

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Alpha-Synuclein and the Immune Response in Parkinson's Disease

Cintia Roodveldt¹, Adahir Labrador-Garrido¹,
Guillermo Izquierdo² and David Pozo¹

¹CABIMER-Andalusian Center for Molecular Biology & Regenerative Medicine
CSIC-University of Seville-UPO-Junta de Andalucía, Seville

²Clinical Neurosciences Management Unit, Virgen Macarena University Hospital,
University of Seville Medical School, Seville
Spain

1. Introduction

In the last few years, it has become evident that the immunological component is of central importance in Parkinson's Disease (PD) pathogenesis and progression. This can also certainly be said about the prominent role that the protein α -synuclein (α Syn) is currently believed to play in the pathobiology of this neurodegenerative disorder. Moreover, the multiple mechanisms through which α Syn might be affecting the immune system appear not to be just a consequence of disease progression, but to actively contribute to the delicate balance between neuroprotection and neurotoxicity that ultimately underlies a given stage of disease.

PD is a proteinopathy, whose pathological hallmark is the presence of deposits of aggregated α Syn in intracellular fibrillar inclusions in neurons of the *substantia nigra pars compacta* (SN) of the brain, known as Lewy bodies (LB) (Croisier et al., 2005; Spillantini et al., 1998), and the loss of dopaminergic neurons (Braak et al., 2003). Three missense mutations, A53T, A30P and E46K, as well as multiple copies of the wild-type (Wt) α Syn gene, are linked to early-onset, familial PD (Gasser, 2005; Kruger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004). Given that α Syn is the major component of LB in both familial and sporadic PD cases (Spillantini et al., 1998), α Syn is considered a critical factor in PD aetiology. Currently, the cellular and molecular mechanisms underlying the pathological actions of α Syn are poorly understood, and the factors contributing to sporadic PD, representing the vast majority of PD cases, are not known.

α Syn, together with β - and γ -synucleins, belong to the family of synucleins, a group of closely related, brain-enriched proteins. This 140 aa-residue protein is largely located in neuronal presynaptic terminals (Kim et al., 2004b) and in the nucleus (Yu et al., 2007). In particular, it is found in the neocortex, hippocampus and SN (Kim et al., 2004b), and in other brain regions, as well as within astrocytes, microglia and oligodendroglia (Austin et al., 2006; Mori et al., 2002; Richter-Landsberg et al., 2000). It is known to interact with a variety of proteins (Jenco et al., 1998; Peng et al., 2005) and lipid membranes (Jo et al., 2000). The physiological functions of α Syn are still being established, but its interaction with pre-

synaptic membranes and lipids suggests a role in the regulation of synaptic vesicle pools including dopamine release control (Perez & Hastings, 2004) and in lipid metabolism (Cabin et al., 2002; Castagnet et al., 2005; Golovko et al., 2009).

Both *in vitro* and *in vivo* in LB, α Syn can self-assemble to form ordered fibrillar aggregates, characterized by a cross β -sheet structure, that are morphologically similar to the amyloid fibrils found in neuritic plaques in Alzheimer's disease (AD) as well as in deposits associated with other amyloidogenic processes (Chiti & Dobson, 2006). The initial phase of the aggregation process is thought to involve the formation of intermediate oligomers and protofibrillar species which, according to accumulating experimental evidence, can be more toxic to cells than the mature fibrils into which they develop (Bucciantini et al., 2002; Stefani & Dobson, 2003). These and other findings suggest a common structure-linked toxicity among pre-fibrillar species, and propose similar mechanisms contributing to pathogenesis for this group of diseases (Baglioni et al., 2006; Bucciantini et al., 2004). Overall, different hypotheses have been proposed that postulate that α Syn induces a 'gain of toxic function' upon aggregation (Bennett, 2005).

While α Syn is typically considered as an intracellular protein, it has also been found to be normally present in extracellular biological fluids, including human cerebrospinal fluid (CSF) and blood plasma (Borghi et al., 2000; El-Agnaf et al., 2003; El-Agnaf et al., 2006; Lee et al., 2006; Tokuda et al., 2006). However, α Syn levels have been found to be elevated in plasma from PD and multiple system atrophy (MSA) patients relative to age-matched controls (Lee et al., 2006), while lower levels than normal have been detected in CSF from PD patients (Tokuda et al., 2006). On the other hand, two studies by El-Agnaf and colleagues showed an elevated content of oligomeric α Syn species present in plasma (El-Agnaf et al., 2006) and post mortem CSF (Tokuda et al., 2010) from PD patients, compared to controls, indicating that changes in the levels and characteristics of extracellular α Syn are associated with the disease (Lee, 2008). Even though membrane permeability from dying cells could be one contributing factor, it has been suggested that vesicle-mediated exocytosis from normal cells is probably the main source of extracellular α Syn (Lee, 2008). By using brain homogenates and neuronal cell cultures, Lee and colleagues (Lee et al., 2005) have shown that both monomeric and aggregated α Syn can be secreted by an unconventional secretory pathway. On the other hand, extracellular α Syn has been shown to be taken up by neuronal and microglial cells in culture, although the nature of the mechanism involved is still controversial (Lee, 2008). In addition, two recent studies have provided strong evidence for a neuron-to-neuron and neuron-to-non-neuronal cell transmission of α Syn aggregates and their associated cytotoxicity, in cellular and mouse models of PD (Danzon et al., 2011; Desplats et al., 2009), highlighting the importance of extracellular α Syn in the pathogenic mechanism of α -synucleinopathies.

2. Neuroinflammation in PD

Another prominent pathological feature of PD brains is the presence of a robust inflammatory response mediated by activated microglia and reactive astrocytes in affected areas of the SN (Glass et al., 2010). Inflammation is the first response of the immune system to pathogens or irritation. In acute conditions, it protects tissue against invading agents and promotes healing. However, a chronic inflammatory state can turn harmful towards the host's own tissue (Gao & Hong, 2008; Kim & Joh, 2006). Microglia are the resident immunocompetent cells in the brain (Aloisi, 2001), capable of antigen presentation to

lymphocytes (Kreutzberg, 1996) and rapid activation in response to immune insults and invading of PD pathogenesis in the central nervous system (CNS) (Kim & Joh, 2006). As a result of pathogen invasion or tissue damage, microglia switch to an activated phenotype and thereby promote an inflammatory response that serves to further engage the immune system by recruiting other cells to the site of brain lesion, and initiate tissue repair (Glass et al., 2010). However, uncontrolled inflammation may result in production of neurotoxic factors that can be highly detrimental (Gao & Hong, 2008; Glass et al., 2010). Indeed, inflammation in the CNS and sustained overactivation of microglia, i.e. reactive microgliosis, are currently believed to be actively involved in the pathogenesis of various neurodegenerative diseases including PD, AD, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) (Gao & Hong, 2008; Glass et al., 2010; Kim & Joh, 2006; Long-Smith et al., 2009).

At present, whether microglial activation ultimately protects or actually exacerbates neuronal loss in the context of PD and other related diseases is still under debate (Delgado & Ganea, 2003; Gao & Hong, 2008; Halliday & Stevens, 2011; Sanchez-Pernaute et al., 2004; Vila et al., 2001; Wu et al., 2002; Wyss-Coray & Mucke, 2002), although the current view favours the second hypothesis. Evidence of microglial attack in PD is supported by findings from epidemiological studies, animal models, and cell culture experiments (McGeer & McGeer, 2008). Epidemiological studies have revealed that taking ibuprofen anti-inflammatory agent regularly is associated with a 35% lower risk of PD (Chen et al., 2005; Chen et al., 2003), supporting the concept that inflammatory attack is contributing to dopaminergic neuronal loss. On the other hand, *in vivo* studies show that the specific early up-regulation of SN microglia in PD correlates with disease severity and dopamine terminal loss (Orr et al., 2005; Ouchi et al., 2005). Overall, studies based on animal models and *in vitro* cell culture, indicate that dopaminergic cells are highly sensitive to inflammatory attack (Castano et al., 1998; Fernagut & Chesselet, 2004) and that microglial cells can be strongly activated to mount such an inflammatory response (Austin et al., 2006). Moreover, it has been recently reported that treatment with CSF from PD patients strongly affects cultured microglial cells, resulting in reduced cell growth, morphological changes, as well as increased content and aggregation of α Syn (Schiess et al., 2010). This illustrates how microglia itself, and not only dopaminergic neurons, can be highly affected by the medium in a PD scenario.

3. α Syn-induced microglial activation

The results gathered thus far using the different PD animal models have substantially increased our understanding of PD's pathogenesis by usually providing different but probably complementary information. Thus, while the MPTP mouse model of PD indicates that inflammation in the SN can be self-propagating and leads to progressive neurodegeneration, the α Syn transgenic animal model demonstrates that overexpression of this endogenous protein can certainly provide a powerful source of inflammation (McGeer & McGeer, 2008). Whether microglial activation is essentially caused by the release of aberrant α Syn species to the extracellular space, (Reynolds et al., 2008b; Wersinger & Sidhu, 2006; Zhang et al., 2005), or otherwise, that neuronal death itself drives microglial immune responses in an α Syn-independent manner (Giasson et al., 2000; Mandel et al., 2005; Przedborski et al., 2001), is still under debate. However, there is ample accumulated

evidence pointing at α Syn as the main trigger of microglial activation in PD (Roodveldt et al., 2008). For example, several studies have demonstrated that extracellular and nigral α Syn- containing aggregates are often surrounded by activated microglia or inflammatory mediators in PD brains (McGeer et al., 1988; Yamada et al., 1992), similarly to what has been described for amyloid plaques in AD (Griffin et al., 2006). Moreover, the extent of microglial activation in the SN from PD patients has been found to be correlated with the degree of α Syn accumulation (Croisier et al., 2005) and with increased α Syn levels as evidenced by *in vitro* (Kim et al., 2009; Klegeris et al., 2008) and *in vivo* (Lee et al., 2009a) studies, strongly supporting the view that the protein has a major role in phenotypic changes of microglia. Up to this point, a considerable number of *in vivo* studies with animal models of PD that directly link α Syn with microglial activation have been reported. It has been demonstrated in mice that overexpression of α Syn alone (by using adeno-associated virus, AAV) is sufficient to trigger neuroinflammation, involving not only classical microglial activation but also stimulation of adaptive immunity, preceding the appearance of overt neurodegeneration signs (Theodore et al., 2008). In line with this finding, a rat AAV-based model for overexpressing the A53T α Syn variant in the SN revealed dramatic changes in cytoskeletal protein levels and activated microglia-mediated neuroinflammation in the striatum (with increased release levels of IL-1 β , IFN- γ , and TNF- α proinflammatory cytokines), well before neuronal loss was evident (Chung et al., 2009). Another recent study using a AAV rat PD model showed that overexpression of Wt α Syn in the SN not only leads to persistent microglia activation, but that depending on the degree of α Syn-induced neuropathology that models either the onset or the late stages of the disease, different microglial responses will occur: upon lower α Syn expression levels where only neurodegeneration occurs, microglia with antigen-presenting capabilities predominate, whereas levels that can induce neuronal cell death correlate with long-term induction of macrophagic microglia (Sanchez-Guajardo et al., 2010), suggesting that microglia may play different roles during disease progression (Sanchez-Guajardo et al., 2010).

Two recent studies have explored the link between neuroinflammation and α Syn dysfunction by lipopolysaccharide (LPS) injection in rat (Choi et al., 2010) or mice (Gao et al., 2011), to trigger systemic and brain inflammation. In the first study, the authors observed increased microglia activation and secretion of proinflammatory cytokines as well as greater nitration of proteins including α Syn, in elderly rats, suggesting that an exaggerated neuroinflammatory response that occurs naturally with aging might contribute to α Syn aggregation and dopaminergic neurodegeneration in PD (Choi et al., 2010). In the second study, the authors evaluated dopaminergic neurodegeneration, α Syn pathology and neuroinflammation in Wt and transgenic A53T α Syn-overexpressing mice (Gao et al., 2011). They observed that, while both models initially displayed acute neuroinflammation, only the latter developed persistent neuroinflammation together with chronic progressive degeneration of nigrostriatal dopamine pathway, accumulation of aggregated, nitrated α Syn, and formation of LB (Gao et al., 2011), suggesting that genetic factors and environmental exposures act synergistically to precipitate the development of PD. On the other hand, microglial cells from α Syn-knockout mice have been shown to exhibit a remarkably different morphology compared to Wt cells (Austin et al., 2006), displaying elevated levels of secreted pro-inflammatory cytokines such as TNF- α and IL-6 after activation, indicating that α Syn plays a critical role in modulating the microglial activation state. More recently, the authors have found that microglial activation in this model is

accompanied by increased protein levels of three enzymes involved in lipid-mediated signalling, which suggests a broader function for α Syn in brain physiology beyond synapsis control (Austin et al., 2011).

In the last few years, several *in vitro* studies have focused on the effects of extracellular α Syn on microglial activation. Zhang *et al.* (Zhang et al., 2005) first reported that exogenous, aggregated α Syn cause activation of microglial cells, which then become toxic towards cultured dopaminergic neurons. Their results indicate that microglial phagocytosis of α Syn and activation of NADPH oxidase, are critical in α Syn-induced microglial activation and neurotoxicity. This finding is highly relevant considering that aggregated α Syn has been shown to be secreted by exocytosis from neuroblastoma and primary neuronal cells (Danzer et al., 2011; Lee et al., 2005), and by stressed neurons (Klegeris et al., 2008). Moreover, following the discovery that α Syn aggregates can be released from neurons and transmitted to neighbouring cells (Desplats et al., 2009), a study has recently shown that α Syn release by SH-SY5Y neuroglioma cells, especially when treated with MPP⁺ neurotoxin, are able to activate the inflammatory response in a microglial cell line (Alvarez-Erviti et al., 2011).

Up to this point, research on α Syn-mediated cell response has focused primarily on the effects on neuroinflammation (Benner et al., 2008) or microglial activation (Cookson, 2009; Reynolds et al., 2008a; Thomas et al., 2007; Zhang et al., 2007; Zhang et al., 2005) of α Syn in its aggregated form. Interestingly, Reynolds and coworkers (Reynolds et al., 2008b) have found that nitrated, aggregated α Syn (N- α Syn) has a stronger stimulating effect on microglia than that of nitrated but non-aggregated α Syn. In addition, several investigations have found that N- α Syn, which has been detected in LB of human brains with PD (Giasson et al., 2000) and has been linked to neurodegeneration in PD mouse models (Benner et al., 2008; Gao et al., 2008), induces a neurotoxic inflammatory microglial phenotype that accelerates dopaminergic neuronal loss (Biasini et al., 2004; Thomas et al., 2007; Zhang et al., 2005; Zhou et al., 2005). By integrating genomic and proteomic techniques, Gendelman and colleagues created a fingerprint of microglial cell activation following its interactions with aggregated N- α Syn in cell culture (Reynolds et al., 2008a), indicating that the activation, which was found to be capable of mediating dopaminergic neurotoxicity, is mainly mediated by the NF- κ B pathway (Reynolds et al., 2008a). However, whether extracellular α Syn contains the same modifications than the protein found in LB (Anderson et al., 2006; Giasson et al., 2000; Hodara et al., 2004), which is a typically pro-oxidative environment, is still uncertain (Lee, 2008).

Over the last few years, certain differential functions for non-aggregated, extracellular α Syn in glia have been reported. It has been observed that, in contrast to the aggregated form, monomeric α Syn enhances microglial phagocytosis (Park et al., 2008). A few investigations that explore the effects of non-aggregated α Syn on the cytokine release profile of potentially relevant cells have been recently done using monocytic (Klegeris et al., 2008) or macrophage (Lee et al., 2009b) cell lines, and primary astrocyte (Klegeris et al., 2006) or microglial (Roodveldt et al., 2010; Su et al., 2009; Su et al., 2008) cultures. Indeed, we have observed a strong innate immune response in primary glial and microglial cell cultures elicited by exogenous, non-aggregated α Syn (Roodveldt et al., 2010). Interestingly, a comparative study using unmodified α Syn has recently shown that exogenous non-aggregated α Syn induces higher TNF- α , IL-1 β and ROS release levels than aggregated α Syn in microglia (Lee et al.,

2010). These and other recent findings point at the importance of exploring the effects on the immune response of aggregated as well as non-aggregated α Syn.

Even though a study using monocytic THP-1 cell line (Klegeris et al., 2008) had shown modest increases in IL-1 β and TNF- α secretion levels after stimulation with A30P, A53T, or E46K α Syn mutants compared to the Wt protein, there is a lack of a comprehensive study of the effect exerted by non-aggregated α Syn, performed with primary cell cultures. With this in mind, we analysed the cytokine release profile of primary microglial cultures—which represents a more comparable physiological environment—after stimulation with Wt or the PD-linked α Syn mutants (Roodveldt et al., 2010). Indeed, we found remarkable differences between the α Syn variants in the interleukin and chemokine release profiles and significant effects on the microglial phagocytic capacity (Roodveldt et al., 2010). In particular, we observed marked differences in IL-6 and IL-1 β pro-inflammatory cytokines, IL-10 immunoregulatory cytokine, as well as IP-10/CXCL10, RANTES/CCL5, MCP-1/CCL2 and MIP-1 α /CCL3 chemokines release levels. Our results indicate that extracellular, non-aggregated Wt α Syn produces a moderate to low pro-inflammatory response in glia, together with a reduction of the immunoregulatory response, and a moderate stimulation of Th1 chemokine secretion. The A30P and E46K pathological variants, on the other hand, can induce strong pro-inflammatory and immunoregulatory responses, together with marked increases in chemokine release levels. This exacerbated innate immune response might explain the earlier onset and more rapid evolution of these two genetic cases of PD as compared to the sporadic kind. Intriguingly, our results from the pathologically-linked A53T variant showed not to provoke a significant innate immune response, which might suggest that other neurodegeneration mechanisms contributing to the pathogenesis of PD, probably involving the adaptive immune response, might exist in this case. Combined with the effect on microglial phagocytosis, our results indicate that these α Syn-induced phenotypes might reflect either a classical (A30P and E36K) or an alternative (A53T) microglial activation state, or a hybrid phenotype (Wt), which could probably explain the different disease progression modes that can occur in PD. Alternative activation of macrophages and microglia is a response to tissue injury that is thought to be involved in tissue repair and restoration (Ponomarev et al., 2007), and has been suggested to play a role in repair and extracellular matrix remodelling in AD (Colton et al., 2006). Currently, there is no other indication that such an activation mode could be operating in the context of PD.

Upon activation, microglia and astrocytes start secreting inflammatory cytokines in order to communicate and mount the immune response to counteract disease or injury. The cytokines TNF- α , IL-1 β , IL-2, IL-4, IL-6, TGF- α , TGF- β 1, TGF- β 2 have all been reported to be increased in the nigrostriatal region and CSF of patients with PD or DLB (Croisier & Graeber, 2006). As a result of α Syn-induced activation of microglia *in vitro*, a few cytokines and metabolites have been shown to be significantly up-regulated (reviewed in (Roodveldt et al., 2008)): IL-6, IL-1 β , ICAM-1, TNF- α , IFN- γ , MCP-1, O₂⁻, iROS, and PEG₂, glutamate, and iCys. In general, disease-linked α Syn variants show a stronger effect on cytokines release than does the Wt protein. Interestingly, analysis of the microglia transcriptome by Gendelman and coworkers (Reynolds et al., 2008a) after stimulation with aggregated N- α Syn, revealed a significant up-regulation of the toll-like receptor 2 (TLR-2) gene. TLRs are known to sense the molecular signatures of microbial pathogens, and play a fundamental role in innate immune responses, inducing the expression of diverse inflammatory genes

(Kawai & Akira, 2007). Therefore, it seems plausible that cells challenged with α Syn, or with certain forms of α Syn, could become hyper-responsive to inflammatory signals.

Activated microglia can also produce substantial amounts of superoxide radicals, which may be the major source of the oxidative stress thought to be largely responsible for dopaminergic cell death in PD. The generation of ROS by microglia activated by α Syn (Thomas et al., 2007) can result in oxidation and nitration of proteins, DNA modifications, and lipid peroxidation, leading to neurotoxicity (Zhang et al., 2005). Oxidation (Ko et al., 2000; Souza et al., 2000) and nitration (Giasson et al., 2000; Souza et al., 2000) of α Syn, in turn, can lead to the formation of more aggregates, which could result in increased cytotoxic effects. Consistent with this, Kelly *et al.* have shown that high levels of oxidized cholesterol metabolites in brains from PD and dementia with LB patients, accelerate α Syn fibrillation (Bosco et al., 2006). On the other hand, McGeer and colleagues (Klegeris et al., 2007a) have reported that α Syn-stimulated microglia, in combination with IFN- γ , produce and increase in the toxicity on human monocytic cells exerted by neurotoxic secretions (Klegeris et al., 2007a). Interestingly, this toxicity can be diminished with specific ligands for ryanodine receptors (Klegeris et al., 2007a), which are known to help mediate the efflux of Ca^{2+} ions from intracellular stores and avoid uncontrolled increases in $[\text{Ca}^{2+}]_i$ that may lead to cell death (Giorgi et al., 2008).

Further insight into the mechanism of pathogenesis might derive from the findings that several proteins which are thought to be linked to PD are up-regulated as a result of α Syn-induced microglial activation. Gendelman and co-workers, by determining the activated microglia proteome profile (Reynolds et al., 2008a), found that aggregated N- α Syn activation of microglia results in differential expression of several proteins. These range from proteins involved in oxidative stress, cell adhesion, glycolysis, growth control, and migration, to cytoskeletal proteins. It is worth noting that two of those proteins found to be most up-regulated, calmodulin and ubiquitin, have been shown to interact with α Syn with possible functional consequences. Calmodulin has been shown, *in vitro*, to bind to α Syn in a Ca^{2+} -dependent manner (Lee et al., 2002) and to inhibit α Syn fibrillation (Martinez et al., 2003). On the other hand, a fraction of α Syn found in LB is mono-ubiquitinated (Hasegawa et al., 2002; Tofaris et al., 2003), but the role of this modification remains unclear. Recently, it has been demonstrated that the ubiquitin-protein isopeptide ligase, seven in absentia homolog (SIAH), directly interacts with and monoubiquitinates α Syn, promoting its aggregation (Lee et al., 2008; Rott et al., 2008) and apoptosis (Lee et al., 2008). In addition, there is also evidence implicating a role for the ubiquitin-proteasome system (UPS) in PD (reviewed in (Lim & Tan, 2007)). Also of interest are the elevated levels of Hsp70 observed upon microglial activation. This central chaperone has been demonstrated to inhibit α Syn aggregation *in vitro* (Dedmon et al., 2005; Huang et al., 2006; Roodveldt et al., 2009), in neuroglioma cells (Klucken et al., 2004) as well as in fly (Auluck et al., 2002) and mouse (Klucken et al., 2004) models of PD, protecting cells from the cytotoxic effects of aggregates.

4. Links between α Syn and astrocytes and oligodendrocytes

Together with microglial cells, astrocytes and oligodendrocytes are part of glia, which normally serve neuroprotective functions but, given adverse stimulation as discussed before, they may contribute to develop chronic neuroinflammation (Halliday & Stevens, 2011; McGeer & McGeer, 2008). Compared to microglia, the functions of astrocytes are

poorly understood. Because they have been shown to produce both pro-inflammatory and anti-inflammatory agents, these cells are thought to have a dual role (McGeer & McGeer, 2008). Many ICAM-1-positive astrocytes are seen in the SN of PD brains and this may attract reactive microglia to the area since microglia carry the counter receptor LFA-1 (Miklossy et al., 2006). Indeed, α Syn has been shown to be capable of both of activating microglia and stimulating astrocytes to produce IL-6 and ICAM-1 (Klegeris et al., 2006). On the other hand, astrocytes have been shown to secrete a number of neurotrophic factors that protect dopaminergic neurons in some models of PD (McGeer & McGeer, 2008), but the mechanisms underlying most of these functions are not yet known. Astrocytes have been shown to express α Syn (Tanji et al., 2001). Interestingly, the presence of α Syn-containing inclusion bodies in astrocytes of sporadic PD brains has been observed (Braak et al., 2007; Terada et al., 2003; Wakabayashi et al., 2000). Finally, a recent study showed that astrocyte expression of A53T α Syn leads to the development of progressed paralysis, strong microglial activation, and neurodegeneration (Gu et al., 2010).

There is still little data on the role of oligodendrocytes in PD. α Syn-containing inclusions have been detected in this cell type in MSA, in DLB, and in PD (Campbell et al., 2001; Wakabayashi et al., 2000). McGeer and colleagues have reported the presence of complement-activated oligodendrocytes in the SN of PD cases (Yamada et al., 1992). Intriguingly, transgenic mice overexpressing Wt α Syn in oligodendrocytes have been observed to develop severe neurological alterations and neurodegeneration (Shults et al., 2005; Yazawa et al., 2005), drawing the attention to a possible role of these glial cells in PD.

5. Expression of α Syn by immunocompetent cells

Given that α Syn expression has been reported also in non-neuronal cells, it is currently thought to play a role besides dopamine release control. While searching for a link between the CNS and peripheral immune system in PD, Kim *et al.* (Kim et al., 2004a) found that α Syn was up-regulated in peripheral blood mononuclear cells (PBMC) at the gene level, in idiopathic PD *vs.* non-PD controls. Moreover, by *in vitro* transfection with Wt, A30P and A53T α Syn genes, they found that α Syn expression is correlated to glucocorticoid-sensitive apoptosis, possibly caused by the enhanced expression of glucocorticoid receptor (GR), caspase activation, CD95 (Fas) up-regulation, and ROS production. However, the increase in ROS production by overexpression of the α Syn mutants was markedly greater than for Wt α Syn. α Syn expression has also been found in cultured human macrophages (Tanji et al., 2002) and its expression levels have been reported to increase by stimulation with pro-inflammatory cytokine IL-1 β or LPS (Tanji et al., 2002), further supporting a role for α Syn in the inflammatory process. Finally, expression of α Syn in cultured human T cells, B cells, natural killer (NK) cells and in monocytes/macrophages, have been reported (Shin et al., 2000). Currently, it is not known whether expression or aggregation, of α Syn in T cells can be regulated by ligand activation of the T cell. This may be relevant as it could represent a key link between regulation of the adaptive immunity and α Syn expression levels.

6. α Syn and the adaptive immune response in PD

In the last few years, mounting evidence has pointed at a possible participation of the adaptive immune system in PD pathogenesis. However, whether this immune response

actually contributes to neurodegeneration, and in that case by which mechanism, remains unknown. The initial observations in PD patients that a small amount of CD8⁺ T-lymphocytes occur in proximity to degenerating nigral neurons (McGeer et al., 1988), and the occurrence in LB of components of the classical or antibody-triggered complement cascade (Yamada et al., 1992) had suggested a possible involvement of the adaptive immunity in the PD process. More recently, the finding of accumulated IgG in the SN of PD patients and increased expression of IgG-binding receptors on activated microglia (Orr et al., 2005), and the detection of anti- α Syn autoantibodies (AAb) in blood serum of PD patients (Papachroni et al., 2007), suggest that the pathological process may involve adaptive immune-mediated mechanisms. In addition, the observation that humoral immune mechanisms can trigger microglial-mediated neuronal injury in animal models of PD (He et al., 2002), and the finding by Standaert and colleagues of IgG deposition in mouse brains following AAV-mediated α Syn overexpression in the SN (Theodore et al., 2008), further support a role of the adaptive immune system in disease progression.

A possible consequence of the initial microglial activation in the affected regions of PD brains is the local permeabilization of the blood-brain barrier (BBB), leading to infiltration to the affected regions by B- and/or T-lymphocytes (Racke et al., 2000). Indeed, a remarkable T- and B-cell infiltration into the SN linked to α Syn overexpression was observed at the early stages, i.e. before the appearance of significant dopaminergic neuronal loss, reaching levels in the SN of up to 10-fold and 5-fold compared to controls (Theodore et al., 2008). A recent study by Hunot and colleagues (Brochard et al., 2009) has shown that CD8⁺ and CD4⁺ T-cells, but not B-cells, had invaded the brain in PD patients and in MPTP-treated mice during the course of neural degeneration. Furthermore, based on these results the authors propose that T-cell dependant toxicity is essentially mediated by CD4⁺ T-cells and requires the expression of FasL (Brochard et al., 2009). Given that the FasL pathway had been shown to produce proinflammatory cytokine responses in macrophages (Park et al., 2003), the authors speculate that the CD4⁺ Th FasL-mediated activation of microglia could participate in neuroinflammation and neurodegeneration processes in PD (Brochard et al., 2009).

Based on results obtained with an MPTP murine model of the disease, Gendelman and colleagues (Reynolds et al., 2010) have suggested that the α Syn-specific regulatory T-cells (Treg cells), which are regulatory components of the adaptive immunity, might be able to counteract the autoaggressive effector T-cell responses that exacerbate neuroinflammation (Benner et al., 2008), and therefore contribute to attenuate neurodegeneration in PD. Indeed, the same group has reported that microglial cells stimulated with N- α Syn are susceptible of essentially opposing immune regulatory responses by Treg cells (CD4⁺, CD245⁺) and effector T-cells (CD4⁺, CD25⁻) in culture (Reynolds et al., 2009a). By analysing an array of cytokines released by treated microglia, the authors found that, while the effector T-cell subset exacerbates microglial-mediated inflammation and may induce neurotoxic responses, Treg cells are able to suppress N- α Syn microglial-induced reactive-oxygen species (ROS) and NF- κ B activation and are proposed to be neuroprotective (Reynolds et al., 2009a). Furthermore, the study indicates that Treg cells can regulate microglial inflammation by inducing Fas-FasL-mediated apoptosis of N- α Syn-stimulated microglial cells (Reynolds et al., 2009a). By using a proteomic analysis, the authors further showed that these Treg cells can significantly alter the microglial protein expression profile for certain proteins linked to cell metabolism, migration, protein transport and degradation, redox biology, and cytoskeletal and bioenergetic

metabolism, to presumably attenuate the neurotoxic phenotype caused by N- α Syn stimulation (Reynolds et al., 2009b).

Thus far, accumulated data demonstrate that in the MPTP model of PD, misfolded and aggregated α Syn are secreted from neurons, which promotes pro-inflammatory M1-type microglia and cytotoxic T-cells, therefore amplifying neuronal damage. In sporadic human PD, it is currently unknown which factor triggers disease onset, but it has been proposed that under certain circumstances, a similar set of temporal and mechanistic events could transform neuroprotective microglia and T cells into cytotoxic cells, thereby accelerating disease progression (Appel et al., 2010). This way, activated microglia and the cytokine milieu that they generate might promote T-cell differentiation into different cell subsets in the context of PD (Appel et al., 2010). Indeed, it has been shown that M1 (pro-inflammatory) cells promote, whereas M2 (non-inflammatory) cells reduce, CD4⁺ Th1 cell proliferation and function (Verreck et al., 2004), but also that, conversely, T-cells can dictate microglial pro- or anti-inflammatory phenotypes (Giuliani et al., 2003; Kebir et al., 2007; Mount et al., 2007). Whether microglia dictate the specific T-cell phenotype or otherwise, that T-cells dictate the specific microglial phenotype (i.e. M1 *vs.* M2), is still unknown (Appel et al., 2010). But overall, the communication established between microglia, T cells and neurons seem to indicate that the immune response is not only a consequence of injury, but that it actively contributes to the balance between neuroprotection and neurotoxicity (Appel et al., 2010; Stone et al., 2009).

To analyse the possibility that humoral immunity may play a role in initiating or regulating inflammation, Orr *et al.* (Orr et al., 2005) analysed the association between nigral degeneration and humoral immune markers in brain tissue of patients with idiopathic or genetic PD and controls. All the patients with PD revealed IgG, but not IgM, binding on dopamine neurons. Moreover, the proportion of IgG-immunopositive neurons showed a negative correlation with the degree of cell loss in the SN, and positive correlation with the number of activated microglia. IgG was found to be concentrated at the cell surface of neurons, but also on their LB, and was shown to co-localize with α Syn. These results, in combination with their finding that activated microglia express high-affinity IgG receptors (Fc γ RI) in both idiopathic and genetic forms of PD, might suggest that the activation of microglia may be induced by neuronal IgG (Orr et al., 2005).

The question regarding the functional importance of antibodies against antigen-specific, disease-associated neuronal proteins still needs to be addressed. It has been demonstrated that an IgG fraction purified from serum of PD patients causes death of dopaminergic neurons *in vivo* following stereotaxic injection in the SN of experimental animals (Chen et al., 1998), and the presence of immunoglobulins in PD brain tissue have been proposed to lead to the targeting of dopaminergic nigral neurons for destruction (Orr et al., 2005). Currently, it remains unknown whether these anti- α Syn AAb are neurotoxic, or on the contrary, they actually have a neuroprotective role, as has been shown in a human α Syn transgenic mouse model of PD (Masliah et al., 2005).

A recent study has assessed the presence of auto-antibodies (AAb) against all three synucleins in the peripheral blood serum of PD patients and healthy controls (Papachroni et al., 2007). While the presence of AAb against β - and γ -Syn showed no association with PD, multi-epitopic AAb against α Syn were detected in 65% of all patients, with a strong correlation with the inherited mode of the disease. In addition, a recent study based on measuring AAb levels against monomeric, oligomeric, and fibrillar α Syn in serum from PD

patients (Gruden et al., 2011), showed that all three AAb specificities reached the highest values after 5-year of disease duration, and subsided in 10-year sufferers. Intriguingly, there was a ca. 15-fold increase in AAb titre values relative to monomeric α Syn (72% of patients), and a ca. 4-fold increase for α Syn oligomers (56% of patients). Moreover, the authors also found a decline in CD3⁺, CD4⁺ and CD8⁺ T-lymphocyte and B-lymphocyte subsets. Based on these results, they suggest that α Syn toxicity elimination by AAb in early PD pathology might be linked with the decline of lymphocyte subsets reflecting the influence of inflammatory and oxidative stress processes (Gruden et al., 2011).

Despite their potential involvement in PD pathogenesis and progression, the role of NK cells in PD has hardly been explored. NK cells are active members of the innate immune system that act as a first-line defence, and also mediate between the innate and adaptive immune systems (Salazar-Mather et al., 1996; Su et al., 2001). Interestingly, a recent study using blood samples from PD patients indicates that the NK activity increases as the disease advances (Mihara et al., 2008). Moreover, the study also showed that the NK cell content among the total lymphocytes of the PD group was higher than in the control group (Mihara et al., 2008).

7. Prospects for α Syn-based immunotherapy in PD

In addition to its well known importance in the pathogenesis of PD, α Syn is becoming a primary target for preventing or controlling the process of PD. In the late few years, vaccination for treating some neurodegenerative disorders has emerged as a potentially useful approach. Thus far, this avenue has been scarcely explored for PD. Importantly, immunization with α Syn was shown to generate a humoral response in a mouse model of PD (Masliah et al., 2005), producing beneficial albeit modest results on histopathological markers of the disease. On the contrary, using N- α Syn as the immunogen proved to elicit strong antigen-specific effector T cell responses in MPTP-intoxicated mice that caused exacerbated neuroinflammation and neurodegeneration (Benner et al., 2008). This response was further shown to be largely mediated by Th17 cells and causing Treg dysfunction (Reynolds et al., 2010). In addition, the authors demonstrated that Treg cells from mice treated with the neuropeptide VIP, known to promote Treg responses (Delgado et al., 2005; Gonzalez-Rey et al., 2006), can efficiently modulate N- α Syn-generated immunity in MPTP mice and confer neuroprotection (Reynolds et al., 2010), suggesting a possible novel therapeutic avenue for PD.

Given that microglial activation can maintain or even aggravate the disease process, blocking inflammation or shifting the balance between pro-inflammatory and anti-inflammatory states in a controlled manner, offers one of the most promising strategies for developing palliative (and maybe preventive) therapies for PD and related disorders. Epidemiological data have identified the non steroidal anti-inflammatory drug (NSAID) ibuprofen as neuroprotective for PD (Klegeris et al., 2007b). NSAIDs are thought to act on dopamine quinone formation and activation by α Syn of both astrocytes and microglia. On the other hand, Gendelman and colleagues demonstrated that T cells from mice immunized with Copolymer-1 (Cop-1), are able to attenuate microglial responses and lead to neuroprotection in a MPTP mouse model of PD (Benner et al., 2004). This neuroprotective effect was later found to be mediated by the CD4⁺ type of T cells, suggesting the possible involvement of Treg cells (Laurie et al., 2007). Later work by the

same group confirmed this hypothesis by showing that passive transfer to MPTP mice of activated Treg cells, but not effector T cells, efficiently suppressed microglial activation and afforded neuroprotection (Reynolds et al., 2007), suggesting that the immunomodulating abilities of Treg cells could potentially be utilized as a therapeutic approach against PD (Stone et al., 2009).

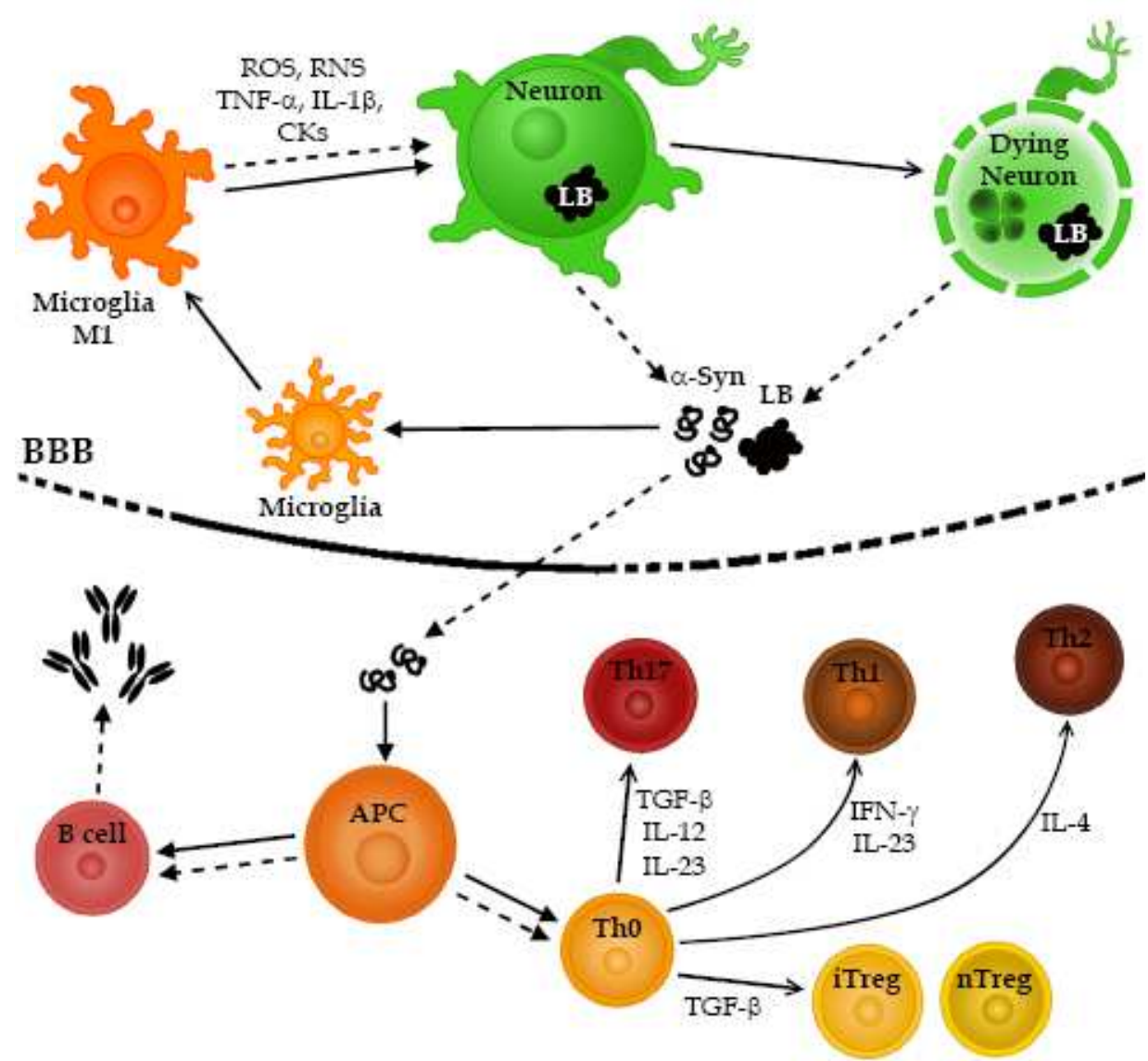


Fig. 1. Links between α Syn and the immune response in PD. Continuous arrows with filled tips: positive effect; continuous arrows with open tips: cell transformation; discontinuous arrows: secretion of cellular factors. APC, Antigen-processing cell; BBB, Blood-brain barrier; CKs, Chemokines; IL, Interleukin; IFN- γ , Interferon γ ; LB, Lewy bodies; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; TGF- β , Tumor growth factor β .

8. Conclusion

It is well established that PD onset and progression are characterized by sustained activation of microglia, linked to significant dopaminergic neuron loss in the SN of the brain. Over the last few years, it has become accepted that overexpression and aggregation of α Syn, an amyloid-like

protein, is linked to neurotoxicity through various proposed mechanisms, and may be one of the primary causes of the immunological abnormalities observed in PD. Recent studies with cellular and *in vivo* models of the disease indicate that increased levels of extracellular α Syn, both in its aggregated and non-aggregated forms, are found in a PD scenario.

Accumulated evidence has now established that aggregated extracellular α Syn is able to trigger the activation of microglia, inducing a highly detrimental cascade of neuroinflammation and neuronal demise. In addition, recent studies have demonstrated that non-aggregated α Syn can also have a substantial impact on microglial phenotype and cytokine release profile, especially in the cases of familial PD α Syn mutants. By releasing toxic factors, or by phagocytosing neighbouring cells, activated microglia and astrocytes are believed to form a self-perpetuating neuronal degeneration cycle. On the other hand, recent findings point at a possible role of the adaptive immune system involving α Syn, and the pathological process in PD. Clearly, further studies in this direction are necessary to help understand the complex immunological mechanisms underlying PD and the key, and possibly multiple, links between α Syn and the immune response in relation to in relation to pathogenesis (Figure 1).

In addition to trying to develop effective tools to prevent α Syn aggregation, modulating the innate immune response by intervening microglial activation, promoting a selective α Syn-specific humoral response, and manipulating the balance between effector and immunomodulatory T-cell populations, may be considered as highly promising therapeutic approaches for the treatment of PD and other synucleinopathies.

9. Acknowledgements

We gratefully acknowledge the financial support provided by the Spanish Ministry of Science and Innovation-Carlos III Institute of Health according to the 'Plan Nacional de I+D+I 2008-2011' (PS09-2252 to DP, and CP10/00527 to CR, with cofunding by FEDER), the Andalusian Ministry of Health (PI-2010-0824 to DP), and the PAIDI Program from the Andalusian Government (CTS-677 to DP). ALG holds a FPU predoctoral fellowship from the Spanish Ministry of Education. We are most indebted to Neuroinvest Foundation for its continuing support.

10. References

- Aloisi, F. (2001). Immune function of microglia. *Glia*, 36, (2), pp. (165-79).
- Alvarez-Erviti, L., et al. (2011). Alpha-synuclein release by neurons activates the inflammatory response in a microglial cell line. *Neurosci Res*, 69, (4), pp. (337-42).
- Anderson, J. P., et al. (2006). Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *J Biol Chem*, 281, (40), pp. (29739-52).
- Appel, S. H., et al. (2010). T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening? *Trends Immunol*, 31, (1), pp. (7-17).
- Auluck, P. K., et al. (2002). Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science*, 295, (5556), pp. (865-8).
- Austin, S. A., et al. (2006). Alpha-synuclein expression modulates microglial activation phenotype. *J Neurosci*, 26, (41), pp. (10558-63).

- Austin, S. A., et al. (2011). Lack of Alpha-Synuclein modulates microglial phenotype In Vitro. *Neurochem Res*, 36, (6), pp. (994-1004).
- Baglioni, S., et al. (2006). Prefibrillar amyloid aggregates could be generic toxins in higher organisms. *J Neurosci*, 26, (31), pp. (8160-7).
- Benner, E. J., et al. (2008). Nitrated alpha-Synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS One*, 3, (1), pp. (e1376).
- Benner, E. J., et al. (2004). Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A*, 101, (25), pp. (9435-40).
- Bennett, M. C. (2005). The role of alpha-synuclein in neurodegenerative diseases. *Pharmacol Ther*, 105, (3), pp. (311-31).
- Biasini, E., et al. (2004). Proteasome inhibition and aggregation in Parkinson's disease: a comparative study in untransfected and transfected cells. *J Neurochem*, 88, (3), pp. (545-53).
- Borghi, R., et al. (2000). Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects. *Neurosci Lett*, 287, (1), pp. (65-7).
- Bosco, D. A., et al. (2006). Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. *Nat Chem Biol*, 2, (5), pp. (249-53).
- Braak, H., et al. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*, 24, (2), pp. (197-211).
- Braak, H., et al. (2007). Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. *Acta Neuropathol*, 114, (3), pp. (231-41).
- Brochard, V., et al. (2009). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest*, 119, (1), pp. (182-92).
- Bucciantini, M., et al. (2004). Prefibrillar amyloid protein aggregates share common features of cytotoxicity. *J Biol Chem*, 279, (30), pp. (31374-82).
- Bucciantini, M., et al. (2002). Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature*, 416, (6880), pp. (507-11).
- Cabin, D. E., et al. (2002). Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. *J Neurosci*, 22, (20), pp. (8797-807).
- Campbell, B. C., et al. (2001). The solubility of alpha-synuclein in multiple system atrophy differs from that of dementia with Lewy bodies and Parkinson's disease. *J Neurochem*, 76, (1), pp. (87-96).
- Castagnet, P. I., et al. (2005). Fatty acid incorporation is decreased in astrocytes cultured from alpha-synuclein gene-ablated mice. *J Neurochem*, 94, (3), pp. (839-49).
- Castano, A., et al. (1998). Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J Neurochem*, 70, (4), pp. (1584-92).
- Colton, C. A., et al. (2006). Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. *J Neuroinflammation*, 3, pp. (27).
- Cookson, M. R. (2009). alpha-Synuclein and neuronal cell death. *Mol Neurodegener*, 4, pp. (9).
- Croisier, E. & M. B. Graeber (2006). Glial degeneration and reactive gliosis in alpha-synucleinopathies: the emerging concept of primary gliodegeneration. *Acta Neuropathol*, 112, (5), pp. (517-30).
- Croisier, E., et al. (2005). Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation*, 2, pp. (14).

- Chen, H., et al. (2005). Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease. *Ann Neurol*, 58, (6), pp. (963-7).
- Chen, H., et al. (2003). Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. *Arch Neurol*, 60, (8), pp. (1059-64).
- Chen, S., et al. (1998). Experimental destruction of substantia nigra initiated by Parkinson disease immunoglobulins. *Arch Neurol*, 55, (8), pp. (1075-80).
- Chiti, F. & C. M. Dobson (2006). Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem*, 75, pp. (333-66).
- Choi, D. Y., et al. (2010). Aging enhances the neuroinflammatory response and alpha-synuclein nitration in rats. *Neurobiol Aging*, 31, (9), pp. (1649-53).
- Chung, C. Y., et al. (2009). Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy. *J Neurosci*, 29, (11), pp. (3365-73).
- Danzer, K. M., et al. (2011). Heat-shock protein 70 modulates toxic extracellular alpha-synuclein oligomers and rescues trans-synaptic toxicity. *FASEB J*, 25, (1), pp. (326-36).
- Dedmon, M. M., et al. (2005). Heat shock protein 70 inhibits alpha-synuclein fibril formation via preferential binding to prefibrillar species. *J Biol Chem*, 280, (15), pp. (14733-40).
- Delgado, M., et al. (2005). Vasoactive intestinal peptide generates CD4+CD25+ regulatory T cells in vivo. *J Leukoc Biol*, 78, (6), pp. (1327-38).
- Delgado, M. & D. Ganea (2003). Neuroprotective effect of vasoactive intestinal peptide (VIP) in a mouse model of Parkinson's disease by blocking microglial activation. *FASEB J*, 17, (8), pp. (944-6).
- Desplats, P., et al. (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci U S A*, 106, (31), pp. (13010-5).
- El-Agnaf, O. M., et al. (2003). Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J*, 17, (13), pp. (1945-7).
- El-Agnaf, O. M., et al. (2006). Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB J*, 20, (3), pp. (419-25).
- Fernagut, P. O. & M. F. Chesselet (2004). Alpha-synuclein and transgenic mouse models. *Neurobiol Dis*, 17, (2), pp. (123-30).
- Gao, H. M. & J. S. Hong (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends Immunol*, 29, (8), pp. (357-65).
- Gao, H. M., et al. (2008). Neuroinflammation and oxidation/nitration of alpha-synuclein linked to dopaminergic neurodegeneration. *J Neurosci*, 28, (30), pp. (7687-98).
- Gao, H. M., et al. (2011). Neuroinflammation and alpha-Synuclein dysfunction potentiate each other driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. *Environ Health Perspect*, pp.
- Gasser, T. (2005). Genetics of Parkinson's disease. *Curr Opin Neurol*, 18, (4), pp. (363-9).
- Giasson, B. I., et al. (2000). Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science*, 290, (5493), pp. (985-9).
- Giorgi, C., et al. (2008). Ca²⁺ signaling, mitochondria and cell death. *Curr Mol Med*, 8, (2), pp. (119-30).
- Giuliani, F., et al. (2003). Vulnerability of human neurons to T cell-mediated cytotoxicity. *J Immunol*, 171, (1), pp. (368-79).
- Glass, C. K., et al. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell*, 140, (6), pp. (918-34).

- Golovko, M. Y., et al. (2009). The role of alpha-synuclein in brain lipid metabolism: a downstream impact on brain inflammatory response. *Mol Cell Biochem*, 326, (1-2), pp. (55-66).
- Gonzalez-Rey, E., et al. (2006). Vasoactive intestinal peptide generates human tolerogenic dendritic cells that induce CD4 and CD8 regulatory T cells. *Blood*, 107, (9), pp. (3632-8).
- Griffin, W. S., et al. (2006). Interleukin-1 mediates Alzheimer and Lewy body pathologies. *J Neuroinflammation*, 3, pp. (5).
- Gruden, M. A., et al. (2011). Immunoprotection against toxic biomarkers is retained during Parkinson's disease progression. *J Neuroimmunol*, pp.
- Gu, X. L., et al. (2010). Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. *Mol Brain*, 3, pp. (12).
- Halliday, G. M. & C. H. Stevens (2011). Glia: Initiators and progressors of pathology in Parkinson's disease. *Mov Disord*, 26, (1), pp. (6-17).
- Hasegawa, M., et al. (2002). Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions. *J Biol Chem*, 277, (50), pp. (49071-6).
- He, Y., et al. (2002). Role of Fc gamma receptors in nigral cell injury induced by Parkinson disease immunoglobulin injection into mouse substantia nigra. *Exp Neurol*, 176, (2), pp. (322-7).
- Hodara, R., et al. (2004). Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. *J Biol Chem*, 279, (46), pp. (47746-53).
- Huang, C., et al. (2006). Heat shock protein 70 inhibits alpha-synuclein fibril formation via interactions with diverse intermediates. *J Mol Biol*, 364, (3), pp. (323-36).
- Jenco, J. M., et al. (1998). Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by alpha- and beta-synucleins. *Biochemistry*, 37, (14), pp. (4901-9).
- Jo, E., et al. (2000). alpha-Synuclein membrane interactions and lipid specificity. *J Biol Chem*, 275, (44), pp. (34328-34).
- Kawai, T. & S. Akira (2007). TLR signaling. *Semin Immunol*, 19, (1), pp. (24-32).
- Kebir, H., et al. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med*, 13, (10), pp. (1173-5).
- Kim, S., et al. (2009). Alpha-synuclein induces migration of BV-2 microglial cells by up-regulation of CD44 and MT1-MMP. *J Neurochem*, 109, (5), pp. (1483-96).
- Kim, S., et al. (2004a). Alpha-synuclein induces apoptosis by altered expression in human peripheral lymphocyte in Parkinson's disease. *FASEB J*, 18, (13), pp. (1615-7).
- Kim, S., et al. (2004b). Alpha-synuclein, Parkinson's disease, and Alzheimer's disease. *Parkinsonism Relat Disord*, 10 Suppl 1, pp. (S9-13).
- Kim, Y. S. & T. H. Joh (2006). Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. *Exp Mol Med*, 38, (4), pp. (333-47).
- Klegeris, A., et al. (2007a). Functional ryanodine receptors are expressed by human microglia and THP-1 cells: Their possible involvement in modulation of neurotoxicity. *J Neurosci Res*, 85, (10), pp. (2207-15).
- Klegeris, A., et al. (2006). Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. *FASEB J*, 20, (12), pp. (2000-8).
- Klegeris, A., et al. (2007b). Therapeutic approaches to inflammation in neurodegenerative disease. *Curr Opin Neurol*, 20, (3), pp. (351-7).
- Klegeris, A., et al. (2008). Alpha-synuclein activates stress signaling protein kinases in THP-1 cells and microglia. *Neurobiol Aging*, 29, (5), pp. (739-52).

- Klucken, J., et al. (2004). Hsp70 Reduces alpha-Synuclein Aggregation and Toxicity. *J Biol Chem*, 279, (24), pp. (25497-502).
- Ko, L., et al. (2000). Sensitization of neuronal cells to oxidative stress with mutated human alpha-synuclein. *J Neurochem*, 75, (6), pp. (2546-54).
- Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci*, 19, (8), pp. (312-8).
- Kruger, R., et al. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet*, 18, (2), pp. (106-8).
- Laurie, C., et al. (2007). CD4+ T cells from Copolymer-1 immunized mice protect dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neuroimmunol*, 183, (1-2), pp. (60-8).
- Lee, D., et al. (2002). alpha-Synuclein exhibits competitive interaction between calmodulin and synthetic membranes. *J Neurochem*, 82, (5), pp. (1007-17).
- Lee, E. J., et al. (2010). Alpha-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. *J Immunol*, 185, (1), pp. (615-23).
- Lee, H. J., et al. (2005). Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci*, 25, (25), pp. (6016-24).
- Lee, J. K., et al. (2009a). Neuroinflammation in Parkinson's disease. *J Neuroimmune Pharmacol*, 4, (4), pp. (419-29).
- Lee, J. T., et al. (2008). Ubiquitination of alpha-synuclein by Siah-1 promotes alpha-synuclein aggregation and apoptotic cell death. *Hum Mol Genet*, 17, (6), pp. (906-17).
- Lee, P. H., et al. (2006). The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple system atrophy. *J Neural Transm*, 113, (10), pp. (1435-9).
- Lee, S. B., et al. (2009b). Identification of the amino acid sequence motif of alpha-synuclein responsible for macrophage activation. *Biochem Biophys Res Commun*, 381, (1), pp. (39-43).
- Lee, S. J. (2008). Origins and effects of extracellular alpha-synuclein: implications in Parkinson's disease. *J Mol Neurosci*, 34, (1), pp. (17-22).
- Lim, K. L. & J. M. Tan (2007). Role of the ubiquitin proteasome system in Parkinson's disease. *BMC Biochem*, 8 Suppl 1, pp. (S13).
- Long-Smith, C. M., et al. (2009). The influence of microglia on the pathogenesis of Parkinson's disease. *Prog Neurobiol*, 89, (3), pp. (277-87).
- Mandel, S., et al. (2005). Gene expression profiling of sporadic Parkinson's disease substantia nigra pars compacta reveals impairment of ubiquitin-proteasome subunits, SKP1A, aldehyde dehydrogenase, and chaperone HSC-70. *Ann N Y Acad Sci*, 1053, pp. (356-75).
- Martinez, J., et al. (2003). Parkinson's disease-associated alpha-synuclein is a calmodulin substrate. *J Biol Chem*, 278, (19), pp. (17379-87).
- Masliah, E., et al. (2005). Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron*, 46, (6), pp. (857-68).
- McGeer, P. L., et al. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology*, 38, (8), pp. (1285-91).
- McGeer, P. L. & E. G. McGeer (2008). Glial reactions in Parkinson's disease. *Mov Disord*, 23, (4), pp. (474-83).
- Mihara, T., et al. (2008). Natural killer cells of Parkinson's disease patients are set up for activation: a possible role for innate immunity in the pathogenesis of this disease. *Parkinsonism Relat Disord*, 14, (1), pp. (46-51).

- Miklossy, J., et al. (2006). Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. *Exp Neurol*, 197, (2), pp. (275-83).
- Mori, F., et al. (2002). Demonstration of alpha-synuclein immunoreactivity in neuronal and glial cytoplasm in normal human brain tissue using proteinase K and formic acid pretreatment. *Exp Neurol*, 176, (1), pp. (98-104).
- Mount, M. P., et al. (2007). Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci*, 27, (12), pp. (3328-37).
- Orr, C. F., et al. (2005). A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain*, 128, (Pt 11), pp. (2665-74).
- Ouchi, Y., et al. (2005). Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol*, 57, (2), pp. (168-75).
- Papachroni, K. K., et al. (2007). Autoantibodies to alpha-synuclein in inherited Parkinson's disease. *J Neurochem*, 101, (3), pp. (749-56).
- Park, D. R., et al. (2003). Fas (CD95) induces proinflammatory cytokine responses by human monocytes and monocyte-derived macrophages. *J Immunol*, 170, (12), pp. (6209-16).
- Park, J. Y., et al. (2008). Microglial phagocytosis is enhanced by monomeric alpha-synuclein, not aggregated alpha-synuclein: implications for Parkinson's disease. *Glia*, 56, (11), pp. (1215-23).
- Peng, X., et al. (2005). Alpha-synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells. *J Cell Sci*, 118, (Pt 15), pp. (3523-30).
- Perez, R. G. & T. G. Hastings (2004). Could a loss of alpha-synuclein function put dopaminergic neurons at risk? *J Neurochem*, 89, (6), pp. (1318-24).
- Polymeropoulos, M. H., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*, 276, (5321), pp. (2045-7).
- Ponomarev, E. D., et al. (2007). CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. *J Neurosci*, 27, (40), pp. (10714-21).
- Przedborski, S., et al. (2001). Oxidative post-translational modifications of alpha-synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *J Neurochem*, 76, (2), pp. (637-40).
- Racke, M. K., et al. (2000). The role of costimulation in autoimmune demyelination. *J Neuroimmunol*, 107, (2), pp. (205-15).
- Reynolds, A. D., et al. (2007). Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. *J Leukoc Biol*, 82, (5), pp. (1083-94).
- Reynolds, A. D., et al. (2008a). Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem*, 104, (6), pp. (1504-25).
- Reynolds, A. D., et al. (2008b). Nitrated alpha-Synuclein and microglial neuroregulatory activities. *J Neuroimmune Pharmacol*, pp. (54-74).
- Reynolds, A. D., et al. (2010). Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol*, 184, (5), pp. (2261-71).
- Reynolds, A. D., et al. (2009a). Nitrated α -synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets. *J Immunol*, 182, (7), pp. (4137-49).
- Reynolds, A. D., et al. (2009b). Proteomic studies of nitrated alpha-synuclein microglia regulation by CD4+CD25+ T cells. *J Proteome Res*, 8, (7), pp. (3497-511).
- Richter-Landsberg, C., et al. (2000). alpha-synuclein is developmentally expressed in cultured rat brain oligodendrocytes. *J Neurosci Res*, 62, (1), pp. (9-14).

- Roodveldt, C., et al. (2009). Chaperone proteostasis in Parkinson's disease: stabilization of the Hsp70/alpha-synuclein complex by Hip. *EMBO J*, 28, (23), pp. (3758-70).
- Roodveldt, C., et al. (2008). Immunological features of alpha-synuclein in Parkinson's disease. *J Cell Mol Med*, 12, (5B), pp. (1820-9).
- Roodveldt, C., et al. (2010). Glial innate immunity generated by non-aggregated alpha-synuclein in mouse: differences between wild-type and Parkinson's disease-linked mutants. *PLoS One*, 5, (10), pp. (e13481).
- Rott, R., et al. (2008). Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. *J Biol Chem*, 283, (6), pp. (3316-28).
- Salazar-Mather, T. P., et al. (1996). NK cell trafficking and cytokine expression in splenic compartments after IFN induction and viral infection. *J Immunol*, 157, (7), pp. (3054-64).
- Sanchez-Guajardo, V., et al. (2010). Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. *PLoS One*, 5, (1), pp. (e8784).
- Sanchez-Pernaute, R., et al. (2004). Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease. *J Neuroinflammation*, 1, (1), pp. (6).
- Schiess, M. C., et al. (2010). CSF from Parkinson disease patients differentially affects cultured microglia and astrocytes. *BMC Neurosci*, 11, pp. (151).
- Shin, E. C., et al. (2000). Expression patterns of alpha-synuclein in human hematopoietic cells and in *Drosophila* at different developmental stages. *Mol Cells*, 10, (1), pp. (65-70).
- Shults, C. W., et al. (2005). Neurological and neurodegenerative alterations in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. *J Neurosci*, 25, (46), pp. (10689-99).
- Souza, J. M., et al. (2000). Dityrosine cross-linking promotes formation of stable alpha-synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J Biol Chem*, 275, (24), pp. (18344-9).
- Spillantini, M. G., et al. (1998). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci U S A*, 95, (11), pp. (6469-73).
- Stefani, M. & C. M. Dobson (2003). Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J Mol Med*, 81, (11), pp. (678-99).
- Stone, D. K., et al. (2009). Innate and adaptive immunity for the pathobiology of Parkinson's disease. *Antioxid Redox Signal*, 11, (9), pp. (2151-66).
- Su, H. C., et al. (2001). NK cell functions restrain T cell responses during viral infections. *Eur J Immunol*, 31, (10), pp. (3048-55).
- Su, X., et al. (2009). Mutant alpha-synuclein overexpression mediates early proinflammatory activity. *Neurotox Res*, 16, (3), pp. (238-54).
- Su, X., et al. (2008). Synuclein activates microglia in a model of Parkinson's disease. *Neurobiol Aging*, 29, (11), pp. (1690-701).
- Tanji, K., et al. (2002). Upregulation of alpha-synuclein by lipopolysaccharide and interleukin-1 in human macrophages. *Pathol Int*, 52, (9), pp. (572-7).
- Tanji, K., et al. (2001). Expression of beta-synuclein in normal human astrocytes. *Neuroreport*, 12, (13), pp. (2845-8).

- Terada, S., et al. (2003). Glial involvement in diffuse Lewy body disease. *Acta Neuropathol*, 105, (2), pp. (163-9).
- Theodore, S., et al. (2008). Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *J Neuropathol Exp Neurol*, 67, (12), pp. (1149-58).
- Thomas, M. P., et al. (2007). Ion channel blockade attenuates aggregated alpha synuclein induction of microglial reactive oxygen species: relevance for the pathogenesis of Parkinson's disease. *J Neurochem*, 100, (2), pp. (503-19).
- Tofaris, G. K., et al. (2003). Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. *J Biol Chem*, 278, (45), pp. (44405-11).
- Tokuda, T., et al. (2006). Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. *Biochem Biophys Res Commun*, 349, (1), pp. (162-6).
- Tokuda, T., et al. (2010). Detection of elevated levels of α -synuclein oligomers in CSF from patients with Parkinson disease. *Neurol*, 75, (20), pp. (1766-72).
- Verreck, F. A., et al. (2004). Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. *Proc Natl Acad Sci U S A*, 101, (13), pp. (4560-5).
- Vila, M., et al. (2001). The role of glial cells in Parkinson's disease. *Curr Opin Neurol*, 14, (4), pp. (483-9).
- Wakabayashi, K., et al. (2000). NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathol*, 99, (1), pp. (14-20).
- Wersinger, C.&A. Sidhu (2006). An inflammatory pathomechanism for Parkinson's disease? *Curr Med Chem*, 13, (5), pp. (591-602).
- Wu, D. C., et al. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci*, 22, (5), pp. (1763-71).
- Wyss-Coray, T.&L. Mucke (2002). Inflammation in neurodegenerative disease--a double-edged sword. *Neuron*, 35, (3), pp. (419-32).
- Yamada, T., et al. (1992). Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins. *Acta Neuropathol*, 84, (1), pp. (100-4).
- Yazawa, I., et al. (2005). Mouse model of multiple system atrophy alpha-synuclein expression in oligodendrocytes causes glial and neuronal degeneration. *Neuron*, 45, (6), pp. (847-59).
- Yu, S., et al. (2007). Extensive nuclear localization of alpha-synuclein in normal rat brain neurons revealed by a novel monoclonal antibody. *Neuroscience*, 145, (2), pp. (539-55).
- Zarranz, J. J., et al. (2004). The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol*, 55, (2), pp. (164-73).
- Zhang, W., et al. (2007). Microglial PHOX and Mac-1 are essential to the enhanced dopaminergic neurodegeneration elicited by A30P and A53T mutant alpha-synuclein. *Glia*, 55, (11), pp. (1178-88).
- Zhang, W., et al. (2005). Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J*, 19, (6), pp. (533-42).
- Zhou, Y., et al. (2005). Microglial activation induced by neurodegeneration: a proteomic analysis. *Mol Cell Proteomics*, 4, (10), pp. (1471-9).



Towards New Therapies for Parkinson's Disease

Edited by Prof. David Finkelstein

ISBN 978-953-307-463-4

Hard cover, 396 pages

Publisher InTech

Published online 02, November, 2011

Published in print edition November, 2011

Parkinson's disease (PD) is characterised clinically by various non-motor and progressive motor symptoms, pathologically by loss of dopamine producing cells and intraneuronal cytoplasmic inclusions composed primarily of α -synuclein. By the time a patient first presents with symptoms of Parkinson's disease at the clinic, a significant proportion of the cells in the substantia nigra have already been destroyed. This degeneration progresses despite the current therapies until the cell loss is so great that the quality of normal life is compromised. The dopamine precursor levodopa is the most valuable drug currently available for the treatment of PD. However for most PD patients, the optimal clinical benefit from levodopa decreases around five to six years of treatment. The aim of the chapters of this book is to work towards an understanding in the mechanisms of degeneration and to develop disease modifying therapies.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Cintia Roodveldt, Adahir Labrador-Garrido, Guillermo Izquierdo and David Pozo (2011). Alpha-Synuclein and the Immune Response in Parkinson's Disease, Towards New Therapies for Parkinson's Disease, Prof. David Finkelstein (Ed.), ISBN: 978-953-307-463-4, InTech, Available from:

<http://www.intechopen.com/books/towards-new-therapies-for-parkinson-s-disease/alpha-synuclein-and-the-immune-response-in-parkinson-s-disease>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen