gao and Hong, 2008; Trapp et al., 2007; Kordower, 2003). However, an uncontrolled chronic neuroinflammatory response will result in the prolonged secretion of pro-inflammatory cytokines, reactive oxygen species and nitric oxide, which are neurotoxic and will exacerbate neuronal injury and death (Gao and Hong, 2008). Therefore, the immune system's capacity to play a role in propagating neuronal dysfunction within the brain could contribute to the progressive nature of PD.

Most proposed immune-modulating therapies to date are rather non-specific in their targeting of the immune/inflammatory response, thus leading to a broad range of adverse effects (Wang et al., 2015). TLRs provide a specific route for therapeutic development. Several TLR compounds have been explored - mostly in animal models of PD - but these agents vary in terms of ease of potential translation into human clinical trials. The three main therapeutic

approaches explored thus far include the use of small molecule inhibitors, repurposed pre-existing drugs, and natural compounds.

Small synthetic molecules, including TLR signalling inhibitors, have recently been explored for their therapeutic capacity in neurodegenerative diseases. TAK242, or resatorvid, is one such small molecule, which specifically suppresses the activation of TLR4 by interacting with the receptor's intracellular TIR domain (Ii et al., 2006; Kawamoto et al., 2008). TAK242 has been used in amyotrophic lateral sclerosis (ALS) and traumatic brain injury (TBI) animal models showing, in both cases, neuroprotective effects and behavioural improvement by blocking TLR4 signalling and thereby reducing neuroinflammation (Fellner et al., 2017; Feng et al., 2017). As previously discussed, oligomeric α-synuclein is hypothesized to exert at least some of its inflammatory effects by binding to TLR4. Hughes et al. (2018) tested whether TAK242 or RSLA (another small molecule inhibitor of TLR4) could inhibit the neurotoxic effects of physiological α-synuclein levels *in vitro*. Pre-treatment with either of these inhibitors significantly reduced α-synuclein-induced oxidative stress and cell death in neuronal cultures. Furthermore, the same authors showed that TNFα production by microglia in culture upon α-synuclein treatment was also significantly attenuated by TAK242 or RSLA treatment. TAK242 has previously been used in a Phase I randomized, double-blind, placebo-controlled clinical trial for the treatment of sepsis, which although negative for its primary outcome, demonstrated the safety and tolerability of this compound, at least for short term use (4 days) (Rice et al., 2010). Although these studies were on disorders other that α-synucleinopathies, they indicate that TAK242 could have beneficial effects in PD through the blocking of TLR4 and subsequent decrease in the neuroinflammatory effects of α-synuclein. CU-CPT22 is another small molecule inhibitor which is selective for TLR1/2. This molecule led to a reduction of NF-κB nuclear translocation with a concomitant decrease in TNFα production in primary microglia exposed to α-synuclein (Daniele et al., 2015) but has not been tested *in vivo*.

In the field of neurodegenerative disease, it is becoming increasingly common for the therapeutic potential of drugs already licenced for other conditions to be explored (so-called "drug repurposing"). One such candidate is vinpocetine which is used clinically in the treatment of cerebrovascular diseases due to its neuroprotective action and its effects on cerebral blood flow (Zhang and Yang, 2014; Patyar et al., 2011; Szilágyi et al., 2005). *In vitro* studies have revealed anti-inflammatory properties of vinpocetine through the inhibition of NF-κB activation (Dastidar et al., 2007; Fan Chung, 2006; Banner and Trevethick, 2004). In a recent double-blind placebo-controlled trial, PD patients were randomised to treatment with vinpocetine or placebo alongside standard levodopa therapy for 14 days, and TLR2, TLR3, and TLR4 mRNA expression levels in peripheral blood monocytes were measured before and after treatment (Ping et al., 2018). The vinpocetine-treated group showed a reduction in TLR2 and TLR4 expression and an increase in TLR3 expression compared to the placebo group, as well as downregulation of MyD88, NF-κB, and TNFα, and a greater improvement in patient cognitive function. It is uncertain whether the improved cognitive function observed here was due to anti-inflammatory or other vinpocetine-induced mechanisms, however, it is an attractive candidate for further study (Ping et al., 2018). Another potential candidate of interest is Candesartan cilexetil, a licensed drug for treating hypertension (angiotensin II receptor blocker), which has recently been shown to suppress TLR expression (specifically TLR2 and TLR4). Dasu et al. (2009) demonstrated that Candesartan treatment reduced the expression of both TLR2 and TLR4, inhibited the secretion of pro-inflammatory mediators such as TNFα, IL-1β and IL6, and was protective against LPS challenge, both *in vitro* in primary human monocytes and *in vivo* in mice. Furthermore, Candesartan has been shown to produce dose-dependent rescue of microglial phenotype and function after α-synuclein stimulation *in vitro* (Daniele et al., 2015). Finally, Candesartan was shown to be neuroprotective and exerted anti-inflammatory effects in a PD animal model study. Rats receiving either Candesartan,

Telmisartan (another angiotensin II receptor blocker), or vehicle for two weeks were then injected with an AAV9 virus expressing α-synuclein. Vehicle-treated α-synuclein animals showed pronounced dopaminergic cell loss in the substantia nigra, increased microglial activation and secretion of pro-inflammatory mediators and showed a significant motor deficit. All these effects were to an extent reversed in both the Candesartan- and the Telmisartan-treated group, though the effect of these two drugs on TLRs was not interrogated (Rodriguez-Perez et al., 2018). Nevertheless, this work suggests that Candesartan may warrant further consideration as a candidate for drug repurposing for PD.

Naturally-derived compounds have also been investigated regarding their use in treating neurodegenerative diseases due to having multiple pharmacological properties. One such naturally-derived compound is Curcumin, an Indian herb extracted from *Curcuma longa* root which has been demonstrated to have anti-inflammatory and anti-oxidative effects *in vitro* and *in vivo* (Monroy et al., 2013). It has been shown to act directly on the TLR4 pathway *in vitro* by inhibiting TLR4 receptor dimerisation and resulting in repression of the TLR4 pathway (Youn et al., 2006). Yu et al. (2016) investigated its effects in an *in vitro* MPTP model of PD and found that pre-treatment with Curcumin inhibited the MPTP-induced activation of primary rat astrocytes in culture, the subsequent production of pro-inflammatory cytokines, and the activation of TLR4 and several components of its downstream signalling pathway. These experiments suggest that Curcumin could work in a neuroprotective manner through the repression of TLR4 signalling and subsequent microglial activation and associated neuroinflammation. It is notable that the suitability of this compound for translation into clinical trials has already been demonstrated in other neurodegenerative diseases, although the results of these studies have been mixed. (Ringman et al., 2012; Baum et al., 2008; Small et al., 2018). Further work is required to demonstrate that the beneficial effects of Curcumin seen in *in vitro* models and in other neurodegenerative diseases can be translated to Parkinson's

disease. Finally, other natural compounds including Asiatic acid, Juglanin, Vitamin D, and Quercetin have been demonstrated to have anti-inflammatory properties and a subset appear to show neuroprotective effects (Lv et al., 2018; Chao et al., 2016; Phan et al., 2010; Zhang and Xu, 2018; Calvello et al., 2016; Zhang et al., 2016). The downregulation of TLRs has been observed in many of these studies, and it has been suggested that the neuroprotective effects of these natural compounds occur through the inhibition of TLRs and subsequent reduction in inflammation (Chao et al., 2016; Zhang and Xu, 2018; Calvello et al., 2016; Zhang et al., 2016). Nevertheless, further work is required to determine whether these naturally derived chemicals are acting directly on the TLR pathways.

Conclusion and future perspectives

Toll-like receptors may play a key role in α-synucleinopathies, including Parkinson's disease, as shown by *in vitro*, animal model, and patient studies. Neuroinflammation is thought to be instrumental in the aetiology and progression of neurodegenerative diseases, and the activation of TLRs by aggregated α-synuclein provides a mechanism by which this neuroinflammation may occur. TLR2 and TLR4 appear to be the most relevant TLRs in α-synucleinopathies and are suggested to be responsible for the detection of aggregated forms of α-synuclein. There remains some dispute in the field over which of these is most critical in mediating neuroinflammation in α-synucleinopathies. Seemingly contrasting data in the literature may reflect differences in model systems used, or in the preparation of α-synuclein aggregates. However, both TLR2 and 4 may contribute in a complimentary way. Recent evidence proposes that TLR4 activation in the periphery may be critical, especially in the gastrointestinal tract, where it may be involved in detecting gut dysbiosis, whilst the role of TLR2 may be more restricted to the brain. Hence TLRs may have tissue-specific roles in α-

synucleinopathies. A number of pre-clinical studies have tested TLR-binding compounds in animal models and demonstrated that these agents could alleviate features of α-synucleinopathies, specifically inflammation, and through this improve behavioural phenotype. Many of these compounds are suitable for clinical trials, but it remains to be seen whether their effects are translatable to human PD. Nevertheless, targeting Toll-like receptors shows potential as a strategy to alleviate disease progression in α-synucleinopathies.

Acknowledgements

The authors are supported by the NIHR Cambridge Biomedical Research Centre Dementia Theme (146281) and the Rosetrees Trust (M369-F1). AK is supported by the Onassis Foundation and the Alborada Trust. CWG is funded by an MRC Clinician Scientist Fellowship (Grant: MR/R007446/1).

Conflict of interest: The authors declare no conflicts of interest.

References

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen Recognition and Innate Immunity. Cell *124*, 783–801.

Alexopoulou, L., Holt, A.C., Medzhitov, R., and Flavell, R.A. (2001). Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature *413*, 732–738.

Banner, K.H., and Trevethick, M.A. (2004). PDE4 inhibition: a novel approach for the treatment of inflammatory bowel disease. Trends in Pharmacological Sciences *25*, 430–436.

Baum, L., Lam, C.W.K., Cheung, S.K.-K., Kwok, T., Lui, V., Tsoh, J., Lam, L., Leung, V., Hui, E., Ng, C., et al. (2008). Six-Month Randomized, Placebo-Controlled, Double-Blind, Pilot Clinical Trial of Curcumin in Patients With Alzheimer Disease: Journal of Clinical Psychopharmacology *28*, 110–113.

Beraud, D., Twomey, M., Bloom, B., Mittereder, A., Neitzke, K., Ton, V., Chasovskikh, S., Mhyre, T.R., and Maguire-Zeiss, K.A. (2011). α-Synuclein alters toll-like receptor expression. Front. Neurosci. *5*, 80.

Bianchi, M.E. (2007). DAMPs, PAMPs and alarmins: all we need to know about danger. Journal of Leukocyte Biology *81*, 1–5.

Brencicova, E., and Diebold, S.S. (2013). Nucleic acids and endosomal pattern recognition: how to tell friend from foe? Front Cell Infect Microbiol *3*.

Brochard, V., Combadière, B., Prigent, A., Laouar, Y., Perrin, A., Beray-Berthat, V., Bonduelle, O., Alvarez-Fischer, D., Callebert, J., Launay, J.-M., et al. (2008). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. Journal of Clinical Investigation.

Brudek, T., Winge, K., Agander, T.K., and Pakkenberg, B. (2013). Screening of Toll-Like Receptors Expression in Multiple System Atrophy Brains. Neurochemical Research *38*, 1252–1259.

Bsibsi, M., Ravid, R., Gveric, D., and Noort, J.M. van (2002). Broad Expression of Toll-Like Receptors in the Human Central Nervous System. Journal of Neuropathology & Experimental Neurology *61*, 1013–1021.

Calvello, R., Cianciulli, A., Nicolardi, G., Nuccio, F.D., Giannotti, L., Salvatore, R., Porro, C., Trotta, T., Panaro, M.A., and Lofrumento, D.D. (2016). Vitamin D Treatment Attenuates Neuroinflammation and Dopaminergic Neurodegeneration in an Animal Model of Parkinson's Disease, Shifting M1 to M2 Microglia Responses. J Neuroimmune Pharmacol 1–13.

Campolo, M., Paterniti, I., Siracusa, R., Filippone, A., Esposito, E., and Cuzzocrea, S. (2018). TLR4 absence reduces neuroinflammation and inflammasome activation in Parkinson's diseases in vivo model. Brain, Behavior, and Immunity.

Carpentier, P.A., Begolka, W.S., Olson, J.K., Elhofy, A., Karpus, W.J., and Miller, S.D. (2005). Differential activation of astrocytes by innate and adaptive immune stimuli. Glia *49*, 360–374.

Chao, P., Lee, H., and Yin, M. (2016). Asiatic acid attenuated apoptotic and inflammatory stress in the striatum of MPTP-treated mice. Food Funct. *7*, 1999–2005.

Cheng, P.-L., Eng, H.-L., Chou, M.-H., You, H.-L., and Lin, T.-M. (2007). Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. Translational Research *150*, 311–318.

Codolo, G., Plotegher, N., Pozzobon, T., Brucale, M., Tessari, I., Bubacco, L., and Bernard, M. de (2013). Triggering of Inflammasome by Aggregated α–Synuclein, an Inflammatory Response in Synucleinopathies. PLOS ONE *8*, e55375.

Conte, C., Roscini, L., Sardella, R., Mariucci, G., Scorzoni, S., Beccari, T., and Corte, L. (2017). Toll Like Receptor 4 Affects the Cerebral Biochemical Changes Induced by MPTP Treatment. Neurochem. Res.

Côté, M., Drouin-Ouellet, J., Cicchetti, F., and Soulet, D. (2011). The critical role of the MyD88-dependent pathway in non-CNS MPTP-mediated toxicity. Brain, Behavior, and Immunity *25*, 1143–1152.

Daniele, S.G., Béraud, D., Davenport, C., Cheng, K., Yin, H., and Maguire-Zeiss, K.A. (2015). Activation of MyD88-dependent TLR1/2 signaling by misfolded α-synuclein, a protein linked to neurodegenerative disorders. Sci Signal *8*, ra45.

Dastidar, S.G., Rajagopal, D., and Ray, A. (2007). Therapeutic benefit of PDE4 inhibitors in inflammatory diseases. Curr Opin Investig Drugs *8*, 364–372.

Dasu, M.R., Riosvelasco, A.C., and Jialal, I. (2009). Candesartan inhibits Toll-like receptor expression and activity both in vitro and in vivo. Atherosclerosis *202*, 76–83.

Deguine, J., and Barton, G.M. (2014). MyD88: a central player in innate immune signaling. F1000Prime Rep *6*.

Doorn, K.J., Moors, T., Drukarch, B., van de Berg, W.D., Lucassen, P.J., and van Dam, A.-M. (2014). Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. Acta Neuropathologica Communications *2*, 90.

Drouin-Ouellet, J., and Cicchetti, F. (2012). Inflammation and neurodegeneration: the story 'retolled.' Trends in Pharmacological Sciences *33*, 542–551.

Drouin-Ouellet, J., Gibrat, C., Bousquet, M., Calon, F., Kriz, J., and Cicchetti, F. (2011). The role of the MYD88-dependent pathway in MPTP-induced brain dopaminergic degeneration. Journal of Neuroinflammation *8*, 137.

Drouin-Ouellet, J., St-Amour, I., Saint-Pierre, M., Lamontagne-Proulx, J., Kriz, J., Barker, R.A., and Cicchetti, F. (2015). Toll-Like Receptor Expression in the Blood and Brain of Patients and a Mouse Model of Parkinson's Disease. International Journal of Neuropsychopharmacology *18*, pyu103–pyu103.

Dzamko, N., Gysbers, A., Perera, G., Bahar, A., Shankar, A., Gao, J., Fu, Y., and Halliday, G.M. (2017). Toll-like receptor 2 is increased in neurons in Parkinson's disease brain and may contribute to alpha-synuclein pathology. Acta Neuropathol *133*, 303–319.

Engen, P.A., Dodiya, H.B., Naqib, A., Forsyth, C.B., Green, S.J., Voigt, R.M., Kordower, J.H., Mutlu, E.A., Shannon, K.M., and Keshavarzian, A. (2017). The Potential Role of Gut-Derived Inflammation in Multiple System Atrophy. J Parkinsons Dis.

Fan Chung, K. (2006). Phosphodiesterase inhibitors in airways disease. European Journal of Pharmacology *533*, 110–117.

Fellner, A., Barhum, Y., Angel, A., Perets, N., Steiner, I., Offen, D., and Lev, N. (2017). Toll-Like Receptor-4 Inhibitor TAK-242 Attenuates Motor Dysfunction and Spinal Cord Pathology in an Amyotrophic Lateral Sclerosis Mouse Model. International Journal of Molecular Sciences *18*, 1666.

Fellner, L., Irschick, R., Schanda, K., Reindl, M., Klimaschewski, L., Poewe, W., Wenning, G.K., and Stefanova, N. (2013). Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. Glia *61*, 349–360.

Feng, Y., Gao, J., Cui, Y., Li, M., Li, R., Cui, C., and Cui, J. (2017). Neuroprotective Effects of Resatorvid Against Traumatic Brain Injury in Rat: Involvement of Neuronal Autophagy and TLR4 Signaling Pathway. Cell Mol Neurobiol *37*, 155–168.

Fitzgerald, K.A., Rowe, D.C., Barnes, B.J., Caffrey, D.R., Visintin, A., Latz, E., Monks, B., Pitha, P.M., and Golenbock, D.T. (2003). LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J. Exp. Med. *198*, 1043–1055.

Fitzner, N., Clauberg, S., Essmann, F., Liebmann, J., and Kolb-Bachofen, V. (2008). Human Skin Endothelial Cells Can Express All 10 TLR Genes and Respond to Respective Ligands. Clin Vaccine Immunol *15*, 138–146.

Frank-Cannon, T.C., Alto, L.T., McAlpine, F.E., and Tansey, M.G. (2009). Does neuroinflammation fan the flame in neurodegenerative diseases? Molecular Neurodegeneration *4*, 47.

Gao, H.-M., and Hong, J.-S. (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends in Immunology *29*, 357–365.

Garcia-Esparcia, P., López-González, I., Grau-Rivera, O., García-Garrido, M.F., Konetti, A., Llorens, F., Zafar, S., Carmona, M., del Rio, J.A., Zerr, I., et al. (2017). Dementia with Lewy Bodies: Molecular Pathology in the Frontal Cortex in Typical and Rapidly Progressive Forms. Front Neurol *8*.

Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., Eggert, K., Oertel, W., Banati, R.B., and Brooks, D.J. (2006). In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. Neurobiology of Disease *21*, 404–412.

Guella, I., Evans, D.M., Szu- Tu, C., Nosova, E., Bortnick, S.F., Goldman, J.G., Dalrymple-Alford, J.C., Geurtsen, G.J., Litvan, I., Ross, O.A., et al. (2016). α- synuclein genetic variability: A biomarker for dementia in Parkinson disease. Annals of Neurology *79*, 991–999.

Gustot, A., Gallea, J.I., Sarroukh, R., Celej, M.S., Ruysschaert, J.-M., and Raussens, V. (2015). Amyloid Fibrils Are the Molecular Trigger of Inflammation in Parkinson's Disease. Biochemical Journal BJ20150617.

Hasan, U., Chaffois, C., Gaillard, C., Saulnier, V., Merck, E., Tancredi, S., Guiet, C., Brière, F., Vlach, J., Lebecque, S., et al. (2005). Human TLR10 Is a Functional Receptor, Expressed by B Cells and Plasmacytoid Dendritic Cells, Which Activates Gene Transcription through MyD88. The Journal of Immunology *174*, 2942–2950.

Hawkes, C.H., Tredici, K.D., and Braak, H. (2007). Parkinson's disease: a dual-hit hypothesis. Neuropathology and Applied Neurobiology *33*, 599–614.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., and Bauer, S. (2004). Species-Specific Recognition of Single-Stranded RNA via Toll-like Receptor 7 and 8. Science *303*, 1526–1529.

Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K., et al. (2000). A Toll-like receptor recognizes bacterial DNA. Nature *408*, 740–745.

Hoebe, K., and Beutler, B. (2004). LPS, dsRNA and the interferon bridge to adaptive immune responses: Trif, Tram, and other TIR adaptor proteins. Journal of Endotoxin Research *10*, 130–136.

Horng, T., Barton, G.M., and Medzhitov, R. (2001). TIRAP: an adapter molecule in the Toll signaling pathway. Nature Immunology *2*, 835–841.

Horng, T., Barton, G.M., Flavell, R.A., and Medzhitov, R. (2002). The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. Nature *420*, 329–333.

Hughes, C.D., Choi, M.L., Ryten, M., Hopkins, L., Drews, A., Botía, J.A., Iljina, M., Rodrigues, M., Gagliano, S.A., Gandhi, S., et al. (2018). Picomolar concentrations of oligomeric alpha-synuclein sensitizes TLR4 to play an initiating role in Parkinson's disease pathogenesis. Acta Neuropathol.

Iannaccone, S., Cerami, C., Alessio, M., Garibotto, V., Panzacchi, A., Olivieri, S., Gelsomino, G., Moresco, R.M., and Perani, D. (2013). In vivo microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. Parkinsonism & Related Disorders *19*, 47–52.

Ii, M., Matsunaga, N., Hazeki, K., Nakamura, K., Takashima, K., Seya, T., Hazeki, O., Kitazaki, T., and Iizawa, Y. (2006). A Novel Cyclohexene Derivative, Ethyl (6R)-6-[N-(2-Chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate (TAK-242), Selectively Inhibits Toll-Like Receptor 4-Mediated Cytokine Production through Suppression of Intracellular Signaling. Mol Pharmacol *69*, 1288–1295.

Ishizawa, K., Komori, T., Sasaki, S., Arai, N., Mizutani, T., and Hirose, T. (2004). Microglial Activation Parallels System Degeneration in Multiple System Atrophy. Journal of Neuropathology & Experimental Neurology *63*, 43–52.

Jack, C.S., Arbour, N., Manusow, J., Montgrain, V., Blain, M., McCrea, E., Shapiro, A., and Antel, J.P. (2005). TLR Signaling Tailors Innate Immune Responses in Human Microglia and Astrocytes. The Journal of Immunology *175*, 4320–4330.

Jang, Y., Koo, J.-H., Kwon, I., Kang, E.-B., Um, H.-S., Soya, H., Lee, Y., and Cho, J.-Y. (2017). Neuroprotective effects of endurance exercise against neuroinflammation in MPTP-induced Parkinson's disease mice. Brain Research *1655*, 186–193.

Jin, M.S., Kim, S.E., Heo, J.Y., Lee, M.E., Kim, H.M., Paik, S.-G., Lee, H., and Lee, J.-O. (2007). Crystal Structure of the TLR1-TLR2 Heterodimer Induced by Binding of a Tri-Acylated Lipopeptide. Cell *130*, 1071–1082.

Kang, J.Y., Nan, X., Jin, M.S., Youn, S.-J., Ryu, Y.H., Mah, S., Han, S.H., Lee, H., Paik, S.-G., and Lee, J.-O. (2009). Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity *31*, 873–884.

Kawai, T., and Akira, S. (2011). Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity. Immunity *34*, 637–650.

Kawamoto, T., Ii, M., Kitazaki, T., Iizawa, Y., and Kimura, H. (2008). TAK-242 selectively suppresses Toll-like receptor 4-signaling mediated by the intracellular domain. European Journal of Pharmacology *584*, 40–48.

Keshavarzian, A., Green, S.J., Engen, P.A., Voigt, R.M., Naqib, A., Forsyth, C.B., Mutlu, E., and Shannon, K.M. (2015). Colonic bacterial composition in Parkinson's disease. Movement Disorders *30*, 1351–1360.

Kim, C., Ho, D.-H., Suk, J.-E., You, S., Michael, S., Kang, J., Joong Lee, S., Masliah, E., Hwang, D., Lee, H.-J., et al. (2013). Neuron-released oligomeric α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nature Communications *4*, 1562.

Kim, C., Rockenstein, E., Spencer, B., Kim, H.-K., Adame, A., Trejo, M., Stafa, K., Lee, H.-J., Lee, S.-J., and Masliah, E. (2015). Antagonizing Neuronal Toll-like Receptor 2 Prevents Synucleinopathy by Activating Autophagy. Cell Reports *13*, 771–782.

Kim, C., Lee, H.-J., Masliah, E., and Lee, S.-J. (2016). Non-cell-autonomous Neurotoxicity of α-synuclein Through Microglial Toll-like Receptor 2. Experimental Neurobiology *25*, 113.

Kim, C., Spencer, B., Rockenstein, E., Yamakado, H., Mante, M., Adame, A., Fields, J.A., Masliah, D., Iba, M., Lee, H.-J., et al. (2018). Immunotherapy targeting toll-like receptor 2 alleviates neurodegeneration in models of synucleinopathy by modulating α-synuclein transmission and neuroinflammation. Molecular Neurodegeneration *13*, 43.

Kim, Y., Zhou, P., Qian, L., Chuang, J.-Z., Lee, J., Li, C., Iadecola, C., Nathan, C., and Ding, A. (2007a). MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. Journal of Experimental Medicine *204*, 2063–2074.

Kim, Y.S., Choi, D.H., Block, M.L., Lorenzl, S., Yang, L., Kim, Y.J., Sugama, S., Cho, B.P., Hwang, O., Browne, S.E., et al. (2007b). A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration via microglial activation. FASEB J *21*, 179–187.

Knott, C., Stern, G., and Wilkin, G.P. (2000). Inflammatory Regulators in Parkinson's Disease: iNOS, Lipocortin-1, and Cyclooxygenases-1 and -2. Molecular and Cellular Neuroscience *16*, 724–739.

Koo, J.-H., Jang, Y.-C., Hwang, D.-J., Um, H.-S., Lee, N.-H., Jung, J.-H., and Cho, J.-Y. (2017). Treadmill exercise produces neuroprotective effects in a murine model of Parkinson's disease by regulating the TLR2/MyD88/NF-κB signaling pathway. Neuroscience.

Kordower, J.H. (2003). In vivo gene delivery of glial cell line–derived neurotrophic factor for Parkinson's disease. Ann Neurol. *53*, S120–S134.

La Vitola, P., Balducci, C., Cerovic, M., Santamaria, G., Brandi, E., Grandi, F., Caldinelli, L., Colombo, L., Morgese, M.G., Trabace, L., et al. (2018). Alpha-synuclein oligomers impair memory through glial cell activation and via Toll-like receptor 2. Brain, Behavior, and Immunity *69*, 591–602.

Lafon, M., Megret, F., Lafage, M., and Prehaud, C. (2006). The Innate Immune Facet of Brain: Human Neurons Express TLR-3 and Sense Viral dsRNA. Journal of Molecular Neuroscience *29*, 185–194.

Latz, E., Schoenemeyer, A., Visintin, A., Fitzgerald, K.A., Monks, B.G., Knetter, C.F., Lien, E., Nilsen, N.J., Espevik, T., and Golenbock, D.T. (2004). TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nature Immunology *5*, 190–198.

Lee, H.-J., Suk, J.-E., Bae, E.-J., and Lee, S.-J. (2008). Clearance and deposition of extracellular α-synuclein aggregates in microglia. Biochemical and Biophysical Research Communications *372*, 423–428.

Liu, S., Liu, Y., Hao, W., Wolf, L., Kiliaan, A.J., Penke, B., Rübe, C.E., Walter, J., Heneka, M.T., Hartmann, T., et al. (2012). TLR2 is a primary receptor for Alzheimer's amyloid β peptide to trigger neuroinflammatory activation. J. Immunol. *188*, 1098–1107.

Loeffler, D.A., Camp, D.M., and Conant, S.B. (2006). Complement activation in the Parkinson's disease substantia nigra: an immunocytochemical study. J Neuroinflammation *3*, 29.

Lv, J., Sharma, A., Zhang, T., Wu, Y., and Ding, X. (2018). Pharmacological Review on Asiatic Acid and Its Derivatives: A Potential Compound. SLAS TECHNOLOGY: Translating Life Sciences Innovation *23*, 111–127.

Ma, Y., Li, J., Chiu, I., Wang, Y., Sloane, J.A., Lü, J., Kosaras, B., Sidman, R.L., Volpe, J.J., and Vartanian, T. (2006). Toll-like receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. J Cell Biol *175*, 209–215.

Maatouk, L., Compagnion, A.-C., Sauvage, M.-A.C., Bemelmans, A.-P., Leclere-Turbant, S., Cirotteau, V., Tohme, M., Beke, A., Trichet, M., Bazin, V., et al. (2018). TLR9 activation via microglial glucocorticoid receptors contributes to degeneration of midbrain dopamine neurons. Nature Communications *9*, 2450.

Mackenzie, I.R.A. (2000). Activated microglia in dementia with Lewy bodies. Neurology *55*, 132–134.

Malek, N., Swallow, D., Grosset, K.A., Anichtchik, O., Spillantini, M., and Grosset, D.G. (2014). Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease – a systematic review. Acta Neurologica Scandinavica *130*, 59–72.

Mariucci, G., Pagiotti, R., Galli, F., Romani, L., and Conte, C. (2018). The Potential Role of Toll-Like Receptor 4 in Mediating Dopaminergic Cell Loss and Alpha-Synuclein Expression in the Acute MPTP Mouse Model of Parkinson's Disease. J Mol Neurosci 1–8.

McCabe, K., Concannon, R.M., McKernan, D.P., and Dowd, E. (2017). Time-course of striatal Toll-like receptor expression in neurotoxic, environmental and inflammatory rat models of Parkinson's disease. Journal of Neuroimmunology *310*, 103–106.

McClure, R., and Massari, P. (2014). TLR-Dependent Human Mucosal Epithelial Cell Responses to Microbial Pathogens. Front Immunol *5*.

McGeer, P.L., Itagaki, S., Boyes, B.E., and McGeer, E.G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology *38*, 1285–1291.

McKeith, I.G., Dickson, D.W., Lowe, J., Emre, M., O'Brien, J.T., Feldman, H., Cummings, J., Duda, J.E., Lippa, C., Perry, E.K., et al. (2005). Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology *65*, 1863–1872.

Miranda-Hernandez, S., and Baxter, A.G. (2013). Role of toll-like receptors in multiple sclerosis. Am J Clin Exp Immunol *2*, 75–93.

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., and Nagatsu, T. (1994a). Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients. Neuroscience Letters *180*, 147–150.

Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., and Nagatsu, T. (1994b). Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neuroscience Letters *165*, 208–210.

Monroy, A., Lithgow, G.J., and Alavez, S. (2013). Curcumin and neurodegenerative diseases. BioFactors *39*, 122–132.

Muzio, M., Bosisio, D., Polentarutti, N., D'amico, G., Stoppacciaro, A., Mancinelli, R., Veer, C. van't, Penton-Rol, G., Ruco, L.P., Allavena, P., et al. (2000). Differential Expression and Regulation of Toll-Like Receptors (TLR) in Human Leukocytes: Selective Expression of TLR3 in Dendritic Cells. The Journal of Immunology *164*, 5998–6004.

Nishiya, T., Kajita, E., Miwa, S., and DeFranco, A.L. (2005). TLR3 and TLR7 Are Targeted to the Same Intracellular Compartments by Distinct Regulatory Elements. J. Biol. Chem. *280*, 37107–37117.

Noelker, C., Morel, L., Lescot, T., Osterloh, A., Alvarez-Fischer, D., Breloer, M., Henze, C., Depboylu, C., Skrzydelski, D., Michel, P.P., et al. (2013). Toll like receptor 4 mediates cell death in a mouse MPTP model of Parkinson disease. Scientific Reports *3*.

Olson, J.K., and Miller, S.D. (2004). Microglia Initiate Central Nervous System Innate and Adaptive Immune Responses through Multiple TLRs. J Immunol *173*, 3916–3924.

Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., and Torizuka, T. (2005). Microglial activation and dopamine terminal loss in early Parkinson's disease. Annals of Neurology *57*, 168–175.

Panaro, M.A., Lofrumento, D.D., Saponaro, C., Nuccio, F.D., Cianciulli, A., Mitolo, V., and Nicolardi, G. (2008). Expression of TLR4 and CD14 in the Central Nervous System (CNS) in a MPTP Mouse Model of Parkinson's-Like Disease. Immunopharmacology and Immunotoxicology *30*, 729–740.

Patyar, S., Prakash, A., Modi, M., and Medhi, B. (2011). Role of vinpocetine in cerebrovascular diseases. Pharmacological Reports *63*, 618–628.

Perez-Pardo, P., Dodiya, H.B., Engen, P.A., Forsyth, C.B., Huschens, A.M., Shaikh, M., Voigt, R.M., Naqib, A., Green, S.J., Kordower, J.H., et al. (2018). Role of TLR4 in the gut-brain axis in Parkinson's disease: a translational study from men to mice. Gut.

Phan, V.K., Nguyen, T.M., Minh, C.V., Nguyen, H.K., Nguyen, H.D., Nguyen, P.T., Nguyen, X.C., Nguyen, H.N., Nguyen, X.N., Heyden, Y.V., et al. (2010). Two new C-glucosyl benzoic acids and flavonoids from Mallotus nanus and their antioxidant activity. Arch. Pharm. Res. *33*, 203–208.

Ping, Z., Xiaomu, W., Xufang, X., and Liang, S. (2018). Vinpocetine regulates levels of circulating TLRs in Parkinson's disease patients. Neurological Sciences.

Poltorak, A., He, X., Smirnova, I., Liu, M.-Y., Huffel, C.V., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., et al. (1998). Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in Tlr4 Gene. Science *282*, 2085–2088.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science *276*, 2045–2047.

Rannikko, E.H., Weber, S.S., and Kahle, P.J. (2015). Exogenous α-synuclein induces toll-like receptor 4 dependent inflammatory responses in astrocytes. BMC Neuroscience *16*, 57.

Ransohoff, R.M. (2016). How neuroinflammation contributes to neurodegeneration. Science *353*, 777–783.

Rice, T.W., Wheeler, A.P., Bernard, G.R., Vincent, J.-L., Angus, D.C., Aikawa, N., Demeyer, I., Sainati, S., Amlot, N., Cao, C., et al. (2010). A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. Crit. Care Med. *38*, 1685–1694.

Ringman, J.M., Frautschy, S.A., Teng, E., Begum, A.N., Bardens, J., Beigi, M., Gylys, K.H., Badmaev, V., Heath, D.D., Apostolova, L.G., et al. (2012). Oral curcumin for Alzheimer's disease: tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. Alzheimer's Research & Therapy *4*, 43.

Roberts, H., and Brown, D. (2015). Seeking a Mechanism for the Toxicity of Oligomeric α-Synuclein. Biomolecules *5*, 282–305.

Rodriguez-Perez, A.I., Sucunza, D., Pedrosa, M.A., Garrido-Gil, P., Kulisevsky, J., Lanciego, J.L., and Labandeira-Garcia, J.L. (2018). Angiotensin Type 1 Receptor Antagonists Protect

Against Alpha-Synuclein-Induced Neuroinflammation and Dopaminergic Neuron Death. Neurotherapeutics.

Roodveldt, C., Labrador-Garrido, A., Gonzalez-Rey, E., Lachaud, C.C., Guilliams, T., Fernandez-Montesinos, R., Benitez-Rondan, A., Robledo, G., Hmadcha, A., Delgado, M., et al. (2013). Preconditioning of Microglia by α-Synuclein Strongly Affects the Response Induced by Toll-like Receptor (TLR) Stimulation. PLoS ONE *8*, e79160.

Ros-Bernal, F., Hunot, S., Herrero, M.T., Parnadeau, S., Corvol, J.-C., Lu, L., Alvarez-Fischer, D., Sauvage, M.A.C., Saurini, F., Coussieu, C., et al. (2011). Microglial glucocorticoid receptors play a pivotal role in regulating dopaminergic neurodegeneration in parkinsonism. PNAS *108*, 6632–6637.

Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., Challis, C., Schretter, C.E., Rocha, S., Gradinaru, V., et al. (2016). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell *167*, 1469-1480.e12.

Scheperjans, F., Aho, V., Pereira, P.A.B., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E., Kaakkola, S., Eerola- Rautio, J., Pohja, M., et al. (2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. Movement Disorders *30*, 350–358.

Shao, Q.-H., Yan, W.-F., Zhang, Z., Ma, K.-L., Peng, S.-Y., Cao, Y.-L., Yuan, Y.-H., and Chen, N.-H. (2018). Nurr1: A vital participant in the TLR4-NF-κB signal pathway stimulated by α-synuclein in BV-2 cells. Neuropharmacology.

Shimazu, R., Akashi, S., Ogata, H., Nagai, Y., Fukudome, K., Miyake, K., and Kimoto, M. (1999). MD-2, a Molecule that Confers Lipopolysaccharide Responsiveness on Toll-like Receptor 4. J Exp Med *189*, 1777–1782.

Shin, W.-H., Jeon, M.-T., Leem, E., Won, S.-Y., Jeong, K.H., Park, S.-J., McLean, C., Lee, S.J., Jin, B.K., Jung, U.J., et al. (2015). Induction of microglial toll-like receptor 4 by prothrombin kringle-2: a potential pathogenic mechanism in Parkinson's disease. Scientific Reports *5*, 14764.

Spillantini, M.G., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R., and Goedert, M. (1997). Alpha-synuclein in Lewy bodies. Nature *388*, 839–840.

Stefanova, N., Reindl, M., Neumann, M., Kahle, P.J., Poewe, W., and Wenning, G.K. (2007). Microglial activation mediates neurodegeneration related to oligodendroglial α-synucleinopathy: Implications for multiple system atrophy. Movement Disorders *22*, 2196–2203.

Stefanova, N., Fellner, L., Reindl, M., Masliah, E., Poewe, W., and Wenning, G.K. (2011). Toll-Like Receptor 4 Promotes α-Synuclein Clearance and Survival of Nigral Dopaminergic Neurons. The American Journal of Pathology *179*, 954–963.

Su, F., Bai, F., Zhou, H., and Zhang, Z. (2016). Microglial toll-like receptors and Alzheimer's disease. Brain, Behavior, and Immunity *52*, 187–198.

Su, X., Maguire-Zeiss, K.A., Giuliano, R., Prifti, L., Venkatesh, K., and Federoff, H.J. (2008). Synuclein activates microglia in a model of Parkinson's disease. Neurobiology of Aging *29*, 1690–1701.

Sun, M.-F., Zhu, Y.-L., Zhou, Z.-L., Jia, X.-B., Xu, Y.-D., Yang, Q., Cui, C., and Shen, Y.-Q. (2018). Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: Gut microbiota, glial reaction and TLR4/TNF-α signaling pathway. Brain, Behavior, and Immunity *70*, 48–60.

Szilágyi, G., Nagy, Z., Balkay, L., Boros, I., Emri, M., Lehel, S., Márián, T., Molnár, T., Szakáll, S., Trón, L., et al. (2005). Effects of vinpocetine on the redistribution of cerebral blood flow and glucose metabolism in chronic ischemic stroke patients: a PET study. Journal of the Neurological Sciences *229–230*, 275–284.

Tang, S.-C., Arumugam, T.V., Xu, X., Cheng, A., Mughal, M.R., Jo, D.G., Lathia, J.D., Siler, D.A., Chigurupati, S., Ouyang, X., et al. (2007). Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. PNAS *104*, 13798–13803.

Trapp, B.D., Wujek, J.R., Criste, G.A., Jalabi, W., Yin, X., Kidd, G.J., Stohlman, S., and Ransohoff, R. (2007). Evidence for synaptic stripping by cortical microglia. Glia *55*, 360–368.

Trudler, D., Farfara, D., and Frenkel, D. (2010). Toll-Like Receptors Expression and Signaling in Glia Cells in Neuro-Amyloidogenic Diseases: Towards Future Therapeutic Application. Mediators of Inflammation *2010*, 1–12.

Venezia, S., Refolo, V., Polissidis, A., Stefanis, L., Wenning, G.K., and Stefanova, N. (2017). Toll-like receptor 4 stimulation with monophosphoryl lipid A ameliorates motor deficits and nigral neurodegeneration triggered by extraneuronal α-synucleinopathy. Mol Neurodegener *12*.

Wang, Q., Liu, Y., and Zhou, J. (2015). Neuroinflammation in Parkinson's disease and its potential as therapeutic target. Translational Neurodegeneration *4*.

Watson, M.B., Richter, F., Lee, S.K., Gabby, L., Wu, J., Masliah, E., Effros, R.B., and Chesselet, M.-F. (2012). Regionally-specific microglial activation in young mice over-expressing human wildtype alpha-synuclein. Experimental Neurology *237*, 318–334.

Wenning, G.K., and Jellinger, K.A. (2005). The role of α-synuclein in the pathogenesis of multiple system atrophy. Acta Neuropathol *109*, 129–140.

Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J., and Mathison, J.C. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science *249*, 1431–1433.

Yamamoto, M., Sato, S., Hemmi, H., Sanjo, H., Uematsu, S., Kaisho, T., Hoshino, K., Takeuchi, O., Kobayashi, M., Fujita, T., et al. (2002). Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. Nature *420*, 324–329.

Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., et al. (2003). Role of Adaptor TRIF in the MyD88-Independent Toll-Like Receptor Signaling Pathway. Science *301*, 640–643.

Yao, C., Oh, J.-H., Lee, D.H., Bae, J.-S., Jin, C.L., Park, C.-H., and Chung, J.H. (2015). Toll-like receptor family members in skin fibroblasts are functional and have a higher expression compared to skin keratinocytes. International Journal of Molecular Medicine *35*, 1443–1450.

Youn, H.S., Saitoh, S.I., Miyake, K., and Hwang, D.H. (2006). Inhibition of homodimerization of Toll-like receptor 4 by curcumin. Biochemical Pharmacology *72*, 62–69.

Yu, S., Wang, X., He, X., Wang, Y., Gao, S., Ren, L., and Shi, Y. (2016). Curcumin exerts anti-inflammatory and antioxidative properties in 1-methyl-4-phenylpyridinium ion (MPP+)-stimulated mesencephalic astrocytes by interference with TLR4 and downstream signaling pathway. Cell Stress and Chaperones *21*, 697–705.

Zarember, K.A., and Godowski, P.J. (2002). Tissue Expression of Human Toll-Like Receptors and Differential Regulation of Toll-Like Receptor mRNAs in Leukocytes in Response to Microbes, Their Products, and Cytokines. The Journal of Immunology *168*, 554–561.

Zecca, L., Zucca, F.A., Wilms, H., and Sulzer, D. (2003). Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. Trends in Neurosciences *26*, 578–580.

Zhang, W. (2005). Aggregated -synuclein activates microglia: a process leading to disease progression in Parkinson's disease. The FASEB Journal *19*, 533–542.

Zhang, L., and Yang, L. (2014). Anti-Inflammatory Effects of Vinpocetine in Atherosclerosis and Ischemic Stroke: A Review of the Literature. Molecules *20*, 335–347.

Zhang, M., Lin, J.M., Li, X.S., and Li, J. (2016). Quercetin ameliorates LPS-induced inflammation in human peripheral blood mononuclear cells by inhibition of the TLR2-NF-κB pathway. Genet. Mol. Res. *15*.

Zhao, J., Han, X., Xue, L., Zhu, K., Liu, H., and Xie, A. (2015). Association of TLR4 gene polymorphisms with sporadic Parkinson's disease in a Han Chinese population. Neurol Sci *36*, 1659–1665.

Zhao, X.-D., Wang, F.-X., Cao, W.-F., Zhang, Y.-H., and Li, Y. (2016). TLR4 signaling mediates AP-1 activation in an MPTP-induced mouse model of Parkinson's disease. International Immunopharmacology *32*, 96–102.

Zhou, P., Weng, R., Chen, Z., Wang, R., Zou, J., Liu, X., Liao, J., Wang, Y., Xia, Y., and Wang, Q. (2016). TLR4 Signaling in MPP. Neural Plasticity *2016*, e5076740.

Zhu, K., Teng, J., Zhao, J., Liu, H., and Xie, A. (2016). Association of TLR9 polymorphisms with sporadic Parkinson's disease in Chinese Han population. Int. J. Neurosci. *126*, 612–616.

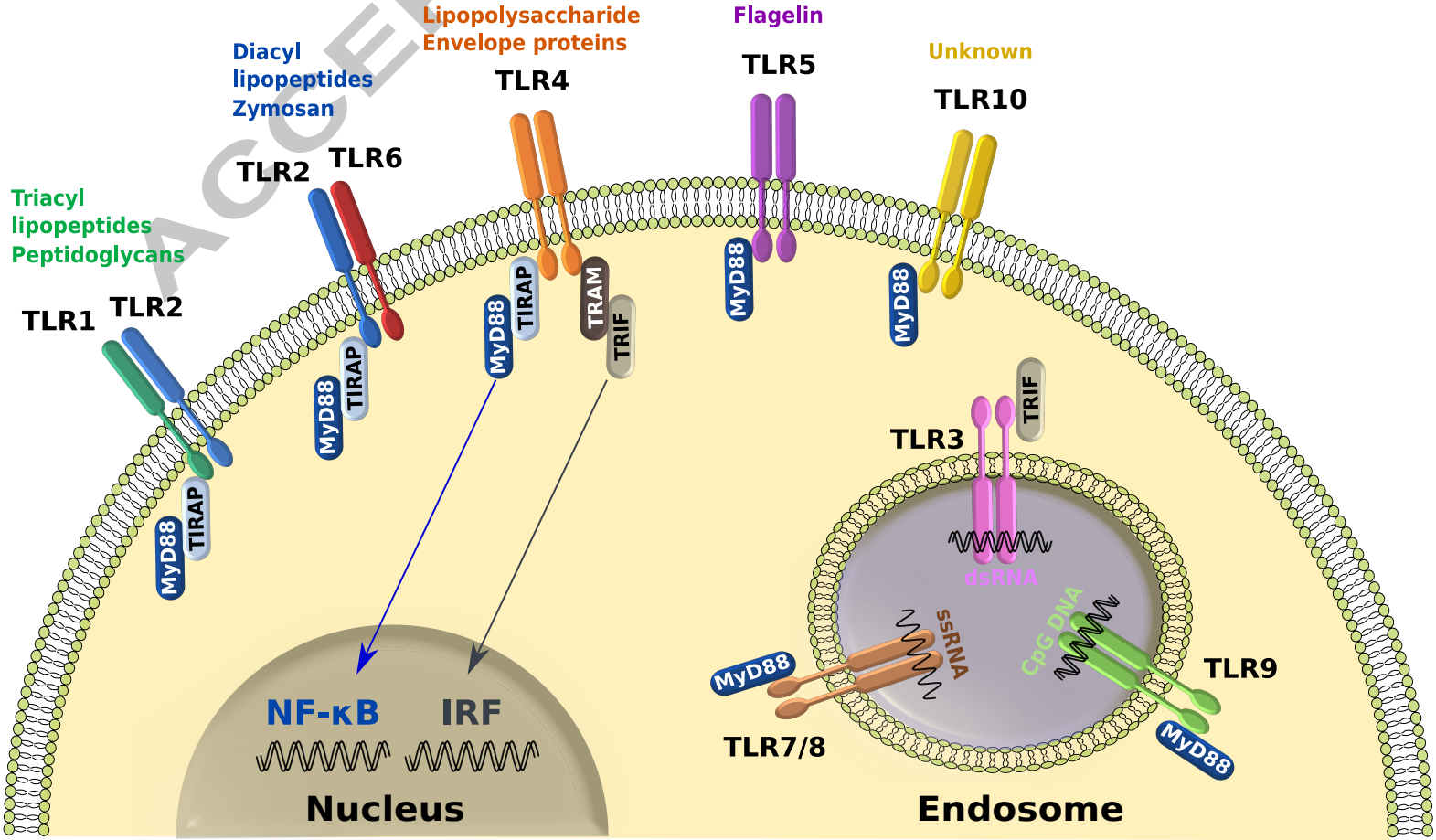
Figure 1 Legend

Figure 1: Cellular localisation and signal transduction of human Toll-like receptors (TLRs). TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 are located on the plasma membrane while TLR3, TLR7, TLR8 and TLR9 are found intracellularly on endosomal or lysosomal membranes. Most TLRs are believed to function in homodimers, though TLR2 forms heterodimers with either TLR1 or TLR6. All TLRs except for TLR3 signal through a MyD88 (myeloid differentiation 88)- dependent downstream pathway. Signalling via TLR2 and TLR4 requires an additional intracellular adapter protein TIRAP (TIR domain-containing adapter protein), which in turn recruits the central adapter MyD88. This triggers a downstream signalling cascade resulting in the nuclear translocation of the transcription factor NF-κB and the expression of pro-inflammatory cytokines such as TNFα, IL-1β and IL6. There is also a MyD88-independent pathway which involves TLR3 and TLR4. Activation of TLR3 engages the adapter protein TRIF (TIR-domain-containing adapter-inducing interferon-β), while TLR4 signalling requires both TRAM (TRIF-related adapter molecule) and TRIF. This results in the downstream activation of the IRF transcription factors (interferon-regulatory factor) which regulate the expression of interferon type 1 genes. Abbreviations: ssRNA: single-stranded RNA, dsRNA: double-stranded RNA, CpG DNA: cytosine-guanine rich DNA.

Figure 2 Legend

Figure 2: The putative role of TLR2, TLR4 and TLR9 in the PD brain.

Oligomeric α-synuclein or other DAMPs activate neuronal TLR2 leading to downstream AKT/mTOR-mediated inhibition of autophagy. This results in accumulation of α-synuclein inside neuronal cell bodies and neurotoxicity. Neuron-released α-synuclein or other DAMPs can activate microglia via TLR2 and TLR4 triggering a signalling cascade that results in the translocation of the transcription factors NF-κB and IRF to the nucleus thereby initiating the production and secretion of pro-inflammatory cytokines and chemokines. These can exacerbate neuronal damage and may also recruit peripheral immune cells to the CNS. Activation of NF-κB could also be triggered by TLR9, though its role in PD is less clear. Microglial TLR4 may play an additional role in promoting the phagocytic clearance of misfolded α-synuclein. Finally, neuron-released α-synuclein may also trigger astrocyte activation via TLR2 and/or TLR4, inducing the production of pro-inflammatory mediators which can further contribute to microglial activation. Abbreviations: TLR: Toll-like receptor; DAMPs: damage-associated molecular patterns; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IRF: Interferon regulatory factor.



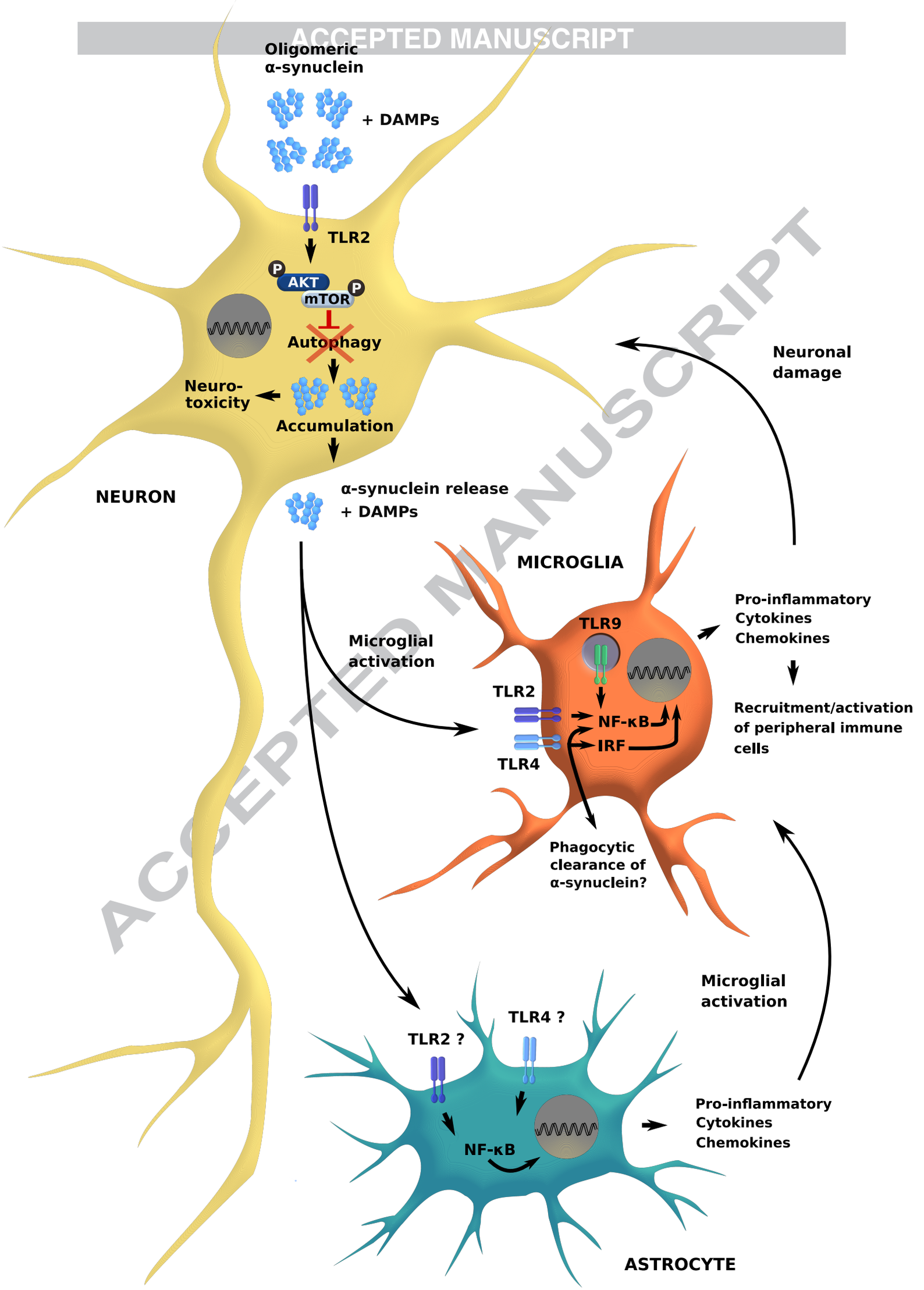


Table 1. Summary of studies evaluating TLRs in PD patients.

References	Study design	Key observation
Dzamko et al., 2017; Drouin-Ouellet et al., 2015	Human PD and control postmortem brains	TLR2 level is increased in the striatum and anterior cingulate cortex of PD brains.
Ping et al., 2018; Dzamko et al., 2017; Doorn et al., 2014; Kim et al., 2013	Human PD and control postmortem brains	Increased co-localization of TLR2 with activated microglia in PD brains. Neuronal TLR2 correlates with disease burden and duration.
Hughes et al., 2018; Drouin-Ouellet et al., 2015; Shin et al., 2015	Human PD and control postmortem brains	TLR4 protein levels are increased in the substantia nigra and caudate/putamen of PD brains.
Ros-Bernal et al., 2011	Human PD and control postmortem brains	TLR9 protein level is increased in the striatum of PD brains.
Ping et al., 2018; Drouin-Ouellet et al., 2015; Shin et al., 2015	Human PD and control PBMCs	TLR2, TLR4, and NF-κB are upregulated in PD PBMCs.
Perez-Pardo et al., 2018	Human PD and control intestinal mucosa biopsies	TLR4 and pro-inflammatory cytokine expression is upregulated in the intestinal mucosa of PD patients compared to controls.
Zhu et al., 2016; Zhao et al., 2015	PD and healthy Chinese Han population	TLR4 and TLR9 polymorphisms are associated with PD.

PD: Parkinson's disease; PBMCs: peripheral blood mononuclear cells

Table 2. Summary of studies evaluating TLR2 in *in vitro* and *in vivo* PD models.

References	Study design	Key observation
Beraud et al., 2011	α-Synuclein-treated BV2 microglia	Upregulation of microglial TLR2.
Kim et al., 2013	α-Synuclein-treated WT & $TLR2^{-/-}$ mouse microglia	TLR2-mediated microglial activation.
Daniele et al., 2015	Mouse microglia treated with α-synuclein and TLR2 antagonists	Inhibition of TLR1/TLR2 ameliorates inflammation.
Gustot et al., 2015	THP1 cells treated with α-synuclein and anti-TLR2 antibodies	Fibrillar α-synuclein activates TLR2.
Codolo et al., 2013	Human monocytes treated with anti-TLR2 antibodies prior to α-synuclein	Both fibrillar and monomeric α-synuclein activate TLR2.
Dzamko et al., 2017	SHSY5Y and IPSC-derived neural cells treated with a TLR2 agonist	Neuronal TLR2 activation leads to increase α-synuclein.
Kim et al., 2015	SHSY5Y cells treated with TLR2 agonist and autophagy inducer or inhibitor	Neuronal TLR2 regulates autophagy.
Kim et al., 2016	WT & $TLR2^{-/-}$ mice	TLR2-mediated microglial activation is neurotoxic.
Kim et al., 2015	$A53T^{+}$ $TLR2^{+/+}$ and $A53T^{+}TLR2^{-/-}$ mice	TLR2 ablation reduces neuronal α-synuclein accumulation and improves motor function.
Chao et al., 2016; Drouin-Ouellet et al., 2011	MPTP mice	MPTP treatment upregulates TLR2.
Kim et al., 2018	Anti-TLR2 immunization of α-synuclein overexpressing mice	Anti-TLR2 immunization alleviates neuroinflammation, neurodegeneration and behavioural deficits.
La Vitola et al., 2018	Anti-TLR2 immunization of WT mice	Anti-TLR2 pre-treatment ameliorates α-synuclein-induced memory deficits.

PD: Parkinson's disease; WT: wild-type; SHSY5Y: human neuroblastoma cell line; BV2: mouse microglia cell line; THP1 cells: human monocyte cell line; PBMCs: peripheral blood mononuclear cells

Table 3. Summary of studies evaluating TLR4 in *in vitro* and *in vivo* PD models.

References	Study design	Key observation
Shao et al., 2018; Fellner et al., 2013	α-Synuclein-treated WT or TLR4$^{-/-}$ mouse microglia	α-Synuclein triggers microglial activation through TLR4.
Rannikko et al., 2015	α-Synuclein-treated WT or TLR4$^{-/-}$ mouse astrocytes	α-Synuclein triggers astrocytic activation through TLR4.
Stefanova et al., 2011	BV2 microglia with anti-TLR4 antibody	TLR4 inhibition impedes microglial α-synuclein phagocytosis.
Zhou et al., 2016	TLR4 knockdown in MPTP-treated BV2 microglia	TLR4 silencing reduces MPTP-induced microglial activation.
Mariucci et al., 2018	WT & TLR4$^{-/-}$ mice	α-Synuclein expression is upregulated in the brain of TLR4$^{-/-}$ mice.
Stefanova et al., 2011	TLR4$^{-/-}$ MSA mouse model	TLR4 ablation exacerbates DA neuronal loss and motor deficits.
Mariucci et al., 2018; Conte et al., 2017; Zhao et al., 2016; Noelker et al., 2013	WT & TLR4$^{-/-}$ MPTP mice	MPTP treatment upregulates TLR4. TLR4 ablation rescues MPTP-induced neuroinflammation, neurotoxicity and motor deficits.
Campolo et al., 2018	WT & TLR4$^{-/-}$ MPTP mice	TLR4 ablation blocks inflammasome activation.
McCabe et al., 2017	WT & 6-OHDA lesioned rats	On 6-OHDA lesioning, striatal TLR4 expression is increased.
Perez-Pardo et al., 2018	WT & TLR4$^{-/-}$ rotenone-treated mice	TLR4 knockout partially rescues dopaminergic cell loss in the substantia nigra and ameliorates the motor deficits.

PD: Parkinson's disease; WT: wild type; BV2: mouse microglia cell line; MSA: multiple system atrophy; 6-OHDA: 6-hydroxydopamine; PBMCs: peripheral blood mononuclear cells

Highlights

- Misfolded proteins like α-synuclein can act as DAMPs leading to activation of TLRs, a major component of the innate immune system.
- TLRs may be a key mediator of neuroinflammatory responses in PD and other α-synucleinopathies.
- Targeting TLRs could be an effective therapeutic strategy for reducing neuroinflammation in PD and slowing down the disease progression.