



## REVIEW ARTICLE

# Evidence of Inflammation in Parkinson's Disease and Its Contribution to Synucleinopathy

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Accumulation of alpha-synuclein ( $\alpha$ Syn) protein in neurons is a renowned pathological hallmark of Parkinson's disease (PD). In addition, accumulating evidence indicates that activated inflammatory responses are involved in the pathogenesis of PD. Thus, achieving a better understanding of the interaction between inflammation and synucleinopathy in relation to the PD process will facilitate the development of promising disease-modifying therapies. In this review, the evidence of inflammation in PD is discussed, and human, animal, and laboratory studies relevant to the relationship between inflammation and  $\alpha$ Syn are explored as well as new therapeutic targets associated with this relationship.

**Keywords** Alpha-synuclein; Astrocyte; Inflammation; Lymphocyte; Microglia; Parkinson's disease.

**INTRODUCTION**

The pathological hallmarks of Parkinson's disease (PD), the second most common neurodegenerative disorder, include Lewy bodies, aggregated alpha-synuclein ( $\alpha$ Syn) protein accumulation in neurons, and dopaminergic neuron degeneration. The pathology of  $\alpha$ Syn-stained Lewy bodies has a spatial progression pattern associated with the disease course, which was proposed by Braak et al.<sup>1</sup> Furthermore, mutations in the *SNCA* gene, which encodes the  $\alpha$ Syn protein, especially copy number variation or aggregation-prone mutations, cause the familial form of PD.<sup>2-5</sup> Furthermore, misfolding and cell-to-cell transmission of  $\alpha$ Syn protein have been evidenced using a fibrillar  $\alpha$ Syn-injected animal model and by the emergence of Lewy body pathology in fetal nigral cells grafted into the striatum of PD patients.<sup>6-9</sup> Accordingly, the hypothesis that  $\alpha$ Syn misfolding and propagation cause PD has become a popular theory by which to explain the pathophysiology of the disease.

Various mechanisms, including autophagy-lysosomal pathway

dysfunction or mitochondrial dysfunction and oxidative stress, are known to be involved in PD pathogenesis.<sup>10</sup> In addition, evidence that neuroinflammation is an important contributor to PD pathogenesis has emerged.<sup>11,12</sup> Epidemiological studies have found that various autoimmune diseases, such as type 1 diabetes, rheumatic disease, Crohn's disease, and ulcerative colitis, are associated with an increased risk of PD development. Conversely, anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs or corticosteroids, can delay or prevent PD onset.<sup>13,14</sup> In a genome-wide association study, pleiotropy was observed between autoimmune disease and PD.<sup>15</sup> Moreover, in genetic studies, more than 90 loci of human leukocyte antigen (HLA) genes encoded by major histocompatibility complex class II (MHC-II) have been identified. These genes participate in antigen presentation during the immune response and are associated with sporadic PD.<sup>16</sup> Thus, there is evidence that the immune response may be involved in the PD process. Although the precise role of inflammation in synucleinopathy is currently unclear, recent research demonstrates that inflammation is not only the result of the PD

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process but also an important contributor to the disease.<sup>11,12,17-19</sup> Therefore, the interaction between the immune response and synucleinopathy may be an important pathomechanism of PD and a possible target for future disease-modifying therapy.

In this review, evidence of activated inflammation in PD is highlighted from autopsy data, in vivo imaging, and laboratory findings. In addition, the temporal and molecular relationship between inflammation and synucleinopathy is evaluated in relation to various aspects of disease progression.

## EVIDENCE OF INFLAMMATION IN PD

Epidemiological and genetic studies have demonstrated that inflammation may be associated with PD.<sup>15,16</sup> In addition, recent studies have reported that an altered gut microbiome in PD and its relevant gut inflammation may contribute to PD pathogenesis.<sup>20</sup> Evidence of inflammation in PD can be concretized by autopsy and in vivo studies. The immune response in the central nervous system (CNS) is distinct from that in the peripheral system in terms of the blood–brain barrier (BBB), resident glial cells (microglia and astrocytes), and absence of an adaptive immune response in itself.<sup>21</sup> However, because  $\alpha$ Syn pathology in PD has been observed from the periphery, such as the enteric nervous system, to the central nervous system, immune responses should be evaluated inside and outside of the CNS in PD. This can be addressed by autopsy studies, in vivo imaging of inflammation, and laboratory analysis of the blood and cerebrospinal fluid (CSF) of patients (Table 1).

### An activated innate immune response was found in the postmortem PD brain

Early studies demonstrated that innate immune cells were found in the postmortem PD brain. Both microglial and astrocytic activation was observed, with consistent microglial activation. Microglia constitute approximately 5%–12% of CNS cells and are capable of self-renewal independent of hematopoietic stem cells.<sup>22</sup> In the CNS, microglia present as major immune mediators; in particular, they perform the functions necessary for the recruitment of the immune system.<sup>22</sup> In PD postmortem studies, microglial activation was observed to varying degrees in different brain regions. Mirza et al.<sup>23</sup> described the activation of microglia labeled with CR3/43 and ferritin in the substantia nigra (SN) but not in the putamen of the PD brain. Moreover, MHC-II-expressing microglia are found at significantly higher levels in the SN, putamen, hippocampus, transentorhinal cortex, cingulate cortex, and temporal cortex as well as in the lymphatic system in PD than in the brain regions of normal controls.<sup>24</sup> Doorn et al.<sup>25</sup> showed differential microglial expression patterns in the PD brain and age-matched control brain: CD68-stained amoe-

boid microglia were identified in the SN and hippocampus, especially in the hippocampal CA2 region, whereas Iba1-stained microglia were significantly increased in the SN of the PD brain. However, such microglia were not found in the subregions of the hippocampus in age-matched control brains.<sup>25</sup> In another study, Kouli et al.<sup>26</sup> characterized microglial responses in various brain regions by human leukocyte antigen–DR isotype (HLA-DR) and Iba1 staining. They found significantly higher microglial activation in the amygdala of demented PD patients than in age-matched controls.<sup>26</sup> Consequently, although microglia exhibited different activation patterns in terms of microglial phenotypic markers and PD brain regions, microglial activation was consistently observed in the postmortem PD brain.

In addition to microglia, astrocytes are the most abundant CNS cell type. They demonstrate diverse morphological and functional characteristics dependent on specific brain areas.<sup>27</sup> Astrocytes are known to contribute to various physiological functions, including maintenance of neurons, formation of the BBB, and regulation of synapse functions.<sup>28</sup> Astrocytic activation in postmortem PD brains was thought not to exist because the differences in the distribution and cellular density of astrocytes stained for glial fibrillary acidic protein (GFAP) or metallothioneins I and II were not observed in the SN and putamen of PD patients.<sup>23</sup> However, astrocytes containing aggregated  $\alpha$ Syn have been found in the PD brain, suggesting that astrocytes play a role in taking up abnormal  $\alpha$ Syn.<sup>29,30</sup> Recently, reported data show that GFAP-positive astrocytes are significantly increased in the SN but are not found in other brain regions of PD subjects relative to age-matched controls.<sup>26</sup> Therefore, human autopsy studies demonstrate that microglial and astrocytic activation is involved in the PD process. However, the data obtained from postmortem brains might reflect the findings of advanced disease status or confounding terminal status affecting inflammation at death. Consequently, the in vivo status of PD must also be investigated.

### In vivo evidence of inflammation in PD patients

In vivo evaluation can reflect the various stages of PD, and compared with autopsy data, it can better exclude the confounding factors affecting inflammation. Positron emission tomography (PET) imaging using ligands targeting microglial or astroglial activation has provided some insights into this respect.

Activated microglia largely exist in two polarized states, namely, the M1 phenotype and the M2 phenotype.<sup>31</sup> The M1 phenotype is associated with the release of proinflammatory cytokines, whereas the M2 phenotype is accompanied by the production of anti-inflammatory molecules.<sup>31</sup> Current PET imaging using 18 kDa translocator protein (TSPO), which is the main target for the development of ligands, can detect activated microglial status because this protein is expressed increasingly when microg-

**Table 1.** Postmortem and in vivo evidence of inflammation in PD

Type of inflammation	Markers for inflammation	Sources	Regions	PD vs. healthy control
Microglia	Iba1	Postmortem brain	SN	Increase <sup>25</sup>
			SN, HIP, ERC, PFC, OTC, PPC, Mesencephalon	No difference <sup>25,26,41</sup>
	HLA-DR		Amygdala	Increase <sup>26</sup>
			SN	No difference <sup>26</sup>
	CR3/43		SN	Increase <sup>23</sup>
			Putamen	No difference <sup>23</sup>
	CD68		SN, HIP	Increase <sup>25</sup>
	TMEM119		Putamen	Increase <sup>43</sup>
	<sup>11</sup> C-PK11195	PET imaging	Temporal cortex, occipital cortex, SN, putamen	Increase <sup>33-35</sup>
			Putamen, caudate nucleus	No difference <sup>35</sup>
	<sup>11</sup> C-DPA713		Temporal cortex, occipital cortex, parietal cortex	Increase <sup>38</sup>
<sup>18</sup> F-FEPPA		Thalamus, caudate, putamen, HIP	Increase <sup>37</sup>	
<sup>18</sup> F-DPA714		Midbrain, frontal cortex, putamen	Increase <sup>38</sup>	
Astrocyte	GFAP	Postmortem brain	SN	Increase <sup>26</sup>
			Amygdala, HIP, ERC, PFC, OTC, PPC, Mesencephalon	No difference <sup>41,83</sup>
	GLAST		Mesencephalon	No difference <sup>41</sup>
	Metallothioneins I and II		SN, putamen	No difference <sup>23</sup>
	MHC-II		SN, putamen, HIP, transentorhinal cortex, cingulate cortex, temporal cortex, lymphatic system, mesencephalon	Increase <sup>24,41</sup>
	<sup>11</sup> C-BU9908	PET imaging	Cortex, brain stem	Increase <sup>39</sup>
Lymphocyte	CD4+	Postmortem brain	SN, amygdala	Increase <sup>26,41,45</sup>
			Blood	PBMC
			Peripheral blood lymphocytes	Lower <sup>48</sup>
	CD8+	Postmortem brain	SN	Increase <sup>44</sup>
			SN, perivascular, amygdala	No difference <sup>26,44,45</sup>
		Blood	PBMC	No difference <sup>42</sup>
	CD45	Postmortem brain	Putamen	Increase <sup>43</sup>
B cells	CD79 $\alpha$ + CD20+	Postmortem brain	SN	No difference <sup>45</sup>
NK cells	CD57+	Postmortem brain	SN	No difference <sup>45</sup>
	CD56+	Blood	PBMC	Increase <sup>47,48,50</sup>
Monocytes	CD14+	Blood	Peripheral blood	Increase <sup>46</sup>
	CD16+		Peripheral blood	No difference <sup>51</sup>
	CD14+/CD16-	CSF	CSF	Lower <sup>51</sup>
	CD14+/CD16+		CSF	Increase <sup>51</sup>
Inflammasome	NLRP3	Blood	PBMC, plasma	Increase <sup>54,56</sup>
			PBMC	No difference <sup>61</sup>
	NLRP1 NLRP4		PBMC	No difference <sup>54</sup>
Cytokines	IL-1 $\beta$	Postmortem brain	SN, frontal cortex	Increase <sup>26</sup>
			Blood	Plasma, serum
	IFN $\gamma$	Blood	PBMC	No difference <sup>42</sup>
			Serum	Lower <sup>55</sup>
	TNF $\alpha$	Postmortem brain	SN, HIP, amygdala, frontal cortex	No difference <sup>26</sup>
			Blood	Serum
		Plasma	Increase <sup>58</sup>	

**Table 1.** Postmortem and in vivo evidence of inflammation in PD (continued)

Type of inflammation	Markers for inflammation	Sources	Regions	PD vs. healthy control
	IL-2	Postmortem brain	Frontal cortex	Increase <sup>57</sup>
	IL-13		Frontal cortex	Lower <sup>57</sup>
	IL-10	Blood	PBMC, plasma	Increase <sup>42,58</sup>
	IL-5		PBMC	No difference <sup>42</sup>
	IL-6	Blood	Plasma	Increase <sup>58</sup>

PD, Parkinson's disease; SN, substantia nigra; HIP, hippocampus; ERC, entorhinal cortex; PFC, prefrontal cortex; OTC, occipito-temporal cortex; PPC, posterior parietal cortex; HLA-DR, human leukocyte antigen DR isotype; PET, positron emission tomography; GFAP, glial fibrillary acidic protein; GLAST, glutamate aspartate transporter; MHC-II, major histocompatibility complex class II; PBMC, peripheral blood mononuclear cell; NK cells, natural killer cells; CSF, cerebrospinal fluid.

lia are activated. However, it is unclear why none of the current TSPO ligands are specific tracers of M1 or M2 microglia.

Using <sup>11</sup>C-PK11195 PET imaging, increased microglial activation was demonstrated in the pons, basal ganglia, frontal cortex, and temporal cortex in PD.<sup>32</sup> With the same ligand, microglial activation in the temporal and occipital regions of PD patients was demonstrated to be increased by 25%–30% compared with microglial activation in these regions in age-matched controls.<sup>33</sup> Increased microglial activation is also shown in the SN and putamen of patients with dementia with Lewy bodies (DLBs) as well as those with PD.<sup>34</sup> Similarly, compared with healthy controls, patients with idiopathic rapid-eye-movement sleep behavior disorder showed microglial activation in the SN.<sup>35</sup> These results indicate that microglia may be activated from the very early or preclinical stages of PD. In addition to <sup>11</sup>C-PK11195 PET, microglial activation has been identified by other PET imaging ligands with improved binding qualities, such as <sup>11</sup>C-DPA713, <sup>18</sup>F-FEPPA, and <sup>18</sup>F-DPA714.<sup>36–38</sup> In a study using <sup>11</sup>C-DPA713, a significant increase in uptake was shown in the occipital, temporal, and parietal cortex of the brains of PD patients.<sup>36</sup> Significantly higher microglial activation was also shown with <sup>18</sup>F-DPA714 in the midbrain, frontal cortex, and putamen of PD patients relative to controls.<sup>38</sup> In summary, microglial activation has been evidenced in vivo by PET studies in the premotor or clinical stages of PD. It should be noted that different activation patterns occur in various brain regions as an effect of the ligand used, which is a limitation of such studies.

From another perspective, increased astrocytic activation in the cortex and brain stem of early PD patients was identified using <sup>11</sup>C-BU9908 PET imaging, which is a marker of the imidazoline 2 binding site and binds reactive astrocytes.<sup>39</sup> Therefore, in vivo PET imaging has provided further evidence of activated inflammation of innate immune cells in PD along with autopsy data.

### Activation of peripheral immune cells in PD

In addition to the activation of innate CNS immune cells, peripheral lymphocyte infiltration has also been observed in the PD brain, which further demonstrates the immune association in

PD.<sup>26,40–44</sup> Increased CD4 and CD8+ T cells but not B cells or natural killer (NK) cells have been observed in the SN of the postmortem PD brain.<sup>45</sup> In a recent study, CD4+ T cell infiltration was identified in postmortem brain sections, especially in the perivascular space and vessel wall directly in contact with astrocytes expressing MHC-II.<sup>41</sup> This suggested that astrocytes were capable of expressing the factors required for T cell infiltration and activation during PD progression.<sup>41</sup> In another study, a significant increase in CD4+ T lymphocytes in the SN was reported in demented PD patients, whereas a correlation existed between the number of CD4+ T cells and activated microglia in the amygdala.<sup>26</sup> In another study, a significant increase in CD8+ T cells but not CD4+ T cells was observed in the SN of PD patients compared with that in the SN of the control group, according to Lewy body staging.<sup>44</sup> The presence of infiltrating lymphocytes in specific brain regions affected by PD, such as the SN and amygdala, suggests that they reach these locations through targeted extravasation rather than through a random event.

In addition, the peripheral blood of PD patients presents altered lymphocytic patterns.<sup>46–48</sup> Increased monocyte<sup>46</sup> or NK cell activation has also been reported in the blood of PD patients.<sup>47–50</sup> Furthermore, Niwa et al.<sup>47</sup> reported an increase in NK cells but not regulatory T cells and type 17 helper T cells in the blood of PD patients. Similarly, a significantly high percentage of NK cells with a lower percentage of CD3+ T cells and CD4+ T cells was identified in PD patients with clinical features relative to healthy controls.<sup>48</sup>

In addition to the blood, the CSF of PD patients has been shown to have higher activation of immune cells, such as T lymphocytes and monocytes, than that of control subjects.<sup>51</sup> Additional evidence of the increased number of monocytes in the CSF of PD patients was recently reported by Pillny et al.<sup>52</sup> These findings indicate that peripheral inflammatory cell activation occurs in the blood and CNS of PD patients and that peripheral immune cell activation may be involved in PD pathogenesis.

However, it is less known how inflammation contributes to selective neuronal vulnerability in PD, but it is postulated that genetic predisposition combined with environmental factors may

increase selective nigral dopaminergic degeneration during chronic inflammation.<sup>53</sup>

### Changes in inflammatory mediators in PD

In addition to the activation of innate CNS immune cells and peripheral immune cells, the levels of various inflammatory cytokines, which are produced by activated immune cells, have been investigated in PD.<sup>44,54-56</sup> The released cytokines may cause endogenous  $\alpha$ Syn to misfold and aggregate. Aggregated  $\alpha$ Syn can induce an inflammatory cell response that continues to release cytokines, which accounts for the continued presence of inflammation and progressive  $\alpha$ Syn pathology.<sup>43</sup>

Altered cytokine expression has been detected in the postmortem PD brain;<sup>26,44,57</sup> cytokine expression was altered in the frontal cortex, with IL-2 levels significantly higher and IL-13 and IL-8 levels lower in PD brains than in controls.<sup>57</sup> In another study, a significant increase in the expression of proinflammatory IL-1 $\beta$  cytokine was observed in the SN and frontal cortex, whereas cytokine levels in other brain regions did not differ in PD patients and healthy controls.<sup>26</sup>

With regard to the inflammatory response in the peripheral system, Eidson et al.<sup>55</sup> found that the levels of two proinflammatory cytokines, namely, TNF $\alpha$  and IFN $\gamma$ , were decreased in the serum of PD patients compared with that in the serum of control subjects. Recently, a significant increase in plasma proinflammatory cytokines, i.e., TNF $\alpha$ , IL-6, and IL-1 $\beta$ , was also found in PD patients.<sup>54,58,59</sup> However, variation in serum cytokine levels over the diurnal cycle must be cautiously interpreted, and various confounding factors, such as the diurnal cycle, drugs, and other medical conditions, must be controlled.<sup>55</sup>

The inflammasome is a cytoplasmic multiprotein complex that controls the inflammatory response and coordinates antimicrobial host defense, for which the essential components are a sensor protein, ASC adapter protein, and the inflammatory protease caspase-1. These components are assembled by the detection of pathogenic signals; inflammatory caspases are activated, and proinflammatory cytokines, i.e., IL-1 $\beta$  and IL-18, are released to induce cell death.<sup>60</sup> Inflammasome activation has been observed in the peripheral blood of PD patients. Expression of inflammasome NLRP3, a type of sensor protein, was found to be significantly increased in the peripheral blood mononuclear cells (PBMCs) of PD patients, whereas other types of inflammasomes (e.g., NLRP1 and NLRP4) did not differ between PD and control subjects.<sup>54</sup> Likewise, NLRP3 plasma levels were significantly higher in PD patients than in healthy controls in another study.<sup>56</sup> However, a recent study that used monocytes from peripheral blood did not find NLRP3 activation despite the detection of altered cytokine levels.<sup>61</sup>

Overall, the determination of cytokines or the inflammasome

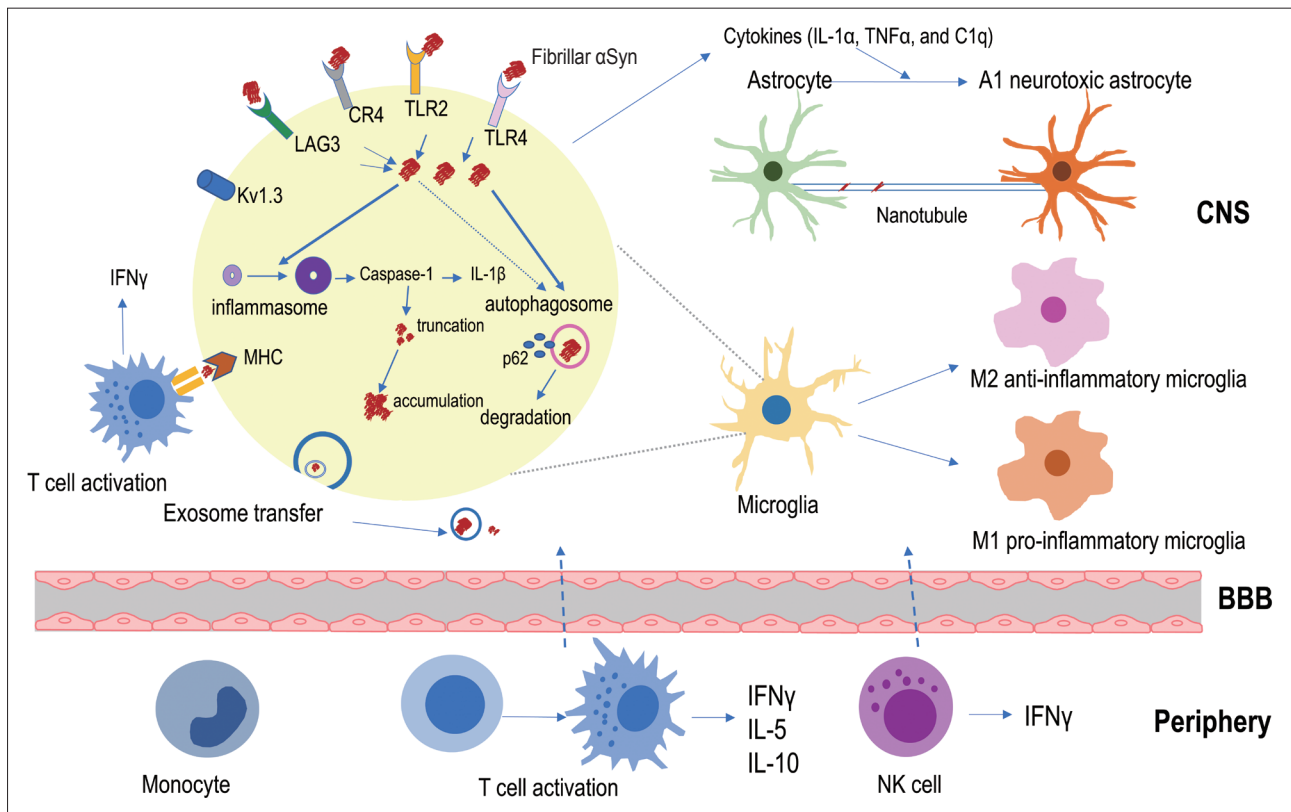
in synucleinopathy might provide new possible evidence of the association of inflammation and  $\alpha$ Syn and could reveal potential biomarkers for PD.

## THE ASSOCIATION OF INFLAMMATION AND SYNUCLEINOPATHY

Given the evidence of an activated inflammatory response from the postmortem PD brain, in vivo PET imaging, and the CSF and blood of PD patients, a question arises: How does inflammation relate to  $\alpha$ Syn propagation? Based on previous autopsy data, it was formerly postulated that inflammation might be the result of PD pathological progression. However, Olanow et al.<sup>43</sup> reported autopsy data from PD patients who received fetal nigral cell transplantation; infiltrating lymphocytes or macrophages and microglia were found around the transplanted cells from the beginning of grafting and even preceded  $\alpha$ Syn accumulation inside the transplanted cells. In addition, Galiano-Landeira et al.<sup>44</sup> recently reported robust CD8+ T cell infiltration, especially in the earliest stage of Lewy body disease, in the comparative analysis of autopsy data from healthy controls, incidental Lewy body disease patients, and PD. A study investigating the relationship between T cell activity in PBMCs and the time of PD diagnosis in longitudinal observation demonstrated that  $\alpha$ Syn-specific T cell reactivity was significantly increased in all periods of PD but was especially highest close to the onset of PD.<sup>42</sup> Overall, these findings indicate the possibility that inflammation can start from an early disease state and possibly precede  $\alpha$ Syn pathology. Therefore, in this section of the review, the association between inflammation and  $\alpha$ Syn pathological progression has been described in three parts: whether 1)  $\alpha$ Syn triggers inflammation, 2) inflammation participates in  $\alpha$ Syn removal, or 3) inflammation contributes to  $\alpha$ Syn propagation (Figure 1).

### Pathological $\alpha$ Syn triggers inflammation

PD animal models can provide evidence of immune cell activation during pathological  $\alpha$ Syn introduction and its propagation.<sup>62-67</sup> After fibrillar  $\alpha$ Syn injection into the brains of mice, thriving microglial and astrocytic activation was observed with  $\alpha$ Syn propagation in these brains (Figure 2).<sup>63,68,69</sup> Moreover, Lee et al.<sup>70</sup> reported that  $\alpha$ Syn accumulation in glial cells produces proinflammatory cytokines and chemokines. In addition, fibrillar  $\alpha$ Syn has been shown to induce an inflammatory response through synthesis of IL-1 $\beta$  through toll-like receptor (TLR) 2 interactions, which thereby leads to the activation of the NLRP3 inflammasome, followed by increased reactive oxygen species production in human monocytes.<sup>71</sup> This suggests that fibrillar  $\alpha$ Syn encounters the TLR2 receptor on the surface of immune cells and triggers a downstream signaling pathway with the subsequent pro-



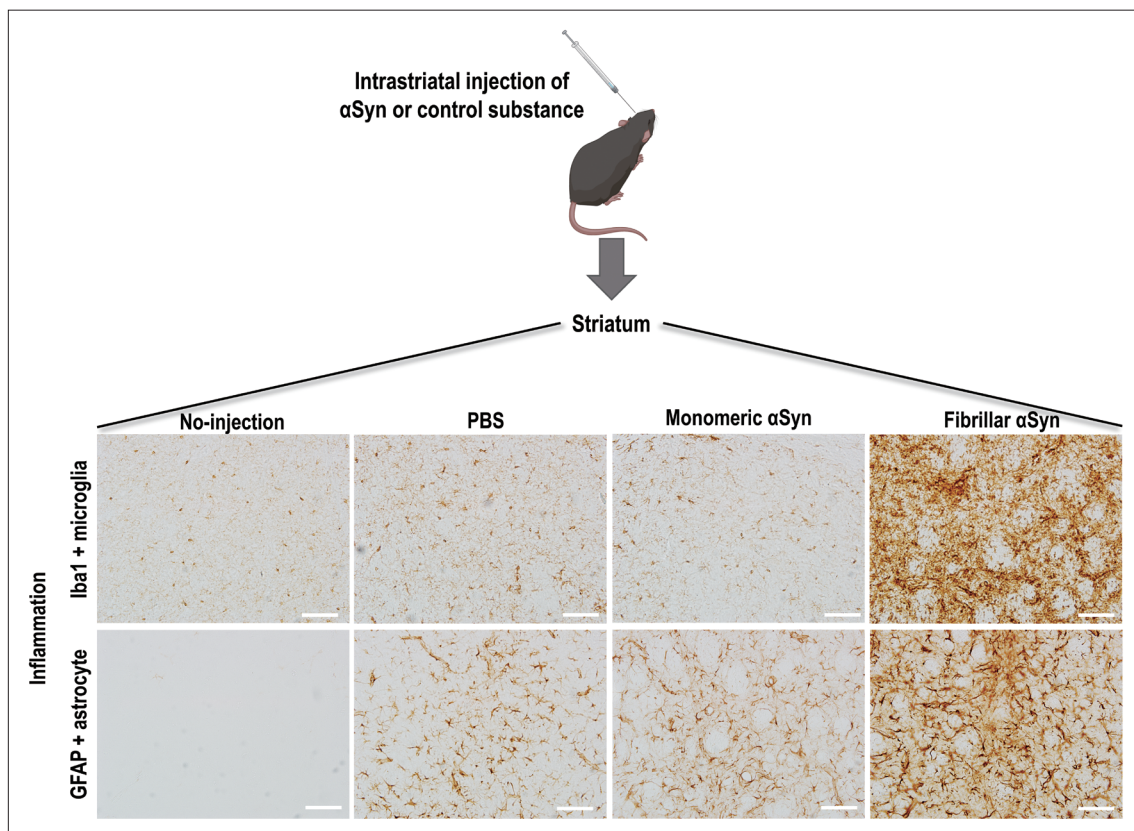
**Figure 1.** The inflammatory response is closely related to pathological  $\alpha$ Syn accumulation and transmission. Various immune receptors and proteins are involved in the internalization of pathological  $\alpha$ Syn or its intracellular signaling, and consequently, the cytoplasmic autophagosome and inflammasome cascade will be activated in immune cells. Responding to pathological  $\alpha$ Syn stimuli, microglia changed their morphology to a proinflammatory phenotype, which enabled the phenotypic conversion of astrocytes to an A1 neurotoxic phenotype. Antigen presentation of processed  $\alpha$ Syn by immune cells can recruit peripheral lymphocytes, and peripheral immune cells can infiltrate the CNS with targeted extravasation. Immune cells can contribute pathological  $\alpha$ Syn to transfer to other immune cells or neurons in various ways, such as direct contact, exosomal transfer, and nanotubules.  $\alpha$ Syn, alpha-synuclein; CNS, central nervous system; LAG3, lymphocyte activation gene 3 protein; CR4, complement receptor 4; TLR, toll-like receptor; MHC, major histocompatibility complex; IL, interleukin; TNF, tumor necrosis factor; BBB, blood–brain barrier; NK cells, natural killer cells.

duction of cytokines and inflammatory molecules. The roles of TLR4 and TLR2 in relation to pathological  $\alpha$ Syn have been identified in several studies.<sup>72-75</sup> The involvement of other immune receptors in pathological  $\alpha$ Syn has also been reported, e.g., cell-surface lymphocyte activation gene 3 protein (LAG3)<sup>76,77</sup> and complement receptor 4 (CR4),<sup>78</sup> indicating that  $\alpha$ Syn triggers an inflammatory response through interactions with various immune receptor-mediated pathways. A recent study identified the upregulation of microglial voltage-gated potassium channel Kv1.3 in response to aggregated  $\alpha$ Syn in primary microglial cultures.<sup>79</sup>

In a recent study by Li et al.,<sup>80</sup> the authors investigated microglial phenotypic polarization against  $\alpha$ Syn and found that monomeric  $\alpha$ Syn modulated microglia toward an anti-inflammatory phenotype after exposure, whereas oligomeric  $\alpha$ Syn led microglia toward a proinflammatory phenotype. This suggests that the pathologic form of  $\alpha$ Syn may contribute to the aggravation of inflammation, unlike monomeric  $\alpha$ Syn.

In addition to the inflammatory response through glial cells, the adaptive immune response through lymphocytes may be af-

ected by  $\alpha$ Syn. Following fibrillar  $\alpha$ Syn injection into the CNS parenchyma, a significant increase in peripheral immune cell infiltration, such as B lymphocyte (CD19+), T helper lymphocyte (CD4+), T cytotoxic lymphocyte (CD8+), activated myeloid cell (CD11+), and NK cell infiltration, was observed in the CNS compared with the effects in a monomer-injected mouse model.<sup>63</sup> Likewise, an alteration of peripheral immune cells was identified in the blood and peripheral organs, including the spleen and lymph nodes, following fibrillar  $\alpha$ Syn injection into the brain.<sup>63</sup> These data suggest that pathological  $\alpha$ Syn triggers immune cell infiltration and activation in the brain as well as in the peripheral system. Moreover, Harms et al.<sup>81</sup> reported that  $\alpha$ Syn overexpression induced MHC-II-mediated antigen presentation on microglia to CD4+ T cell expression; thus, the interaction between microglia and CD4+ T cells led to potent cytokine production. In agreement with this, a recent study demonstrated that  $\alpha$ Syn triggers incremental MHC-II expression associated with an accompanying T cell response; therapeutic targeting of T cell disruption in animal models was found to reduce MHC-II expres-



**Figure 2.** Fibrillar  $\alpha$ Syn injection induced inflammation in the mouse brain. Iba1-positive microglia and GFAP-positive astrocyte immunoreactivity were significantly increased in the striatum of fibrillar  $\alpha$ Syn-injected mice compared to monomeric  $\alpha$ Syn-injected and PBS-injected mice at 7 days after injection. DAB staining immunohistochemistry. Scale bar: 100  $\mu$ m.  $\alpha$ Syn, alpha-synuclein; GFAP, glial fibrillary acidic protein; PBS, phosphate buffered saline; DAB, 3,3'-diaminobenzidine.

sion as well as CNS myeloid cells in relation to  $\alpha$ Syn expression.<sup>82</sup>

To understand the differential contribution of various peripheral immune cells in synucleinopathy, Iba et al.<sup>83</sup> evaluated T lymphocyte populations in 10–11-month-old transgenic mice overexpressing human  $\alpha$ Syn, which demonstrated considerable accumulation of  $\alpha$ Syn in the cortical and subcortical regions. The authors found an increase in the number of CD3 and CD4+ T cells in mouse brains, and CD3+ T cells were detected in close proximity to glial cells (Iba1 and GFAP) and human  $\alpha$ Syn (SYN211)-positive neurons in the neocortex, hippocampus, and striatum, which supports previous observations in the human brain.<sup>40,41</sup> Furthermore, an accompanying increase in IFN $\gamma$  and CD1d-restricted NK T cells was identified in this animal model.<sup>83</sup> In another study, when  $\alpha$ Syn oligomer was increased using an injection of adeno-associated virus coding  $\alpha$ Syn in the T cell-competent rat brain, the presence of CD4+ and CD8+ T cell infiltration was correlated with the upregulation of MHC-II-expressing microglia. However, this correlation was not observed in T cell-deficient rats.<sup>69</sup> Taken together, these studies imply that pathological  $\alpha$ Syn contributes to the recruitment of lymphocytes as well as the activation of innate immune cells.

### Inflammation opposes $\alpha$ Syn toxicity

One of the primary roles of microglia and astrocytes is their macrophage-like function.<sup>84,85</sup> Lee et al.<sup>86</sup> explored the efficiency of clearing extracellular  $\alpha$ Syn in different brain cell types, including neurons, astrocytes, and microglia, and found that microglia exhibited the highest rate of  $\alpha$ Syn clearance. Supporting evidence by Loria et al.<sup>87</sup> also indicated that astrocytes were able to efficiently clear fibrillar  $\alpha$ Syn more efficiently than neurons. A recent study by Tsunemi et al.<sup>88</sup> also emphasized the importance of lysosomal function in astrocytes for  $\alpha$ Syn degradation during the pathogenesis of the disease. Although neurons and astrocytes can clear  $\alpha$ Syn, microglia are the major immune cells that quickly engulf and degrade  $\alpha$ Syn to maintain brain health by changing their morphological state to M1 (the proinflammatory phenotype) or M2 (the antiinflammatory phenotype).<sup>89</sup> Choi et al.<sup>74</sup> suggested that the neuroprotective function of microglia occurs through ingestion and degradation of neuron-released  $\alpha$ Syn through a selective autophagy mechanism. Therefore, a better understanding of microglial function in relation to  $\alpha$ Syn will likely contribute to the identification of therapeutic targets for inflammation in synucleinopathies.

In studies of the cellular mechanism underlying  $\alpha$ Syn clearance, in vitro research has identified that  $\alpha$ Syn alters microglial function through interactions with TLR.<sup>72,73,75,90</sup> Stefanova et al.<sup>72</sup> demonstrated that TLR4 deficiency in  $\alpha$ Syn-overexpressing transgenic mice led to significantly increased levels of  $\alpha$ Syn in the midbrain and forebrain compared with  $\alpha$ Syn-overexpressing transgenic mice with intact TLR4 expression, indicating that impairment of TLR4 influences extracellular  $\alpha$ Syn clearance through microglia. In accordance with this study, Fellner et al.<sup>73</sup> indicated that TLR4 is required for the  $\alpha$ Syn-dependent activation of glial cells. In addition, TLR4 was recently linked to selective autophagic degradation of  $\alpha$ Syn in synucleinopathy,<sup>74</sup> whereas oligomeric  $\alpha$ Syn interacts with TLR2 to induce microglial activation.<sup>90</sup> Therefore, the modulation of the TLR response to  $\alpha$ Syn can be a possible treatment target for the improvement of  $\alpha$ Syn propagation.

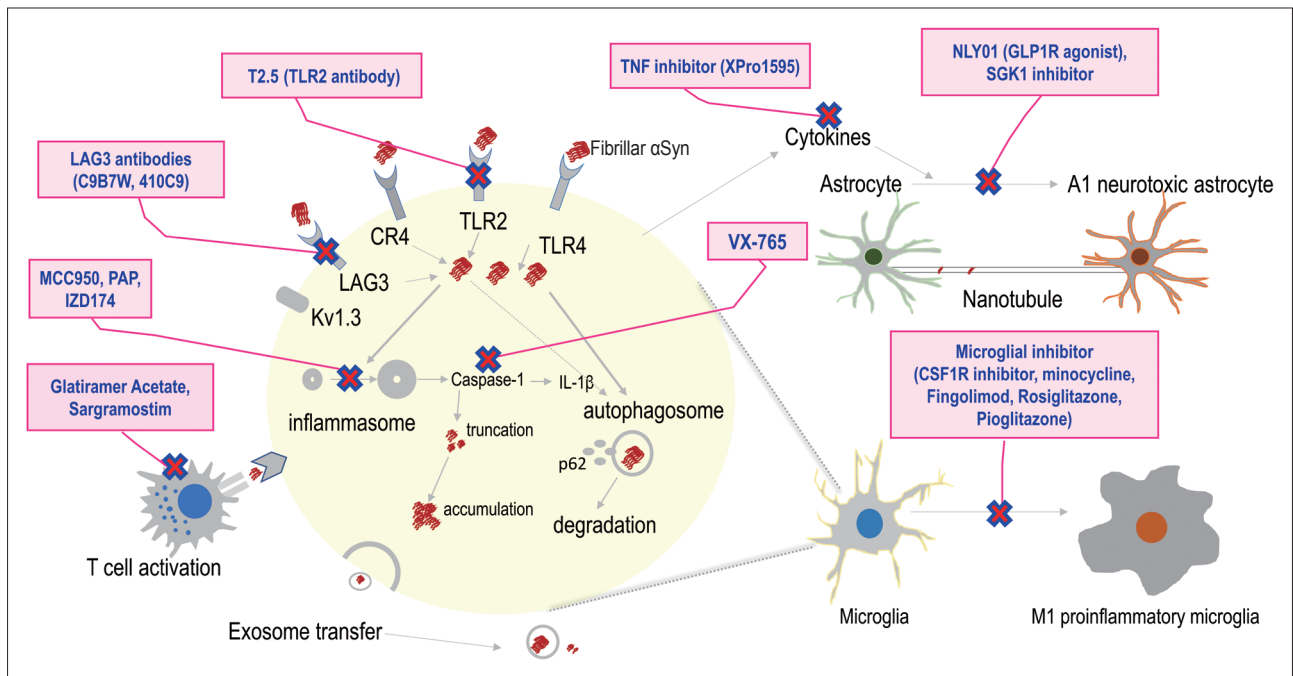
Disruption of the BBB in synucleinopathy has been observed in a PD animal model;<sup>91</sup> this process may allow the entrance of peripheral immune cells into the brain for pathological  $\alpha$ Syn clearance. A recent study demonstrated the protective role of NK cells (granular lymphocytes of the innate immune system) in efficiently internalizing and degrading extracellular  $\alpha$ Syn; the systemic depletion of NK cells in a PD mouse model exacerbated motor abnormalities and  $\alpha$ Syn propagation compared with the effects in a control group.<sup>92</sup> In another study, reconstitution of T cell populations reduced  $\alpha$ Syn accumulation and resulted in persistent

microgliosis in the striatum of mice with an immune system impaired by  $\alpha$ Syn preformed fibril injection.<sup>93</sup> These data support that NK cells, T lymphocytes, microglia, and astrocytes can play protective roles in the  $\alpha$ Syn pathology of PD.

### Inflammation contributes to $\alpha$ Syn propagation

Accumulating evidence now supports the case that glial activation contributes to  $\alpha$ Syn propagation. Microglial exosomes also contribute to the progression of  $\alpha$ Syn pathology through exosomal transfer of  $\alpha$ Syn from cell to cell.<sup>17,19</sup> In addition, reactive microglia enhance the transmission of exosomal  $\alpha$ Syn through TLR2.<sup>94</sup> Recently, Izco et al.<sup>95</sup> investigated the temporal relationship between  $\alpha$ Syn and inflammation in a mouse model of PD. They found that the peak expression of Iba1-positive microglia, GFAP-positive astrocytes, and IL-1 $\beta$  occurred before  $\alpha$ Syn accumulation.<sup>95</sup> Furthermore, several researchers have been able to block or suppress microglial activation using pharmacological drugs and thereby have identified decreases in  $\alpha$ Syn pathology in a mouse model.<sup>17,94</sup>

There is also evidence that astrocytes actively transfer aggregated  $\alpha$ Syn to healthy astrocytes through direct contact or tunneling nanotubes.<sup>41,96</sup> Ngolab et al.<sup>97</sup> demonstrated that DLB brain-derived exosomes trigger  $\alpha$ Syn accumulation in astrocytes in the mouse brain, suggesting the participation of astrocytes in  $\alpha$ Syn transmission. In addition, activated microglia have been shown



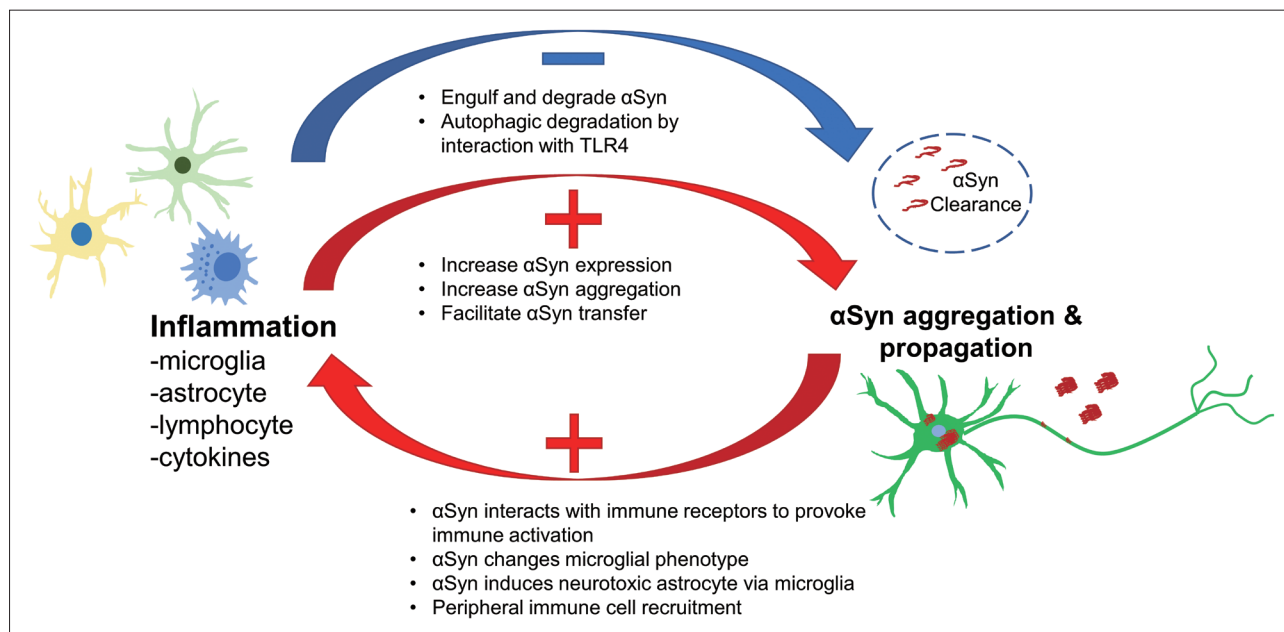
**Figure 3.** Ongoing therapeutic option targeting the interaction of inflammation and  $\alpha$ Syn. Therapeutic targets currently under evaluation are described in the figure, but all are in the early stages. Efforts to explore new therapeutic targets based on this mechanism will be required.  $\alpha$ Syn, alpha-synuclein; PAP, papaverine; LAG3, lymphocyte activation gene 3 protein; CR4, complement receptor 4; TLR, toll-like receptor; IL, interleukin; TNF, tumor necrosis factor; GLP-1R, glucagon-like peptide-1 receptor; SGK1, serum and glucocorticoid-regulated kinase 1 inhibitor; CSF, cerebrospinal fluid.



**Table 2.** Therapeutic trial for inflammation and its interaction with  $\alpha$ Syn in PD

Category	Compound	Description	Findings	Status
Microglia	CSF1R inhibitor (PPLX3397-Pexidartinib)	Inhibits microglia/macrophage	Depletion of microglia suppressed $\alpha$ Syn aggregation and transmission in mice <sup>17,94</sup>	Preclinical
	Mincycline (Mino)	Reduces the proliferation/activation of resting microglia	Prevention of the dopaminergic neurons loss, increased the dopamine level, decreased the Lewy body pathology in mice <sup>104</sup>	Phase 2 NINDS NET-PD
	Fingolimod	Blocks T cell egress from lymph nodes (prevents T-cell entry to the brain)	Decreased $\alpha$ Syn pathology in enteric nervous system of A53T transgenic mice <sup>105</sup>	Preclinical
	Rosiglitazone	PPAR $\gamma$ inhibits microglial release of TNF $\alpha$	Reduced $\alpha$ Syn pathology and prevented loss of dopaminergic neurons <sup>106</sup>	Preclinical
	Pioglitazone	PPAR $\gamma$ agonists, inhibits microglia activation	Modifies progression in early PD <sup>107</sup>	Phase 2
Astrocyte	NLY01 (GLP-1R agonist)	Blocks A1 neurotoxic astrocyte generation by microglia	Reduced $\alpha$ Syn pathology in A53T transgenic mice <sup>99</sup>	Preclinical
	SGK1 inhibitor	SGK1 is negatively regulated by Nurr and Fox2 in glial cells	Phase 1, the drug was found to be safe and well-tolerated	Phase 2 NCT03672604
TLR2	T2.5 antibodies	Blocks TLR2	Ameliorated neuronal $\alpha$ Syn aggregation and protected dopaminergic neuron loss <sup>108</sup>	Preclinical
LAG3 receptor	LAG3 antibodies (C9B7W and 410C9)	Blocks LAG3 receptor	Decreased $\alpha$ Syn pathology and inflammation in mice <sup>109</sup>	Preclinical
T lymphocytes	GA	Attenuates the activation of CD4 <sup>+</sup> T cells and the pro-inflammatory response	Reduced $\alpha$ Syn transmission <sup>77</sup>	Preclinical
	Sargramostim (Leukine)	Human recombinant granulocyte-macrophage colony-stimulating factor affects myeloid recovery	Improved the motor function and restored the $\alpha$ Syn level in the midbrain and striatum of MPTP-treated mice <sup>110</sup>	Preclinical
Inflammasome	MCC950	Blocks ATP and nigericin dependent NLRP3 activation	Sargramostim treatment in PD is well-tolerated <sup>111</sup>	Phase 1 NCT010882010
	PAP	Selective inhibitor of phosphodiesterase 10A activity	Prevented inflammasome activation by fibrillar $\alpha$ Syn, and led to less neuron loss and better dopaminergic signaling <sup>112</sup>	Preclinical
	VX-765 (caspase-1 inhibitor)	Inhibits proteolytic processing of IL-1 $\beta$ and IL-18 to secreted forms	Inhibited $\alpha$ Syn aggregation and neuronal cell death results from MPTP/P mice model <sup>113</sup>	Preclinical
	Inzomelid (IZD174) (NLRP3 inhibitor)	Inhibitor of inflammasomes containing NLRP3, or nod-like receptor family, pyrin domain-containing protein 3	Inhibition of caspase-1 rescued BE(2)-M17 human dopaminergic neuroblastoma cells from the toxic effects of $\alpha$ Syn <sup>114</sup> Reduced $\alpha$ Syn pathology in transgenic mouse model of MSA <sup>115</sup>	Preclinical
Cytokines	XPro1595 (TNF inhibitor)	Targeted soluble TNF	The treatment was well tolerated in double-blind evaluations in healthy volunteers	Phase 1 NCT04338997
			Reduced the $\alpha$ Syn protein level <sup>116</sup> Neuroprotective effects in rat model <sup>117</sup>	Preclinical

$\alpha$ Syn, alpha-synuclein; PD, Parkinson's disease; NINDS NET-PD, National Institute of Neurological Disorders and Stroke Neuroprotection Exploratory Trials in Parkinson's Disease; PPAR $\gamma$ , peroxisome proliferator activated receptor gamma; GLP-1R, glucagon-like peptide-1 receptor; SGK1, serum and glucocorticoid-induced kinase 1 inhibitor; TLR, toll-like receptor; GA, glatiramer acetate; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ATP, adenosine triphosphate; PAP, papaverine; IL, interleukin; MSA, multiple system atrophy; TNF, tumor necrosis factor.



**Figure 4.** An overview of inflammation and the  $\alpha$ Syn interaction.  $\alpha$ Syn, alpha-synuclein; TLR, toll-like receptor.

to induce neurotoxic A1 astrocytes by secreting IL-1 $\alpha$ , TNF $\alpha$ , and C1q complements.<sup>98</sup> Thus, Yun et al.<sup>99</sup> demonstrated that microglia-induced inhibition of A1 astrocytes led to a decrease in pathological  $\alpha$ Syn in an animal model.

A recent study using intranasal infusion of endotoxin–lipopolysaccharide (LPS) to induce olfactory bulb (OB) inflammation in mice found that LPS triggered microglial activation, inflammatory cytokine expression, and phosphorylated  $\alpha$ Syn accumulation in the OB and SN through IL-1 $\beta$ /IL-1 receptor 1-dependent signaling.<sup>100</sup> This finding suggests that inflammation, particularly IL-1, plays a role in mediating the initiation and propagation of  $\alpha$ Syn pathology from the OB.<sup>100</sup>

In relation to systemic inflammation, La Vitola et al.<sup>101</sup> developed a PD mouse model through systemic administration of LPS followed by intracerebroventricular injection of an  $\alpha$ Syn oligomer as well as an  $\alpha$ Syn-overexpression transgenic mouse administered LPS. The authors demonstrated that peripherally induced neuroinflammation influences the action of  $\alpha$ Syn oligomers to potentiate a detrimental effect.<sup>101</sup> The influence of the adaptive immune system on  $\alpha$ Syn has also been explored.<sup>102</sup> By crossing human  $\alpha$ Syn-overexpressing transgenic mice and lymphocyte-lacking mice, Sommer et al.<sup>102</sup> determined that the presence of T lymphocytes modulated toward a proinflammatory M1 phenotype was associated with an increased number of  $\alpha$ Syn aggregates in the SN and striatum. Their findings indicate that the inflammatory response contributes to  $\alpha$ Syn transmission through various pathways.

### Therapeutic strategies targeting the interaction between inflammation and $\alpha$ Syn in PD

As discussed above, extensive evidence suggests that inflammation plays a critical role in  $\alpha$ Syn-associated PD pathogenesis (Figure 1). Therefore, researchers have explored treatment targets by modulating the immune response to ameliorate  $\alpha$ Syn-associated PD pathology (Figure 3). This has led to numerous preclinical and clinical trials, as presented in Table 2.<sup>103-117</sup>

## CONCLUSION

In conclusion, inflammation plays important roles in  $\alpha$ Syn-related pathological processes and vice versa. Based on the current understanding, CNS innate immune cells, i.e., microglia and astrocytes, are activated in response to pathological  $\alpha$ Syn, promote the propagation of  $\alpha$ Syn, and clear abnormal  $\alpha$ Syn. In addition, peripheral immune cells, such as lymphocytes and NK cells, interact with CNS innate immune cells responding to abnormal  $\alpha$ Syn and contribute to  $\alpha$ Syn propagation (Figure 4). A vicious cycle is thereby created through the interaction between activated inflammation and  $\alpha$ Syn propagation, which may support prolonged PD progression. Thus, blocking this cycle using anti-inflammatory methods could represent a promising option for future therapy.

### Conflicts of Interest

The authors have no financial conflicts of interest.

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