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Review

The Role of Microglia and Astrocytes in the Pathomechanism of Neuroinflammation in Parkinson's Disease—Focus on Alpha-Synuclein

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Abstract

Glial cells, including astrocytes and microglia, are pivotal in maintaining central nervous system (CNS) homeostasis and responding to pathological insults. This review elucidates the complex immunomodulatory functions of glial cells, with a particular focus on their involvement in inflammation cascades initiated by the accumulation of alpha-synuclein (α-syn), a hallmark of Parkinson's disease (PD). Deriving insights from studies on both sporadic and familial forms of PD, as well as animal models of PD, we explore how glial cells contribute to the progression of inflammation triggered by α-syn aggregation. Additionally, we analyze the interplay between glial cells and the blood-brain barrier (BBB), highlighting the role of these cells in maintaining BBB integrity and permeability in the context of PD pathology. Furthermore, we delve into the potential activation of repair and neuroprotective mechanisms mediated by glial cells amidst α-syn-induced neuroinflammation. By integrating information on sporadic and familial PD, as well as BBB dynamics, this review aims to deepen our understanding of the multifaceted interactions between glial cells, α-syn pathology, and CNS inflammation, thereby offering valuable insights into therapeutic strategies for PD and related neurodegenerative disorders.

Keywords: α-synuclein; Parkinson's disease; microglia; astrocytes; neuroinflammation; blood-brain barrier; peripheral lymphocytes

1. Introduction

1.1 Parkinson's Disease Forms and Relation to α-Synuclein Aggregation

Parkinson's disease (PD) manifests in two primary forms: familial and sporadic. The familial variant is linked to genetic mutations, particularly in the α-synuclein (α-*syn*) gene, such as *A30P* [1], *A53T* [2], *E46K* [3], *H50Q* [4,5], and *G51D* [6], which account for about 15% of all cases [7]. The exact cause of sporadic PD remains elusive, although both genetic and environmental factors are believed to play a role. Certain pesticides like rotenone and paraquat [8], as well as toxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [9], have been associated with sporadic PD. Recent population-based studies indicate that air pollutants generated by urban transportation significantly increase the risk of disease [10–12]. Moreover, variations in PD incidence across different ethnic groups also hint at genetic influences [13]. Recent research underscores the significance of environmental toxins in sporadic PD development [14]. The interplay between genetic and environmental factors, along with processes like neuroinflammation, oxidative stress, and α-syn misfolding, likely contributes to sporadic PD pathogenesis. Addressing α-syn aggregation has emerged as a potential therapeutic strategy, indicating the complex nature of sporadic PD etiology.

Research efforts in PD rely heavily on understanding its prodromal stages and identifying accessible markers for staging. The Braak hypothesis, proposed by Braak *et al.* [15] in 2006, offers a conceptual framework that outlines the potential sequence of PD development. According to this hypothesis, PD pathology may initially emerge in olfactory structures and enteric nerves in the gut, possibly years or even decades before affecting the substantia nigra pars compacta (SNpc). Subsequent neurodegeneration in the SNpc causes dopaminergic (DA) neuron loss, and motor symptoms appear. Research conducted in 2019 by Kim *et al.* [16] seems to confirm this theory, and the vagus nerve has been proposed as a potential conduit of α-syn aggregates (α-syn AGs) from the enteric nervous system to the brain. This research also found that truncal vagotomy prevented the transmission of pathological α-syn as well as behavioral and motor deficits in mice. While the hypothesis has sparked debate due to the diverse nature of clinical PD presentations and contradictory pathological findings, it suggests a compelling idea: in some PD cases, the disease may originate in the peripheral nervous system. Moreover, it implies that many non-motor symptoms observed in PD patients could be inherent to the disease's early development and natural progression.

The onset of PD symptoms is linked to neuropathological changes in basal ganglia structures (BG) that manifest as motor and non-motor symptoms. Considering the intricate pathophysiology of PD, two phenotypes are currently distinguished, known as "brain-first" and "body-first" [17]. These subtypes suggest contrasting routes of α-syn pathology propagation [18,19]. In the "body-first" subtype, initial pathology may emerge in the enteric or peripheral au-

tonomic nervous system before spreading to the medulla oblongata via the vagus nerve. This ascending pathology affects the pons, leading to rapid eye movement (REM) sleep behavior disorder (RBD) before involving the SNpc [20]. In the "brain-first" subtype, pathology may originate in the amygdala or olfactory bulb and then spread to the brainstem and cortex [21]. A chronic inflammatory state in the central nervous system (CNS) appears to be a common feature linking both subtypes of PD. Neuroinflammation is believed to accompany progressive neurodegeneration triggered by abnormal α-syn accumulation in neurons. Glial cells, particularly microglia and astrocytes, actively participate in PD-related neuroinflammation, displaying diverse pro- and anti-inflammatory functions. They contribute to both the spread of neuroinflammation within the CNS and the protection of DA neurons. Glial cells maintain constant communication with each other, with neurons, and with peripheral immune cells.

1.2 α-Syn in PD

Alpha-synuclein is a leucine-rich, 14 kDa protein encoded by the synuclein alpha (*SNCA)* gene, of which the aforementioned variants (*A30P, A53T* [22], *E46K* [23], *H50Q*, and *G51D*) are associated with familial PD [7,24]. In pathological states, α-syn may aggregate (which is equivalent to toxic gain of function and results in neurotoxicity in PD), be secreted from neurons into the extracellular space, transferred as free protein or by extracellular vesicles, and detected in the blood and cerebrospinal fluid (CSF) of PD patients [7,25]. Increased α-syn expression may be a result of *SNCA* gene multiplications [26,27]. Different structural forms of α-syn can occur, including monomeric (which is nontoxic and soluble), fibrillar, and oligomeric. They are recognized by microglial membrane receptors, resulting in pro-inflammatory microglial activation. This can indicate that α-syn not only exists as a pathological marker of PD but also contributes to inflammation [7,28]. It is thought that, overall, aggregated forms of α-syn are involved in a number of pathologies, e.g., neuroinflammation [29], mitochondrial dysfunction [30], and endothelial degeneration [31,32]. Monomeric α-syn induces microglial phagocytosis and, conversely, α-syn AGs can inhibit these processes [7].

Some strains of α-syn are considered to be mediators for spreading pathological forms of aggregates in the brain, being able to pass through the blood-brain barrier (BBB) and between different brain cells [33,34]. The BBB is a natural barrier formed by astrocytes, pericytes, microglia, and also metabolically active endothelial cells (ECs), which is connected with junctional proteins [35] and basement membranes (BMs), creating parenchymal (astroglial) and endothelial BMs [36].

Aggregated forms of α-syn can interact with neuronal membranes, causing their disruption [37], and reduce microglial phagocytosis by binding to Fc gamma receptor IIB (FcγRIIB). This possibly impairs the clearance of aggregated molecules, which further exacerbates neuroinflammation and, thus, neurodegeneration [38]. However, research has also shown that a fibrillar form of α-syn has a higher potential to elevate levels of the pro-inflammatory cytokines secreted by BV-2 microglial cells compared with other forms of α-syn [39–41]. This research also indicated that the fibrillar form of α-syn preferentially underwent phagocytosis by these cells. In its fibrillar forms, α-syn can alter autophagy processes, as well as the functionality of mitochondria in microglial cells [42]. Fibrils of α-syn might also impair communication between ECs and neurons as, in the study of Kuan *et al.* [43], pathological forms of α-syn in EC-neuron co-cultures lead to endothelial dysfunction.

Change in α-syn structure acts as a damage-associated molecular pattern (DAMP) that leads to the activation of microglial immune receptors, e.g., toll-like receptor 2 (TLR2). α-Syn seems to interact with TLR2 receptors, while these, in turn, appear to induce the polarization of pro-inflammatory phenotype (M1) microglia [28,44,45]. However, TLR2 is not the only receptor in the family that interacts with α-syn, resulting in microglial activation. In 2011, Stefanova *et al.* [46] showed that TLR4 mediates the phagocytosis of recombinant α-syn (via translocation of nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) cells). Fibrillar α-syn can also activate the NF-κB pathway in microglia [47], as well as nod-like receptor protein 3 (NLRP3) inflammasomes in human microglial cells [48]. NLRP3 can be found in astrocytes and microglia, and drives the extracellular secretion of pro-inflammatory interleukin-1β (IL-1β), leading to exacerbated autophagy and neuronal damage [48,49]. α-Syn can also bind to CD11b integrin, resulting in Rho signaling pathway activation and, subsequently, to the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX2), which is a critical factor for microglial activation [50]. Overall, pathological misfolded α-syn acts both as an effector and a regulator of glial functionality and is the main factor contributing to neuronal death in PD. In patients with PD, CNS inflammation is aggravated by the induction of glial cell activation, pro-inflammatory cytokines, reactive oxygen species (ROS), and nitric oxide (NO) secretion and release, leading to ongoing neurodegeneration.

In this article, we explore the immunomodulatory roles of glial cells, with a focus on their involvement in inflammation progression caused by α-syn accumulation, as well as their potential activation of repair and neuroprotective mechanisms. In addition, we compile scientific evidence confirming the dual role played by microglia and astrocytes in the propagation of inflammation within the CNS in PD. Furthermore, we discuss scientific data highlighting the crucial role of α-syn in the mechanisms involved in recruiting peripheral immune responses in PD.

2. Role of Astrocytes

The role of astrocytes in the CNS includes anti-inflammatory and neuroprotective functions [51]. These cells have numerous delicate processes that extend from around 80% of their cell membrane surface and maintain constant contact with blood vessels, neuronal synapses, and other glial cells. Two primary types of astrocytes exist – fibrous and protoplasmic – both of which are present in the white and gray matter of the spinal cord and brain, though they differ in morphology [51]. Astrocyte morphology can vary depending on the phenotype they assume, either pro-inflammatory or anti-inflammatory. Moreover, astrocytes differ between brain regions, e.g., striatal astrocytes differ in morphology from those in the hippocampus [52,53], and their functional capabilities decrease with age [54]. The differences in functionality include electrophysiological properties, astrocyte-synapse proximity, and Ca^{2+} signaling [55]. Such differences could be a reason that specific brain regions are vulnerable to damage in PD, as demonstrated by the latest research conducted by Bondi and collaborators [56]. This study confirmed the age- and location-dependent density and morphology of astrocytes in mouse SNpc. From the perspective of neuronal survival and maintaining the integrity of the BBB, the involvement of astrocytes in the release of neurotrophic factors, such as mesencephalic astrocyte-derived neurotrophic factor (MANF), cerebral dopamine neurotrophic factor (CDNF) [57,58], brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF) [59], is crucial. In response to CNS damage or inflammation, astrogliosis occurs, resulting in a change of morphological forms among astrocytes [e.g., radial, fibrous, and protoplasmic] [60]. The neurotoxic activity of astrocytes, which contributes to the death of neurons and oligodendrocytes, can also be triggered by classically activated microglial cells, particularly by IL-1α, tumor necrosis factor (TNF), and complement component 1q (C1q) secretion [61], as well as by the release of pathological α-syn, even though its expression in astrocytes is less pronounced than in neurons [62].

Astrocytes and microglia are in constant contact, mutually influencing each other's functions. Astrocytes can respond to inflammatory signals by regulating the activation of microglia, but they can also react to inflammation resulting from microglial activation and adopt neurotoxic rather than neurotrophic functions. Astrocytes are found in the *post-mortem* brains of patients with PD and other neurodegenerative disorders, as well as in α-syn pre-formed fibril (PFF) injected mice [61,63].

While astrocytes secrete pro-inflammatory factors like chemokines (e.g., C–C motif chemokine ligand 2 (CCL2), CCL5, C-X-C motif chemokine ligand 1 (CXCL1), CXCL10, and CXCL12 [64]), it is essential to note that their anti-inflammatory properties outweigh the pro-inflammatory properties [65]. Astrocytes primarily support brain homeostasis by upregulating channels, like Kir4.1 (inwardly rectifying K+ 4.1) [66], and transporters, like glutamate-aspartate transporter (GLAST), excitatory amino acid transporter 1 (EAAT1) [67], to remove potassium and glutamate ions from damaged neurons. Additionally, they regulate oxidative stress by producing glutathione (GSH), which helps counteract the neurodegeneration induced by ROS [68,69]. Animal study suggest that transient GSH depletion in the SNpc triggers an inflammatory reaction with a response from the astroglia [70]. Thus, the weakened protective role of astrocytes, which may result from their age-related decline in cell number in the SNpc, could promote inflammatory processes and neurodegeneration. Astrocytes encircle endothelial cells directly, secreting factors that boost and maintain the integrity of the BBB [71] and can promote barrier properties and functionality, even in non-neural ECs [72].

Astrocytes serve as energy suppliers for neurons by acting as the brain's glycogen reservoir and providing energy substrates such as lactate and ketone bodies [73]. Due to the progressive neurodegeneration seen in PD, neurons require more energy, and the relatively low density of astrocytes in the SNpc makes DA neurons in this region particularly vulnerable [73]. Astrocytes also demonstrate neuroprotective properties through their influence on α-syn spreading. Evidence suggests that astrocytes can endocytose neuron-derived α-syn AGs and transport them to lysosomes for degradation, as is seen with microglia [74,75]. Astrocytes degrade α-syn AGs more effectively than neurons, probably due to the higher abundance of lysosomes in astrocytes [74].

Apart from their neuroprotective roles, astrocytes activate genes associated with immune functions in response to the transfer of α-syn from neuronal cells [76]. To delve into this phenomenon, Lee *et al.* (2010) [76] exposed primary astrocyte cultures to a conditioned medium from α-syn-expressing SH-SY5Y neuronal cells and analyzed gene expression changes via microarray analysis. They found a significant upregulation in genes associated with pro-inflammatory cytokines and chemokines upon exposure to extracellular α-syn, indicating the induction of an inflammatory response in astrocytes. α-Syn, which is typically confined within neurons, can be released through unconventional exocytosis, particularly under stress [77]. This release is accompanied by an increased presence of α-syn AGs outside neuronal cells. These aggregates, once in the extracellular space, are capable of being internalized by both neurons and glial cells via endocytosis [78,79]. Research conducted by Lee *et al.* [76] showed that α-syn formed inclusion bodies in astrocytes that take up the protein, indicating that astrocytes could be involved in the accumulation of α-syn released from nearby neurons. Such aggregation can disrupt the functions of astrocytes and increase microglial activation (especially in the mid-brain, spinal cord, and brainstem), thereby contributing to neu-

roinflammation [80]. Expanding upon these observations, recent studies have demonstrated the transfer of α-syn between neurons through successive exocytosis and endocytosis events [81,82]. This transfer process results in the formation of Lewy body-like structures within recipient neurons, ultimately leading to their demise [83]. α-Syn accumulation inhibits autophagy by microglia, and, at the same time, intracellular transportation of α-syn is intensified by disrupted autophagic processes [84]. Such intercellular transmission may serve as a fundamental mechanism driving the spread of Lewy body pathology during the progression of PD. More than that, non-neuronal cells can also take part in α-syn propagation as astrocytes can contain α-syn inclusions and are possibly able to mediate the transfer of this protein between the cells of the neurovascular unit (NVU) [85].

Astrocytes exposed to neuron-derived α-syn induce the production of the pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, tumor necrosis factor-α (TNF-α)) and chemokines that can activate microglia [76,86–89]. In addition, astrocytes play a pivotal role in amplifying neuroinflammation by responding to pro-inflammatory signals, such as IL-1α, TNF-α, and C1q, as well as to fragmented mitochondria from activated microglia [61,90]. In MPTP-treated monkeys, elevated expression of interferon (INF)-γ receptor was observed on astroglia, along with an increased TNF-α immunoreactivity associated with astroglia. This suggests that overactivation of astroglial cells might have a significant impact on the advancement of PD [91]. In an inducible mouse model expressing the mutant A53T α-syn variant, specifically in astroglial cells, the primary observation was the combination of microgliosis and rapidly progressing paralysis, as well as widespread astrogliosis. Additionally, the overexpression of the mutant α-syn in astroglial cells disrupted the normal functions of astrocytes. This disruption resulted in compromised integrity of the BBB, disturbed homeostasis of extracellular glutamate, and ultimately, led to significant loss of DA neurons in the midbrain and motor neurons in the spinal cord [80]. Astrocytes derived from induced pluripotent stem cells (iPSCs) of PD patients and containing α-syn AGs exhibit a heightened reactive state and secrete increased levels of pro-inflammatory cytokines, including IL-6 and CCL-5, when subjected to inflammatory stimuli [92]. Upon exposure to transmitted α-syn, astrocytes engage in both receptor-mediated endocytosis and interactions with receptors on the cell membrane. TLR2, recognized as a pattern recognition receptor, plays a role in the uptake of α-syn in astrocytes, while TLR4 does not participate in this process [88,93–95]. Both TLR2 and TLR4 are implicated in mediating the pro-inflammatory effects of transmitted α-syn. In a separate investigation, a PD mouse model that overexpresses mutant α-syn, exhibited both structural and functional changes in astroglial mitochondria, along with disrupted secretion of factors crucial for neuronal differentiation [96]. These findings imply that the buildup of α-syn in astroglial cells may play a role in the onset of PD.

A recent study demonstrated that PD mutant mice that overexpress both human α-syn and transglutaminase 2 (TG2) exhibited increased α-syn aggregation and heightened astroglial activation compared with mice that overexpress only α-syn. This suggests that TG2 may play a significant role in the accumulation of α-syn and the development of PD and related disorders, offering a novel target for therapeutic interventions [97].

Although astrocytes secrete factors that promote BBB integrity and maintenance, they also produce factors that can disrupt these. For example, vascular endothelial growth factor (VEGF), typically known for promoting vascular growth, impairs BBB integrity under unfavorable conditions. In pathological states, astrocytes secrete VEGF and act adversely towards endothelial integrity [98]. Moreover, the elevated reactive astrocyte count observed in some diseases is considered to be associated with BBB disruption [72]. In the human induced pluripotent stem cells (iPSC)-derived BBB co-culture model, BBB dysfunctions may arise as a result of TNF triggering astrocytes to adopt an inflammatory reactive state. This occurs, among other mechanisms, through the activation of signal transducer and activator of transcription 3 (STAT3), which is correlated with vascular inflammation in *post-mortem* human tissue [72]. Vascular changes have been observed in both males and females in the *post-mortem* substantia nigra (SN) [99]. Another study by Jackson *et al*. [100] demonstrated that astrocyte-derived apolipoprotein E4 (APOE4) is associated with BBB disruption, leading to BBB leakage and impaired tight junctions. The removal of astrocyte-derived APOE4 alleviated these phenotypes [100]. *In vitro* modeling of the BBB with astrocytes carrying the leucine rich repeat kinase 2 (LRRK2) G2019S mutation showed modified function and morphology of vessels, altered possibly through mitogen-activated protein kinase kinase 1 and 2 (MEK1/2). Importantly, inhibiting MEK1/2 improved vessel integrity. Overall, astrocytes carrying this mutation have a pro-inflammatory profile and reduce the integrity of the BBB [99].

While microglia are traditionally recognized as the primary cells for activating inflammasomes in the brain, astrocytes can also express and activate these inflammatory signaling complexes [101–103]. In PD model mice overexpressing mutant human A53T α-syn, NLRP3 inflammasomes are activated within the midbrain to produce IL-1β, although this process largely relies on the microglial uptake of α-syn [102]. Furthermore, astrocytes engage in crosstalk with various other cell types, including oligodendrocytes, endothelial cells, and peripheral immune cells, during neuroinflammatory responses [49,104].

Overall, astrocytes play a crucial role in maintaining neuronal balance, mitigating neuroinflammation, and supporting neuroregeneration and neuronal renewal.

3. Role of Microglia

Microglial cells occur most densely in regions such as the SN, hippocampus, BG nuclei, and olfactory bulb; the SNpc contains a notably high concentration of microglia, which makes DA neurons particularly susceptible to degradation [105]. Research conducted by De Biase *et al*. [106] in 2017 suggests that microglial cells in the SNpc specifically may be predisposed to malfunctioning in pathological states and may differ from those within the BG, including in such features as anatomy, transcriptomes, lysosome content, and membrane properties. This study also showed that neuronal phenotypes differ between the SNpc and the ventral tegmental area (VTA), which seems to confirm the specific susceptibility of neurons in the SNpc to damage [106]. Microglial activity changes with aging as these cells become more predisposed to transition into inflammatory phenotypes [107,108].

Within the CNS, microglia serve as an innate immune system and first line of defense against harmful factors such as viruses and bacteria, which may be removed by phagocytosis [7]. During phagocytosis, foreign particles and cells are recognized, engulfed, and digested by microglia. This represents one of the major pathways for clearing misfolded α-syn in PD, with the involvement of, for example, TLRs [109] and the complement system [7,110]. Microglia display diverse morphological variations within a heterogeneous population and possess both pro- and anti-inflammatory characteristics [111,112].

Microglial cells account for about 7% of non-neuronal brain cells of different species, depending on the brain region [113]. They exhibit distinct morphological forms and are categorized into two primary phenotypes. The M1 phenotype is activated via the classical pathway, e.g., by lipopolysaccharide (LPS) [114,115], which prompts the microglia to adopt a pro-inflammatory stance [28]. Activation of microglia can also be induced by α-syn [116], which leads to ROS production and results, in turn, in neurotoxicity [28].

Conversely, alternative activation, e.g., by IL-4 [114, 115], leads to the anti-inflammatory (M2) phenotype that is characterized by anti-inflammatory properties. Microglia take on a branched morphology, featuring a small, elongated cell body and numerous extended processes that contract and stretch, thereby facilitating exploration of the surrounding environment. The M2 phenotype can be divided further into M2a, M2b, and M2c types [114,117].

When exposed to abnormal α-syn, pathogens, or remnants of damaged neurons, microglia transition from a resting state to an active state, resulting in alterations in cell morphology, gene expression, and the production and expression of various factors [116,118].

Microglia are also observed in embryos [119] where they secrete growth factors to promote the survival of newly formed neurons, but can also eliminate abnormally formed nerve cells [120]. Importantly, major histocompatibility complex class II (MHC class II) molecules are expressed on microglial cells, enabling them to present antigens to other microglial cells, as well as to immune cells (such as T lymphocytes), which leads to the initiation of immune response mechanisms [115,120]. For this reason, microglia are considered to be antigen-presenting cells (APCs). Sporadic PD with late onset is correlated with polymorphism in the MHCII locus – human leukocyte antigen – DR isotype (HLA-DR) [121]. MHCII molecules are also expressed on macrophages [122] and monocytes [123] (whereas MHCI are expressed on neurons), and are responsible for antigen-presenting, T cell recruitment, and promoting immune responses to specific stimuli. MHCI and II molecules are considered to be the bridge between innate and adaptive immunity [124].

Microglia are in constant contact with other cells from their environment, including neurons. An example of communication between a nerve cell and microglia is that which is associated with type I membrane glycoprotein (CD200) – a glycoprotein expressed on neurons, ECs, and astrocytes, which has a specific receptor (CD200R) located on microglia [125,126]. Proper communication between CD200 and its receptor maintains the microglia in a resting state (under physiological conditions) [126–128]. Damaged neurons (e.g., under inflammatory conditions) may not be able to secrete adequate CD200, with IL-4 being a key component in this mechanism [120,129]. The abnormal interaction between CD200 and CD200R leads to exacerbated DA neuronal death, increased microglial activity and increased production of the pro-inflammatory cytokines IL-6 and TNF-α. This was demonstrated in a 2011 study by Zhang *et al*. [127] on a rat model of PD induced by 6-hydroxydopamine (6-OHDA) administration. Thus, it can be concluded that, via their contact with microglia, neurons can regulate microglial function and activation. Microglia can also phagocytose components of the NVU, such as endothelial cells [130].

Furthermore, microglia that exhibit the M1 phenotype impact BBB permeability [131], leading to the infiltration of peripheral immune cells [132] and contribute to the release of a plethora of pro-inflammatory cytokines, ROS [133], and NO [134]. They also contribute to the elimination of pathogens and/or damaged neurons [135], and produce pro-inflammatory factors that can alter BBB integrity [99]. There is evidence indicating that classical microglial activation can trigger the infiltration of peripheral immune cells into the CNS, thereby exacerbating and prolonging the inflammatory state within the brain [136]. A study by Haruwaka *et al*. [130] suggests the presence of an accumulation of microglial cells in the proximity of cerebral vessels in the very early stages of inflammation, preceding any alterations to BBB integrity. Interestingly, it seems that such contact, with ECs as well, leads to BBB protection, while an extended state of systemic inflammation is associated with higher activation of microglia and

Table 1. The involvement of astrocytes, microglia, and endothelial cells in alpha-synuclein aggregation-dependent neurodegeneration in Parkinson's disease. The presented compilation arises from studies using *in vitro* (cell cultures), *in vivo* (animal models), and *post-mortem* (tissue samples from PD patients) methods.

Cell type	Function/phenotype	Alpha-syn-mediated changes	*In vitro*/*in vivo*/*post-mortem* model	References
Astrocytes	Ability to form functionally proper BBB	Deterioration of structural integrity	Human PD donors, cell culture	[99]
		decreased expression of the water channel protein aquaporin-4	iPSC-derived astrocytes	[89]
	Cooperation with endothelial cells during maintenance of the BBB	Abnormal accumulation of Glut1 and vWF, and the redistribution of aquaporin-4 to the soma of astrocytes	α-Syn transgenic mice	[80]
	Neurotoxic phenotype	Microglia-mediated conversion	α-Syn PFF mouse model	[63]
	Pro-inflammatory phenotype	NLRP3 inflammasome-dependent; increase in caspase-1 and IL-1β levels, ASC protein levels, and the number of GFAP+ cells	Mouse model; primary culture	[49]
		TLR4-dependent gene expression; cylooxygenase-2, NO synthase mRNA; phosphorylation of p38 mitogen-activated protein kinase and c-Jun N-terminal kinase, and NF-κB1 nuclear translocation	Primary $TLR4^{+/+}$ and $TLR4^{-/-}$ mouse astrocytes	[88]
		Induction of pro-inflammatory genes for IL-1, IL-6, TNF-α; secretion of CCL2, CCL20, CXCL1, CX3CL1; involvement of TLR-2 signalling, NF-κB1, Fc receptor signalling, antigen processing (TAPBP and CD74)	Cell line SH-SY5Y; primary astrocytes from rat	[76]
		Increased IL-6, CXCL1, and IL-8 secretion	iPSC-derived astrocytes	[89]
		Increased IL-6 and CCL5 secretion	iPSCs from PD	[92]
		Increased IL-6 and TNF-α secretion	Cell culture	[40,41]
		TG2-dependent pro-inflammatory phenotype; increased number of GFAP cells	$TG2^{KO}/Syn^{Tg}$ double-modified mice	[97]
	Antigen-presenting phenotype	Elevated expression of HLA-DMA and MHC class II proteins	Human iPSC-derived astrocytes	[86]
	TLR2 – dependent pro-inflammatory responses	Overexpression of TLR2; increased IL-6, TNF-α, and IL-1β secretion	α-Synuclein transgenic mice	[94]
	Uptake of α-syn oligomer/fibrils by cells	Pronounced	Human iPSC-derived astrocytes	[74,75]
Microglia	Production and secretion of pro-inflammatory cytokines	Increased TNF-α and IL-1β production and secretion	BV2 cell culture	[39]
	TLR4 – dependent pro-inflammatory responses	Increased phagocytic activity, pro-inflammatory cytokine release (TNF-α, IL-6); CXCL and ROS production	TLR4 deficient ($TLR4^{-/-}$) mice, microglial; cell culture	[93]
		Increased phagocytic activity, activated NF-κB and p38 pathways	Cell culture	[116]
	TLR2 – dependent pro-inflammatory responses	Increased proportion of microglia displaying an ameboid and reactive morphology	α-Syn transgenic mouse model	[95]
		Increased IL-1β secretion dependent on NLRP3 inflammasome assembly and caspase-1 activity	Human PD donors, cell culture	[48]
		Increased percentage of microglial cells with amoeboid morphology and production of IL-6, IL-1β, and TNF-α	Primary rat microglia and $TLR2^{-/-}$ mice	[45]

Table 1. Continued.

Cell type	Function/phenotype	Alpha-syn-mediated changes	*In vitro*/*in vivo*/*post-mortem* model	References
	Pro-inflammatory gene induction	Elevated expression of TNF-α, IL-1β, IL-6, and COX-1	α-Syn transgenic mice	[80]
		Elevated expression of CXCL10, Rt1-a2, Grn, Csf1r, C3, C1qa, Tyrobp, Serping1, and Fcer1g in Cd74+; i.e., genes involved in complement protein expression, phagocytosis, T cell recruitment and activation, cytokine and chemokine release, and inflammasome signalling	α-Syn PFF injected rats	[143]
	NLRP3 inflammasome-dependent pro-inflammatory phenotype	Increased level of cleaved caspase-1 and adaptor protein ASC; microgliosis	*Post-mortem* brains of patients with PD; α-syn PFF-injected mice	[102]
	Pro-inflammatory MHCII response	Infiltration of CCR2+ and Ly6C+ peripheral monocytes into the SNpc	AAV2-SYN transduced mice	[151,152]
	Uptake of α-syn aggregates by cells; phagocytosis	Pronounced	BV2 microglial cells; rat microglia cells	[79]
		Inhibition of the uptake of α-syn aggregates in the absence of T lymphocytes	BV2 microglia	[147]
		Inhibition of phagocytosis	A53T α-syn transgenic mice	[38]
	Infiltration of peripheral T CD4 lymphocytes into the brain	MHCII and IL-4-dependent; induction of MHCII+ on microglia; increased IL-4 production by CD4 lymphocytes	α-Syn overexpressing mouse model	[148]
		MHCII-dependent microgliosis and peripheral blood T lymphocyte transfer into striatum and SNpc	α-Syn PFF injected immunocompromised (NSG) mice	[150]
	Pro-inflammatory phenotype	FcγR-mediated conversion	Primary cultures from mice	[47]
	Astrocyte-mediated anti-inflammatory phenotype	Activation of the p38/ATF2 (microglia) and the NF-κB (astrocytes) pathways	Transgenic A53T mice, mouse primary microglia	[144]
	Microglia and astrocyte interaction	Increased number of astroglia and microglia in close proximity to CD3 positive cells during T cell infiltration; higher IFN-γ and significantly higher *TNF*-α mRNA level	α-Syn TG transgenic mice	[149]
		Downregulation of CD200-CD200R1 and CX3CL1-CX3CR1 pathway	rAAV-hSYN-injected mice	[128]
Endothelial	BBB structure	Deterioration of BBB integrity	Brain tissue from PD patients	[32]
	Deterioration of BBB integrity	Downregulated expression of tight junction proteins	hCMEC/D3 human brain endothelial cells; brain tissue from PD patients	[43]
		Upregulation of LRP1-ICD	Mouse model	[34]
		Pathological activation of pericytes	Transgenic mice	[145]
		Elevated release of IL-1β, IL-6, MCP-1, TNF-α, and MMP-9 by pericytes	RBECs co-cultured with rat brain pericytes	[146]

The list of abbreviations: ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; ATF2, activating transcription factor-2; BBB, blood-brain-barrier; CCL2, C-C motif chemokine ligand 2; CCL20, C-C motif chemokineligand 20; CCL5, C-C motif chemokine ligand 5; CCR2, C-C chemokine receptor type 2; CD200, type I membrane glycoprotein; CD200R1, CD200 receptor 1; COX-1, cyclooxygenase-1; CX3CL1, C-X3-C motif chemokine ligand-1; CX3CR1, C-X3-C motif chemokine receptor-1; CXCL1, C-X-C motif chemokine ligand 1; FcγR, Fc gamma receptor; GFAP, glial fibrillary acidic protein; Glut1, glucose transporter 1; HLA-DMA, major histocompatibility complex, class II, DM alpha; IFN-γ, interferon gamma; IL-1, interleukin 1; IL-1β, interleukin 1 beta; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; iPSC, induced pluripotent stem cells; LRP1-ICD, low-density lipoprotein receptor-related protein-1; Ly6C, lymphocyte antigen 6 complex, locus C1; MCP-1, monocyte chemotactic protein-1; MHC, major histocompatibility complex; MMP-9, matrix metalloproteinase-9; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NLR family pyrin domain containing 3; PD, Parkinson's disease; RBECs, rat brain endothelial cells; ROS, reactive oxygen species; SNpc, substantia nigra pars compacta; TAPBP, TAP-associated glycoprotein, tapasin; TG2, transglutaminase-2; TLR2, toll-like receptor 2; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; vWF, von Willebrand factor; α-syn, alpha-synuclein; PFF, preformed fibril; Rt1-a2, mature alpha chain of major histocompatibility complex class I antigen; Grn, progranulin; Csf1r, macrophage colony-stimulating factor 1 receptor; AAV2-SYN, adeno-associated virus serotype 2 vector-mediated α-syn expression; rAAV2-hSYN, recombinant adeno-associated viral vector-mediated human α-syn expression; hCMEC/D3, human cerebral microvascular endothelial cell line.

weakened BBB functioning [130]. Moreover, microglial activation has been shown to exist even before the death of DA neurons in a rat 6-OHDA model of PD [137], indicating that microglia are activated and assume pro-inflammatory functions prior to the degeneration of DA neurons, as observed in PD.

Neuroinflammation within the CNS can yield positive outcomes and aid in neutralizing threats. Microglial cells exhibiting the M2 phenotype play a crucial role in this process by secreting anti-inflammatory factors and expressing specific genes that facilitate the repair and regeneration of impaired neurons [28,138]. Microglia take part in releasing anti-inflammatory cytokines, growth factors like transforming growth factor (TGF)-β1 [139], and neurotrophins, all of which are crucial for promoting neuroregeneration within the SNpc [118]. Microglial cells also express FcγR [134,140], which enables them to eliminate pathological forms of α-syn that are associated with immunoglobulin G (IgG) complexes [141]. Anti-inflammatory IL-4 can stimulate microglial cell proliferation and induce genes prompting microglia to activate in an alternative manner, thus promoting neuroprotective actions and inhibiting pro-inflammatory properties [115,142]. In order to shield nerve cells from toxicity and inflammation, it is vital to maintain proper microglial function and CNS homeostasis, along with the interaction between microglia and neurons [141].

Recent research by Stoll *et al.* [143] on rats treated with α-syn pre-formed fibril (PFF) injections presents a new perspective on glial gene expression in the case of α-synucleinopathy. Several gene expressions were analyzed, associated with astrocytes of both pro-inflammatory (e.g., *C3*, *Gbp2*, *Ggta1*, *Serping1*) and anti-inflammatory functions (*Tm4sf1*, *Emp1*, *S100a10*). Even though the experiment showed that the upregulation of pro-inflammatory-associated genes was far greater than that of anti-inflammatory-associated genes, the expression of genes associated with all different phenotypes was increased. The authors highlight how context plays a huge role in glial phenotype changes; astrocytes and microglia work together and swap their functions in response to inflammatory signals. For example, the expression of C3 (complement component 3) differed between microglia and astrocytes, localizing initially in microglia at 2–4 months post PFF injection and then shifting to astrocytes at 6 months post injection. Researchers concluded that the main pathways recognized by gene expression upregulation in α-synucleinopathy are complement, phagocytosis, T cell recruitment and activation, and cytokine/chemokine and inflammasome signaling [143]. This appears to be the latest and broadest view and also confirms the involvement of the innate immune system in PD synucleinopathy. The latest study by Leandrou *et al.* (2024) [144] identifies specific molecular pathways involved in microglial and astrocytic responses to α-syn oligomers, such as the p38 mitogen-activated protein kinases/activating transcription factor 2 (p38/ATF2) signaling pathway in microglia and the NF-κB pathway in astrocytes, that opens new possibilities of influence on both glial cell types in PD. Table 1 (Ref. [32,34,38–41,43,45,47–49,63,74–76,79,80,86,88,89,92–95,97,99,102,116,128,143–152]) shows the results of experimental studies using *in vivo* and *in vitro* methods, as well as brain *post-mortem* analyses in PD patients, highlighting changes in the function and phenotype of astrocytes, microglia, and endothelial cells induced by α-syn aggregation.

By assuming both pro- and anti-inflammatory phenotypes, microglia play a pivotal role in initiating and perpetuating neuroinflammation, as well as in the neuroprotective and regenerative processes within the CNS. The interactions between DA neurons, microglia, and astrocytes during α-syn-induced neurodegeneration in the SNpc are summarized in Fig. 1.

4. Involvement of Glial Cells in Recruiting Peripheral Immune Mechanisms in PD

When the body's internal balance is maintained, the CNS enjoys a state of immunological privilege. However, conditions like those observed in PD lead to ongoing inflammation in the CNS and compromise the integrity of the BBB. This breach allows activated pro-inflammatory lymphocytes and monocytes to infiltrate regions of the CNS affected by neurodegeneration [28]. It is noteworthy that ECs are also known to be responsible for trafficking immune cells [153]. The disruption of the BBB integrity and functionality of its components leads to enhanced neuronal loss as a result of severe neuroinflammation and α-syn accumulation. In addition, the permeability of the BBB in PD has been shown to be significantly increased [145,154]. BBB dysfunction can also be caused by changes in the functionality of ECs, to which α-syn-induced inflammation contributes. This leads to the activation of ECs and the release of pro-inflammatory molecules. The ECs appear to shrink, and gaps appear between them, increasing the permeability of the EC layer. Ultimately, this results in the recruitment of leukocytes, which are involved in the progression of neuroinflammation [155]. EC structure and functionality can also be modulated by vascular endothelial growth factor A (VEGFA), which is released by astrocytes and stimulated by microglia that produce IL-1 in chronic inflammation states [156,157]. It is thought that VEGF can mediate BBB breakdown [158]. In addition, α-syn-activated pericytes lead to BBB dysfunction by releasing pro-inflammatory factors [146]. Elabi *et al.* [145] showed that the activation of pericytes at an early stage of the disease in the human α-syn overexpression mouse model resulted in vascular alterations associated with BBB altered permeability.

Research suggests that, under conditions of chronic CNS inflammation, peripheral immune cells can migrate into the CNS [147,159]. Various communication mechanisms between microglia and neurons are implicated in this

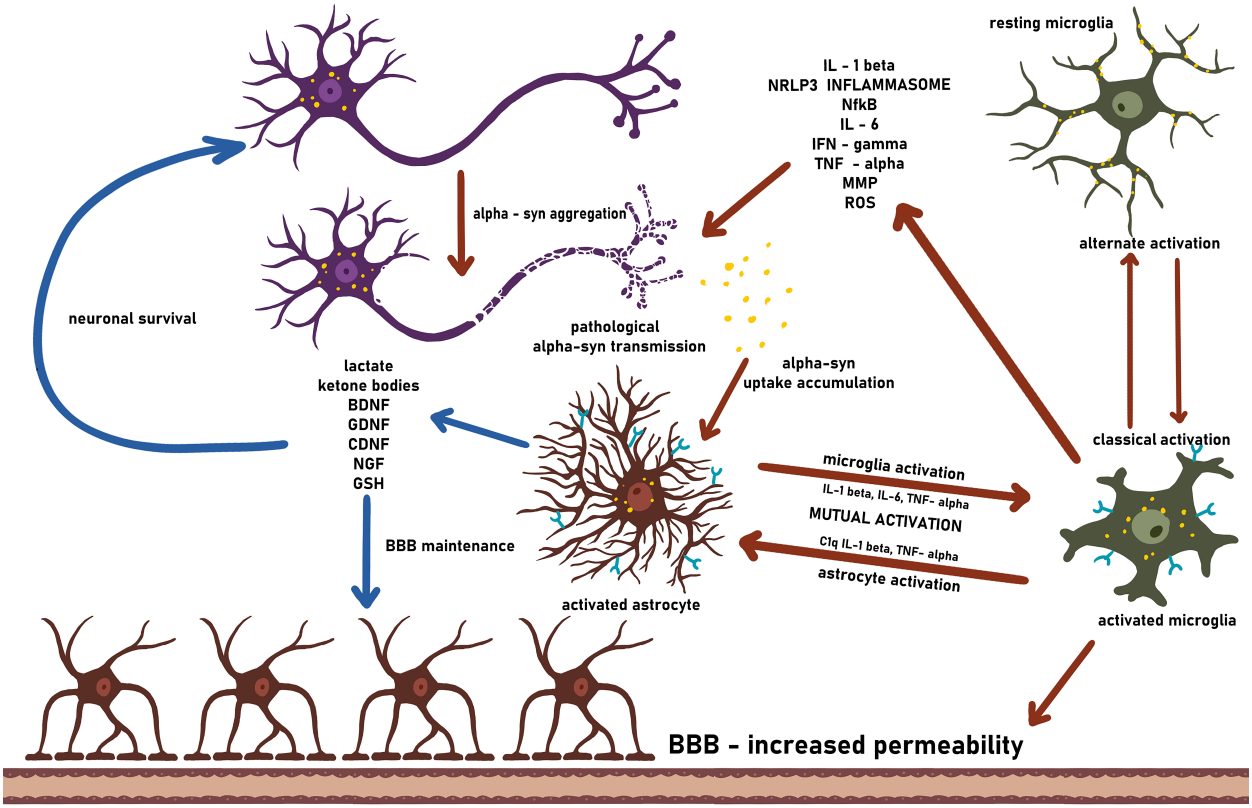

Fig. 1. Cooperation between astrocytes and microglia leads to neurodegenerative processes that are triggered by the aggregation of pathologically altered α-syn (depicted by red arrows), while also involving glial cells in neuroprotective mechanisms (shown by blue arrows). The diagram illustrates the interplay among neurons, microglia, and astrocytes, with detailed explanations provided above. All abbreviations have been explained in the text. The figure was created using Clip Studio Paint Pro v 2.0 (license number: L6136027487). BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; CDNF, cerebral dopamine neurotrophic factor; GSH, glutathione; BBB, blood-brain barrier; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ROS, reactive oxygen species; NGF, nerve growth factor; NRLP3, NOD-like receptor protein 3; IFN, interferon; IL, interleukin; C1q, component 1q.

process. The shift in microglial profile to an active state also contributes to this phenomenon [136]. Effective interaction between neurons producing fractalkine glycoprotein (C-X3-C motif chemokine ligand-1, CX3CL1) and its receptor on microglia (C-X3-C motif chemokine receptor-1, CX3CR1) [160] typically modulates microglial cell phenotype [161]. However, dysfunction in this mechanism can lead to the increased release of pro-inflammatory factors and neuroinflammation [162]. The pro-inflammatory microglia profile is considered to take part in immune cell recruitment [163].

Damaged neurons release the pathological form of α-syn into the environment, prompting microglia to adopt a pro-inflammatory response to counter the threat to the CNS. Exogenous α-syn can induce this microglial response directly, which, in turn, leads to the release of pro-inflammatory cytokines, increases phagocytic activity, and further aggravates microglial-induced inflammation. This may result in the transmission of α-syn in a prion-like way [7]. Thus, α-syn contributes to BBB disruption indirectly – by promoting inflammation [164]. The aberrant protein may possibly enter cervical lymph nodes, initiating the activation of macrophages [165] and thus effector T cell responses. Subsequently, T helper (TCD4) and T cytotoxic (TCD8) lymphocytes breach the BBB and migrate to the site of inflammation. α-Syn peptides bound to MHCI on neurons and MHCII on microglia re-stimulate peripheral immune cells, which incites inflammatory reactions [148,166]. Helper T cells (CD3/CD4+) are found near activated astrocytes, blood vessels, and places of high expression of pro-inflammatory cytokines, with CD3+ cells co-expressing interferon gamma (IFN-γ) [149]. While pro-inflammatory factors released by lymphocytes and microglia aid in removing pathological proteins, they also harm neighboring neurons, which leads to the further release of α-syn. These interconnected mechanisms, which perpetuate the continuous presence of the protein in the extracellular space and sustain inflammatory processes, are referred to as a self-propelling loop of neuroinflammation [167].

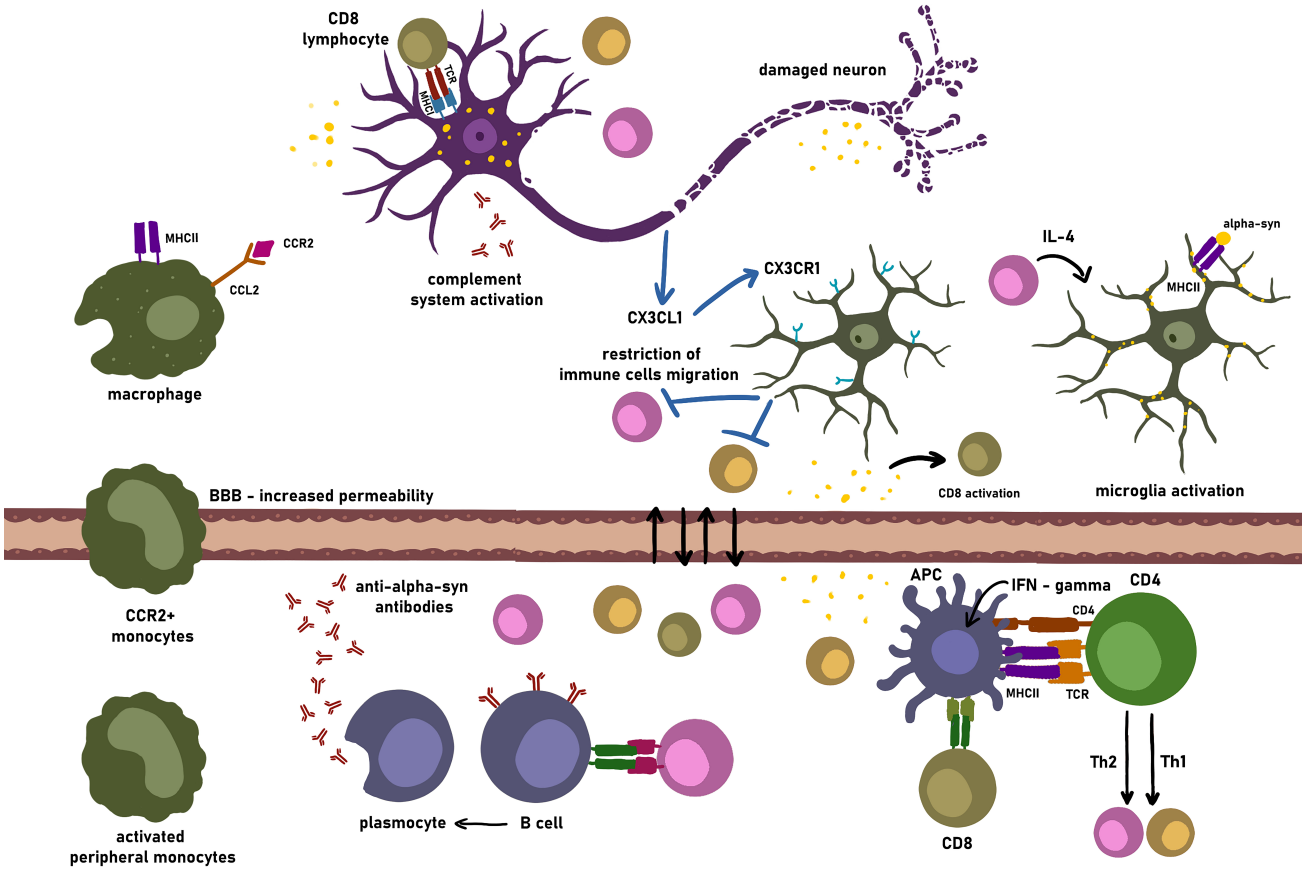

Fig. 2. Peripheral immune system cells are recruited through a compromised BBB because of α-syn presentation to peripheral lymphocytes. Depending on their activation type, microglial cells can either intensify or mitigate (blue lines) the inflammatory response. Further details are discussed in the accompanying text. All abbreviations have been explained in the text. The figure was created using Clip Studio Paint Pro v 2.0 (license number: L6136027487). APCs, antigen-presenting cells;TCR, T-cell receptor; Th1, type 1 helper T cells; Th2, type 2 helper T cells.

A recent study by Hourfar *et al.* [164] was conducted to explore the impact of α-syn AGs on BBB integrity in the human cerebral microvascular endothelial cell line (hCMEC/d3). The results indicated that α-syn AGs have a direct and damaging impact on ECs in the BBB (causing their dysregulation and mitochondrial dysfunction), with astrocytes playing a shielding role due to their ability to interact with pathological aggregates. However, in inflammatory states, it seems that the functions are reversed and, together with microglia, astrocytes take part in BBB disruption [164].

Outside the CNS, Th2 lymphocytes prompt B cells to generate anti-α-syn antibodies. These antibodies cross the BBB, bind to α-syn epitopes on neuron surfaces, and activate the complement system, resulting in antibody-dependent cytotoxicity and acute inflammation [166]. However, with disease progression, the T cell subsets change, resulting mainly in an imbalance between Th (Th2 and Th17) and Treg cells, and contributing to neurodegeneration by pushing the pro-inflammatory phenotypes [124]. The infiltration of peripheral immune cells into the CNS not only triggers the secretion of pro-inflammatory factors by activated immune cells, but also exerts anti-inflammatory effects [150]. Treg cells that are able to release, for example, IL-10 and TGF-β, can modulate both the activity of M1 microglia (since Treg depletion has been shown to result in activation of microglia via the STAT3 pathway, enhancing inflammation after spinal cord injury [168]) and other immune cells as well [169]. Additionally, factors secreted by Th2 cells, such as IL-5 and IL-4, prompt pro-inflammatory microglia to transition to the anti-inflammatory/resting phenotype – M2 [166].

The advancement of PD is closely tied to the activity of glial cells, including astrocytes and microglia. These cells can take on different phenotypes that determine their pro- or anti-inflammatory behaviors. When glial cells become pro-inflammatory, they release cytokines and chemokines that fuel the onset and continuation of neuroinflammation in the CNS. Conversely, shifting to an anti-inflammatory phenotype aids in shielding neurons and other brain cells by expressing factors that promote their protection and regeneration. Astrocytes, microglia, and neu-

rons interact with each other, influencing their functioning through various forms of intercellular communication. Several different pro- and anti-inflammatory factors are found in the CSF and/or blood of PD patients, including TNF-α, IFN-γ, TGF-β, IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10 [170–172].

Although neuroinflammation in the CNS is generally associated with pro-inflammatory microglial activity, research conducted by Harms *et al*. [151] cast the neuroinflammatory properties previously attributed to microglia in a new light. Researchers suggested that the activation of resident immune cells in the CNS depends strongly on peripheral monocytes infiltrating the brain in pathological states [151,152]. The study showed the recruitment of pro-inflammatory peripheral monocytes with C-C chemokine receptor type 2 (CCR2) (induced by α-syn exacerbated expression) as a main and crucial component in the response of α-syn-dependent CNS cells in a mouse model of PD [151]. CCR2 is a chemokine receptor, located on myeloid cells from the periphery, and the interaction between CCR2 and its ligand CCL2 seems to be fundamental for monocytes entering brain tissue. CCL2-CCR2 signaling induces monocyte infiltration through the BBB, where they facilitate anti- and pro-inflammatory reactions as macrophages [151]. The study also showed that inhibiting such infiltration of CCR2+ monocytes reduces the inflammation mediated by α-syn. CCR2-knockout mice exhibited attenuated MHCII expression in the SNpc [152]. As previously shown by the researchers, modulation of MHCII diminished α-syn neurotoxicity [152]. High expression of MHCII (HLA-DR) in the CNS, in the proximity of α-syn and thus of dying neurons, seems to indicate that APCs and adaptive immune processes play pivotal roles in neurodegeneration in PD [124,173].

Monocytes are divided into classical (CD14+/CD16–; differentiating into dendritic cells and macrophages), non-classical (CD14–/CD16+), or integral (CD14+/CD16+) types, and they vary in terms of the particles released, which include IL-6, IL-10, CCL2, IL-1β, IL-8, and TNF [124, 174–176]. In PD patients, monocytes undergo changes in their functionality, including their activation process, proliferation, and phagocytosis, with the duration of the disease as an affecting factor [124].

An overview of the mechanism of lymphocyte recruitment involving microglial cells through a compromised BBB to sites affected by neurodegeneration resulting from α-syn aggregation is shown in Fig. 2.

5. Conclusions

The latest data indicates that PD does not only impact the SNpc dopaminergic neurons but also affects other brain structures and the peripheral nervous systems, often manifesting as non-motor symptoms before motor signs appear. The inflammation triggered by activated microglia and astrocytes is believed to drive these diverse symptoms. The aggregation of α-syn exacerbates neuroinflammation in PD, worsening the loss of neurons. However, crucial details are still missing about the specific structural forms of α-syn that activate microglia and astrocytes. Neuroinflammation in the CNS compromises the integrity of the BBB, increasing its permeability and allowing peripheral immune cells to infiltrate and migrate towards the inflamed areas. Components of the immune system, along with glial cells, work to neutralize pathological α-syn, thus clearing the extracellular space. In addition, they trigger antibody-dependent immune responses and activate other cells, which exacerbates inflammation. Understanding the factors that can alter the immunomodulatory properties of glial cells, especially in connection with their reparative abilities, offers promising avenues for developing therapies that can slow down the progression of PD.

Author Contributions

OH, BG equally contributed to the analysis of selected articles for the text, manuscript, table and figures preparation. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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