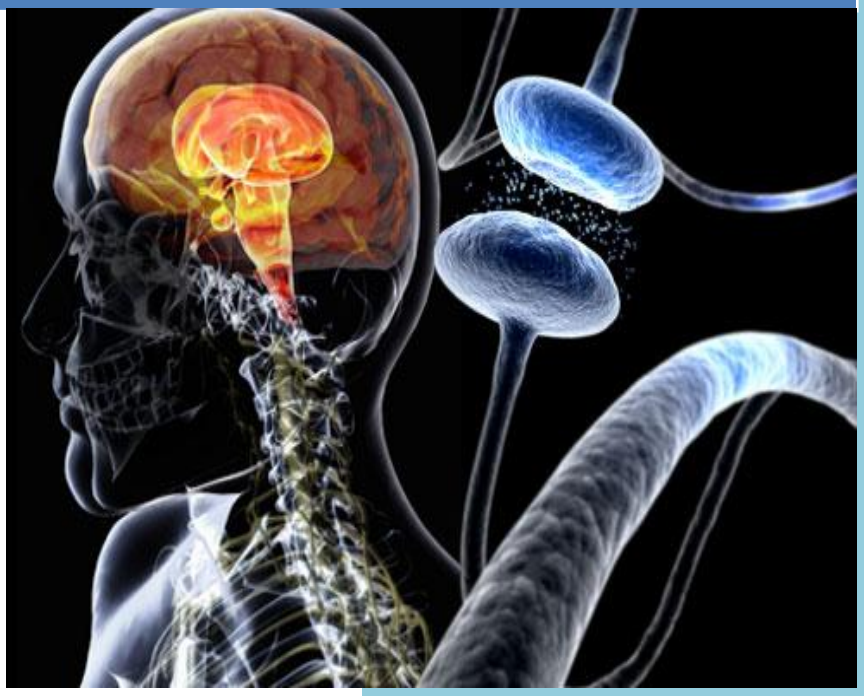
Modulation of microglia-T cell interaction as treatment of Parkinson's Disease



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Abstract

Parkinson's disease (PD) is a neurodegenerative disease characterized by damage and loss of dopaminergic neurons in the substantia nigra. Motor dysfunction is a result of damage and loss of these dopaminergic neurons. The cause of PD is unknown and therefore no cure is available. The only treatment of PD is symptomatic treatment by inducing the level of dopamine. Accumulation of α-synuclein and neuroinflammation are known to be major factors in the pathogenesis of PD.

Accumulation of the pre-synaptic protein α-synuclein is the main protein found in lewy body inclusions. These lewy bodies are settled in the central nervous system and in the gastro intestinal tract (GI tract) in PD patients. In PD the α-synuclein is misfolded and this leads to different structures, reports indicate an oligomer structure to be the most toxic. α-synuclein activates microglia and triggers neuroinflammation.

Microglia are the residential macrophages in the CNS. Peripheral macrophages are activated into a classical activated phenotype being pro-inflammatory or an alternative activated phenotype which is anti-inflammatory. Data indicate that also a pro- and an anti-inflammatory phenotype of microglia exist. The classical activated phenotype is induced by IFN-γ and TNF-α and the alternative activated phenotype is induced by IL-10 and/or IL-4. Phagocytic activity is an important mechanism in clearance of infectious agents, the alternative activated microglia phenotype possess a higher phagocytic activity than the classical activated phenotype. The α-synuclein-activated microglia in PD secrete the pro-inflammatory cytokines TNF-α, IL-6, IL-1β and IFN-γ and the pro-inflammatory mediator nitric oxide resulting in neuron damage and death. This indicates α-synuclein to activate the classical activated microglia phenotype in PD. Furthermore the microglia in PD express the surface marker MHC-II; indicating that microglia can activate T cells. Microglia are not only neurotoxic they are also able to secrete the anti-inflammatory cytokine IL-10 and TGF-β and BDNF which is a neuroprotective factor. This indicates that microglia are both neurotoxic and neuro-protective. There might be a balance between pro- and anti-inflammatory microglia and in PD this balance is skewed to the pro-inflammatory phenotype.

Inflammation of the substantia nigra and striatum is detected in PD patients with infiltration of both $CD4^+$ and $CD8^+$ T cells. $CD4^+$ T cells were found to be more essential in the pathogenesis of PD than $CD8^+$. T cells are activated by microglia but also by α-synuclein in the cervical lymph node. After activation the α-synuclein specific T cells migrate to the CNS and activate microglia. Th1 and Th17 might be the $CD4^+$ T cells subsets involved in PD as the cytokines IL-1, IL-6, IL17 and IFN-γ secreted by these cells stimulate classical activated microglia to produce pro-inflammatory cytokines, reactive oxygen species and nitric oxide. This indicates that the balance of the $CD4^+$ T cell subsets is skewed towards a pro-inflammatory response, thus Th1 and Th17. Indications showed Tregs to be involved in PD, but the exact role is unknown. Although data on the number and function of Tregs in PD is inconsistent, reports indicate Tregs to decrease the activation of microglia and the secretion of pro-inflammatory cytokines by induced production of IL-10 and TGF-β.

As α-synuclein is detected in the GI tract and it is known that PD patients have GI tract problems decades before PD onset, a hypothesis exist that PD starts in the enteric nervous system and affect the CNS through the vagus nerve. A report showed inflammation of the GI tract in PD patients and up-regulation of enteric glial markers indicating that also these cells are involved in PD. Although

accumulation of α-synuclein is found in the GI tract, more research is necessary to investigate the consequences and to clear which neurons are damaged.

As only symptomatic treatment is available it is important to find a way to stop accumulation of α-synuclein and to stop the inflammation. Both accumulation of α-synuclein and inflammation need to be stopped to reduce the progression of PD. The inflammation might be stopped by modulation of the microglia-T cell interaction. By inducing IL-10 producing Tregs, the phenotype of microglia can be changed to the alternative activated microglia, in addition the balance of T cell subsets is skewed towards equilibrium being less pro-inflammatory. Tregs can be induced by adoptive transfer of Tregs or by administration of VIP. VIP also induces motility of the GI tract in PD patients and is therefore a promising treatment. In addition to VIP treatment, medical food like poly-unsaturated fatty acids are found to possess neuroprotective properties and to suppress Th17 activities in the mucosal immune system and might be beneficial in PD. More research is necessary to reveal the role of Tregs in PD both in the CNS and in the ENS, but I am confident that induction of Tregs will reduce the neuroinflammation in PD patients.

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD) (1). 1.2 million people are diagnosed with PD in Europe and that number is still increasing (2-4). Two types of PD exist; the familial is about 10% of the PD cases and characterized by early onset and show mutations in several genes like parkin and leucine-rich repeat kinase 2 (LRRK2). 90% of the PD cases are sporadic and diagnosed later than the familial PD patients (5).

PD is a neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra and striatum. The nigrostriatal pathway projecting from the substantia nigra pars compacta to the putamen in the striatum is composed of dopaminergic neurons. Degeneration of these dopaminergic neurons results in less secreted dopamine from the pre-synaptic neuron, consequently, the post-synaptic neuron is not activated and causes tremor, rigidity and bradykinesia (6). These symptoms worsen as the disease progresses. In addition to dopaminergic neurons, also adrenergic neurons are damage. The synthesis of noradrenaline, a neurotransmitter secreted from adrenergic neurons, is hydroxylated dopamine, indicating that when dopaminergic neurons are damage adrenergic neurons are also damaged (7). The cause of PD is unknown, though it is known that α-synuclein and neuroinflammation play a role. However, it still remains to be solved if neuroinflammation is causing dopaminergic loss or is a consequence of the neurodegeneration. In brain regions of PD patients lewy bodies are found, these are inclusions of protein aggregation mainly consistent of α-synuclein. α-synuclein is a protein found in the brain (8). The physiologic function of α-synuclein is not very well known but on the basis of mouse models α-synuclein is thought to play a role in constructing vesicles for dopamine storage in the synapsis of neurons (8). α-synuclein binds to phosphor membranes and as a result the secondary structure of α-synuclein changes and the vesicle is formed. When no vesicles are formed dopamine cannot be stored resulting in auto-oxidation and forming of free radicals. These free radicals can damage the neurons and oxidize α-synuclein making it even less functional (8). The current treatment for PD is Levodopa (which is transformed into dopamine in the brain), dopamine agonists and dopamine breakdown inhibitors, these drugs increase the level of dopamine, but this is only symptomatic treatment, for this reason it is important to find the cause of PD to cure this disease or find a way to inhibit the progression of the disease.

Neuroinflammation in the brain is a major factor in PD pathophysiology with microglia and $CD4^+$ T cells as main players (9) (figure 1). $CD4^+$ T cells are differentiated into several subsets; T helper (Th)1, Th2, Th17 and regulatory T cells (Treg) (10). These subsets affect each other by cytokine secretion and expression of transcription factors; hereby the subsets are capable of regulating the differentiation of each other. These regulatory mechanisms establish a balance between the subsets. Under healthy circumstances the balance between these subsets is in equilibrium but in allergy the balance is Th2 skewed and in chronic inflammations Th1 skewed. The balance between Th17 and Tregs is often skewed towards Th17 in autoimmune diseases. Mounting evidence show that Tregs is an essential immune regulator in both health and disease, Tregs keep the homeostasis of the immune system in healthy situations and prevent development of autoimmune diseases by inhibiting the survival of auto-reactive T cells (10-13). In several diseases like multiple sclerosis and arthritis Tregs are less functional and a skewed balance occur between the $CD4^+$ subsets and therefore makes it difficult to cure these diseases (12, 14).

In addition to inflammation in the brain and motor dysfunction PD patients often have gastro-intestinal tract (GI tract) problems which occur decades before diagnosis (15). In the past decade and it still is today a highly debated subject whether the disease initiates in the enteric nervous system (ENS) or not (16-19). Among others detection of α-synuclein in the GI tract and inflammation in the colon of PD patients show that the ENS is involved in PD, but if the settling of α-synuclein and colon inflammation is the beginning of the disease or a consequence of the neurodegeneration in the substantia nigra and striatum, remains to be clarified (20).

As it is known that neuroinflammation is involved in PD, the purpose of this literature review was to answer the question: Is it possible to treat Parkinson's disease by modulating the interaction of α-synuclein activated microglia and $CD4^+$ T cells by inducing Tregs in the brain and in the GI tract?

To answer this question the current knowledge of the action of microglia and $CD4^+$ T cells in PD is described. The first part of the review describes the different phenotypes of microglia, the following part describes the role of T cells and the interaction between T cells and microglia and in the third part is the effect of PD in the ENS discussed. At the end the current and experimental treatments of PD are lined out.

α-synuclein activation of microglia in PD

α-synuclein is a pre-synaptic protein found in the central nervous system (CNS) in dopaminergic and adrenergic neurons both intracellular and extracellular (21, 22). According to post-mortem analysis α-synuclein and activated microglia are present in the substantia nigra of PD patients (23). Aggregated and un-aggregated α-synuclein is found in lewy bodies in synapses of PD patients but also in the GI tract, spinal cord, vagus nerve sympathetic ganglion and sciatic nerve (16, 21, 24, 25). Reports indicate that aggregation of α-synuclein is necessary to activate microglia to be neurotoxic (26-29).

In PD α-synuclein is misfolded, the cause of the misfolding of α-synuclein in human is unknown. Some mutations are found to cause misfolding of α-synuclein , but these mutations are only seen in patients with early onset PD (30). The mutations A53T and A30P are known to cause misfolding of α-synuclein in PD animal models (31). Several structures of α-synuclein are detected, monomers associate and form dimers which attach to other dimers and form oligomers and protofibrils and finally grow into aggregates (32). It is still unknown which structure is the most toxic, although oligomers caused the most death of dopaminergic neurons and might be the most toxic structure (33).

Recently Beraud et al. (34) reported up-regulation of Toll-like receptor (TLR)2 and TLR3 expression on microglia by α-synuclein and that microglia were activated through these receptors. α-synuclein can also activate microglia through receptors like TLR4, CD36 and integrin αM. A peptide of α-synuclein is presented in the major histocompatibility complex-II (MHC-II) making the microglia able to activate T cells (35-37). Hereby α-synuclein activates microglia directly and triggers neuroinflammation in absence of neurodegeneration (26, 38-40). This indicates that neuroinflammation might cause the neurodegeneration in PD. Forsyth et al. (17) found a strong correlation between α-synuclein and oxidative stress. Also the pro-inflammatory microglia phenotype and production of oxidative stress is caused by a specific structure of misfolded α-synuclein and other studies found monomeric α-synuclein to induce a stronger pro-inflammatory response than aggregated α-synuclein (28, 37, 41). Based on expression, production and release of pro-inflammatory mediators, it is suggested that α-synuclein activates the classical phenotype of microglia (28, 38). The phenotypes of microglia are discussed in the next chapter. In addition to microglia activation in the CNS, α-synuclein is able to diffuse to the cerebral lymph node (CLN) where antigen presenting cells (APCs) are present and activate α-synuclein specific T cells (figure 1). These newly activated T cells migrate to the CNS where they secrete cytokines and re-activate microglia and the inflammation is started (42).

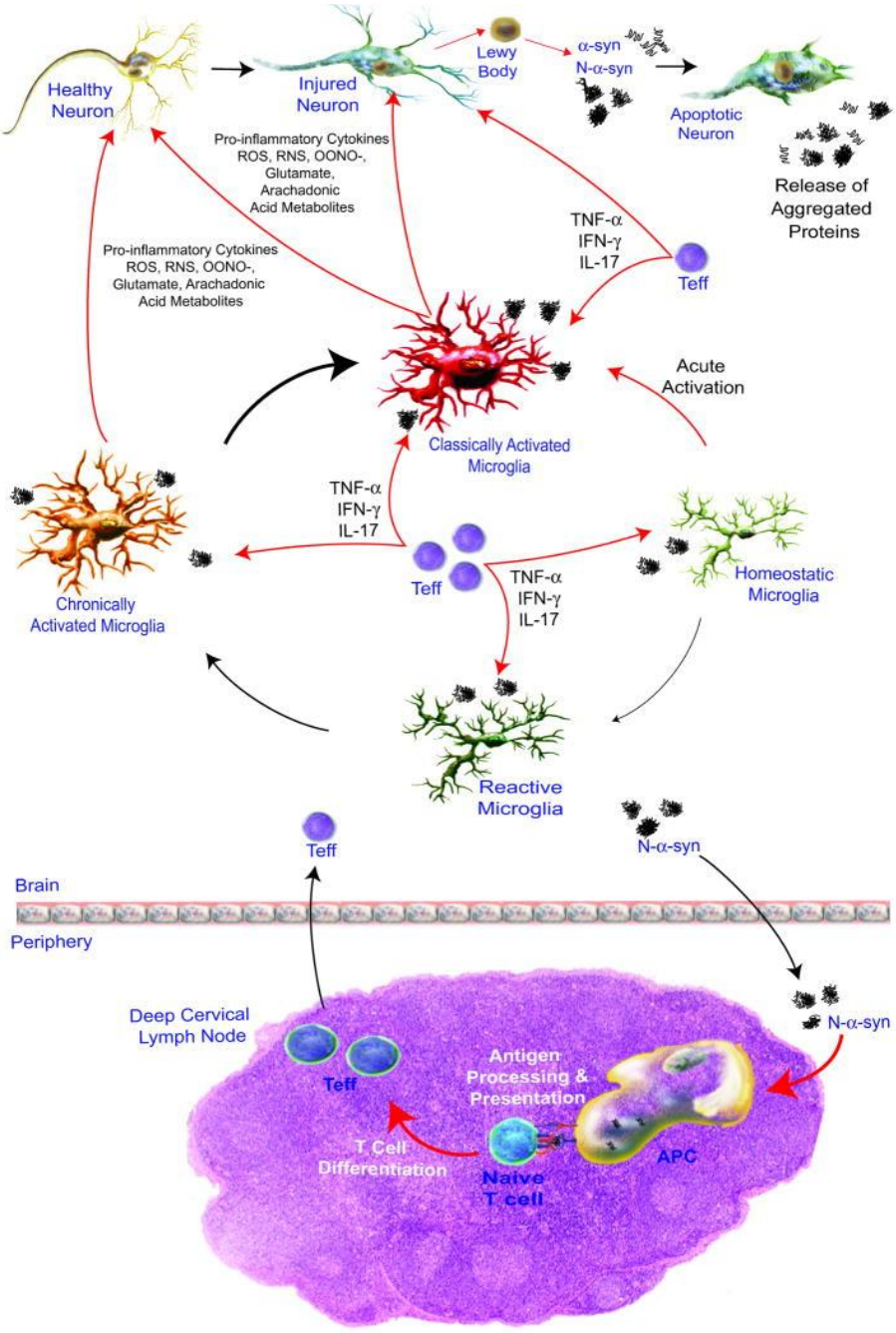

Figure 1. In PD pathophysiology microglia are activated by aggregated α-synuclein. Classical activated microglia secrete pro-inflammatory mediators like cytokines and free radicals which damage neurons. α-synuclein is always present and this results in chronic activation of microglia. α-synuclein also diffuses to the cervical lymph node where α-synuclein peptides are presented to T cells by APCs. α-synuclein specific T cells are migrating to the CNS where pro-inflammatory cytokines are secreted and induce chronic activation of microglia and damage of neurons. (141)

Role of microglia in PD

Microglia are the resident macrophages in the CNS and are thought to play an essential role in the pathophysiology of PD. Ouchi et al. (43) showed by positron emission tomography (PET) markers that accumulation of activated microglia in the midbrain is higher in PD patients than in healthy subjects, furthermore they reported a positive correlation between activated microglia and age in healthy subjects but not in PD patients, which might be because of the highly increased severity of microglia activation in PD patients. Also, a positive correlation between activated microglia and disease severity in PD patients was found (43). In contrast, Gerhard et al. (44) found no correlation between microglia activation and severity of disease but did find increased microglia activation in PD patients compared to healthy subjects in the following brain regions: basal ganglia including the striatum striatum, pons and cortex.

Microglia phenotypes

In the periphery macrophages observe the surroundings for foreign invaders. In the CNS this is the task of microglia. Macrophages are activated into different phenotypes; the classical activated, the alternative activated and the wound-healing phenotype (Table 1). These three phenotypes can be illustrated as a circle where the phenotypes overlap each other, in other words, it is possible to encounter types of macrophages that show a phenotype in between the three phenotypes mentioned above (45, 46). The classical activated macrophage is the pro-inflammatory phenotype induced by Interferon-γ (IFN-γ), Tumor necrosis factor-α (TNFα) and TLRs and as a result of activation this phenotype increases the expression of MHC-II, production of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) and the levels of secreted pro-inflammatory cytokines like TNF-α, Interleukin (IL)-1β, IL-23, IL-6 and IL-12 (45, 47). The wound-healing phenotype is induced by IL-4 and/or IL-13 and expresses MHC-II, IL-10 and IL-1ra. The alternative activated phenotype is anti-inflammatory and induced by IL-10 and glucocorticoid hormones, this is the only phenotype that do not express MHC-II, but secrete anti-inflammatory cytokines like IL10, transforming growth factor-β (TGF-β) and IL-1ra and express the receptor antagonist arginase-1 (reviewed in (45, 47)).

Table 1. The macrophage phenotypes, which cytokines induce the phenotype and the mediators they secrete

	Induced by	Secrete
Classical activated MΦ	IFN-γ, TNF-α	TNF-α, IL-1β, IL-6, NO, IL-12
Alternative activated MΦ	IL-10	IL-10, TGF-β, IL-1ra
Wound-healing MΦ	IL-4, IL-13	IL-10, IL-1ra

As microglia have a similar role in the CNS as macrophages have in the periphery, it is likely that different phenotypes of activated microglia exist as well, although up to date this is not well established, but reports indicate that different phenotypes of microglia are involved in inflammation, neurodegeneration and neuroprotection of the CNS (48-53). Perry et al. reviewed the role of microglia in neurodegenerative diseases and indicated that microglia adopt to several phenotypes when activated, the activated phenotype depends on the disease, the stage of the disease and composition of the microenvironment. The phenotypes range from one extreme to the other from pro-inflammatory phenotypes to anti-inflammatory, like the phenotypes of macrophages (46). In this review the terms classical and alternative activated microglia phenotypes will be used as the range of

microglia phenotypes is from pro- to anti-inflammatory. Durafourt et al. (52) found that microglia are similar to macrophages but the phenotypes are not exactly the same. Classical activated macrophages and microglia were generated by IFN-γ activation of granulocyte-macrophage colony-stimulating factor (GM-CSF) treated monocytes and microglia, respectively. The microglia showed similar phenotypic properties as classical activated macrophages both expressing the surface marker CD80 and secreting the cytokine IL-12 and TNF-α. In contrast, the microglial phenotype adopted after stimulation with IL-4 and IL-13 express other surface markers than the alternative activated macrophage phenotype adopted after IL-4 and IL-13 activation. This suggests that different phenotypes of microglia exist although different form the macrophage phenotypes. On the other hand, Michelucci et al. (51) found phenotypes of pro- and anti-inflammatory microglia when treated with IFN-γ and IL-4/IL-10, respectively. Microglia treated with IFN-γ showed high expression of IL-6 and TNF-α suggesting a pro-inflammatory phenotype. Michelucci (51) looked at specific anti-inflammatory markers, which indicates that microglia adopt an anti-inflammatory phenotype when treated with IL-4 expressing reduced levels of TNF-α, IL-1β and induced level of the alternative activation transcription factor arginase I. Durafourt (52) only used expression of IL-12 and IL-10 to determine pro- and anti-inflammatory functional microglia and found the alternative activated phenotype to be neither pro- or anti-inflammatory. The discrepancy between the two studies might be because Durafourt et al. only looked at IL-12 and IL-10 to determine if the phenotype was pro- or anti-inflammatory.

Phagocytosis by microglia

Phagocytosis is a very important mechanism in clearing infectious agents in the body; phagocytosis is a function of macrophages. As soon as an infectious agents is passing the physical barrier phagocytic cells like macrophages bind this agent by receptors like TLRs, lipopolysaccharide (LPS) receptor and scavenger receptor and consequently the infectious agent is ingested and killed (54). Michelucci et al. (51) clearly found phagocytic activity of microglia to be increased when microglia were treated with IL-10, but no difference when treated with IL-4 compared with non-stimulated microglia. In addition, classically activated microglia are less phagocytic than alternative activated microglia. This is consistent with the findings of Durafourt et al. (52), where classical activated microglia were less phagocytic than non-activated microglia and alternative activated microglia treated with IL-4. They also found microglia to be more phagocytic than macrophages. This suggests that IL-10 can induce phagocytosis of microglia. In PD aggregated α-synuclein is the component that is thought to cause the neuron damage and thus the agent that is recognized by microglia to be phagocytized. An *in vitro* study showed that monomeric α-synuclein induces phagocytic activity of microglia but aggregated α-synuclein reduces the phagocytic activity of microglia (55). If this is the case in PD patients then the phagocytic activity of microglia is inhibited by aggregated α-synuclein and α-synuclein is not cleared and consequently increases the level of aggregated α-synuclein. That microglia are less phagocytic in the presence of aggregated α-synuclein agrees with the indication that classical activated microglia have reduced phagocytic activity. Inducing alternative activated microglia could be used as a possible treatment of PD as the phagocytic activity is induced and aggregated α-synuclein is cleared.

Microglia in PD

In a healthy situation microglia survey the CNS and detect foreign invaders. Depending on the type of invader/damage the microglia will change into a pro- or anti-inflammatory phenotype; this change is also dependent on the composition of the microenvironment (56). The microglia fight and clear the invader by phagocytosis and activate the adaptive immune system to support the clearance of the

invader and to protect the neurons (56, 57). After clearing the pathogen microglia return into the surveying state (56, 58). In PD microglia are continuously activated and do not return to their resting state (44).

Activated microglia enhance MHC-II expression in PD animal models (48, 50, 59). The MHC-II expression is also enhanced in the substantia nigra of PD patients (60, 61). A very small expression of MHC-II is found in healthy subjects (60). The expression of MHC-II on microglia after activation indicates that they can act as APCs. This theory is supported by Depboylo et al. (50) as they found 80% of the infiltrated $CD3^+$ T cells to be close by or directly bound to MHC-II^+ microglia.

The activated microglia in the substantia nigra of PD patients secrete the pro-inflammatory cytokines TNF-α, IL-6, IL1-β and IFN-γ, furthermore is also intercellular adhesion molecule 1 (ICAM-1) expressed in the substantia nigra (38, 51, 62-65). α-synuclein activated microglia showed no change in expression of IL-4, IL-13 and Arginase I, the markers for alternative activated microglia (38). This indicates that α-synuclein activates microglia to adopt a classical activated phenotype.

Pro-inflammatory cytokines secreted from microglia cause neuron damage and death, what makes these microglia neurotoxic. These secreted pro-inflammatory cytokines might bind to receptors on neurons which activate cyclooxygenase 2 (COX-2) and results in neuron death (9, 66, 67). In addition to secretion of pro-inflammatory cytokines, NO and superoxide released by α-synuclein microglia also damage neurons and play a role in neurodegeneration (figure 1) (68-72). Conversely, microglia are also neuroprotective when neurotrophic factors like brain-derived neurotrophic factor (BDNF) and anti-inflammatory cytokines like IL-10 and TGF-β are secreted. In early PD there might be “a steady state” (equilibrium) between pro- and anti-inflammatory microglia. At a certain point during the disease, the neurotrophic microglia are not able to keep up with the neurotoxic microglia as they are constantly activated by accumulated α-synuclein and the balance is skewed towards the classical/neurotoxic microglia phenotype. This is suggested by Li et al. (49) as they reported the effect of microglia to depend on the intensity of the stimulation; low stimulation makes the microglia act neurotrophic and high stimulation results in less viability of the neurons. This might simulate early and late stage of PD, respectively.

T cells in PD

Inflammation of the substantia nigra, striatum and cerebrospinal fluid is detected in both PD animal models and in PD patients with infiltration of both $CD4^+$ and $CD8^+$ T cells (38, 50, 73-76). Infiltration of T cells is detected in both early and late stage of PD suggesting T cells to play a role in both neurodegeneration and neuron cell death (38, 48). Natural killer (NK) cells are not involved and the role of B cells is debatable as some reports show B cells do play a role whereas others claim that they do not (38, 50, 73). The role of B and NK cells is beyond the purpose of this review and will not be discussed further. In PD animal models the density of $CD8^+$ T cells is higher than $CD4^+$ in the substantia nigra and striatum (50, 73, 77). In $CD8^+$ deficient mice neurodegeneration still occurs in the substantia nigra, whereas the neurodegeneration in the substantia nigra in $CD4^+$ deficient mice is not different from saline treated mice; indicating the role of $CD4^+$ T cells to be more essential in neurodegeneration and neuron death in the substantia nigra than $CD8^+$ T cells in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model (50, 73). Beers et al. (78) found $CD4^+$ T cells to be neurotoxic but also neuroprotective in Amyotrophic lateral sclerosis (ALS), another neurodegenerative disease similar to PD. Later Beers et al. (79) showed that Tregs, a $CD4^+$ T cell subtype was responsible for the neuroprotective properties of $CD4^+$ T cells. The role of Tregs in PD is discussed further down.

The role of Th1 and Th17 in neuroinflammation in PD

Brocard et al. (73) showed that T cells are infiltrated in the CNS and actively participating in damaging dopamine neurons in MPTP treated mice after microglia activation, but also Benner et al. (42) showed $CD4^+$ T cells to be a main player in neurodegeneration by using MPTP injected severe combined immune deficiency syndrome (SCID) mice (B and T cell deficient mice). Brocard et al. (73) found the $CD4^+$ T cells to be harmful independently of IFN-γ, which might indicate that Th1 is not the main player, on the other hand it has been reported that Th1 can mediate cytotoxicity independently of IFN-γ (80). The damage in the substantia nigra and striatum might be due to other cytokines like TNF-α (74, 81). Moreover Th1 cells secrete IFN-γ which promote classical activated microglia and play a role in neurodegeneration (82). In addition to Th1, Th17 might play a role in the neuroinflammation in PD. The up-regulated expression of the Th17 transcription factor, retinoic acid-related orphan receptor-γt (RORγt), and increased secretion of the Th17 cytokine, IL-17, indicate Th17 to be an important participant in other neurodegenerative diseases (83-85). Furthermore, a report showed that Th17 cells are able to disrupt the blood-brain barrier (BBB) and promote CNS inflammation, additionally Th17 are able to kill neurons by expression of granzyme B (85). The role of Th17 in PD is not well established, only a few reports indicate the participation of Th17 in PD (73, 86, 87). Reynolds et al. (86) reported increased production of IL-17 and IL-6 by T cells in α-synuclein immunized mice. In addition, an outrageous number of dying neurons occurred when Th17 cells were adoptively transferred to MPTP treated mice; the percentage surviving neurons was only 5% of the control group treated with PBS (86). As the expression of Th17 markers increased, a decrease in Th1 (IFN-γ), Th2 and Treg (IL-2) markers in α-synuclein immunized mice were detected (86). This indicates Th17 to be involved in the neuroinflammation in PD patients (figure 1) (86) and the balance of T cell subsets to be skewed towards Th17.

Th1 and Th17 cells are producers of pro-inflammatory cytokines and secrete IL-1, IL-6, IL17 and IFN-γ which stimulate microglia to produce pro-inflammatory cytokines, reactive oxygen species and NO (figure 1) (51, 62-64, 68, 69, 72, 82, 86, 88). Baba et al. (89) showed the percentage of IL-4 producing

T cells in peripheral blood of PD patients to be decreased and the percentage IFN-γ producing T cells was unchanged. The ratio of IFN-γ and IL4 producing T cells was increased, this indicates a skewed balance towards Th1 response, but this is not possible as the IFN-γ is unchanged. This might indicate that another subset like Th17 is involved to decrease the IL4 producing T cells, unfortunately the IL-17 producing T cells was not evaluated.

T cells and microglia interaction

Microglia and T cells interact and are able to activate each other in a positive feedback mechanism. As previously discussed T cells can be activated in the CLN by other APCs than microglia, these activated T cells migrate to the CNS and activate microglia (42). Classical activated microglia promote proliferation and function of Th1 cells (90). Th1 cells secrete IFN-γ which promotes classical activated microglia and production of IFN-γ and IL-6 (82, 91, 92). Also Th17 might influence microglia by secreting IL-17 (85). Furthermore, alternative activation of microglia reduces proliferation and function of Th1 cells and in addition Th2 cells secrete IL-4 which inhibits the production of free radicals by microglia (93).

Regulatory T cells

Mounting evidence show that T effector cells are damaging in neurodegenerative diseases (9, 73, 86, 94), but Beers et al. (78) suggested $CD4^+$ T cells to be neuroprotective in ALS. The markers for classical microglia activation were increased and alternative microglia activation markers were decreased in a T cell depleted mouse model indicating that a subtype of $CD4^+$ T cells skews the classical microglia activation into alternative activated microglia which are neuroprotective (78, 79). Tregs are responsible for the neuroprotective function (79). Tregs are able to induce alternative activated microglia and reduce the speed of the progression of ALS. Tregs reduce the inflammation and try to keep the progression of the disease at a stable level by skewing the microglia phenotype to alternatively activated microglia. As the disease accelerates Th1 induces classical activated microglia and pro-inflammatory factors are present and the Tregs are not able to skew the phenotype back to alternative activated microglia (79). This is consistent with the fact that the number of Tregs is decreased in blood from ALS patients; this is probably because they migrate to the CNS to try to modulate the immune response (79, 95, 96). At a later stage of the disease the number of Tregs is increased, maybe because the phenotype of microglia is skewed to the classical/pro-inflammatory phenotype and the Treg are not recruited to the CNS anymore (95). The number of Tregs in blood is also decreased in PD patients compared with healthy controls. This indicates that the immune response going on in the CNS of PD patients is similar to the response in ALS patients, as described above, but this has to be confirmed (89). Contradictory, Saunders et al. (94) reported no difference in the number of Tregs in blood PD patients compared with controls; this is reported by others as well (97). Reynolds et al. (86) reported Tregs from α-synuclein immunized mice to be less functional than Tregs from naïve mice. This is in agreement with the findings of Saunders et al. (94), that Tregs are less suppressive in PD thus less functional compared with controls but Rosenkrants et al. (97) reported the suppressive function of Tregs in PD to be higher than controls. As not much is known about Tregs in PD neuroinflammation and the results are inconsistent more research is necessary before a clear mechanism can be purposed but it is clear that Tregs are involved in the pathophysiology of PD.

Dr. Gendelman's Lab is one of the few groups publishing on the role of Tregs in PD. Reports from this group showed Tregs to decrease the activation of microglia induced by MPTP and the production of

H_2O_2, ROI and the pro-inflammatory cytokines TNF-α, IFN-γ, IL-6 and IL-1. They also showed Tregs to induce the expression of Forkhead box protein 3 (Foxp3), TGF-β and IL-10 in the midbrain of MPTP mice (91). The midbrain contains the substantia nigra where dopaminergic neurons degenerate in PD. This indicates that Tregs inhibit the function of microglia in PD. Furthermore, Tregs induced apoptosis of microglia through FasL-Fas interactions (92) and induced neuron survival; the percentage of surviving neurons was 80% in MPTP treated mice with adoptive transfer of Tregs from α-synuclein immunized mice compared with 34% survival in mice treated with MPTP only (86, 91). Tregs modulated microglia to be actively phagocytic and secrete regulatory factors to maintain CNS homeostasis (98). All these results indicate that Tregs convert microglia into a neuroprotective phenotype, the alternative activated microglia (92). Furthermore, Tregs also increased the secretion of the neuroprotective factors BDNF and glial cell-line derived neurotrophic factor (GDNF) (91).

FasL-Fas pathway probably has a significant role in neurodegeneration, as Saunders et al. (94) found Fas expression on T cells to be significantly more in PD patients than in controls and the FasL-Fas pathway was found to play a role in the damage and/or death of neurons (73, 92, 94). MPTP injected mice showed expression of Fas on neurons; 40% of the dopaminergic neurons expressed Fas, noteworthy is that Fas was not expressed on microglia in the substantia nigra (99) and this is inconsistent with the findings of Reynolds et al. (92). In addition, FasL is expressed on 80% of Tregs in presence of α-synuclein activated microglia, this demonstrate that the regulating role of Tregs might occur through the FasL-Fas pathway (92). This is notable as it indicates Tregs to kill the Fas expressing neurons through the FasL-Fas pathway, this is not agreeable with the findings of Reynolds et al. that Tregs are neuroprotective (92). A hypothesis is that when neurons are damaged they express Fas and are turned into apoptosis by Tregs. On the other hand, Fas is also expressed on effector T cells whereas Tregs might kill these damaging cells as well. It is clear that more research is needed to find out the exact mechanism of the FasL-Fas pathway in PD.

Parkinson's disease in the GI tract

It is well established that PD patients often have GI tract problems like constipation. These GI tract problems are known to occur decades before the onset of motor dysfunction symptoms (15). Levodopa can cause constipation in the first year of use (100), this could explain constipation in dopamine treated patients but do not explain the GI tract problems years before diagnosis.

Stages of PD

Braak et al. (101) suggested PD to develop in six stages (figure 2). The brain stem is the first to be affected and then the pathology spreads to the substantia nigra and in the final stage the neocortex is affected. The classification of the stages is based on detected lesions, α-synuclein containing lewy bodies or neurites, in the different regions of the brain; in stage 1 the dorsal motor nucleus of the vagal nerve is affected, lesions are detected in the medulla oblongata and pontine tegmentum (part of brain stem) in stage 2, when the progression of PD enters stage 3 or 4 the mesencephalic and prosencephalic nuclei (part of the midbrain) are affected and the patient is clinically diagnosed. In the last stages 5-6 is the cerebral cortex affected.

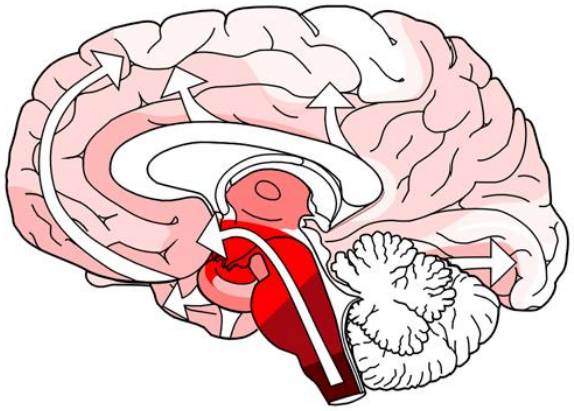

Figure 2. Braaks theory of the stages in the progression of PD in the brain. The dark red part is stage 1, the area affected first and the very light area is affected in the latest stage 5-6. (101)

Braak et al. (102) indicate that the pathophysiology of PD starts in the gut; that an undetermined pathogen, which could be LPS (103), enters the human body and in some way finds its way through the intestinal mucosal and induces aggregation of α-synuclein in terminal axons of neurons affecting the ENS and via projections the CNS (16). The ENS is directly connected with the dorsal motor nucleus of the vagal nerve and α-synuclein lesions are detected in this nucleus, this indicates that lesions might be present in the ENS before they are detected in the dorsal motor nucleus. In addition, lesions are detected in the ENS as early as stage 2 (16), but it is unknown if the lesions are present earlier (102). This topic is much debated as no well-documented data is yet present. Blandini et al. (18) reported a decrease in fecal output in rats with a substantia nigra lesion, suggesting that damage to neurons in substantia nigra cause alterations in the fecal output, indicating constipation; this was also reported by Colucci et al. (19). The mechanism of the connection between substantia nigra lesion and fecal output is further unknown. Supporting Braaks hypothesis is the fact that the intestinal permeability is increased in PD patients compared with controls. The patients participated in this study were early diagnosed and biopsies were taken before any treatment was started, indicating that the increased permeability is not caused by drug treatment like Levodopa (17). If

aggregated α-synuclein was caused by inflammation then it probably would be detectable in inflammatory bowel disease (IBD) patients as well, suggesting that the aggregated α-synuclein is caused by something else (17) and that might also cause the inflammation of the colon (20). In addition to increased permeability a positive correlation was found between the permeability and α-synuclein indicating that the component causing α-synuclein aggregation might also cause the increased permeability of the intestine barrier (17).

α-synuclein in the GI tract

Lewy bodies and α-synuclein are found in all segments of the gastro intestinal tract, spinal cord, vagus nerve sympathetic ganglion and sciatic nerve in PD patients (24, 25). α-synuclein is detected in untreated PD patients as well indicating that treatment is not causing the α-synuclein in PD patients (104). Annerino et al. (25) found α-synuclein pathology to be most prominent in the stomach, duodenum and proximal jejunum and less prominent in the ileum and colon. They also reported that the most α-synuclein was detected in the myenteric plexus but also in the submucosa plexus (figure 3). According to Annerino et al. (25) is only 3% of the α-synuclein aggregates related with dopaminergic neurons in the GI tract, the rest of the aggregates may be related with cholinergic neurons or α-synuclein may down-regulate the neuron markers but this was not tested and is unknown. Braak et al. (16) also detected α-synuclein in both plexuses of the gut walls through the whole GI tract from the stomach to the end of the colon. α-synuclein was found in the submucosa of the colon in untreated PD patients (104); this was also reported by Lebouvier et al. (105, 106). An important finding is that α-synuclein is detected in all ages whereas phosphorylated α-synuclein increased by age (107). Phosphorylated-α-synuclein was present in the colon of 72% of PD patients while 0% in controls (106). According to Bottner et al. (107) is p-α-synuclein not usable as pathological marker for PD as it seems to be a regular finding in adults but this is inconsistent with the findings of Lebouvier et al. (106). In addition, the patients with lewy neurites had a higher frequency of constipation than the control group and patients without lewy neurites. Finally they found a strong correlation between lewy neurite burden and disease severity (106).

Loss of neurons in the GI tract in PD

Singaram et al. (108) reported a significant decrease in dopamine neurons in the myenteric plexus in PD patients compared with controls but no differences in the submucosal plexus. Lebouvier et al. (105) suggested in consistence with Singaram et al. (108) that no differences were detected in the dopaminergic neurons in the submucosal plexus in the PD patients compared with controls. Contradictory to Singaram et al. (108) and Lebouvier et al. (105) Annerino et al. (25) concluded that there is no loss of myenteric neurons in PD patients. Forsyth et al. (17) suggest that if neuron loss is responsible for dysfunction of GI tract it might be the submucosa plexus neurons. Annerino et al. (25) indicate that it is more likely that the dysfunction of the GI tract is caused by neuropathology in the submucosal plexus and/or in the dorsal motor nucleus of the vagus and not caused by damage of the myenteric plexus.

Anderson et al. (109) suggest that MPTP to be a neurotoxin for dopaminergic neurons in the ENS and to cause changes in the motility of the GI tract. This is based on results showing that the density of dopaminergic neurons in the ENS decreased in MPTP treated mice compared with control mice. These MPTP treated mice had increased colon motility, which is contradictory with the findings in PD patients (106); Anderson et al. (109) say it might be because of differences between mice and man. Contradictory, Kuo et al. (110) showed that 2 types of transgenic mice with 2 different mutations in

α-synuclein A53T and A30P both had ENS abnormalities after 3 months; the motility of the colon was decreased in the A53T type and the whole gut transition time was increased in both types compared with controls. Colucci et al. (19) discovered the expression of the D2 dopamine receptor in the colon to decrease in 6-hydroxydopamine (6-OHDA) injected rats compared with controls. More research is needed to determine the role of α-synuclein and which neurons are damaged in the GI tract of PD patients.

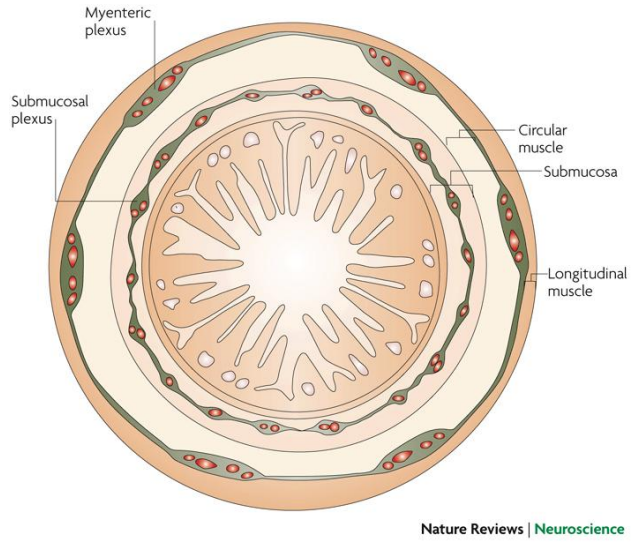

Figure 3. Transverse section of the small intestine. The submucosal plexus and the myenteric plexus which contain the ganglia of the ENS. (170)

Inflammation of the gut in PD

Singaram et al. (108) detected inflammatory cells in the myenteric plexus in PD patients and these were absent in controls. Recently Devos et al. (20) detected inflammation in the colon of PD patients. This is the first time inflammation in the GI tract of PD patients is reported. They found increased levels of the glial cell markers glial fibrillary acidic protein (GFAP) and Sox-10 and the cytokines TNF-α, IL-6, IFN-γ and slightly increased levels of IL-1β in colon biopsies from PD patients compared with controls (20). The cytokine profile in the colon of PD patients was similar to the cytokine profile found in Crohn's disease (CD) patients (20). GFAP is an intermediate filament protein in astrocytes and enteric glial cell, Sox-10 is a transcription factor in enteric glia cells (111). A significant correlation between the expression of the enteric glial cell markers and the mRNA of the pro-inflammatory cytokines was found (20). These findings indicate that enteric glial cells and T cells also are involved in the inflammation in the ENS in PD patients.

Enteric glial cells

Enteric glial cells are associated with astrocytes in the CNS (112). Astrocytes are an important contributor in the maintenance of the BBB (113) but indications exist that the BBB is damaged when astrocytes are exposed to misfolded α-synuclein (114). α-synuclein accumulation is detected in astrocytes *in vitro* and in PD animal models and α-synuclein exposed astrocytes show increased gene expression of pro-inflammatory cytokines like IL-1, IL-6 and TNF-α (115). In contrast Gu et al. (114) found no up-regulation of the pro-inflammatory cytokines in astrocytes. They found microglia to be activated by α-synuclein exposed astrocytes; furthermore they showed microglia activated by astrocytes to secrete the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and through this activation of microglia Gu et al. (114) showed astrocytes to contribute to neurodegeneration. Like

astrocytes enteric glial cells possess protective capabilities (116-120). Enteric glial cells secrete GDNF (Bar 1997) which prevent death of epithelial cells (116, 119, 121). In addition *in vivo* and *in vitro* studies showed enteric glial cells to be wound-healing by secretion of pro-epidermal growth factor (proEGF) (120). Also, absence of enteric glial cells *in vivo* increases the permeability of the intestinal epithelial barrier (IEB) (117, 118). These data indicate enteric glia cells to be an important component in the protection of the IEB like astrocytes are important in the maintenance of the BBB. This means that if enteric glia cells or astrocytes are reduced so is the functionality of the IEB or BBB, respectively, and invaders can pass the barrier and cause inflammation. Because of the data discussed above enteric glial cells might be involved in the pathogenesis of PD in the gut. A hypothesis is that inflammation of the gut causes dysfunction of enteric glial cells with increased permeability of the IEB as a result. Another hypothesis is that something causes dysfunction of the enteric glial cells resulting in increased permeability of the IEB and following causes inflammation of the gut.

Inflammatory mediators like IFN-γ are shown to increase activation and proliferation of enteric glial cells *in vitro* (122, 123) but not *in vivo* (124). Furthermore, IFN-γ activates enteric glial cells to produce the pro-inflammatory cytokine IL-6 (125) and the free radical NO. Contradictory, enteric glial cells can also inhibit inflammation by secretion of nerve growth factor (NGF) (126). These pro-inflammatory and protecting properties of glial cells indicate that different phenotypes exist of enteric glial cells.

Residential mucosal macrophages are different from other macrophages. They have no expression of cytokines, CD14, CD11b and other surface markers normally expressed by macrophages reviewed in (127). Moreover, residential mucosal macrophages express TLR3-8 and through these receptors they perform phagocytosis of invaders. In case of an invader circulating monocytes are recruited to the inflamed mucosa and these cells generate pro-inflammatory mediators to fight the invader (127). Furthermore it is unknown if residential mucosal macrophages present antigens and if they polarize into classical and alternative activated macrophages (127). Residential mucosal macrophages and enteric glial cells are different, glial cells express MHC-II and secrete IL-6 when activated by IFN-γ and LPS *in vitro* (123), which indicates their ability to activate T cells, but mucosal macrophages do not secrete cytokines and it is unknown if they express MHC-II. On the other hand, residential mucosal macrophages are phagocytic but to my knowledge, it is unknown if enteric glial cells possess phagocytic activity.

CD is an autoimmune disease, where the GI tract or part of it is inflamed. The inflammation can occur in the whole GI tract from mouth to anus. Previously, Th1 was thought to be the $CD4^+$ subset to be chronically activated causing the inflammation, but recently Th17 was demonstrated to be the main subset involved in CD (128, 129). In healthy situations a balance between pro- and anti-inflammatory factors is present, but in diseases like CD and probably PD this balance is disturbed. The function of Th17 is in balance with the function of Tregs, when Th17 is chronically activated, like in CD, Tregs are not able to reduce the inflammatory response. The number of Tregs decreases in the peripheral blood as the Tregs migrate to the inflamed region. The Treg in the inflamed mucosa of CD patients enter apoptosis and the number of Tregs stays decreased (130). Reports showed the number of Tregs to increase when CD patients were treated with Infliximab, an anti-TNF-α antibody, and the disease severity was reduced with Infliximab treatment (130, 131). As TNF-α also plays a role in the pathophysiology of PD and was detected in the inflamed colon of PD patients (20), Infliximab might

be a possible treatment in PD. The question is if Infliximab can cross the BBB. If the GI tract problems in PD patients are caused by inflammation, anti-TNF-α treatment might be a solution.

Different phenotypes of macrophages are also detected in the intestine. The mucosal macrophage phenotype found in the colon of IBD patients expressed higher levels of CD14, CD16, HLA-DR, CD11b and CD11c compared to healthy colons (132, 133). This is further confirmed by Smythies et al. (134); they showed resident macrophages not to be pro-inflammatory. This is probably because macrophages in the intestine in CD are recruited from the blood and classically activated (127). Moreover, reports indicate that the phenotype of macrophages can change and by inducing alternative activated macrophages inflammation in IBD patients might be reduced (135-137). Little is known about the role of enteric glial cells in CD, but studies indicate that dysfunction of enteric glial cells has a role in inflammation of the gut (138, 139).

So far very little is known about GI tract inflammation in PD patients, but that inflammation not only occurs in the CNS but in the GI tract as well is a very important finding for future research to discover the pathology of PD and how the interaction between the ENS and CNS takes place. Still a lot of work needs to be done to find out the role of enteric glial cells in PD and which neurons are affected in which parts of the ENS, but it is definitely an important part of the investigation of PD.

Current and future treatment

Currently no therapy is available to inhibit the progression of PD. The current treatment is only relief of symptoms. The used drugs are increasing the level of dopamine in the CNS. These drugs do that in different ways;

- Levodopa is transformed into dopamine
- Dopamine agonist bind directly on the postsynaptic dopamine receptors
- Catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) B inhibitors decrease the breakdown of dopamine.
- Anticholinergic drugs block the effect of acetylcholine but these are rarely used because of the side effects.
- Amantadine is an N-Methyl-D-aspartic acid (NMDA) antagonist and stimulate dopamine indirectly

An additionally therapy is deep brain stimulation but this is only used when the progression of PD is at a stage when little or no response to dopamine is present anymore and this surgery only affect the levodopa responsive symptoms. The patient can use complementary therapy like herbs and vitamins in combination with levodopa but this is not approved by the FDA as a treatment. (100, 140)

PD is an inflammatory disease which suggests that nonsteroidal anti-inflammatory drugs (NSAID's) can be used as treatment of PD (141, 142). No studies in PD patients with NSAID's but *in vitro* and *in vivo* studies showed NSAID's to be neuroprotective in PD (143-145). Traditional NSAID's have side effects as both COX1 and COX2 are inhibited and moreover a higher dose is necessary as the drugs have to cross the BBB. Inhibition of COX1 is paired with cardio protective effects and these protective effects are absent in COX2 specific inhibitors. A prevention study in Alzheimer's disease patients was discontinued because specific COX2 inhibitor caused increased risk of cardiovascular events in the patients (146). Vioxx, a COX2 inhibitor was taken off the market because it showed increased incidence of myocardial infarction in rheumatoid arthritis patients (147). Minocycline is a lipophilic third generation tetracycline analog (antibiotic) which easily cross the BBB. Minocycline inhibits activation and proliferation of microglia, secretion of pro-inflammatory cytokines and chemokines. Minocycline is able to reduce PD in animal models (148, 149), but in clinical trials minocycline seems to have no effect although the sample size was small (150). Minocycline was also tested in ALS patients in a larger group, but showed a negative effect as the disease accelerated compared with placebo treated patients; this makes minocycline a non-beneficial drug. The reason for the negative effect of minocycline on ALS is unknown but possible because the used animal model is a poor representative of the disease or the approach to translational neuroscience is inadequate (151). Because of these results use of minocycline in other neurodegenerative diseases need to be reconsidered.

Glucocorticoids (GC) are known to induce alternative activated macrophages which produce IL-10 and perform high phagocytic activity (45, 47). The glucocorticoid receptor (GR) is dysfunctional in PD, the expression of the receptor in the substantia nigra is decreased and the level of cortisol is highly increased in PD patients, but Ros-Bernal et al. (152) showed the GR on microglia to be important in the survival of neurons. This is consistent with the fact that GC induce alternative activated microglia, indicating that using GC as treatment in PD might be beneficial although only tested in the MPTP

animal model. In the MPTP mouse model dexamethasone treatment showed protection of dopamine damage (153).

Neuraltus Pharmaceuticals performed clinical trials to determine the effects of the small molecule NP001 on PD, AD and ALS patients. NP001 is a drug skewing the phenotype of activated macrophages to the alternative activated phenotype. In October 2012 Neuraltus announced the results of the phase 2 trial in ALS and is starting phase 3 trials (154).

Other experimental treatments investigated at the moment are stem cell therapy and vasoactive intestinal peptide (VIP). Mesenchymal stem cells were applied intravenously and induced neuroprotection in α-synuclein transgenic mice (155). Mesenchymal stem cells can differentiate into neuron- and glia-like cells, and as a result a reduced secretion of pro-inflammatory cytokines linked to microglia activation reduced the damage of dopaminergic neurons.

VIP is a neuropeptide and is known to be immune regulatory and induce Tregs in Experimental autoimmune encephalomyelitis (EAE) and arthritis (156, 157). According to Reynolds et al. (86) this is also the case in PD. They showed VIP to reduce the neuroinflammation and induce the survival of neurons in MPTP treated animals. VIP inhibited the expression of Th17 cytokines like IL-6 and IL-17 and induced the expression of Treg markers like Foxp3 and IL-10 and in addition VIP induced the suppressive capacity of Tregs (86). The anti-inflammatory cytokine IL-4 induces the neuron survival and reduces the nitrate/nitrite and superoxide levels *in vitro* (93). VIP induces differentiation of Th2 cells and is secreted by Th2 cells as well (158). VIP injected into the substantia nigra of MPTP treated mice decreased activation of microglia, production of TNF-α, IL-1β and ROI and increased survival of neurons (159). When VIP is injected i.p. in PD animal model, the survival of neurons increased as well (160). In AD mouse model VIP inhibits microglia activation by blocking three inflammatory pathways p38 MAPK, p42p44 MAPK and NFkB (161). Subjects suffering from constipation have a reduced number of VIP positive neurons and increased NO positive neurons (162). This indicates that VIP treatment might reduce neuroinflammation by inducing Tregs, which induce alternative activated microglia by secreting IL-10 and additionally VIP treatment might reduce constipation in PD patients.

Discussion

The ideal treatment would be curing PD, but as the causer of the disease is not known, this is impossible. To help PD patients it is not satisfactorily to treat the symptoms as is done today. To slow down the progression of this neurodegenerative disease it is important to stop or reduce the neuroinflammation but the accumulation of α-synuclein has to be stopped as well. If the accumulation of α-synuclein is not stopped, the microglia will still be chronically stimulated by α-synuclein. If only the accumulation is stopped I doubt that the microglia are able to change phenotype without any anti-inflammatory stimulation. The microglia are already activated by α-synuclein and even though no α-synuclein is present to constantly activate new microglia, previously activated microglia and T cells are present and continue the inflammation as a positive feedback mechanism resulting in activation of each other by secretion of cytokines. Microglia induce proliferation and function of Th1 cells and activate T cells by secretion of IFN-γ and T cells secrete IL-1, IL-6, IL17 and IFN-γ which in turn activate microglia. The balance of the T cell subsets is pro-inflammatory skewed thus adjusting this dysbalance by modulating the microglia-T cell interaction could be a possible way to reduce the inflammation and to equilibrate the balance of T cell subsets. I think the chance is higher to reduce the progression of PD, when both accumulation of α-synuclein and inflammation are stopped. To stop the inflammation, the phenotype of microglia needs to be changed from the pro-inflammatory to the anti-inflammatory phenotype. This can be realized by administration of IL-4 which induces alternative activated microglia which are anti-inflammatory and more phagocytic and suppress Th1 functions. Alternative activated microglia and thus phagocytic activity can also be induced by IL-10 secreted by Tregs. Induction of Tregs can be done by adoptive transfer of Tregs but also by administration of VIP. The balance of the T cell subsets is also skewed towards equilibrium being less pro-inflammatory when Tregs are induced. Moreover, VIP might reduce the decreased GI tract motility in PD patients. Finally, VIP induces secretion of IL-4 by Th2 cells, indicating that treatment of PD by administration of VIP induces alternative activation of microglia directly but also through induction of Tregs, this is a promising treatment with beneficial outcomes.

Inflammation of the colon has newly been detected in PD patients and several reports indicate that the ENS is affected and the permeability of the IEB is changed, therefore medical foods might be beneficial in the treatment of PD. Several reports document compounds used as medical foods to modulate the immune system; prebiotics like oligosaccharides induce Tregs but also Th1 (163), the probiotic lactobacillus is also shown to skew the immune response towards a Treg and/or Th1 response (164). Different probiotics exist with different effects, a systemic review show no evidence that use of probiotic is beneficial in CD (165), as the cytokine profile of the colon inflammation in PD is similar to the inflammation in PD probiotics might not be beneficial in PD. Contrary, glutamine and whey protein are showed to improve the intestinal permeability in CD patients (166). Also poly-unsaturated fatty acids (PUFAs) like omega-3 and 6 fatty acids are shown to suppress Th17 cells in the mucosal immune system (167), furthermore an *in vitro* study showed an omega-6 fatty acid derivative to possess neuroprotective properties in the ENS (168). Considering the effects of PUFAs these might be beneficial in PD patients. Souvenaid is a medical nutrition product launched recently, as supplement for patients with mild AD. The nutrient combination contains phospholipids among others and is designed to improve memory and to support the formation of synapses (169). Fatty acids are derivatives of phospholipids so maybe Souvenaid also has an effect in PD patients.

In conclusion, by administration of VIP, GI tract motility increases, the number and function of Tregs induces as well as the IL4 secretion by Th2 cells, resulting in induction of alternative activated microglia and inhibiting the damaging effects of the classical activated microglia. Additionally use of medical food like PUFAs decreases the permeability of the IEB and might modulate the skewed balance of Th17/Treg towards Tregs. More research is necessary to reveal the role of Tregs in PD both in the CNS and in the ENS, but I am confident that induction of Tregs will reduce the neuroinflammation in PD patients.

Abbreviations

AD…… Alzheimer's disease

ALS ……..Amyotrophic lateral sclerosis

APC…… Antigen presenting cell

BBB…… Brain-blood barrier

BDNF……..Brain-derived neurotrophic factor

CD…… Crohn's Disease

CLN……..Cervical lymph node

CNS…… Central nervous system

COMT……Catechol-O-methyltransferase

COX…… Cyclooxygenase

EAE……..Experimental autoimmune encephalomyelitis

ENS……..Enteric nervous system

Foxp3…….Forkhead box protein 3

GC……Glucocorticoid

GDNF…… Glial cell-line derived neurotrophic factor

GFAP…… Glial fibrillary acidic protein

GI tract…… Gastro-intestinal tract

GM-CSF…… Granulocyte-macrophage colony-stimulating factor

GR……Glucocorticoid receptor

IBD……Inflammatory bowel disease

ICAM-1…… Intercellular adhesion molecule 1

IEB…… Intestinal epithelial barrier

IFN-γ……Interferon-γ

IL……Interleukin

LPS……Lipopolysaccharide

LRRK2…….. Leucine-rich repeat kinase 2

MAO-B…….. Monoamine oxidase B

MHC-II…….. Major histocompatibility complex-II

MPTP…….. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NGF…….. Nerve growth factor

NK……Natural killer cells

NMDA…… N-Methyl-D-aspartic acid receptor

NSAIDs……..Nonsteroidal anti-inflammatory drugs

6-OHDA…… 6-hydroxydopamine

NO…… Nitric oxide

PD……Parkinson's disease

PET…….. Positron emission tomography

proEGF……..Pro-epidermal growth factor

PUFA…….. Poly-unsaturated fatty acids

RNI…….. Reactive nitric intermediates

ROI…….. Reactive oxygen intermediates

ROR-γt…….. Retinoic acid-related orphan receptor-γt

SCID……Severe Combined Immune Deficiency syndrome

TGF-β…….. Transforming growth factor β

Th…….. T helper cell

TLR…….. Toll-like receptor

TNF-α……Tumor necrosis factor α

Treg……Regulatory T cell

VIP……Vasoactive intestinal peptide

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