Role of Glia in Parkinson's Disease

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Layman's summary

Parkinson's disease (PD) is a disorder of the motor system and it is characterized by symptoms such as tremor, bradykinesia, rigidity, and postural instability. In addition, patients with PD also suffer from symptoms that are not related to the motor system such as dementia, anxiety, sleeping disorders, and gastrointestinal problems. An important hallmark of the disorder in the brains of PD patients in the loss of nerve cells in a particular brain regions namely the substantia nigra. Also, patients have accumulations of a specific protein namely alpha-synuclein in certain brain regions.

Recently, research has shown that simultaneously with the changes mentioned above, activation of a certain cell type in the brain, namely glial cells, occur. Glial cells are support cells in the brain and they serve important functions for normal functioning of nerve cells and inflammation in the brain. Also, glia cells are present in the gut. The question arises if the activation of these glial cells are the result of the loss of the nerve cells or if the activation of these glial cells may contribute to initiation and progression of the disorders. More and more research accumulates that activation of glial cells are involved in initiation and progression of the disorder.

In this review, research into the involvement of activation of glial cells in initiation and progression of PD will be discussed. Firstly, results from human and animals studies into the location of activated glial cells in the brain and body will be described. Furthermore, studies on how these activated glial cells may contribute to initiation and/or progression of PD will be described. Also, it will be discussed which substances are produced by these activated glial cells and if these substances might be used to diagnose PD in an early stage of the disease for example by taking blood samples. In addition, it will be studies whether genes which are previously shown to be involved in PD are also expressed in glial cells. At last, research into new treatment of PD that are targeting activation of glial cells will be discussed.

Different type of glial cell exist in the brain namely microglia, which are the brain scavenger cells, astrocytes, which are important support cells that surround all the nerve cells, and oligodendrocytes, which play an important role in fast conduction of signals in nerve cells and keeping the nerve cells healthy. All glial cells are important mediators of inflammation in the brain. It is evident that especially microglia and astrocytes play an important role in PD. Activated microglia and astrocytes are already present in the brain before loss of nerve cells in the substantia nigra occur indicating that they might be involved in initiation of PD. Activated microglia and astrocytes can cause death of nerve cells by production of certain substances that induce inflammation or induce damage to the nerve cells. The role of oligodendrocytes in initiation and/or disease progression is not evident. Research with anti-inflammatory substances in animal models of PD further support the role of glial cells in initiation and progression of PD. Targetting glial cells to block inflammation or change the balance toward a less inflammatory state to treat PD might be a new effective treatment.

Contents

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder which is characterized by motor symptoms such as tremor, bradykinesia, rigidity, and postural instability. In addition to the classic motor symptoms, PD patients also suffer from non-motor symptoms such as dementia, anxiety, sleeping disorders, and gastrointestinal problems which seriously impairs their quality of life. An important hallmark of the disorder is the degeneration of dopamine neurons in the substantia nigra (SN) pars compacta and the presence of Lewy bodies (LB) and Lewy neurites (LN) in particular brain areas including the brainstem or cortical regions.

Recently, research has shown that simultaneously with the pathological changes glial reactions occur. Controversy remains if these glial reactions occur in response to neurodegeneration in PD or if these glial reactions contribute to neurodegeneration in PD. New insights show that glial reactions might be involved in the initiation and progression of pathology in PD. Glial cells are present in the brain but also in the autonomous system such as in the gastrointestinal system. If glial cells are not merely a response to degeneration in PD and are indeed involved in disease initiation and progression, glial cells in the substantia nigra (SN) and striatum might be involved in the occurrence of motor symptoms while glia in cortical regions might be involved in non-motor symptoms such as dementia and/or anxiety. Glial cells that are present in the gastrointestinal system might be involved in gastrointestinal problems.

In this review, research into involvement of glial cells in initiation and progression of PD will be discussed. The presence and location of reactive glia in the brain will be described using animal and human studies. Furthermore, studies on how these reactive glia might contribute to initiation and/or progression of PD will described. Also, it will be discussed which substances are produced by reactive glia and if these might be used as (early) biomarkers for the disease. In addition, it will be studied whether genes that are known to be involved in PD are expressed in glial cells. At last, research into new treatments of PD by inhibiting activation of glial cells will be discussed.

It is evident that especially microglia and astrocytes play an important role in PD. It is suggested that these activated phagocytic microglial cells contribute to loss of dopamine neurons in the SN through oxidative stress and production of pro-inflammatory cytokines. Microglial activation occurs before SN dopaminergic neuronal loss occurs which indicates that microglia might be important initiators of PD. However some questions remain to be elucidated. For example the underlying mechanisms how phagocytic microglia selectively target dopaminergic neurons. Astrocytes also appear to play an important role in the initiation of PD. Astrocytes are able to take up alpha-synuclein. Since astrocytes are not able to degrade alpha-synuclein, astrocytes start to produce inflammatory cytokines and ROS. Especially, the ability of astrocytes to recruit microglia makes astrocytes an important link in disease initiation and disease progression. The role of oligodendrocytes remain to be elucidated but evidence suggest that oligodendrocytes are not involved in initiation but might only be involved in progression of the disorder due to demyelination of neurons. Results of studies with anti-inflammatory agents in animal models of PD further support the involvement of glia in the initiation and progression of PD. In addition, to better study the role of inflammation in PD, research should be performed in animal models for PD that are not induced by inflammatory stimuli, for example the alpha-synuclein overexpressing model, since this might bias findings. In the search for effective disease-modifying treatments for PD, a new strategy might be to block inflammation or change the balance towards a more anti-inflammatory state.

Introduction

Parkinson's disease (PD) is a well-known progressive neurodegenerative disorder. The disorder is characterized by motor symptoms such as tremor, bradykinesia, rigidity, and postural instability [1]. In addition, patients with PD also display non-motor disabilities such as cognitive disabilities [1], gastrointestinal problems [2], and psychiatric symptoms such as depression [3], anxiety [4, 5], and insomnia[6]. In Europe, the overall prevalence of PD for persons over the age of 65 is 1.8% [7].

An important hallmark of the disorder is the degeneration of dopamine neurons in the substantia nigra (SN) pars compacta and the presence of Lewy bodies (LB) and Lewy neurites (LN) in particular brain areas including the brainstem or cortical regions [1, 8, 9]. These LB's and LN's contain accumulated alpha-synuclein aggregates in respectively cell bodies and cell axons in the central and peripheral nervous system [8, 9]. Recently, research has shown that simultaneously with the pathological changes in neurons, glial reactions occur. Newer concepts suggest that these glial reactions might be involved in the initiation and progression of pathology in PD [8]. Many research has been done to study the role of glia cells in PD but also in other degenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, and multiple system atrophy [10].

In this review, I will only focus on the role of glial cells in PD related to motor symptoms, dementia, and gastrointestinal problems. Firstly, I will first give an introduction on PD. Secondly, I will describe the different types of glial cells in the central and peripheral nervous system and their function. After that, I will focus on the role of these different types of glial cells in PD related to the abovementioned symptoms of PD. Then, glial cells as possible target for treatment of PD will be described. At last, I will summarize all the previous parts and draw some conclusions.

Parkinson's Disease

PD is a progressive neurodegenerative disorder which is characterized by motor symptoms such as tremor, bradykinesia, rigidity, and postural instability [1]. The first description of the disorder was made by James Parkinson in 1817. PD affects 1% of the general population and 2% of those above the age of 65 [7, 11]. In addition to the classic motor symptoms, PD patients also suffer from non-motor symptoms which seriously impairs their quality of life [12]. Examples of non-motor symptoms are neuropsychiatric symptoms (e.g. depression, anxiety, dementia), sleep disorders, autonomic symptoms (e.g. sexual difficulties, sweating), gastrointestinal symptoms (e.g. constipation, nausea), sensory symptoms (e.g pain, olfactory/visual dysfunction), and other symptoms such as fatigue [12]. Studies have shown that these psychiatric and non-motor symptoms influence disability in PD in a negative fashion [13, 14]. Patients suffering from major depression showed increased impairments in activities of daily living compared to patients with minor or no depression [14]. In the following section, underlying mechanisms, etiology, and current en new developments in treatments of PD will be discussed.

Underlying mechanisms

In the last decades, many research has been done to study the underlying mechanisms in PD. The pathological hallmark of PD is degeneration of dopamine neurons in the SN pars compacta resulting in motor problems. The loss of these neurons is accompanied by formation of LB's and LN's [1]. The main component of these LB's and LN's are aggregates of misfolded alpha-synuclein [8]. Alpha-synuclein is a protein which is mostly located in the axon and axon terminals [15]. The protein has a high affinity for membranes of synaptic vesicles [16] and it is suggested to be involved in terminating dopamine neurotransmission by altering dopamine transporter-mediated uptake of synaptic dopamine [17]. Most of the neurons contain alpha-synuclein but not all cells. Therefore, only neurons containing (enough) alpha-synuclein are vulnerable to become involved in PD [15]. In PD and also in some other neurological disorders such as diffuse Lewy body disease, alpha-synuclein has changed from conformation from an α-helical conformation to a β-sheet-rich structure [17, 18]. The altered form self-aggregates with other similar altered forms of alpha-synuclein and additional proteins resulting in inclusions in cell bodies and axons, respectively called LB's and LN's. The neurons are unable to degrade altered alpha-synuclein by ubiquitination and proteasomal recycling. This altered protein handling results in accumulation which leads to neurodegeneration (see Figure 1) [15, 19]. However, the reason underlying the non-functioning degrade system remains to be

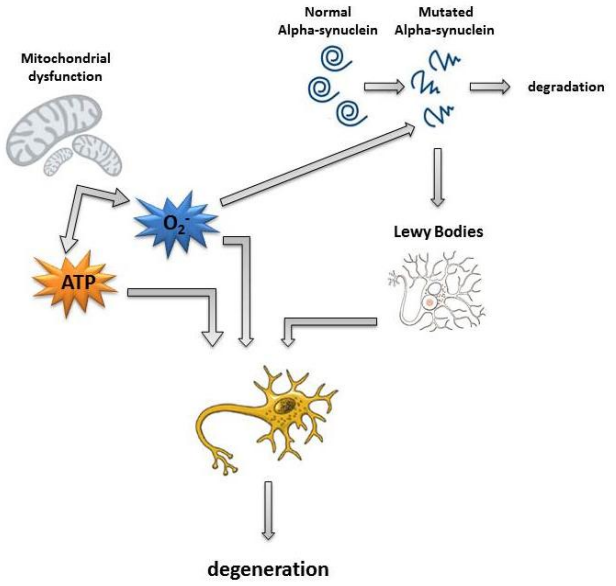


Figure 1 Pathological mechanisms underlying Parkinson's Disease. Altered alpha-synuclein accumulates into Lewy Bodies resulting in degeneration of neurons. Mitochondrial dysfunction and oxidative stress results in cell damage and eventually neurodegeneration.

elucidated.

Also, research has shown that mitochondrial dysfunction and oxidative stress is involved in PD pathology, but the underlying mechanisms controlling these events remain unclear. If the level of reactive oxygen species (ROS) reaches toxic levels, a state of oxidative stress is reached. This state might be reached due to increased ROS production or reduced cell-buffering mechanisms [20]. ROS are able to damage molecules in the cell and increased oxidative damage of lipids, proteins and DNA have been reported in post-mortem brain studied of PD patients. Also other markers of oxidative stress have been reported in brains of PD patients, such as reduced expression of the antioxidant protein glutathione (GSH) and decreased mitochondrial complex I activity in the SN of PD patients [20, 21].

Research also suggests that PD might be a prion-like disease. Prions arise when normal cellular proteins undergo a conformational change to a misfolded isoform. These misfolded forms triggers misfolding of other normal folded proteins. Together, these misfolded proteins aggregate into plaques [22], also known as LB's and LN's containing alpha-synuclein in PD. As mentioned above, neurons are unable to degrade altered alpha-synuclein in LB's and LN's resulting in accumulation which leads to neurodegeneration [15]. Research on post-mortem tissue from advanced PD patients that received transplantation of fetal nigral mesencephalic cells many years earlier showed that also in these transplanted cells LB's and LN's are present. This suggests that the misfolded alpha-synuclein is able to 'infect' unaffected healthy neurons [18]. Also, in vitro and animal studies support the prion hypothesis. It was shown that affected cells are able to secrete alpha-synuclein via exocytosis. In addition, unaffected cells are able to take up this alpha-synuclein via endocytosis [18]. Also, it was shown that injection of insoluble alpha-synuclein aggregates from older and clinically affected transgenic mice overexpressing human A53T mutant alpha-synuclein in young transgenic mice resulted in accelerated disease progression [23].

Etiology

Some PD patients have a family history of PD and these familial forms of PD are caused by only genetic factors. However, the vast majority of the PD cases are sporadic and caused by a complex interplay between genetic and environmental factors. Genetic screening of patients with a familial form of PD revealed several genes that are involved in PD. For five genes, namely SNCA, PARK2, PINK1, PARK7 and LRRK2, a causal association has been found with PD (See Table 1) [24]. The biological function of alpha-synuclein is not completely understood but it is thought that alpha-synclein is involved in neurotransmitter release and vesicle turnover at the presynaptic terminals. Also the function of leucine-rich repeat kinase remains unclear. The function of the other three proteins have been studied intensively. Parkin is involved in targeting proteins for degradation and maintaining mitochondrial function. PTEN-induced putative kinase 1 is involved in the oxidative stress response and maintaining mitochondrial function. DJ-1 is an anti-oxidant and is also an important redox sensor [24]. These genes have toxic or protective functions and mutations in these genes cause neurodegeneration either by increased toxic function (e.g. mutation in the genes for alpha-synuclein or LRRK2) or decreased protective function (e.g. mutation in the genes for PARK2, PINK1 and PARK7/DJ-1) [25]. In these genes, different types of mutations have been found such as missense mutations, duplications, triplications but also whole depletions [26].

Gene	Protein	Function	Inheritance*
SNCA	Alpha-synuclein	Neurotransmitter release	AD
PARK2	Parkin	Target proteins for degradation, maintenance of mitochrondrial function	AR
PINK1	PTEN-induced putative kinase 1	Oxidative stress response, maintenance of mitochondrial function	AR
PARK7	DJ-1	Redox sensor, antioxidant	AR
LRRK2	Leucine-rich repeat kinase 2	Unknown	AD

Table 1 Genes involved in familial PD. *Autosomal dominant (AD), autosomal recessive (AR).

Although most PD cases are sporadic, only a few environmental factors have been identified. One important risk factor for PD is aging. After the age of 60, the incidence increases massively [27]. Studies have shown that this increase continues up to 85 years of age [28, 29]. Another risk factor for PD is pesticides [30]. A higher prevalence of PD was reported in Canadian rural agriculture regions [31]. Also, repeated intraperitoneal injection of the herbicide paraquat in mice resulted in age- and dose-dependent loss of dopamine neurons in the SN, but not in striatal regions [32]. In contrast to the adverse effects of smoking and alcohol, these substances are inversely related to PD risk [27]. Nicotine, which is a substance in cigarettes, is likely to be the cause of the protective effect of smoking since it stimulates dopaminergic neurons, has neuroprotective effects, and also relieves symptoms of PD [33]. Studying the protective mechanisms underlying the action of nicotine and alcohol could provide new insights on therapeutic strategies. Examples of risk factors that (weakly) increase the risk for PD are head trauma, vascular disease, diabetes, and lack of exercise [27].

Treatments

Current treatment of PD successfully alleviates the symptoms and improves the quality of life of PD patients. However, these treatments are symptomatic and no disease modifying treatments are available that delay or prevent the progression of the disease [34]. Treatments that are often used are for example levodopa, dopamine receptor agonists and COMT inhibitors [35]. All drugs result in increased dopamine levels in the brain. Dopamine agonists increase the availability of dopamine and these agonists have a longer half-life than Levodopa [35]. Levodopa, which is the major pharmacotherapy for PD, is used to replace dopamine in the brain since the dopamine precursor levodopa is able to pass the blood brain barrier. MAO inhibitors and COMT inhibitors are often administrated together with Levodopa to extend its action by reducing metabolic degradation of Levodopa. However, long-term Levodopa treatment has an important and disabling complication in almost all PD patients namely Levodopa-induced dyskinesia characterized by abnormal involuntary movements [36].

Currently, research is aiming at finding targets for neuroprotection in PD that delay or prevent the progression of the disease. Examples are antioxidant therapies, anti-apoptotic agents and trophic factors [34]. However, none of the human clinical trials so far have revealed any disease-modifying effects possibly due to limitations in trial design and heterogeneity and time course of the

disorder [34]. More research should be done to find neuroprotective factors since it could massively increase treatment outcomes.

Furthermore, research suggests that degeneration of SN dopaminergic neurons and alpha-synuclein depositions occurs preclinically [8]. Therefore, research is trying to identify biomarkers that can diagnose PD preclinically. Current markers are not yet validated in preclinical stages and are now used in patients in advanced stages of PD. Also, the current markers are not specific and expensive [37]. Finding reliable and less expensive biomarkers in tissues that are more easily accessible would make early and more effective treatment of PD possible. Current research revealed a greater diagnostic accuracy when evaluating multiple independent biomarkers compared to single biomarkers [37].

Glia in the Brain and Body

More than just support cells

Different types of glia cells exist in the brain but also in the body. Generally, they are divided into astrocytes, microglia and oligodendrocytes. Over the last decades, glial cells have been known for their role as passive support cells for neurons in the brain. However, glial cells have a much wider range of brain functions and the functions differs over the different types of glial cells [8].

Astrocytes

Astrocytes cover neurons in the brain. Initially, astrocytes were thought to function as passive support cells that take up neurotransmitters and maintain extracellular ion levels [8]. However, it has been shown that they are involved in many more processes such as controlling synaptogenesis and plasticity, regulating blood flow, and promoting myelination [8].

Two types of astrocytes are present in the brain namely protoplasmic astrocytes, which are found in the grey matter, and fibrous astrocytes, which are found in the white matter [8, 38]. Identification of astrocytes can be done using immunohistochemical staining of glial fibrillary acid protein (GFAP) (See Figure 2A). Most astrocytes have no detectable expression of GFAP and astrocyte processes do not overlap. However, astrocytes can become reactive in response to CNS injury and disease and these reactive astrocytes are involved in neuronal survival and regeneration. Reactive astrocytes are characterized by molecular, cellular and functional changes. These changes cause alterations in molecular expression (e.g. increased GFAP expression), cellular hypertrophy and sometimes also proliferation and scar formation. When astrocytes proliferate, astrocytes extend their processes beyond their individual domain resulting in overlapping of neighboring astrocytes processes (See Figure 2B) [38].

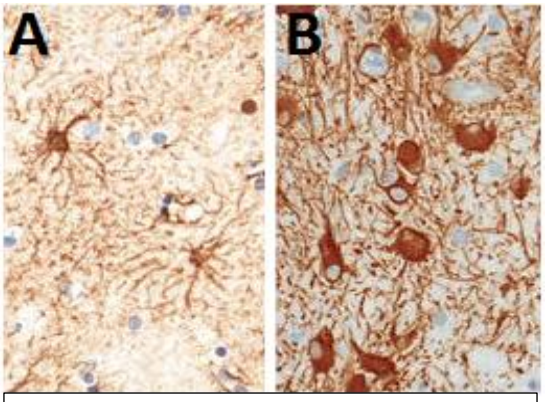


Figure 2 Astrocyte morphology in human tissue (GFAP staining counter counterstained with haematoxylin) (A) Morphology of astrocytes **(B)** Reactive gliosis with increased GFAP expression and overlapping astrocyte processes [38]

Microglia

Microglia have already been identified in 1919 by Del Rio Hortega as a mesodermal cell with phagocytic function. Indeed, research revealed the phagocytic function of microglia [10] and also the role of microglia in the innate immune systems of the CNS [8]. Microglia make up about 10 – 20% of all the glial cells [8, 10]. Microglia are constantly active sampling the extracellular environment, each microglia having its own area [10].

Microglia are rapidly activated and activation results in proliferation into an amoeboid state facilitating the migration of the activated microglia [39]. Activated microglia identification can be achieved using macrophage markers such as major histocompatibility complex I and II, Iba1 and GLUT5 and these activated microglia secrete cytokines and chemokines [8]. In the first stage of activation, microglia morphology resembles the morphology of resting microglia and the microglia are non-phagocytic [40]. In the second stage, different pathological stimuli such as infection, inflammation [41], cytokines and alpha-synuclein aggregation [42], neuronal death, mechanical injury, and toxins are able to transform these activated microglia into (potentially cytotoxic) phagocytes resembling the morphology of macrophages, namely amoeboid (See Figure 3) [40].

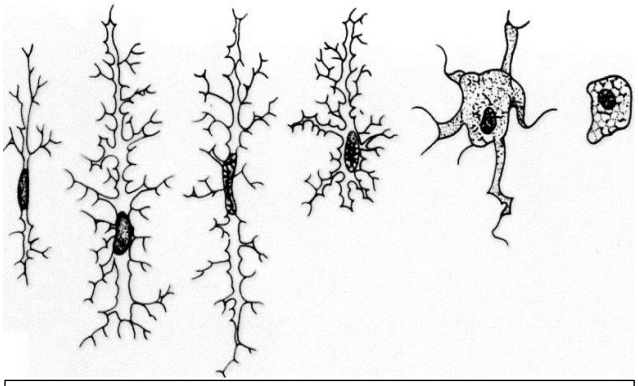


Figure 3 Gradual activation of microglia into microglia with phagocytic function. From left to right Resting microglia, activated microglia, transformed microglia [39].

Oligodendrocytes

Nonmyelinating and myelinating oligodendrocytes have been identified. Nonmyelinating oligodendrocytes reside in the gray matter while myelinating oligodendrocytes reside in the white matter thereby providing support to neurons [8]. The main function of oligodendrocytes is the production of myelin and neurotrophic factors such as hepatocyte growth factor (HGF), activin A, transforming growth factor-beta2 (TFG-beta2), and BDNF [43].

Mature myelinating oligodendrocytes originate from oligodendrocyte precursor cells (OPCs), not only during development but also during adulthood and in response to CNS injury and disease. OPCs are present in the whole CNS and account for 5 – 10% of all glia [44].

Involvement of Glia in Parkinson's Disease

Accumulating evidence has shown that chronic inflammation is involved in several neurodegenerative diseases such as PD. One important characteristics of chronic inflammation is the presence of activated microglia and reactive astrocytes in the CNS [45]. Studies have shown that, in addition to the presence of LB's and LN's and the degeneration of dopaminergic neurons in the SN, glial reactions are indeed present [10]. Glial reactions might be the result of degeneration of dopaminergic neurons. However, it is suggested that these glial reactions might also be important for disease progression and even for disease initiation [8]. Since glial cells are abundant throughout the whole CNS, these glial cells might be involved in multiple clinical characteristics of PD. Glia in the SN and striatum are possibly involved in motor symptoms, glia in the cortical regions might be involved in non-motor symptoms for example dementia, and involvement of enteric glia might result in gastrointestinal problems. In the following section, the role of glia in the different regions of the CNS related to clinical characteristics will be described.

Microglia

It is now well established that activated phagocytic microglia are present in the SN of patients of PD. Evidence comes from research on postmortem brain tissue from patients with PD. It was shown that large numbers of reactive microglia were present in the SN of patients with PD [46] and that these reactive microglia expressed macrophage markers [8]. Additional evidence comes from PET studies in which they found increased amounts of activated microglia in PD affected brain regions such as the nigrostriatal pathway [47], the pons, basal ganglia and frontal and temporal cortical regions [48]. Microglial activation is also supported by research in animal models of PD in which it has been shown that activated microglia are abundant in the SN [49].

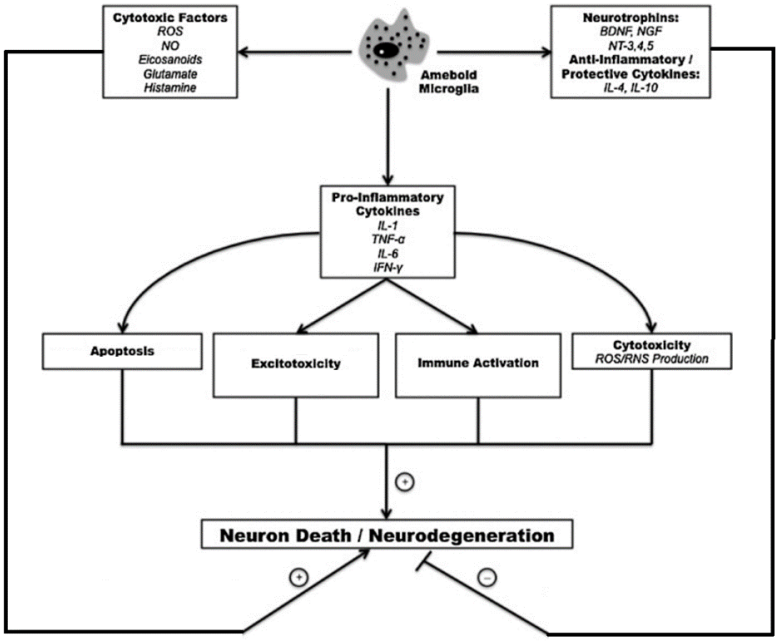
However, how these glial cells contribute to initiation and/or progression of PD is still unknown. It has been shown in animal models that microglial activation already occurs before any SN dopaminergic neuronal loss occurs, indicating that microglia might be involved in initiation of dopaminergic loss in PD [8]. However, if microglia are involved in initiation of PD, the question arises how microglia selectively target these dopaminergic neurons in the SN. Recent research in mice revealed an important role of dopamine in the recruitment of immune cells to the dopaminergic neurons in the SN. Using the 6-OHDA model, they found that the increased 6-OHDA-induced microglial activation could be reduced by depletion of dopamine by alpha-methylparatyrosine [50].

Several mechanisms have been suggested how these activated glial cells are involved in the progression of PD. It is suggested that these activated microglial cells contribute to loss of dopamine neurons in the SN through oxidative stress and production of pro-inflammatory cytokines [10, 41, 49]. Activated microglia are able to produce superoxide radicals thereby contributing to oxidative stress, which is thought to be important in loss of dopaminergic neurons in PD. Oxidative stress might result in oxidation of dopamine into a quinone product which is able to damage mitochondria and other cell components [10]. The role of superoxide radicals produced by microglia is further supported by research into mechanisms of the 6-OHDA model. It is suggested that not only the toxin directly induces ROS via 6-OHDA auto-oxidation and effects on the mitochondrial respiratory chain but also depends on microglial activation and NADPH-derived free radicals. It was observed that 6-OHDA injection also resulted in a significant increase in NADPH subunit expression which could be inhibited

by the NADPH inhibitor apocynin. Also, low doses of 6-OHDA did not result in dopamine cell loss nor did it result in increased NADPH subunit expression, microglial activation and ROS. However, the NADPH complex activator angiotensin II resulted in a significant increase in the former [51]. Activated microglia are also able to produce pro- and anti-inflammatory cytokines. Examples of pro-inflammatory cytokines are tumor necrosis factor alpha, interleukin-1beta, interleukine-6 and interferon-gamma [39, 41]. Initially, the function of the production of these pro-inflammatory cytokines is to prevent further damage [39]. However, it might also result in increased and maintained neuroinflammation, which eventually might be toxic to dopaminergic neurons and other glial cells in the SN [41]. In the CSF of patients with PD, elevated levels of pro-inflammatory cytokines have been found [42]. In addition, it has been shown that microglial activation leading to loss of dopamine neurons is dependent on the pro-inflammatory cytokine IL-1 [52]. In Figure 4, the mechanisms by which activated microglia contribute to neurodegeneration in PD and other neurodegenerative disorders is shown.

As described in the introduction, five genes namely SNCA (alpha-synuclein), PARK2 (Parkin), PINK1 (PTEN-induced putative kinase 1), PARK7 (DJ-1) and LRRK2 (Leucine-rich repeat kinase 2) have been found to be involved in PD. A study by Trudler et al. (2013) has shown that DJ-1 down-regulation in microglia resulted in increased microglial neurotoxicity to dopaminergic neurons. Also, increased monoamine oxidase (MAO) activity was found in DJ-1-deficient microglia resulting in augmented levels of ROS, nitric oxide, and pro-inflammatory cytokines. As a result, dopaminergic neurotoxicity was increased. The increased neurotoxicity and pro-inflammatory microglia phenotype could be reduced by the MAO inhibitor rasagaline [53]. Also, in vitro culturing of midbrain glia from parkin null mice revealed dysfunction of parkin null glia cells [54].

Concluding, evidence from human postmortem brain tissue, animal models and genetic studies shows that microglia might be involved in disease initiation and progression. However, mechanisms should be further investigated for example how activated microglia selectively target dopaminergic neurons. In addition, some models used to study microglial activation use inflammatory stimuli which might result in biased findings in these studies. These studies should be replicated in an alpha-synuclein overexpression model.



Astrocytes

Also reactive astrocytes have been reported in the SN of patients of PD. Postmortem studies revealed increased number of astrocytes and GFAP immunoreactivity in the SN [43, 45]. Damier et al. (1993) revealed a 30% increase in the density of astrocytes in the SN of PD patients which was also inversely correlated with degeneration of dopaminergic neurons [55], indicating that dopaminergic neurons are more vulnerable for degeneration when less astrocytes are present. Astrogliosis in the SN and striatum has also been demonstrated in rat and mice models of PD [56, 57]. The response of astrocytes in the MPTP model occurs later namely after 5 days after exposure compared to the response of microglia which occurs after 2 days of exposure [56] which indicates that astrocytes are involved somewhat later in the initial phase than microglia.

Reactive astrogliosis is an important mechanism that protects the CNS through several mechanisms for example the production of protective factors [8] such as nerve growth factor or scavenging excitotoxic agents such as glutamate and calcium [58]. However, astrocytes might become dysfunctional when they are subject to excessive oxidative stress and aberrant production of cytotoxic mediato[illegible]Astrocytes are then able to produce c[illegible]rons [58]. Recently, it has been shown t[illegible]t as cytokine in PD [9]. As a result, astr[illegible]e SN or to promote recovery, thereby increasing the vulnerability of dopaminergic neurons in the SN [59]. The neuroprotective and neurodegenerative functions of astrocytes depends on the molecules it secretes and takes up from the extracellular space. Since astrocytes are more abundant cell type in the brain, the role of astrocytes might even be more important than the role of microglia [58].

Figure 4 Involvement of activated phagocytic microglia in neurodegenerative disorders such as PD. Activated microglia are able to produce cytotoxic factors and pro- and anti-inflammatory cytokins and chemokines thereby increasing or decreasing neurodegeneration [39].

It is suggested that astrocytes are able to take up altered alpha-synuclein that have escaped axon terminals [60]. A study by Lee et al. (2010) showed that altered alpha-synuclein can be transferred to astrocytes *in vitro*. Subsequently, in contradiction to microglia that degrade altered alpha-synuclein after internalization [8], these altered alpha-synuclein accumulates in astroctyes. As a result, astrocytes start to produce pro-inflammatory cytokines and chemokines thereby activating microglia [60]. Furthermore, a study by Khan et al. (2014) showed that glia maturation factor (GMF) deficiency in astrocytes resulted in an increased antioxidant state, a reduced production of ROS and decreased levels of pro-inflammatory cytokines in response to MPP^+-induced toxicity *in vitro* compared to wild-type astrocytes [61]. This indicates that GMF in astrocytes is important for the toxic effects of MPP^+ and that astrocytes play an important role in the regulation of oxidative stress and pro-inflammatory states which are shown to be involved in PD.

The genes involved in PD are also expressed by astrocytes namely the genes for PINK-1, parkin and DJ-1 [8]. It has been shown in primary cultures that DJ-1 knockout astrocytes produce 10 times more NO than controls. When reintroducing DJ-1 with lentivirus this excessive NO response was restored [62]. Furthermore, cultured midbrain glia from parkin knockout mice revealed less astrocytes compared to wild-type [54]. Also, it was shown that PINK-1 expression is the most abundant in astrocytes [63].

Concluding, astrocytes possess neuroprotective and neurodegenerative properties and it is very likely that astrocytes are involved in initiation and progression of PD. In PD, astrocytes might

become dysfunctional and finding methods to replace astrocytes for dopaminergic recovery could be a new future treatment for PD.

Oligodendrocytes

Even though oligodendrocytes play an important role in neuronal support and neurotrophic function, the role of oligodendrocytes remains poorly understood. Alpha-synuclein accumulations have been found in oligodendrocytes in the SN and outside the SN in the brains of patients with PD [64]. Oligodendrocytes do not express alpha-synuclein but it has been shown *in vitro* and *in vivo* that oligodendrocytes are able to internalize alpha-synuclein [65, 66]. The neuron-to-oligodendrocyte transfer of alpha-synuclein might play a role in the pathogenesis of PD. Also, oligodendrocytes express LRRK2 which is a protein that is shown to be involved in PD (see page 6) [10].

Substantia nigra, striatum and motor dysfunction

In PD, dopaminergic neurons in the SN degenerate. These neurons primarily project to the striatum and degeneration of these neurons in PD results in decreased levels of dopamine in the striatum. The direct and the indirect pathway together regulate movements. The direct pathway, which involves the basal ganglia, thalamus and cortex, normally facilitates movements and gets activated by dopaminergic input from the SN. The indirect pathway, which involves the globus pallidus externa and the subthalamic nucleus, normally represses movements and gets inhibited by the dopaminergic input from the SN. However in PD, dopaminergic input from the SN is decreased resulting in net increased inhibition of the direct pathway and net decreased inhibition of the indirect pathway. Overall, this leads to suppression of initiation of movements resulting in typical motor dysfunction seen in PD [67].

As mentioned above, activated microglia and reactive astrocytes are present in the SN of patients with PD [46-48]. These results are also seen in animal models of PD. Minocycline is an antimicrobial agents but it has also been proven to inhibit microglial activation and attenuate degeneration of nigrostriatal dopamine neurons in the MPTP model for PD [49].

Cortical regions and dementia

Patients with PD also develop non-motor symptoms for example dementia. The prevalence of dementia in PD patients is around 40% and almost 80% if people survive 20 years with the disease. Important hallmarks of Parkinson's Disease Dementia (PDD) are impairments in short-term recall, attention, visuospatial functions and executive functions such as decision making [68]. Frontal lobe dysfunction are thought to be the cause of these impairments [69] but the underlying mechanisms remain unclear.

Much research has been done to also study the underlying mechanism that lead to non-motor symptoms in PD such as dementia. To delineate the underlying mechanisms in the development of dementia in PD, studies have been performed in PD patients with and without dementia [68, 69]. Analysis of microglial activation in PDD and PD patients, revealed significant cortical microglial activation for both PDD and PD patients compared to control subjects. Also, there was a significant reduction in glucose metabolism in PDD and PD patients [68]. These differences were detectable in an early stage of the disease and also in patients that did not yet develop dementia,

indicating that microglial activation might be an important factor in progression of the disease and a possible early indicator for the development of dementia later on [68, 69]. Another study by Van den Berge et al. (2012) investigated the involvement of astrocytes in cortical regions in dementia. However, this study revealed that alpha-synuclein pathology did correlate with dementia in PD but cortical astrogliosis did not [1].

Enteric glia and gastrointestinal problems

Many PD patients also suffer from gastrointestinal problems, such as delayed gastric emptying or decreased motility of the gut. Most interestingly, these gastrointestinal problem precede motor symptoms for many years [70].

LB's and LN's are present in the enteric nervous system and the dorsal motor nucleus of the vagus and they are thought to play a major role in gastrointestinal problems in PD [70]. In another study, mRNA expression levels of pro-inflammatory cytokines and glial marker and the presence of Lewy pathology was assessed in biopsies from PD and control patients. Results showed an increase in pro-inflammatory cytokines and glial marker in PD compared to controls. This data indicate that enteric inflammation occurs in PD [71]. More recently, research has been done to study the mechanisms underlying neuroplastic changes in the enteric nervous system evoked by inflammation and it was shown that enteric glia and neurotrophins deserve special attention in understanding mechanisms underlying ENS plasticity [72]. Another study showed that ghrelin, a circulating orexigenic signal that controls energy homeostasis by stimulating appetite and body weight, is able to enhance dopaminergic survival via reduced microglial response and enhanced mitochondrial function [73]. The precise involvement of enteric glia in gastrointestinal problems remains to be elucidated. Future research should determine if enteric glia are likewise involved in degeneration of neurons in the enteric nervous system or if another mechanism is involved.

In the search for early biomarkers for PD, searching for biomarkers in enteric tissue might be very interesting since this tissue is readily accessible. It has been shown that LB's and LN's are present in the enteric nervous system and evidence shows that these are also present in early and prodromal PD [74]. These findings indicate that these alpha-synucelin deposits in the enteric nervous system might be used as an early biomarker for PD. However, more research has to be done to apply alpha-synuclein depositions in the enteric nervous system as an early diagnostic biomarker for example which amount of alpha-synuclein in the enteric nervous system is evaluated as positive. Also, it should be studied how early in the disease alpha-synuclein deposits occur in the enteric nervous system and if this indeed precedes deposition in the CNS and thus might be used as a biomarker for prodromal PD [74]. However, the possibility of using the enteric nervous system for early diagnosis deserves further study since early diagnosis could massively increase treatment outcomes.

Targeting Glia to Treat Parkinson's Disease

Since research has shown that glial cells are involved in Parkinson's Disease, glial cells might be targets for new treatments to delay or even prevent disease progression. Over the last decades, much research has been focusing on identifying these new targets, especially since current treatments, such as levodopa, are symptomatic. In the following section, research on using glial cells as new targets to treat PD will be discussed.

Since it is becoming more evident that neuroinflammation plays an important role in PD, anti-inflammatory agents are being studied for their effect on glial cells. Most of these anti-inflammatory agents are already in common use for other diseases. Minocycline is an example of an agent with anti-inflammatory properties. Minocycline is an antimicrobial agents but it has also been proven to inhibit microglial activation and attenuate degeneration of nigrostriatal dopamine neurons in the MPTP model for PD. In addition, it also prevents formation and activation of three important microglial-derived cytotoxic mediators [75].

Exposure to methamphetamine results in dopaminergic neurotoxicity in striatal regions, which is also seen in PD. In addition, it is thought that astrocytes are mediators of methamphetamine neurotoxicity. Studies have shown that methamphetamine interacts with sigma receptors and treatment with sigma receptor antagonists in rodent models resulted in attenuation of methamphetamine neurotoxicity [76]. In a study by Robson et al. (2014), they investigated whether these antagonists also alter glial responses to methamphetamine in mice. Results showed that pretreatment with the sigma receptor antagonist SN79 resulted in attenuated methamphetamine induced astrocyte activation [76].

In a study by Gosha et al. (2007), effects of NF-kappaB on glial activation have been studied. NF-kappaB is a transcription factor that is thought to induce transcription of genes that are involved in vascular inflammation including interleukines and cytokines. In the MPTP mouse model of PD, it has been shown that inhibition of NF-kappaB by i.p. injection of NF-kappaB essential modifier-binding domain (NBD) peptide resulted in reduced activation of NF-kappaB in the SN, inhibited activation of microglia in the SN. In addition, nigrostriatal axis and neurotransmitters were protected and motor function of MPTP intoxicated mice was improved [77].

Another study investigated possible neuroprotective effects of granulocyte macrophage colony stimulating factor (GM-CSF) in the MPTP mouse model. Pretreatment with GM-CSF (i.p) significantly protected dopaminergic neurons in the SN and reduced microgliosis. These findings suggest that GM-CSF pretreatment prevents MPTP-induced neurodegeneration and reduces microglial activation which might lead to enhanced neuronal survival [78].

Ghrelin, which is an endogenous hormone produced and released by the stomach, is also thought to have neuroprotective properties by inhibiting apoptotic mechanisms. Also, it is able to suppress MPTP-induced microglial activation *in vitro* and *in vivo* in the SN and striatum [79].

Concluding, these findings indicate that these agents might possess neuroprotective properties by suppressing microglial activation in PD. Therefore, these agents might be valuable as new therapeutic targets for PD and also other neurodegenerative diseases.

Conclusion and discussion

Much research has been done to understand the underlying mechanisms in the initiation and progression of PD. It has already been proven that degeneration of dopamine neurons in the SN pars compacta is the cause of the characteristic motor symptoms in PD. In addition to the loss of these neurons, LB's and LN's containing aggregates of misfolded alpha-synuclein are formed. Besides, accumulating evidence shows that also glial reactions are present in PD and other neurodegenerative disorders. It is now more widely accepted that inflammation is an important progressor or even initiator of PD. Glial cells are important inflammators since they are able to produce pro-inflammatory cytokines. Blocking activation of glial cells might lead to decreased inflammation in the brain and maybe prevent disease progression.

Different types of glial cells exist all having distinct functions. It is evident that especially microglia and astrocytes play an important role in PD. Microglia serve an important role as innate immune system for the brain and they possess phagocytic functions. Human and animal studies have shown that activated phagocytic microglia are present in the nigrostriatal pathway but also in the pons, basal ganglia and frontal and temporal cortical regions. Microglia get activated by many types of changes in the environment. It is suggested that these activated phagocytic microglial cells contribute to loss of dopamine neurons in the SN through oxidative stress and production of pro-inflammatory cytokines. The neuroprotective and neurodegenerative functions of astrocytes depends on the molecules it secretes and takes up from the extracellular space. In PD, astrocytes are able to take up altered alpha-synuclein which subsequently accumulates in the cytosol. In response, astrocytes produce cytokines and chemokines thereby activating microglia. Especially, the ability of astrocytes to recruit microglia makes astrocytes an important link in disease initiation and disease progression. Oligodendrocytes are able to internalize alpha-synuclein. However, the role of oligodendrocytes in PD remains poorly understood. In addition, multiple anti-inflammatory agents have been identified to possess neuroprotective properties. These findings support the role of glia in the initiation and progression of PD. Further studying these agents might result in new disease-modifying treatments for patients with PD.

Studies also showed that glial reactions occur preclinically and these glial reactions could be used to diagnose PD in an early stage. However, before glial cells can be used to diagnose PD preclinically, methods should be more specific, less expensive and applicable on tissues that are easily accessible. More research has been focusing on enteric glia, since the gastrointestinal system is a readily easy accessible tissue to take biopsies. However, the predictive value of enteric biopsies should be further determined. Also, if biopsies in the gastrointestinal system might be used preclinically to diagnose PD should be investigated. This could massively improve treatment outcomes since PD will be diagnosed very early.

Concluding, studies have shown that microglia and astrocytes are important initiators and progressors of PD. Microglial activation occurs before SN dopaminergic neuronal loss occurs which indicates that microglia might be important initiators of PD. However some questions remain about the underlying mechanisms for example how phagocytic microglia selectively target dopaminergic neurons. In addition to the role of microglia in the initiation of PD, astrocytes also appear to play an important role in the initiation of PD. Astrocytes are able to take up alpha-synuclein. Since astrocytes are not able to degrade alpha-synuclein, astrocytes start to produce inflammatory cytokines and ROS. Subsequently, these factors might result in activation of microglia thereby contributing to

initiation and progression of PD. The role of oligodendrocytes remain to be elucidated but evidence suggest that oligodendrocytes are not involved in initiation but might only be involved in progression of the disorder. Results of studies with anti-inflammatory agents in animal models of PD further support the involvement of glia in the initiation and progression of PD. Most of these agents have not yet been studied in patients with PD, while some are already used to treat other disorders. In the future, the most effective agents should be tested in patients with PD to test if these agents might have disease-modifying effects. In addition, to better study the role of inflammation in PD, research should be performed in animal models for PD that are not induced by inflammatory stimuli since this might bias findings. The alpha-synuclein overexpression model might be a good alternative. Studying the mechanisms underlying glial involvement in PD might increase treatment outcomes and therefore deserves special attention.

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