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INVESTIGATING IMMUNOMODULATING FACTORS AND THEIR ROLE IN TREATMENT FOR PARKINSON'S DISEASE

THE EFFECTS OF ALPHA SYNUCLEIN, TUMOUR NECROSIS FACTOR ALPHA, INTERLEUKIN 1 BETA AND INTERLEUKIN 6 ON NEURO-INFLAMMATION IN PARKINSON'S DISEASE

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Abstract

With no cure available, Parkinson's disease (PD) is a complex disease and the second most common neurodegenerative disorder. Displaying both motor and non-motor symptoms, PD is an invasive disease for both patient and caretakers. Hitherto, the cause of PD is attributed to the death of dopaminergic neurons (DANs) in the substantia nigra (SN) in the basal ganglia, as the death of these neurons is the cause of tremors, muscle rigidity and other distinctive PD symptoms. DANs produce dopamine, a compound of vital importance in signal transmission in the brain. DAN loss can be attributed to a variety of cellular processes: microglia activation, which also causes excessive cytokine production (tumour necrosis factor alpha [TNF-α], interleukin [IL]-1β, IL-6, and more), and alpha-synuclein aggregates forming Lewy bodies, which leads to oxidative stress and mitochondrial impairment. All three mentioned cytokines have proven to aid the pro-inflammatory signal released by activated M1 microglia or astrocytes, however neuroprotective responses are also witnessed by M2 microglia, TNF-α, IL-1β and IL-6. This review also describes current available treatments amongst them levodopa/carbidopa use, and deep brain stimulation. Prospects of the potential new PD treatment agents phloretin, sirtuin 1 (SIRT1) and cystamine/cysteamine are also discussed briefly. Concluding that PD is a complex disease with an interesting pathology, further research remains necessary to provide a cure by understanding its aetiology.

List of contents

List of abbreviations

Table 1 List of abbreviations in alphabetical order found in this thesis.

Abbreviation	Definition
A2AR	A2A receptor
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
α-syn	Alpha synuclein
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
Ca^{2+}	Calcium (ions)
Caspase	Cysteine-aspartic protease; cysteine aspartase; cysteine-dependent aspartate-directed protease
CCL	Chemokine (C-C motif) ligand
cIAP	Cellular inhibitor of apoptosis protein
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
COX-2	Cyclooxygenase-2
CSF	Cerebrospinal fluid
CXCL	Chemokine (C-X-C motif) ligand
DA	Dopamine
DAN(s)	Dopaminergic neuron(s)
DBS	Deep brain stimulation
DD(s)	Death domain(s)
DLB	Dementia with Lewy bodies
EDS	Excessive daytime sleepiness
EGF	Epidermal growth factor
ER	Endoplasmic reticulum
eSNCA	Extracellular alpha synuclein
ETC	Electron transport chain
FADD	Fas-associated death domain
FGF	Fibroblast growth factor
GAD	Generalized anxiety disorder
GDNF	Glial cell-derived neurotrophic factor
Gp130	Glycoprotein 130
IFN-γ	Interferon gamma
IKK	Inhibitor of IκB kinase
IL	Interleukin
IL-1R1	Interleukin type I receptor 1
IL-1RAP	IL-1 receptor accessory protein
IRAK	IL-1 receptor-associated kinase
IκB	Inhibitor of 'kappa B' (κB)
LB(s)	Lewy body (bodies)
L-DOPA / L-dopa	Levodopa
LPS	Lipopolysaccharide
MAO-B	Type B monoamine oxidase
MAPK	Mitogen-activated protein kinases
MCI	Mild cognitive impairment
MCP1	Monocyte chemoattractant protein 1

Abbreviation	Definition
MGluR	Metabotropic glutamate receptor
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Multiple sclerosis
MSA	Multiple system atrophy
mTNF-α	Transmembrane tumour necrosis factor alpha
MTS	Mitochondrial targeting sequence
MYD88	Myeloid differentiation primary response gene 88
NE	Norepinephrine
NEMO	NF-κB essential modifier
NF-κB	Nuclear factor kappa(-light-chain-enhancer of activated) B (cells)
NGF	Nerve growth factor
NIK	NF-κB-inducing kinase
NMDA	N-methyl-D-aspartic acid
NMDAR	N-methyl-D-aspartic acid receptor
NO	Nitric oxide
PD	Parkinson's disease
PHL	Phloretin
PI	Phosphatidyl inositol
PI3K	Phosphoinositide-3OH kinase
PLAD	Preligand binding assembly domain
RBD	REM sleep behaviour disorder
RIP1	Receptor-interacting protein 1
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
sIL-6R	Soluble interleukin 6 receptor
SIRT1	Sirtuin 1
SN	Substantia nigra
SNCA	Alpha synuclein
SNpc	Substantia nigra pars compacta
sTNF-α	Soluble tumour necrosis factor alpha
TAB	TAK1-binding protein
TAK	TGF-β-activated kinase
TGF-α	Transforming growth factor alpha
TGF-β	Transforming growth factor beta
TIM	Translocase of the inner membrane
TIR	Toll-and IL-1R–like
TNFR	Tumour necrosis factor receptor
TNF-α	Tumour necrosis factor alpha
TOLLIP	Toll-interacting protein
TOM	Translocase of the outer membrane
TRADD	Tumour necrosis factor receptor (type …)-associated death domain
TRAF	Tumour necrosis factor receptor-associated factor

Introduction

Issue

Parkinson's disease (PD) is a complex neurological disease and the 2nd most common neurodegenerative disorder in aging (Mhyre, Boyd, Hamill, & Maguire-Zeiss, 2012) (Tomiyama, Lesage, Tan, & Jeon, 2014) with no cure available (Hassanzadeh & Rahimmi, 2018) (Kempuraj, et al., 2016). It is most commonly known by the symptoms muscular rigidity, tremors, postural and gait impairment and bradykinesia, but displays both motor and non-motor symptoms (Mhyre, Boyd, Hamill, & Maguire-Zeiss, 2012) (Kalia & Lang, 2015) (Jankovic, 2008), see Figure 1. The non-motor symptoms usually have an earlier onset than the more distinguished motor symptoms and as the disease progresses, more symptoms are known to develop or worsen. A meta-analysis of 27 studies from 2001 to 2014 showed PD to be more common in males (40y+, 61.21 patients per 100,000) than females (40y+, 37.55 patients per 100,000) (Hirsch, Jette, Frolkis, Steeves, & Pringshei, 2016), and has a higher incidence rate when age progresses (Mhyre, Boyd, Hamill, & Maguire-Zeiss, 2012) (Hirsch, Jette, Frolkis, Steeves, & Pringshei, 2016) (Johns, 2014). The incidence of PD is approximately 1% at the age of 65, whereas at the age of 85 the incidence is approximately 5% (Reece, et al., 2011). In approximately 5-15% of PD cases the disease is inherited, or familial, due mutations in one of the genes associated with Parkinson´s disease (Kaur, Gill, Bansal, & Deshmukh, 2017) (Meissner, et al., 2011), being the genes *Parkin* (*PARK*), *SNCA*, *PINK1, DJ-1, LRRK2, ATP13A2, PLA2G6, VPS35*, or other genes which are not (yet) confirmed to be of influence on PD onset when mutated (Tomiyama, Lesage, Tan, & Jeon, 2014) (Pan, et al., 2017) (Steger, et al., 2016) (Klein & Westenberger, 2012) (Houlden & Singleton, 2012) (Rocha, De Miranda, & Sanders, 2017). The role of genetics in identifying a gene that, when mutated, causes PD in any form, be it familiar, early onset or ´regular´, still is not always clear amongst studies. For example the *EIF4G1* gene is a gene in which different study results do not lead to consensus amongst scientists (Klein & Westenberger, 2012) (Nichols, et al., 2015) (Chartier-Harlin, et al., 2011). Other causes of the onset of PD besides inherited mutations have been discovered such as pesticides (Mhyre, Boyd, Hamill, & Maguire-Zeiss, 2012) (Agnihotri & Aruoma, 2019), metals and solvents (Agnihotri & Aruoma, 2019). Also, other environmental factors such as the use of caffeine and nicotine have shown to provide a neuroprotective function in PD,

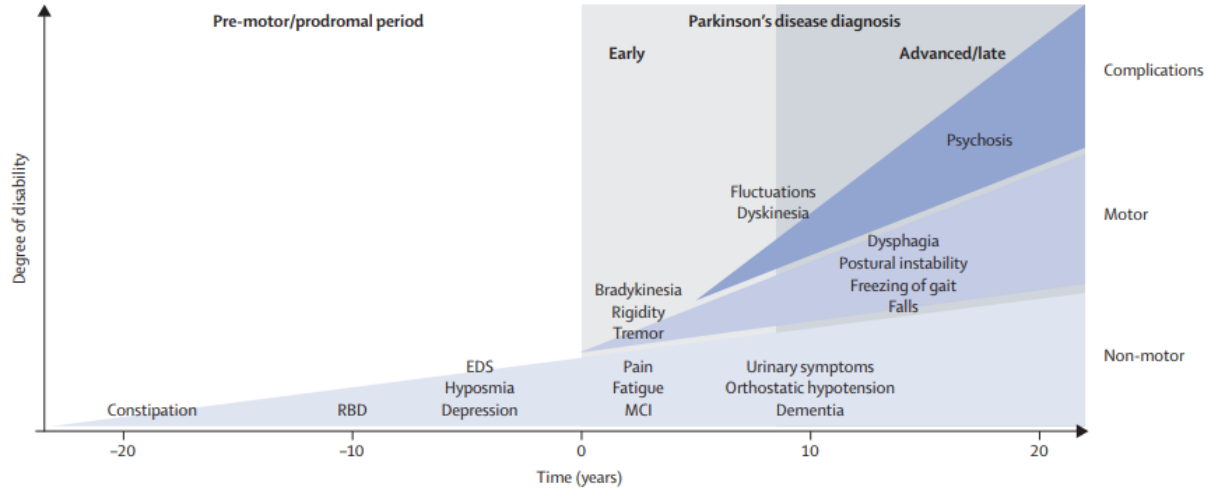

Figure 1 Schematic overview of symptom progression of patients with Parkinson's disease (PD). Two symptom types can be distinguished, motor and non-motor symptoms. Also complications can occur in advanced stages of the disease. Diagnosis is usually made at motor symptoms onset, displayed by '0 years' on the x-axis. As is shown, symptoms occur earlier than when diagnosis usually made, in the pre-motor/prodromal period, leaving much room for improving diagnostic utilities. RBD=REM sleep behaviour disorder, EDS=excessive daytime sleepiness, MCI=mild cognitive impairment. Source image: *(Kalia & Lang, 2015)*

and tobacco has shown to confer 'protective' benefits to decreasing PD incidence (Mhyre, Boyd, Hamill, & Maguire-Zeiss, 2012) (Wirdefeldt, Adami, Cole, Trichopoulos, & Mandel, 2011) (Quik, 2004) (Kalda, Yu, Oztas, & Chen, 2006).

When investigating the cause of PD symptoms, biology leads the way to the basal ganglia, the area of the brain that controls goal-directed movement, learning habits and innate motoric actions, such as locomotion (Grillner, 2018). Studies show that the death of dopaminergic neurons (DANs) in the substantia nigra (SN) in the basal ganglia is the cause of tremors, muscle rigidity and other PD symptoms (Leal, Casabona, Puntel, & Pitossi, 2013) (Wang, Gao, Duan, & Yang, 2019). DANs are important in transmitting signals via the nigrostriatal pathway from the substantia nigra pars compacta (SNpc) to the striatum in the basal ganglia, present in the core of the human brain. 60% of the DANs in the SNpc may have already been lost when motor symptoms, in particular tremors, arise (Wang, Gao, Duan, & Yang, 2019). Dopaminergic neurons are neurons that produce the neurotransmitter and chemical compound 3,4-dihydroxyphenylethylamine, or dopamine (DA) (Carlberg, 1990), which is of vital importance in signal transmission in the brain. In the absence of DA, DANs cannot transmit signals from the SNpc to the dorsal striatum conducted by the nigrostriatal pathway. This pathway is necessary for signal transduction that leads to automatic muscle movement used in every-day-life (Rice, Patel, & Cragg, 2011). When less dopamine is available, as is the case with PD patients, conscious initiation of movement is needed for continuous muscles direction. This is seen in one of the PD patients' symptoms which is the sudden 'freeze' of action, also known as akinesia (Agnihotri & Aruoma, 2019). Muscle movement spontaneously halts and needs conscious direction from the brain to continue movement, making the patient seem 'robot-like'.

Environmental factors have also shown to be of importance in the cause of PD onset, but are not the only cause for PD. For example, pesticides have proven to induce the misfolding of the protein alpha-synuclein (α-syn), as well as causing this protein to form aggregates and create Lewy bodies that are harmful for the central nervous system (CNS) (Chin-Chan, Navarro-Yepes, & Quintanilla-Vega, 2015).

Aim

Research has shown that neuro-inflammation is intertwined in the cause of neurodegenerative disorders (Lee, Tran, & Tansey, 2009). Many studies have detected that inducing the misfolding of the α-syn protein as well as causing it to form aggregates are most likely part of the cause of neuro-inflammation induction in PD (Gao, et al., 2008). Activated microglia (activation due to e.g. α-syn aggregation) have been implicated to be part of the onset of neuro-inflammation in PD as well (Sanchez-Guajardo, Tentillier, & Romero-Ramos, 2015).

The goal of this thesis is to create an overview of the current knowledge regarding the role of α-syn in neuro-inflammation in PD, as well as exploring the option of cytokines in mediating neuro-inflammation onset due to α-syn and the current status in treatment research. As research has showed, cytokines can produce an immunomodulating response that could provide useful in treating (symptoms of) neurodegenerative diseases such as PD (Chatterjee & Kordower, 2019). The cytokines that will be reviewed in this thesis are tumour necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6), as these cytokines have proven to be of importance in previous research (Chatterjee & Kordower, 2019) (Leal, Casabona, Puntel, & Pitossi, 2013) and will now be investigated in their functions in PD. Current and future treatments are investigated to provide an overview of the current status of PD treatment and treatment research.

The role of α-synuclein in neuro-inflammation in Parkinson's disease

Indicated to be part of the onset of PD, the pathogenic form of the α-syn protein is harmful for the CNS. (Devi, Raghavendran, Prabhu, Avadhani, & Anandatheerthavarada) showed that mutated α-syn present in the mitochondria of cells in the SNpc and striatum of PD patients´ brain can cause DAN loss, elevated reactive oxygen species (ROS) production and other reactions, harmful for the patients´ CNS.

A-syn is a protein that is made up of 140 amino acids and is found in the brain. The α-syn protein is made up of three general segments, an amphipathic lysine-rich N-terminus crucial in modulating membrane interactions, a transmembrane middle section that has similarities to Alzheimer's disease's amyloid-β plaques, and a disordered acidic C-terminus which is most likely used in nuclear localization and interactions with metals, small molecules and proteins (Lashuel, Overk, Oueslati, & Masliah, 2012). The function of native α-syn is still unknown, but it is suggested that α-syn is functional in the regulation of neurotransmitter release and it would play an important role in plasticity due to its vesicle-binding properties (Wang, Gao, Duan, & Yang, 2019) (Lashuel, Overk, Oueslati, & Masliah, 2012) (Thakur, Hua Chiu, Roeper, & Goldberg, 2019).

A-synuclein aggregates make up most of the Lewy bodies

In PD patients misfolded α-syn is found in large quantities in the cytoplasm of neurons. Different forms of α-syn are detected in PD patients' brain and indicate towards a process which leads up to the formation of α-syn aggresomes, or aggregates (Rabenstein, et al., 2019). The factors that cause the accumulation of α-syn aggregates are not fully known, however some studies show very plausible results such as mutations in the *SNCA* (synuclein alpha) gene, or misfolded α-syn proteins that act prion-like and turn the native α-syn protein into the misfolded shape (Lashuel, Overk, Oueslati, & Masliah, 2012) (Maiti, Manna, & Dunbar, 2017).

Misfolded α-syn proteins are present in different shapes, however the monomeric and oligomeric forms of α-syn are maintained at an equilibrium. A-syn monomers are predominantly present in α-helical confirmations. When quantity increases, α-syn monomers cleave together to create unstable α-syn dimers. These dimers eventually lead up to oligomers of all shapes and sizes such as β-sheet-like, spherical or ring-like structures. These oligomeric forms of α-syn are toxic and each form most likely has its own role in mediating α-syn toxicity in neurons and perhaps also in glial cells (Lashuel, Overk, Oueslati, & Masliah, 2012). These toxic oligomeric forms may be influenced by oxidative and nitrative stress (Souza, Giasson, Chen, Lee, & Ischiropoulos, 2000), phosphorylation of the serine group of α-syn on amino acids S129 and S87 (Oueslati, Paleologou, Schneider, Aebischer, & Lashuel, 2012), truncation (Oueslati, Fournier, & Lashuel, 2010), or by interactions with small molecules or lipids (Lashuel, Overk, Oueslati, & Masliah, 2012). After oligomer formation, fibrils are created out of the oligomers by the addition of monomers to the oligomers. The fibril structures are amyloid-shaped and progress from small to 'mature' amyloid fibrils when more monomers are cleaved. The cleaving of these amyloid fibrils make up for Lewy bodies (LB) (Lashuel, Overk, Oueslati, & Masliah, 2012).

Pathogenic α-syn aggregates can spread from pathogenic neurons to yet unharmed neurons. The aggregates can be transported from pathogenic to healthy neurons through direct penetration of the healthy neuron, through membrane receptors, trans-synaptically, or via endocytosis using exosomes (Lashuel, Overk, Oueslati, & Masliah, 2012). In theory, this spread of aggregates can

cause a cascade that could develop exponentially in PD patients' brain, where more harm would follow.

Mitochondrial dysfunction is induced by α-synuclein aggregates cleaving mitochondria

The aggregates of α-syn oligomers and amyloid fibrils are prone to cleaving organelles and therefore are highly toxic in neurons. A-syn cleaved to mitochondria can e.g. lead to mitochondrial impairment, by α-syn using its own mitochondrial targeting sequence (MTS) located at the N-terminus of the protein (Devi, Raghavendran, Prabhu, Avadhani, & Anandatheerthavarada, 2008) (Chinta, Mallajosyula, Rane, & Andersen, 2010) to be recognized by the translocase of the outer membrane (TOM) receptors present on the mitochondria. MTS directs the transport of nuclear-encoded mitochondrial proteins into the mitochondrion. After these proteins have penetrated the TOM complex, they are allowed access to the translocase of the inner membrane (TIM) complex of the mitochondrion, which leads to the core of this organelle. By being able to interact with the TOM receptors on the outer membrane of mitochondria, α-syn can inhibit protein uptake by the mitochondria by cleaving TOM20, a type of TOM receptors, leading up to negative effects such as mitochondrial impairment and ROS production (Rocha, De Miranda, & Sanders, 2017) (Di Maio, et al., 2016) (Chinta, Mallajosyula, Rane, & Andersen, 2010).

Dopaminergic neuron loss is induced by mitochondrial dysfunction and oxidative stress

(Wang, et al.) state that mitochondrial dysfunction is sufficient to cause DAN loss in the SNpc. This would indicate that as α-syn aggregation leads up to mitochondrial dysfunction, theoretically it also would be responsible for DAN loss. Several studies show that mitochondrial dysfunction is not the only probable cause for DAN loss, as it is aided in its causative role by mutations and multiplications of the *SNCA* gene that would cause familial PD (Rocha, De Miranda, & Sanders, 2017). *SNCA* polymorphisms are more associated with idiopathic PD, which again emphasizes the intricate relation between α-syn and DANs (Wang, et al., 2019).

The relationship of α-syn with DANs is a well-known hallmark of α-synucleinopathies which include PD, dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) (Goedert, Jakes, & Spillantini, 2017). Another cause written to DAN loss is oxidative stress (Guo, Zhao, Li, Li, & Liu, 2018) (Kim, Kim, Rhie, & Yoon, 2015).

Oxidative stress is characterized by an imbalance in the production and scavenging of ROS. In the case of such an imbalance where production is increased and scavenging is not increased (as much) or even decreased, ROS are present in excessive quantities which leads to uncontrolled reactions with its surroundings within a cell such as organelles, proteins, macromolecules, lipids and nucleic acids. By interacting with these factors, ROS can induce an inflammatory response by activating pathways which in turn could cause mitochondrial dysfunction as well as cellular dysfunction, neuro-inflammation and DNA injuries (Guo, Zhao, Li, Li, & Liu, 2018). To understand what causes ROS production imbalance, (Guo, Zhao, Li, Li, & Liu) stated that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) plays an important role in ROS production. MPTP can efficiently cross the blood-brain barrier (BBB), where once inside the brain it is oxidized by type B monoamine oxidase (MAO-B) into the metabolite 1-methyl-4-phenylpyridinium (MPP+). MPP+ is a substrate for the dopamine transporter, selectively taken up by DANs, and due to its accumulation function in the mitochondria is also toxic for DANs (Dias, Junn, & Mouradian, 2013) (Guo, Zhao, Li, Li, & Liu, 2018). MPP+ inhibits the respiration complex I of the mitochondrial electron transport chain (ETC) in DANs (Guo, Zhao, Li, Li, & Liu, 2018). The function of the respiration complex I of

mitochondria regards the transportation of electrons and proton gradient generation across the mitochondrial inner membrane to power ATP production (Mimaki, Wang, McKenzie, Thorburn, & Ryan, 2012). The function of the ETC is to cause a proton gradient difference across a membrane (e.g. the mitochondrial matrix). Therefore, with inhibition of complex I and ETC, ROS are produced. Studies show that ROS have two relationships with mitochondria, they are produced by the mitochondria, but mitochondria are also the target for excessive ROS to bind to, which would lead up to a negative outcome of the interaction (Puspita, Chung, & Shim, 2017).

In situations where excessive ROS quantities are present in cells, ROS can activate pathways that eventually lead up to the degeneration of DANs. The activated pathways cause immune cell activation (such as microglia and astrocytes) which in turn stimulates pro-inflammatory actions such as cytokine release, which in the case of PD can eventually result in neuronal cell death (Guo, Zhao, Li, Li, & Liu, 2018). Losing these DANs to oxidative stress caused by excessive ROS production would also impact DA levels in a negative way.

Activated microglia cause pro-inflammatory actions in response to excessive reactive oxygen species quantities and α-synuclein aggregates

Different cell types play their own role in the human brain. E.g. neurons are responsible for transmitting information by signal transmission (Lodish, et al., 2000) and astrocytes provide homeostasis in the CNS by acting as chemosensors, transporting major protons and ions, and control the BBB (Verkhratsky & Nedergaard, 2017). Oligodendrocytes are the myelinating cells that produce the insulating sheath around axons to improve signal transduction speed (Bradl & Lassmann, 2009) and ependymal cells line the walls of the ventricular system, and separate the cerebrospinal fluid (CSF) from the parenchyma of the brain as well as control the CSF flow. Ependymal cells also mediate the osmotic regulation, and regulate the dispersion of signalling molecules through the cilia present on their cellular membrane (Vidovic, Davila, Gronostajski, Harvey, & Piper, 2018). Microglia are the immune cells of the innate immune system of the CNS that use phagocytosis to clear unwanted residue or factors, which when left unhandled could cause unwanted pathway activation. Microglia are also known as the 'brain's resident myeloid cells' (Schetters, Gomez-Nicola, Garcia-Vallejo, & Van Kooyk, 2018), or the 'macrophages of the brain', and can present antigens, produce cytokines and are capable of chemotaxis (Michell-Robinson, et al., 2015). They also mediate the communication between immune cells and the CNS (Kempuraj, et al., 2016).

Activated microglia have been identified to be important in PD neuro-inflammation as well as other hallmarks for neuro-inflammation such as reactive astrocytes, but also an increase in the production of cytokines, prostaglandins, ROS or reactive nitrogen species (RNS), chemokines and complement cascade proteins (Flood, et al., 2011) (Lee, Tran, & Tansey, 2009) (Ransohoff & Perry, 2009). Increased levels in ROS and RNS can both individually cause disruption of the BBB and can result in activation of the adaptive immune system (Lee, Tran, & Tansey, 2009) (Ransohoff & Perry, 2009).

Activated microglia have the ability to stimulate both neurodegeneration and neuroprotection in response to neuro-inflammation

During neuro-inflammation, microglia are activated by T-lymphocytes and other complementary systems (Tang & Le, 2015). When activated, microglia produce and secrete ROS (Fischer & Maier, 2015) and cytokines that aid the already present pro-inflammatory response such as TNF-α, interferon gamma (IFN-γ) and a variety of interleukins (Kaur, Gill,

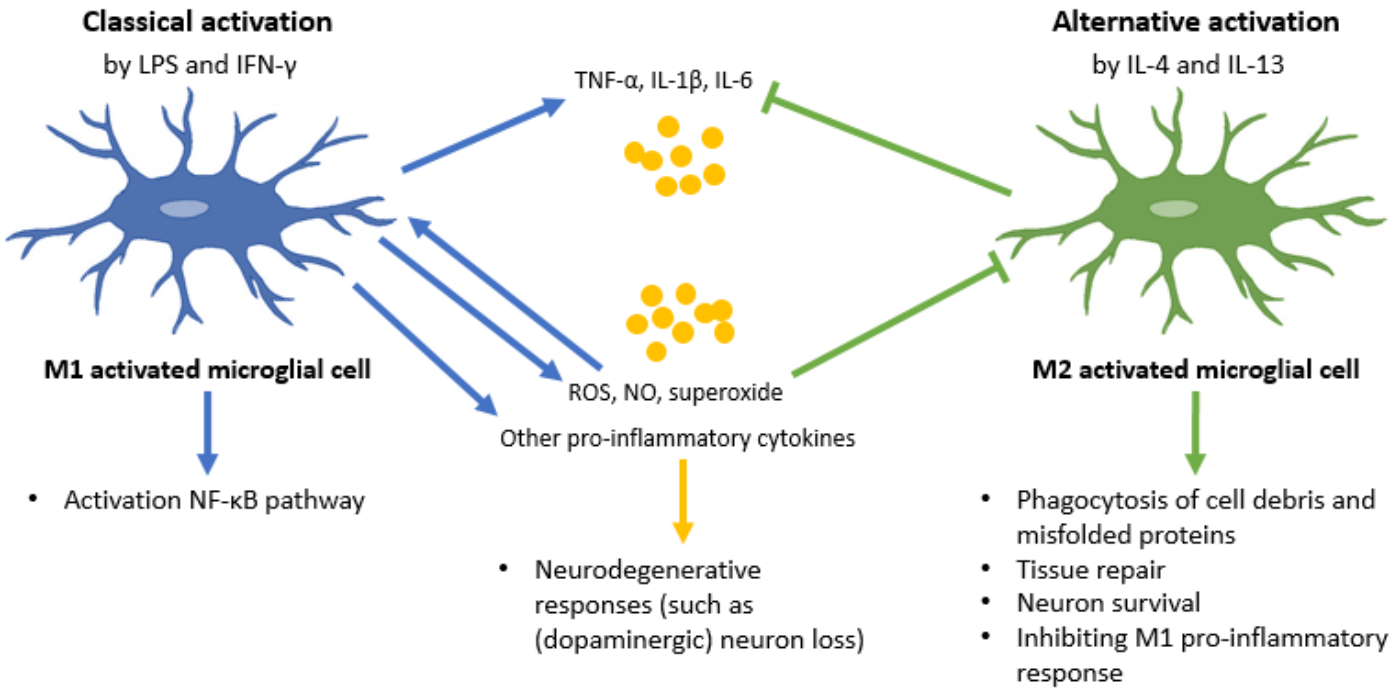

Figure 2 Schematic overview of M1 and M2 microglia activation and the impacts on and of cytokines and other molecules. M1 microglia are activated through classical activation which is performed by the presence of the molecules lipopolysaccharide (LPS) and interferon gamma (IFN-γ) *(Orihuela, McPherson, & Harry, 2016) (Tang & Le, 2015)*. M1 microglia induce neurodegenerative responses when activated. They release pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, but are also stimulated by ROS, nitric oxide (NO) and superoxide. Activated M1 microglia also activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway which in turn can lead to neuron loss *(Tang & Le, 2015)*. M2 microglia are activated through alternative activation which is induced by interleukins 4 and 13 (IL-4 and IL-13) *(Orihuela, McPherson, & Harry, 2016) (Tang & Le, 2015)*. Once activated, M2 microglia perform neuroprotective responses such as phagocytosis, tissue repair and M1 pro-inflammatory response inhibition (cytokines et cetera). M2 microglia however are inhibited by ROS, NO and superoxides *(Tang & Le, 2015)*. Source image 'microglia cell': *(Microglia, sd)*

Bansal, & Deshmukh, 2017). Microglia are also stimulated in their pro-inflammatory cytokine secretion by the presence of ROS, nitric oxide (NO) and superoxide. However, research has shown that microglia can be activated in two different types, namely M1 and M2 activated microglia. The previous mentioned actions of activated microglia are characteristic for M1 activated microglia. Interestingly, where M1 microglia create a neurodegenerative response, M2 activated microglia show a neuroprotective response by inhibiting pro-inflammatory cytokines released by M1 activated microglia, stimulate phagocytosis of misfolded proteins (e.g. α-syn) and cell debris, as well as repairing tissue and other processes (see Figure 2) (Tang & Le, 2015) (Orihuela, McPherson, & Harry, 2016) (Kaur, Gill, Bansal, & Deshmukh, 2017). During PD's chronic inflammatory state, constant inflammation signals from M1 activated microglia can make the healthy balance between M1 and M2 activated microglia shift (Cherry, Olschowka, & O'Banion, 2014). This could lead to a lack of necessary activated M2 microglia, resulting in a boost of the already present inflammation state.

Understanding that simply activating or deactivating microglia is not a sole solution to PD symptoms treatment or even to provide a cure, no conclusions can yet be drawn based on the currently obtained and available knowledge on this matter.

The role of cytokines TNF-α, IL-1β and IL-6 in neuro-inflammation

When inflammation sets in and M1 microglia are activated, cytokines are released into the extracellular space as a response to PD pathology (see Figure 2). Apart from difference in M1 and M2 microglial activation, studies

show opposite results regarding the effect of cytokines on inflammatory conditions as well (Chaparro-Huerta, Rivera-Cervantes, Torres-Mendoza, & Beas-Zárate, 2002). Both neurodegenerative and neuroprotective qualities are seen in e.g. TNF-α when it binds to either of its two receptors, tumour necrosis factor receptor (TNFR) 1 and TNFR2 respectively.

When investigating post-mortem brains of PD patients, (Hirsch & Hunot) found increased concentrations of TNF-α, IL-1β and IL-6, as well as other cytokines with increased concentrations (IL-2, epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), TGF-β and β2-microglobulin). These elevations would indicate that the mentioned cytokines play a role in PD pathology, making this selection interesting for research. This thesis will however limit itself to reviewing the current status of TNF-α, IL-1β and IL-6 in relation to PD neuro-inflammation.

Tumour necrosis factor alpha plays a role in dopaminergic neuron loss in relation to Parkinson's disease

TNF-α is a pro-inflammatory cytokine that is important in cellular signal transduction. It is a 233-amino acid long protein found in the extracellular space (soluble TNF-α, or sTNF-α), but is also found to be an integral component of membranes (transmembrane TNF-α, or mTNF-α) (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015) (UniProtKB - C1K3N5 (C1K3N5_HUMAN), 2019). The role of TNF-α in PD remains difficult to grasp as the function of this cytokine is diverse. Among the functions of TNF-α, inducing cellular cytokine secretion (Delves, Martin, Burton, & Riott, 2011) likely seems to be the most important and relevant function related to PD. As stated earlier, TNF-α can bind to two receptors, TNFR1 and TNFR2 (Fischer & Maier, 2015). TNFR1 is usually bound by either sTNF-α or mTNF-α, whereas the form of TNF-α that binds to TNFR2 is not clear as (Dong, Dekens, De Deyn, Naudé, & Eisel) state mTNF-α only binds to TNFR2, and (Probert) states both forms of TNF-α are able to bind TNFR2. When bound to TNFR1, TNF-α displays a pro-apoptotic function, whereas when bound to TNFR2 the opposite can be seen and cell survival and proliferation is induced. However, when bound to TNFR2, TNF-α can still display pro-apoptotic functions when it is in cooperation with TNFR1 (Fischer & Maier, 2015) (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015).

When looking at TNF-α and its two receptors in a general setting (unrelated to PD), TNFR1 has both intracellular and extracellular death domains (DDs) of which the intracellular DDs distinguishes TNFR1 from TNFR2. The extracellular DD is the preligand binding assembly domain, or PLAD. PLAD is responsible for preventing auto-activation of the receptor and, as the name indicates, is also essential for ligand binding (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015). When TNF-α extracellularly binds to TNFR1, one of the intracellular DDs, the TNFR-associated death domain (TRADD), or core adapter, is recruited. TRADD initiates the recruitment of signalling complex I, which consists of receptor-interacting protein 1 (RIP1) with added non-degradative poly-ubiquitin chains, TNFR-associated factor 2 (TRAF2), inhibitor of apoptosis protein (cIAP) 1, and cIAP2. Signalling complex I in turn later activates the IKK complex, consisting of the IκB kinase (IKK)α and IKKβ subunits and the regulatory subunit nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) essential modifier (NEMO), also known as IKKγ. The function of the IKK complex is to either inhibit or stimulate the classical/canonical, NF-κB pathway which depends on the binding of NEMO (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015) (Legarda-Addison, Hase, O'Donnell, & Ting, 2009). When NEMO is bound to IKKα and IKKβ, the IKK complex is activated and phosphorylates IκB, an inhibitory molecule, which is degraded by the ubiquitin-proteasome upon its phosphorylation (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015). Degradation of IκB results in the translocation of transcription

factors of NF-κB to the cell's nucleus causing anti-apoptotic gene transcription and thus activation of the classical NF-κB pathway.

However, when NEMO is not bound to the IKKα and IKKβ subunit, IκB is not phosphorylated and degraded, which leads to no translocation of the NF-κB transcription factors and no protein translation, therefore the protective classical NF-κB pathway remains inactive, preventing apoptosis-protection and thus leads to cell death (Legarda-Addison, Hase, O'Donnell, & Ting, 2009). However, another option for this classical NF-κB pathway to result in apoptosis, is for RIP1 not to be ubiquitinated and degraded. RIP1 then forms signalling complex II with Fas-associated death domain (FADD), TRADD, and TRAF2, of which FADD activates pre-cysteine-aspartic protease (caspase)-8. Caspases are a protein family that degrade essential proteins upon activation as well as activating other caspases. Pre-caspase-8 activates caspase-8 which in turn activates a caspase cascade eventually resulting in apoptosis (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015). In the case of PD, activated caspase-8 would result in death of neurons and/or microglia in the SNpc which leads to the pathology seen in PD. Necroptosis, directed necrosis, can also be induced by TNFR1 use (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015).

Compared to TNFR1, TNFR2 has no DDs and results in cellular survival. Whether sTNF-α or mTNF-α is bound to TNFR2, activation within the cell still follows and NF-κB is activated by either the non-canonical NF-κB pathway or the phosphoinositide-3OH kinase (PI3K), or phosphatidyl inositol (PI) 3-kinase/Akt, pathway (not explained) (Probert, 2015) (Ortí-Casañ, et al., 2019) (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015). In the non-canonical NF-κB pathway, when TNFR2 is not bound to TNF-α, it is followed by the recruitment of TRAF2 which causes TRAF1, cIAP1 and cIAP2 to be recruited instantly after itself. After TRAF2-cIAP1/2 complex activation TRAF3 recruits it to NF-κB-inducing kinase (NIK) which is then degraded by the proteasome. However, when TNF-α is bound to TNFR2, NIK is stabilized due to degraded TRAF3. Now present, NIK activates IκBα which causes phosphorylation and activation of IKKα. IKKα in turn activates the precursor protein of NF-κB, namely p100 which is followed by NF-κB2 p52 and RELB production and p100 C-terminal IκB-like structure degradation (Dong, Dekens, De Deyn, Naudé, & Eisel, 2015) (Sun, 2010). NF-κB2 p52 and RELB are two heterodimers that are functional in inducing target gene transcription (Sun, 2010).

When looking at the functions of TNF-α in the CNS, TNF-α mediates responses of neurons to N-methyl-D-aspartic acid (NMDA), an ionotropic glutamate receptor (Carlson, et al., 1999) which is frequently expressed in the SNpc as well as the rest of the basal ganglia (Gaki & Papavassiliou, 2014). Research shows that excessive activation of the NMDA receptors leads to neuron death through a process called excitotoxicity. Excitotoxicity is a result of an uncontrolled increase in intracellular calcium (Ca^{2+}), which enters through the NMDA receptors (Carlson, et al., 1999). Ca2+ modulates mitochondrial function as well as endoplasmic reticulum function. Ca^{2+} also stimulates DA synthesis, which is connected to the SNpc DANs which use Ca^{2+} channels to have Ca^{2+} ions enter its cytoplasm (Gaki & Papavassiliou, 2014). Therefore, it can be stated that Ca^{2+} is a key factor in maintaining DA levels in DANs. Excessive Ca^{2+} in neurons causes an increase in basal mitochondrial oxidant stress (Surmeier, et al., 2017) (Gaki & Papavassiliou, 2014). When the elevated level of Ca^{2+} over time is maintained, the mitochondria can show dysfunction seen in an increase in mitophagy (autophagy of mitochondria). Increase in mitophagy would result in an elevated expression of proteins such α-syn (Surmeier, et al., 2017) as the mitochondria can no longer clear misfolded proteins, which would lead to LB creation, causing oxidative stress, and more mitochondrial dysfunction, as explained earlier (Figure 2). Increase in mitophagy would therefore result in a perpetual circle that

includes repetitive pro-inflammatory actions which is consistent with the expected pathological image of PD. (Leal, Casabona, Puntel, & Pitossi) stated that maintained or high levels of TNF-α, but also IL-1β, will lead to neurodegeneration which regarding TNF-α is supported by (Chertoff, et al.).

Research is currently still investigating the role of TNF-α and both its receptors in their incomplete-known function and the pathways and its factors involved (Probert, 2015). This thesis has aimed to provide as much clarity on the subjects as currently possible, however due to potentially unknown factors, it is likely that the information today is still not yet (fully) understood nor complete to provide a full report on the investigated matters.

The effect of interleukin 1 beta (IL-1β) on neuro-inflammation in Parkinson's disease

As research states, interleukins play an important role in general inflammation, but in neuro-inflammation as well. IL-1β is a member of the interleukin 1 cytokine family and is an important factor in mediating inflammatory responses. It also functions in cell proliferation and differentiation as well as apoptosis. IL-1β is produced and secreted solely by myeloid cells such as macrophages and microglia as an inactive 31kDa precursor protein named pro-IL-1β. Pro-IL-1β is converted to its active form by the caspase-1 protein (Mantovani, Dinarello, Molgora, & Garlanda, 2019) (Lopez-Castejon & Brough, 2011) (IL1B interleukin 1 beta [Homo sapiens (human)], 2008). Under certain conditions related to astrocytes, IL-1β has shown to increase BBB permeability, which according to (Rothhammer & Quintana), led to an increased influx of chemokine (C-C motif) ligand (CCL) 2 (also known as monocyte chemoattractant protein 1 [MCP1]), CCL20, and chemokine (C-X-C motif) ligand (CXCL) 2, which caused BBB disruption and in so doing promoted the influx of peripheral inflammatory signals. The production of the same chemokines is also a result of IL-1β in relation to the NF-κB pathway (Rothhammer & Quintana, 2015).

In the setting of general inflammation, once secreted and present in the extracellular space, IL-1β can bind to its receptor and activate the NF-κB pathway as well as the mitogen-activated protein kinases (MAPK) pathway (not explained). Prior to activating either of these two pathways, IL-1β first binds to its interleukin type I receptor (IL-1R1), and with the aid of the IL-1 receptor accessory protein (IL-1RAP) it forms a trimeric complex. The Toll-and IL-1R–like (TIR) domains present on the intracellular side of IL-1R1 and IL-1RAP move close together enabling the recruitment of myeloid differentiation primary response gene 88 (MYD88), Toll-interacting protein (TOLLIP) and IL-1 receptor-associated kinase (IRAK) 4. Five components, IL-1β, IL-1R1, IL-1RAP, MYD88 and IRAK4, form a stable complex. After this complex is formed, the phosphorylation of IRAK1, IRAK2, and IRAK4 is triggered by MYD88, after which TRAF6 is recruited and oligomerized. Subsequently, after dissociation TRAF2 and phosphorylated IRAK1 and IRAK2 migrate to the membrane where they associate with TGF-β-activated kinase (TAK) 1 and TAK1-binding protein (TAB) 1 and TAB2. With this association, the TRAF6-TAK1-TAB1-TAB2 complex is formed and transported back to the cytosol (Ozbabacan, Gursoy, Nussinov, & Keskin, 2014). There TRAF6 is ubiquitinated and phosphorylation of TAK1 follows. From now on both the MAPK pathway and the NF-κB pathway can be activated by TAK1. Regarding the NF-κB pathway, phosphorylated TAK1 activates IKKβ (Ozbabacan, Gursoy, Nussinov, & Keskin, 2014) where subsequent steps follow and NF-κB is transported into the nucleus for the activation of pro-inflammatory protein translation and transcribing genes encoding for pro-inflammatory cytokines production and release such as IL-1β, TNF-α, and IL-6 (Flood, et al., 2011).

Little is known about the effect of IL-1β on PD as little research effort has been put into

this connection. When looking at neuro-inflammation in general, a study has been performed on IL-1β relating to Alzheimer's disease (AD) which stated that chronic expression of IL-1(β) in the striatum led to extensive striatal demyelination (Ferrari, et al., 2004). This is also a very possible scenario in PD pathology. However, (Ferrari, et al.) also stated that maintained IL-1(β) levels did not affect the remyelination process, making the striatal demyelination less of an issue as the regeneration process was not impaired. Another study implicated that IL-1β in neurodegenerative diseases, such as PD but also AD, and multiple sclerosis (MS), is thought to display a modulatory role in Ca^{2+} signalling. It would stimulate the glutamate-dependent NMDA receptor (NMDAR) expression and disrupt Ca^{2+} homeostasis. Disrupting the Ca^{2+} homeostasis to a state of calcium ion overflow can, as explained earlier, lead to excitotoxicity and eventually neuronal death. However, the disequilibrium of Ca^{2+} in the cells can also lead to endoplasmic reticulum (ER) stress, which can lead to excitotoxicity and neuron death in the hippocampus as described by (Dong, Kalueff, & Song) in a study that was focussed on AD, not PD in particular. However, in a study relating PD to IL-1β, elevated levels of the cytokine were observed. In this particular study, (Couch, Alvarez-Erviti, Sibson, Wood, & Anthony) investigated the differences in the neuro-inflammatory response on LPS and on extracellular α-syn (eSNCA) to the brain. Both microglial-like cell cultures (murine BV2 cell line) (Henn, et al., 2009) and mouse models were used to determine that the injection of eSNCA directly into the SNpc lead to an acute inflammatory response where upregulation of the mRNA of pro-inflammatory cytokines such as IL-1β, as well as that of IL-6 and TNF-α was witnessed 24 hours after injection. Elevated levels of the anti-inflammatory cytokines TGFβ and cyclooxygenase-2 (COX-2) were found 24 hours after eSNCA injection as well, making these cytokines also interesting for further research (Couch, Alvarez-Erviti, Sibson, Wood, & Anthony, 2011).

Interleukin 6 and its role in neuro-inflammation in Parkinson's disease

IL-6 is a 23.7kDa cytokine that is involved in many cellular processes such as the differentiation of B-cells, lymphocytes, monocytes and the generation of T(h)17 cells. It acts on hematopoietic progenitor cells, B- and T-cells, and CNS cells, and can induce nerve cell differentiation (UniProtKB - P05231 (IL6_HUMAN), 2019). IL-6 has pro-inflammatory properties, but can act as a defensive mechanism against inflammatory factors as well (Gabay, 2006). It can bind to both a soluble receptor (alternative IL-6 signalling) and a membrane-bound receptor (classical IL-6 signalling). When IL-6 binds to its soluble receptor, named sIL-6R, it can interact with glycoprotein 130 (gp130) of cells lacking membrane-bound IL-6 receptors, and in so doing modulate a large variety of immune cells (Neurath & Finotto, 2011). When IL-6 is bound to sIL-6R, together they can dictate the transition of an acute inflammation state to a chronic inflammation status, resulting in the transition of active neutrophils to active monocytes and/or macrophages in a general environment (Gabay, 2006). The chronic inflammation state can be compared to what is seen in neurodegenerative diseases such as AD and PD. The mechanism that drives the transition from acute to chronic inflammation is still poorly understood. In the acute inflammation state, secreted cytokines promote the production of so-called 'acute phase proteins'. Mostly IL-6 promotes this protein production, but is joined by cytokines IL-1β, TNF-α, TGF-β, and potentially also IL-8. These cytokines are secreted mostly by macrophages, microglia in the CNS, and monocytes. The acute phase proteins are produced by hepatocytes after stimulation by usually IL-6, and are functional in presenting anti-inflammatory responses (Gabay, 2006).

As stated earlier, (Couch, Alvarez-Erviti, Sibson, Wood, & Anthony) showed IL-1β, IL-6 and more cytokines' mRNA expression levels were elevated 24 hours after injection of

eSNCA into the SNpc of mice. This research group also investigated the cytokine levels after LPS injection as both seem to induce an inflammation state in the brain resembling PD pathology. (Couch, Alvarez-Erviti, Sibson, Wood, & Anthony) discovered that LPS injection resulted in even higher levels of IL-6, IL-1β and COX-2 mRNA expression. Note that the microglia, that most-likely are producing these cytokines, were first 'primed' by SNCA to produce a more extravagant response to LPS once injected. Another observation made in this study was neuronal cell death followed after SNCA injection, but not after LPS. It can therefore most likely be concluded that IL-6, but also IL-1β and COX-2, production by microglia is most likely stimulated through both α-syn and LPS injection in the SNpc (Couch, Alvarez-Erviti, Sibson, Wood, & Anthony, 2011). This fortifies the connection between activated microglia producing pro-inflammatory cytokines leading to continuously induced chronic neuro-inflammation. It also contributes to the dogma related to PD that states neuronal cell death, amongst them DANs, is a result of cytokine-induced chronic neuro-inflammation through microglia activation.

Treatments for Parkinson's disease

As stated earlier, unfortunately no cure is available for PD. Despite the current impossibility to halt PD pathology progress, symptoms can be supressed to aid PD patients in maintaining their quality of life as high and for as long as possible. Treatment options regarding symptom control vary from standard treatment which includes dopamine replacement therapy (Hasegawa, Kobayashi, & Ishiyama, 2020), to a treatment where patients undergo invasive surgery for deep brain stimulation (Little & Brown, 2020). Also, researchers continuously move to develop new treatments for a cure and to improve symptom alleviation as best as possible.

Current symptom suppression treatments in Parkinson´s disease

Generally speaking, current treatments of PD provide two common options, drug treatment and surgery. The first option, drugs, can be divided into two categories, dopaminergic drugs, and non-dopaminergic drugs (Maiti, Manna, & Dunbar, 2017). The most well-known drug is the dopaminergic drug named levodopa (3,4-dihydroxy-L-phenylalanine), or L-dopa, a precursor of dopamine (LeWitt & Fahn, 2016). Where dopamine cannot transcend the blood-brain-barrier, levodopa can transcend this barrier and subsequently be converted into the functional dopamine through its carboxylation. As levodopa is given as an oral medication, preventing it from being converted into dopamine before reaching the CNS through the BBB is very important. Therefore, the drug carbidopa is usually administered alongside levodopa to execute this necessary function of inhibiting premature conversion (Maiti, Manna, & Dunbar, 2017). Without carbidopa it would be impossible for levodopa to be useful in high enough concentrations in the brain. Once inside the CNS, levodopa is transported to the synaptic area of neurons in the SNpc and striatum to promote signal transduction, and in so doing promote cognitive functions such as (subconscious) movement initiation, therefore aiding the patient's quality of life. Research has showed that levodopa/carbidopa administration reduces patients' motor symptoms (Okun, 2012).

Other medications that are administered to PD patients for symptom suppression are MAO-B-, catechol-O-methyltransferase (COMT)- and NMDAR inhibitors, as well as DA agonists, and anti-cholinergics (Radhakrishnan & Goyal, 2018). Of these five mentioned drugs, only the latter is a non-dopaminergic drug, whereas the first four are all dopaminergic drugs. As described earlier, MAO-B plays an important role in ROS production, as it converts MPTP to MPP+. When MPP+ is taken up by DANs, it

accumulates in the mitochondria which eventually leads to the unwanted ROS production. The excessive ROS production allow the degeneration of DANs, which in turn cause DA levels to decrease. Therefore, by inhibiting MAO-B, oxidative stress should subside to a certain level and DA levels should be maintained (Guo, Zhao, Li, Li, & Liu, 2018) (Maiti, Manna, & Dunbar, 2017). Rasagiline and selegiline are examples of administered MAO-B inhibitors. MAO can also break down DA to dihydroxy phenyl acetate which is aided by COMT to turn into homovanillic acid (Maiti, Manna, & Dunbar, 2017). As COMT indirectly aids in DA degradation, COMT-inhibitors would therefore help maintain DA levels. Common medications of this inhibitor are tolcapone and entacapone. The side-effect of these two medications is that they can reduce the sensitivity for L-dopa (Maiti, Manna, & Dunbar, 2017). DA agonists mimic the response of DA produced by the DA receptors. DA agonists can bind to the DA receptors and therefore aid in increasing DA(-like) responses (Brooks, 2000).

As the disease progresses, changes in drug supplementation can be made. For example, when taking in the medications at set times becomes problematic, usually in a later stage of the disease, e.g. transdermal patches, subcutaneous injections, and intrajejunal infusions can be administered to aid the patients in their treatment (Radhakrishnan & Goyal, 2018).

The second common option of PD treatment is surgery. The most common surgery is deep brain stimulation (DBS), where one or more electrodes are placed inside the brain with an impulse generator attached to it. The most used sites in the PD brain are the subthamalic nucleus and the internal segment of the globus pallidus, both of which are nuclei located in the basal ganglia. In PD degeneration is not contained to the SNpc, but also present in other regions such as the two mentioned. How DBS exactly results in positive effects on PD pathology such as increased blood flow, promotion of neurotransmitter release (locally), extra calcium release by astrocytes, and stimulation of neurogenesis, is incompletely understood. The electrodes provide electric stimuli that disrupt 'wrong' neural signalling patterns within its region. However, the effect of the electrical stimuli is not limited to the electrode region, as DBS

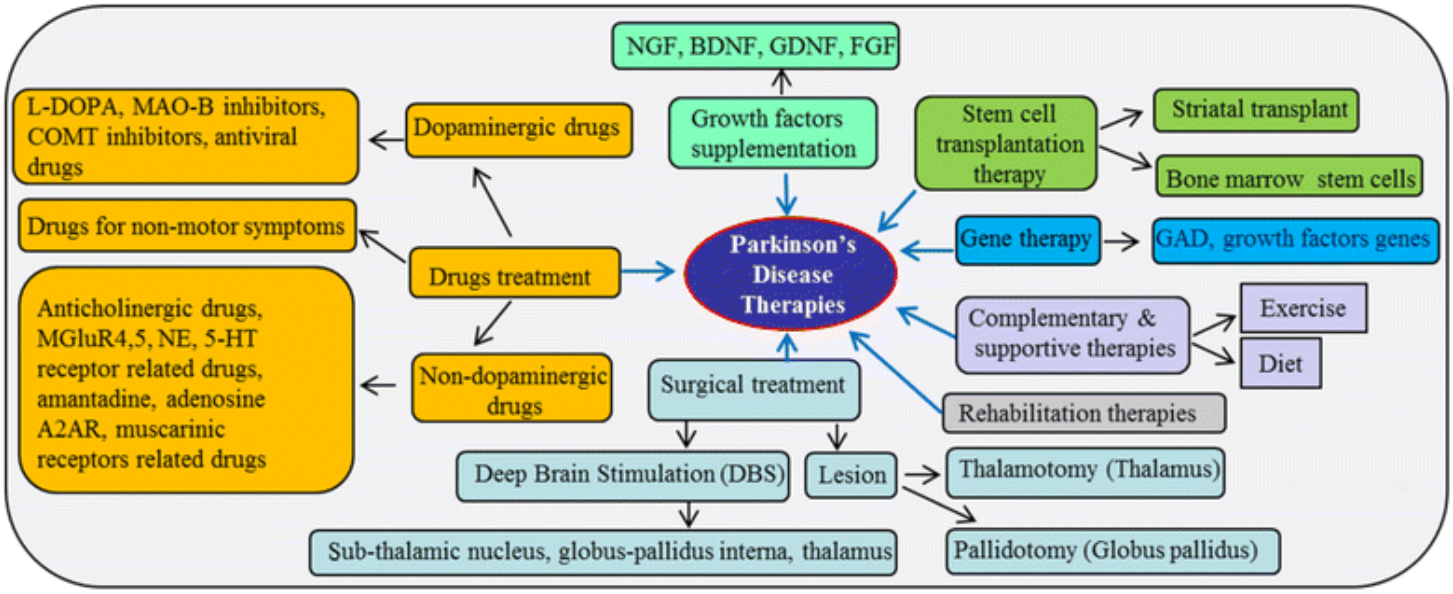

Figure 3 Schematic overview of treatment options up to ≤2017 of symptoms of Parkinson's disease. Seven different colours are given the possible treatment options, being surgical-, and drugs treatment, growth factor supplementation, stem cell transplantation-, gene-, complementary and supportive-, and rehabilitation therapies. L-DOPA=levodopa; MAO-B=type B monoamine oxidase; COMT=catechol-O-methyltransferase; MGluR=metabotropic glutamate receptor; NE=norepinephrine; 5-HT=5-hydroxytryptamine (serotonin); adenosine A2AR=adenosine A2A receptor. NGF=nerve growth factor; BDNF=brain-derived neurotrophic factor; GDNF=glial cell-derived neurotrophic factor; FGF=fibroblast growth factor; GAD=generalized anxiety disorder. Source image: *(Maiti, Manna, & Dunbar, 2017)*.

radiates electrical, chemical, and neural-network influences, met with in the entire brain (Okun, 2012). Possible negative effects of DBS on PD patients entail depression, social adjustment issues, and cognitive disturbances (Müller, 2012). As DBS is an invasive surgery, patients need to meet certain standards before they can be opted for DBS. Therefore, not every patient has a pathology suitable for DBS and might need different therapies when previous mentioned drugs start to 'wear out'.

Besides the two common treatment options, drug treatment and surgery, other therapies also have potential in aiding PD patients (see Figure 3). Other therapies for example include growth factor supplementation, stem cell transplantation therapy, gene therapy, and rehabilitating therapies. Among the rehabilitating therapies is the effect of exercise on PD pathology. (Feng, et al.) reviewed that studies where animal models were used to examine the effect of exercise was positive as it showed improvement in both motor (instability, gait impairment, et cetera) and non-motor (sleep disorders, cognitive functioning) symptoms. The studies showed beneficial effects of exercise training on autophagy, apoptosis, as well as increased neuroplasticity, regulating neurotrophic factors, and protection of neurons against brain damage (Feng, et al., 2020).

Prospects in treatment research for Parkinson's disease

Many PD treatments are focussed on replacing the loss of dopamine, removing (debris of) misfolded proteins, in particular that of α-syn, and maintaining the DANs still present in the SNpc and striatum of PD patients' brains, as well as maintaining DA levels. In this chapter a small selection of the current prospects are elucidated.

For example, (Zhang, Yang, & Liu) examined the effect of the flavonoid phloretin (PHL) on PD pathology by using an MPTP-inducing-PD mouse model. PHL has displayed various PD-beneficial activities in both cell cultures and animal models, such as neuroprotective effects as well as anti-inflammatory-, anti-apoptotic-, anti-cancer-, and antioxidant effects (Chang, Huang, & Liou, 2012) (Zhang, Yang, & Liu, 2019). The mouse models used by (Zhang, Yang, & Liu) through MPTP administration, resulted in the mice displaying PD-like symptoms and pathology, namely oxidative stress, activation of glial cells through neuro-inflammation, DAN loss in the SNpc, and depleted DA levels. MPTP administration also showed to increase TNF-α, IL-1β, and IL-6 levels in the used mouse model. However, with administration of PHL, all three cytokine levels decreased, noting towards the probable anti-inflammatory ability of PHL. PHL also showed an increase in DA level when administered with MPTP (Zhang, Yang, & Liu, 2019). Another study that investigated the role of PHL in inflammation used LPS-activated macrophages as a biological basis. (Chang, Huang, & Liou) also detected the inhibition of TNF-α and IL-6 levels when RAW264.7 cells were pre-treated with PHL compared to the control group. The role of PHL in PD involved pathways remains poorly understood. However, PHL indicates to be a membrane-binding flavonoid which has influence on dipole creation and gradient current across membranes (Ostroumova, Efimova, & Malev, 2015), as it can lower the membrane potential (Fong, 2015). This is likely as several studies state that PHL is able to cross the BBB (Fong, 2015). This is of course an important and necessary feature for PHL to be a good therapeutic agent for PD.

Another molecule that is investigated in its effect on neuro-inflammation is sirtuin 1 (SIRT1). In this study by (Ye, et al.) results show that LPS increased TNF-α and IL-6 release in BV2 cells, and simultaneously inhibited SIRT1. When the cells were treated with resveratrol, a SIRT1 activator, the LPS effect was reversed and neither cytokine level was increased. Also, when the cells were treated with sirtinol, a SIRT1 inhibitor, the opposite results were

witnessed, indicating SIRT1 plays a role in TNF-α and IL-6 release. How both resveratrol and sirtinol influence SIRT1 activation is unclear and thus asks for further research before more conclusions can start to be drawn on the influence of SIRT1 on PD pathology. It is however implied that the mechanism by which SIRT1 would provide a neuroprotective role would be related to involvement in the p53-caspase-3-dependent apoptotic pathway (Ye, et al., 2013).

Both phloretin and sirtuin 1 are still being investigated in the laboratory and have not come to the clinical trial phase. However, the research of molecule cystamine, and its reduced form cysteamine, as a potential treatment for PD has shown a promising future. Both molecules have gone through clinical trials before for other clinical indications such as Huntington's disease (Cicchetti, David, Siddu, & Denis, 2019) (Cisbani, et al., 2015). Both molecules have also shown good prospects, as toxin-induced animal model studies of PD presented that cystamine is neuroprotective. It can also cross the BBB (Bousquet, et al., 2010) which is an important feature to have in PD treatment agents, and partly by increasing brain-derived neurotrophic factor (BDNF) levels, it prevents cell death. Cystamine as well as cysteamine have both shown results in toxin-induced chronic lesion mouse models of PD where they stop and reverse ongoing neurodegenerative processes (Cisbani, et al., 2015). Cystamine/cysteamine administration as a treatment for PD seems to become more promising as research continuous.

Discussion

In this thesis the impact of α-syn aggregates to PD pathology and the influence of cytokines in PD involved pathways such as the NF-κB and MAPK pathway was reviewed. Results clearly showed a well-studied α-syn to be of vital importance in PD pathology. This protein is also frequently called a hallmark of this life-changing disease. Research also showed α-syn to induce M1 microglia activation, which in turn conducted further inflammatory actions. Activated microglia produce pro-inflammatory cytokines (such as TNF-α, IL-1β, and IL-6, but more as well) which lead up to mitochondrial dysfunction, oxidative stress and eventual cell death. Promotion of Ca^{2+} influx in mitochondria caused by over-stimulus of NMDA receptors was attributed to TNF-α release and resulted in cellular death. Furthermore, the role of TNF-α, IL-1β, and IL-6 were investigated more deeply as they showed great complexity due to mediating both neuro-inflammatory responses as well as neuroprotective responses by stimulating pathway activation through receptor binding. Lastly, PD treatments were elucidated and represent solely symptom suppression as a cure remains elusive. Hopes for new treatments remain, as many potential agents are being tested in cell cultures, animal models as well as starting their clinical trials.

Many attempts on creating an anti-inflammatory drug have been made, however, research does not guarantee success. An honourable mention is the NEMO-binding domain (NBD) inhibitor (NBDI). An *in vitro* (264.7 cells, a murine macrophage cell line) and *in vivo* (*mdx* mice) study of acute lung injury treatment (Zhao, et al., 2018) and another study of Duchenne muscular atrophy (Reay, et al., 2011) have provided results regarding the functionality of this inhibitor in different forms. As the name states, NBDI binds to the NBD, which is the part of NEMO of which it attaches to IKKβ. By inhibiting this domain, further activation of the NF-κB pathway is inhibited, thereby preventing pro-inflammatory gene mRNA expression to occur. Studies showed that the NBDI SR12343 inhibited TNF-α (and others) expression through the classical NF-κB pathway, but left the non-canonical pathway 'untouched' (Zhao, et al., 2018). Regardless of the efficacy of the drug, it was poor pharmacokinetics such as the serum's half-time, that prevented it from being useful in clinical trials. New research is being performed

to try to improve the pharmacokinetics, however, positive results have yet to be booked, as disappointing results have already been obtained (Rhodes, et al., 2018). Despite the unsuccessful attempt, limitations in clinical use, and the difference in tested disease pathologies, SR12343 still shows great capability of being useful in aiding PD treatment when problems in its pharmacokinetics are overcome.

Compared to the most common neurodegenerative disease being AD, PD and AD show a resemblance as both diseases have problems with proteins malfunctioning in the brain, α-syn in PD and amyloid beta (Aβ) plaques in AD. Both diseases also show a variety of unwanted cytokines being released in the brain, indicating pathology would show similarities as well. However, results regarding elevated cytokine levels show a different situation. (Chen, Hu, Cao, Liu, & Cheng) performed a meta-analysis which captured results of cytokines in CSF and blood from AD, PD and amyotrophic lateral sclerosis (ALS) patients, from 71 articles describing a total of 2629 patients with 2049 controls. Similarities in elevated cytokine levels in the CSF samples unfortunately remained absent, indicating to fewer similarly used pathways between these two diseases than expected. However, the group of Chen also stated that the lack of similar elevated cytokine levels regarding TNF-α, is most likely due to smaller groups used. When larger groups are used, it can be more expected to see similar results in TNF-α results which were not significantly present in either CSF sample results.

Regarding the same meta-analysis performed by (Chen, Hu, Cao, Liu, & Cheng), blood and CSF samples of the same patients showed a different outcome of cytokines' levels being elevated, or in some cases even diminished. Because of the difference in results of CSF and blood samples, it is important to note that elevated cytokine levels should be second-guessed even more than the ones showing similar results in both blood and CSF, to promote unified conclusions to be drawn. However, another point of view could argue that especially the results that are similar in both blood and CSF should be checked more so, as IL-1β has shown to be able to improve BBB permeability. This phenomenon could implicate potential crossing of periphery cells and/or periphery-originated cytokines and other factors through the BBB, theoretically contaminating the obtained CSF data. However, these chances are most likely very slim, but still important to note as it theoretically is a possibility. It is therefore advisable to re-check data and studies on which sample type had been used and on which sample type the data was based. Knowing that *in vitro* tests can only provide a limited amount of information, animal models need to be prepared well on the potential differences in different samples being collected as (Chen, Hu, Cao, Liu, & Cheng) showed.

Research has shown that the functions of cytokines are not simple, but quite the opposite, they are very diverse molecules, interacting with important biological processes. The cytokines themselves are not per se neurodegenerative, but it is the receptor they bind to that initiates further negative (neurodegenerative) or positive (neuroprotective) cascades. Therefore, 'simply' inhibiting these cytokines would not only inhibit the 'neurodegenerative receptors' of the cytokine, but also the 'neuroprotective receptor'. I therefore move to have research focus more on preventing 'negative receptor' inhibition and stimulating the positive side of the cytokines, alongside the attempts of inhibiting elevated TNF-α, IL-1β, and IL-6 cytokine levels. Although the road to accomplishing these features is not easily done, research is a limitless field of work and the backbone of our society as health organizations rely on new approaches originating from this field.

In conclusion, PD pathology is hallmarked by DAN loss and aggregation of α-syn in LBs. This is seen in many PD studies,

including oxidative stress and mitochondrial dysfunction. Causes are hard to find; however familial PD leads the way to mutations in PD-related genes. Hitherto, idiopathic PD has, as the name states, an unknown aetiology which drives researchers to continue to explore PD. Research leads the way to α-syn activating microglia and astrocytes. Many indications are made that these cells are responsible for pro-inflammatory cytokine release which in turn would stimulate oxidative stress, cause excessive calcium levels, activate pathways such as that of NF-κB, and more pro-inflammatory processes. Current PD treatments are limited to symptom suppression as no cure is yet available. New studies show positive prospects as they prepare for eventual clinical trials, as well as performing cell culture and animal model tests. To provide a cure for PD, more research into the aetiology of PD is required, as well as testing potential new treatment targets.

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