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Immunological features of α-synuclein in Parkinson's disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized pathologically by the presence, in the brain, of intracellular protein inclusions highly enriched in aggregated α-synuclein (αSyn), known as Lewy bodies. The onset of PD is accompanied by a local immune reaction in regions of the brain affected by the inclusions, although the mechanism that leads to pathogenesis is far from clear. It is, however, established that disease onset and progression are characterized by sustained activation of microglia, which is linked to significant dopaminergic neuron loss in the *substantia nigra*. A recent body of evidence indicates that aggregated or modified αSyn can indeed trigger the activation of microglia, inducing a lethal cascade of neuroinflammation and eventually, neuronal loss, pointing at aggregated and modified forms of αSyn as a primary cause of PD pathogenesis. By releasing toxic factors, or by phagocytosing neighbouring cells, activated microglia and astrocytes may form a self-perpetuating cycle for neuronal degeneration. Additional findings suggest a link between αSyn and humoural-mediated mechanisms in PD. In this review, we attempt to recapitulate our current understanding of PD physiopathology focused on αSyn and its links with the immune system, as well as of novel and promising therapeutic avenues for the treatment of PD and of other synucleinopathies.

Keywords: Parkinson's disease • α-synuclein • aggregation • amyloid • immune response • microglia • neurodegeneration • physiopathology • therapy

Introduction

Parkinson's disease (PD), a progressive neurodegenerative disorder characterized by resting tremor, muscular rigidity and gait disturbances [1, 2], is pathologically characterized by the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and their termini in their dorsal stratium [3]. The pathological hallmark of PD is the presence of deposits of aggregated α-synuclein (αSyn) in intracellular inclusions known as Lewy bodies (LB) [4, 5]. Three missense mutations, A53T, A30P and E46K, as well as multiple copies of the wild-type (Wt) αSyn gene, are linked to familial PD, which is often manifested in early onset of the disease [6–9]. However, the factors contributing to sporadic PD, which represents the majority of PD cases, are not known, and in either case, the cellular and molecular mechanisms underlying the pathological actions of αSyn are not well understood.

αSyn, together with β- and γ-synucleins, belong to the expanding family of synucleins, a group of closely related, brain-enriched

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proteins. αSyn is a 140-amino acid protein that is highly expressed in pre-synaptic terminals, in particular in the neocortex, hippocampus and SN [10], but is also found in other regions of neurons as well as within astrocytes and oligodendroglia [11, 12]. It is known to interact with a variety of proteins [13, 14] and also with lipid vesicles [15], and it may be involved in lipid metabolism [16, 17]. In its free state αSyn is intrinsically disordered, with no well-defined structure as determined *in vitro*, although NMR studies have shown long-range interactions between the acidic C-terminal region and the amyloidogenic central region [18–20]. Interactions with acidic phospholipids membranes result in induction of helical conformation in its N-terminal region [21, 22].

The physiological functions of αSyn are still being established. Its interaction with pre-synaptic membranes suggests that one function may be the regulation of synaptic vesicle pools, including dopamine control [23]. A role as a molecular chaperone, assisting in the folding and refolding of certain synaptic proteins, was also proposed [24]. Although αSyn is normally considered as a cytoplasmic protein, it has also been found to be present in extracellular biological fluids, including human cerebrospinal fluid and blood plasma [25, 26]. One mechanism that leads to the presence of extracellular αSyn is thought to be membrane permeability as a result of cell death, although it has also been reported that monomeric and aggregated αSyn may be secreted by an unconventional endoplasmic reticulum/Golgi-independent exocytosis pathway [26].

αSyn can self-assemble *in vitro* to form ordered fibrillar aggregates, characterized by a cross β-sheet structure, that are morphologically similar to the aggregates found in LB, in neuritic plaques in Alzheimer's disease (AD) as well as in deposits associated with other amyloidogenic processes (reviewed in [27]). A significant international effort has been made to elucidate the biophysical basis for the aggregation of αSyn [28, 29]. The initial phase of the aggregation process is thought to involve the formation of oligomeric species which, according to accumulating experimental evidence, are more toxic to cells than the mature fibrils into which they develop [30, 31]. These and other findings suggest a common structure-linked toxicity among prefibrillar species, and it has been proposed that similar mechanisms may in general contribute to pathogenesis for this group of diseases [32, 33]. Overall, many hypotheses have been put forward that propose that αSyn induces a 'gain of toxic function' upon aggregation [27].

Importance of inflammation processes in PD pathology

Inflammation is the first response of the immune system to pathogens. In acute conditions, it protects tissue against invading agents and promotes healing. However, when sustained chronically, it can cause serious damage to the host's own tissue [34]. Although the central nervous system (CNS) has been traditionally seen as an immune-privileged organ, it has become increasingly evident that inflammation is actively involved in the pathogenesis of many degenerative diseases including multiple sclerosis (MS), AD, and PD (see references in [34]). A robust and highly localized inflammatory response mediated by reactive microglia and reactive astrocytes is prominent in affected areas of the SN in PD brains (reviewed in [34]).

Microglia are the main immunocompetent cells within the CNS [35], capable of antigen presentation to lymphocytes [36] and rapid activation in response to pathological change in the CNS [34]. Microglial cells are evenly distributed throughout the normal brain, in close proximity to neurons and astrocytes. At the site of inflammation, activated microglia change their morphology, express increased levels of major histocompatibility complex (MHC) antigens and become phagocytic [37, 38]. In addition, they start releasing inflammatory cytokines that amplify the inflammatory response by activating and recruiting other cells to the brain lesion [34]. Microglia can also release potent neurotoxins, which may cause neuronal damage, and, indeed, sustained overactivation of microglia has been observed in a variety of neurodegenerative diseases [34].

Evidence of microglial attack in PD is supported by findings within three different areas of research: epidemiological studies, animal models and cells in culture [39]. Epidemiological studies that investigated the effects of using anti-inflammatory agents showed that taking ibuprofen regularly was associated with a 35% lower risk of PD [40, 41], supporting the concept that inflammatory attack is contributing to dopaminergic neuronal loss. *In vivo* findings show that the specific early up-regulation of SN microglia in PD correlates with disease severity and dopamine terminal loss, but not with disease duration [42, 43]. This correlation may not be unexpected if one considers that dopaminergic cells of the CNS are highly vulnerable to oxidative and inflammatory attack. Indeed, the animal models of PD currently in use are based on oxidative stress or inflammatory stimulation to the SN area (reviewed in [39]).

The animal models of PD are generally of either of two types; 'type 1' is based on the administration of oxidizing compounds that are preferentially taken up by dopaminergic cells (*e.g.* rotenone, 6-hydroxydopamine); and 'type 2' is based on localized administration of inflammatory agents, mainly lipopolysaccharide (LPS), 1-methyl-4-phenyl-1,2,3,4-tetrahyfropyridine (MPTP) or αSyn [39]. The MPTP model indicates that inflammation in the SN can be self-sustaining whereas the αSyn model indicates that overexpression of this endogenous protein can provide a source of inflammation [39]. In addition, the transgenic mouse models for PD that have been described utilize neuron-specific promoters to overexpress Wt or mutant αSyn locally (reviewed in [44, 45]), and have been shown to capture major features of PD such as locomotor defects, the formation of inclusion-like structures and neurotoxicity. Studies with both animal model and cells in culture have shown, albeit indirectly, that dopaminergic cells are highly sensitive to inflammatory attack [45, 46] and that microglial cells can be activated to mount such an attack [47].

Stimulation of microglia by αSyn

Several studies have demonstrated that extracellular and nigral aggregates immunoreactive to αSyn are often surrounded by activated microglia or inflammatory mediators [48, 49]. This phenomenon mirrors what has been described in AD, where amyloid plaques are usually co-localized with clusters of activated microglia [50]. Microglial cells from αSyn knockout mice have been shown to exhibit a remarkably different morphology compared to Wt cells [47]. Moreover, after activation, the microglial cells secrete elevated levels of pro-inflammatory cytokines, such as tumour necrosis factor (TNF)-α and interleukin (IL)-6 [47], indicating that αSyn plays a critical role in modulating the activation state of microglia. Still, the mechanisms underlying microglial activation in PD, and how the latter affects neuronal survival remains poorly understood. One line of investigation posits that neuronal death itself drives the microglial immune response [51–53], but others have proposed that activation could occur as a consequence of release of aggregated protein from the cytosol or within LB to the extracellular space. In such a situation, the death of dopaminergic neurons would lead to the release of protein aggregates that would in turn activate microglia, inducing a lethal cascade of neuroinflammation and neuronal demise [54–56]. Therefore, although PD is not an autoimmune disease, evidence of localized attack by microglia places it in the autotoxic category [39].

Several recent *in vitro* studies have focused on the effects of extracellular αSyn on microglial activation (Table 1). Zhang *et al.* [54] first reported that exogeneous, aggregated αSyn activates microglial cells, which then become toxic towards cultured dopaminergic neurons. This is particularly relevant, because aggregated αSyn has been shown to be secreted by exocytosis from neuronal cells [26] although it might also be released by membrane permeability from dead cells [57]. The study found that microglial phagocytosis of αSyn and activation of NADPH oxidase were critical in microglial activation induced by aggregated αSyn, and neurotoxicity [54]. The toxicity level was lower in mice null for NADPH oxidase, indicating that oxygen-free radicals generated by the activated microglia, are likely to play a significant role in neurotoxicity. It was also reported that induction of NADPH oxidase is linked to direct activation of the Mac-1 receptor, and not by αSyn internalization *via* a scavenger [58]. Finally, it was proposed that nigral neuronal damage, regardless of its aetiology, might release aggregated αSyn, which could then lead to persistent and progressive neuronal damage [58].

Several works have concluded that the mutated, disease-causing forms of αSyn are more potent stimuli of microglial activation than the Wt protein, indicating a possible molecular mechanism for the increased toxicity of the αSyn mutants linked to familial PD [57]. Likewise, it has been shown that aggregated αSyn has a stronger stimulating effect on microglia [56] than that of non-aggregated αSyn (Table 1). Recent investigations demonstrated that aggregated αSyn induces a neurotoxic inflammatory microglial phenotype that accelerates dopaminergic neuron loss [54, 56, 59, 60]. By integrating genomic and proteomic techniques, Gendelman and coworkers [61] created a fingerprint of microglial cell activation following its interactions with aggregated, nitrated N-αSyn (N-αSyn) – previously found to form oligomers through dityrosine crosslinking [62]. They observed a neuroinflammatory phenotype that was capable of mediating neuronal toxicity that correlates with human disease (Table 1). These results appear relevant because αSyn proteins nitrated at four tyrosine (Tyr) positions have been detected in LB of human brains with PD [51]. It would be interesting to pursue analogous studies with other αSyn forms that are post-translationally modified and also found in LB, *e.g.* C-terminally truncated, or serine (Ser)^{129}-phosphorylated αSyn (reviewed in [63]).

αSyn-triggered stimulation of the innate immune system

Upon activation, microglia and astrocytes can secrete neurotoxic products and inflammatory cytokines [39]. The latter ones are produced in order to communicate and orchestrate the immune response to disease, or injury, often by inducing proliferation [64]. The cytokines TNF-α, IL-1β, IL-2, IL-4, IL-6, tumour growth factor (TGF)-α, TGF-β1, TGF-β2 have all been reported to be present at higher levels in the nigrostriatal region and cerebrospinal fluid of patients with PD or dementia with LB ([64] and references therein). Activated microglia may also produce large amounts of superoxide radicals, which may be the major source of the oxidative stress believed to be largely responsible for dopaminergic cell death in PD.

A number of cytokines and metabolites have been shown to be significantly up-regulated as a result of αSyn-induced activation of microglia *in vitro* (Table 1), including IL-1β, IL-6, intercellular adhesion molecule (ICAM)-1, TNF-α, interferon (IFN)-γ, MCP-1, O_2^-, iROS, and PEG_2, glutamate and iCys. Activation appears to be mainly mediated by the mitogen-activated pathway (MAP) kinase, NADPH (shown for stimulation with aggregated N-αSyn), and NF-κB, pathways (Table 1). In general, disease-linked αSyn mutants show a stronger effect on cytokine release than does the Wt protein. It may also be relevant that, under some conditions, αSyn tested variants require the presence of IFN-γ in the medium to effectively induce microglial activation or cytotoxicity (Table 1), indicating a synergy between this cytokine and αSyn. Contrary to the increase in nitric oxide species (or nitric oxide synthetase) observed for LPS-stimulated neurons or microglial cells [65–67], aggregated αSyn-treatment of microglia did not seem to significantly alter nitrite levels [54] (Table 1). Interestingly, analysis of the microglia transcriptome by Gendelman and coworkers [61] after stimulation with aggregated N-αSyn, revealed a significant up-regulation of the toll-like receptor 2 (TLR-2) gene. TLRs sense the molecular signatures of microbial pathogens, and play a fundamental role in innate immune responses, inducing the expression of diverse inflammatory genes (for a review, see [68]). It therefore seems plausible that cells challenged with αSyn, or at least with certain forms of αSyn, could become hyper-responsive to inflammatory signals.

Table 1 Activation profile of αSyn-stimulated glial cells

αSyn	Co-stimulation	Cytokines, receptors or proteins affected	Pathways involved	Stimulated cells	Effect	Ref.
Wt, A30P, E46K, A53T	IFN-γ	↑ ICAM-1, ↑ IL-6	P38, JNK, ERK1/2, MAPK	Human astrocytes and U-373 MG astrocytoma cells		[93]
Wt, A30P, E46K, A53T, Δ71–82	IFN-γ	↑ TNF-α (but only A53T w/o IFN-γ), ↑ IL-1β		Human microglia	Reduced monocytic cell viability, but only with IFN-γ	[57]
Wt	IFN-γ		P38, JNK, ERK1/2, MAPK	Human microglia	↓ Viability dopaminergic cells	[57]
Aggregated Wt		↑ Extracellular O_2^-, ↑ Intracellular ROS, ↑ PEG_2	NADPH oxidase	Rat primary mesenchepalic neuron-glia cell culture	↓ Dopamine uptake, cell loss, morphological alterations of dopaminergic cells	[54]
Wt A30P A53T			NADPH oxidase Binding Mac-1	Rat primary mixed neuron-glial cell culture	↑ O_2^- ↑ Intracellular ROS	[58]
Aggregated, nitrated Wt				Microglia (C57BL/6J mice)	↑ H_2O_2	[60]
Aggregated *versus* non-aggregated, nitrated Wt		↓ Actin, galectin 3 and 14–3-3 sigma	NF-κB	Microglia (C57BL/6J mice)		[56]
		↑ Biliverdin reductase calmodulin and ferritin light chain				
		↑ Glutamate and extracellular Cys				
		↓ Intracellular glutamate and intracellular Cys and GSH (No changes with unaggregated N-αSyn)				
Aggregated, Nitrated Wt		↑ TNF-α ↑ IL-6, ↑ MCP-1, ↑ IFN-γ	NF-κB (↑ mRNA of *Tnf, Ccl2, Il6, Il1-β, Nfkb*) MAPK (↑ mRNA of *Fos, Raf1*)	Microglia (C57BL/6J mice)	↑ Dopaminergic cell death (less for non-nitrated, only with aggregated αSyn)	[61]
			↑ Hsp70, SOD, Peroxiredoxins 1, 4, and 5			
			↓ Aconitase and ↑ calmodulin			
			↓ β-actin, L-plastin, α-tubulin			

The generation of reactive oxygen species (ROS) by microglia activated by αSyn [60] (or other stimulants) can result in oxidation and nitration of proteins, DNA modification, and lipid peroxidation, leading to neurotoxicity [54]. Oxidation [62, 69] and nitration [51, 62] of αSyn can in turn, lead to the formation of more aggregates, and hence result in increased cytotoxicity. Consistent with this, Bosco *et al.* have shown that high levels of oxidized cholesterol metabolites in brains from PD and dementia with LB patients, accelerate the conversion of soluble αSyn into amyloid fibrils [70].

Recently, McGeer and coworkers [71] found that human microglia constitutively express ryanodine receptors (RyRs), which help to mediate the efflux of Ca^{2+} ions from intracellular stores. Elevated levels of free intracellular Ca^{2+} ($[Ca^{2+}]_i$) lead to Ca^{2+} signals that may initiate both short- and long-term cellular responses, and indeed sustained and uncontrolled $[Ca^{2+}]_i$ increases can lead to cell death (for a review, see [72]). Interestingly, αSyn stimulation of microglia, in combination with IFN-γ, has been found to induce toxicity of human monocytic cells by producing neurotoxic secretions, and this toxicity can be diminished with specific RyR ligands [71].

Other proteins up-regulated by αSyn-triggered microglial activation

Reynolds *et al.* [61], by determining the activated microglia proteome profile, found that aggregated N-αSyn activation of microglia results in differential expression of several proteins (Table 1). These range from proteins involved in oxidative stress, cell adhesion, glycolysis, regulation of growth, and migration, to proteins of the cytoskeleton. It is intriguing that two of those proteins found to be particularly highly up-regulated, calmodulin and ubiquitin, have been shown to interact with αSyn with possible functional consequences. Calmodulin has been shown, *in vitro*, to bind to αSyn in a Ca^{2+}-dependent manner [73] and to inhibit fibrillation of αSyn [74]. Several studies have reported that a fraction of αSyn found in LB is mono-ubiquitinated [75, 76], but the role of this modification remains unclear. Recently, it has been demonstrated that the ubiquitin–protein isopeptide ligase, seven in absentia homologue, directly interacts with and monoubiquitinates αSyn, promoting its aggregation [77, 78] and stimulating apoptosis [78]. There is also evidence implicating a role for the ubiquitin–proteasome system (UPS) in PD (reviewed in [79]), linking some parkin mutations to UPS aberrations and altered protein degradation. The role of αSyn in UPS impairment is less clear, although it has been reported that overexpression of αSyn (in particular the disease-associated mutants) or an aggregated form of Wt αSyn, can inhibit the proteasome function [80–83]. Also of interest in activated microglia expression profile are the elevated levels of Hsp70. This chaperone has been demonstrated to inhibit αSyn aggregation *in vitro* [84], in neuroglioma cells [85], as well as in fly [86] and mouse [85] models of PD, protecting cells from the cytotoxic effects of aggregates.

αSyn and apoptosis of immune cells

In PD patients, disturbed cellular and humoural functions in the peripheral immune system have been described, including the occurrence of auto-antibodies (AAbs) against neuronal structures and the presence of a high number of microglial cells expressing the histocompatibility leukocyte (antigen HLA-DR) in the SN [87]. In addition to cytokines, apoptosis-related proteins are elevated in the stratium of PD patients [88, 89].

While searching for a link between the CNS and the peripheral immune system in PD, Kim *et al.* [90] observed that αSyn was up-regulated in peripheral blood mononuclear cells at the gene level, in idiopathic PD *versus* non-PD controls. Moreover, by *in vitro* transfection with Wt, A30P and A53T αSyn genes, they found that αSyn expression is correlated to glucocorticoid-sensitive apoptosis, possibly caused by the enhanced expression of glucocorticoid receptor, caspase activation, CD95 (Fas) up-regulation and ROS production. However, the increase in ROS production by overexpression of the αSyn mutants was markedly greater than for the Wt protein. It has also been reported that overexpression of C-terminally truncated αSyn in transfected astrocytes, especially when treated with TNF-α, induces cell death by apoptosis [91].

Links between αSyn and astrocytes or oligodendrocytes

Compared to microglia, the functions of astrocytes are poorly understood. These cells migrate to a site of injury and develop hypertrophic morphology. As opposed to microglia, they are thought not to attack a pathological target, but rather to seal it off. Because they have been shown to elaborate both pro- and anti-inflammatory agents, these cells appear to have a dual role in the immune homeostasis [39]. Many ICAM-1 positive astrocytes are seen in the SN of the brains of PD patients and this phenomenon may attract reactive microglia to the area because microglia carry the counter receptor LFA-1 [92]. Indeed, αSyn is capable of stimulating astrocytes to produce IL-6 and ICAM-1 [93] (Table 1). The action of αSyn on astrocytes is believed to be through receptors, but the identity of the latter is currently unknown; however, antagonists of such putative αSyn receptors might constitute novel PD-specific anti-inflammatory agents. Finally, astrocytes have also been shown to secrete a number of neurotrophic factors that protect dopaminergic neurons in some models of PD ([39] and references therein), but the mechanisms underlying most of these functions are not yet known.

There is very little data on oligodendrocytes in PD, although Yamada *et al.* have reported the presence of complement-activated oligodendrocytes in the SN of PD cases [49]. As in astrocytes [94], αSyn-containing inclusions have been reported in oligodendrocytes [94, 95], both in dementia with LB and in PD.

αSyn and the humoural immune system in PD

The observation in PD patients that small numbers of $CD8^+$ T lymphocytes occur in proximity to degenerating nigral neurons [48] and that components of the classical or antibody-triggered complement cascade occur in LB [49], suggests that the pathological process may involve humoural-mediated mechanisms [43]. In addition, humoural immune mechanisms can trigger microglial-mediated neuronal injury in animal models of PD [96]. To analyse the possibility that humoural immunity may play a role in initiating or regulating inflammation, Orr *et al.* [43] analysed the association between nigral degeneration and humoural immune markers in brain tissue from patients with idiopathic or genetic PD and controls. All the patients with PD had significant levels of immunoglobulin G (IgG), but not of 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 a positive correlation with the number of activated microglia. IgG was found to be concentrated at the cell surfaces of neurons, but also on their LB, and was shown to co-localize with αSyn. These results, in combination with the finding that activated microglia express high-affinity IgG receptors (FcγRI) in both idiopathic and genetic forms of PD, could suggest that the activation of microglia may be induced by neuronal IgG [43]. Even though the identity of the antigen or antigens responsible for IgG binding to dopamine neurons remains unknown, it is possible to argue that IgG binding to dopamine neurons in PD may result in their selective targeting and subsequent destruction by activated microglia [43].

A possible consequence of the initial microglial activation in the affected regions of PD brains is the local permeabilization of the blood-brain barrier, leading to infiltration to the affected regions by B and/or T lymphocytes, and believed to constitute a critical step in the development of autoimmune reactions [97]. To explore the possible involvement of αSyn in steps that go beyond the initiation of the local immune response in PD, Papachroni *et al.* [98] have assessed the presence of AAbs against all three synucleins in the peripheral blood serum of PD patients and of healthy control individuals. Although the presence of AAbs against β- and γSyn showed no correlation with PD, AAbs against αSyn were detected in 65% of all patients. Moreover, the presence of these AAbs strongly correlated with inherited forms of the disease, but not with the sporadic form. The observation that the AAbs generated are multi-epitopic, confirms that the entire αSyn molecule is auto-immunogenic, and eliminates the possibility that the observed immune reaction could be the result of cross-reactivity with another, similar antigen [98].

The question regarding the functional importance of antibodies against disease-associated neuronal proteins remains wide open. It has been demonstrated that an IgG fraction purified from the serum of PD patients causes the death of dopaminergic neurons *in vivo* following stereotaxic injection into the SN of experimental animals [99], and the presence of immunoglobulins in PD brain tissue could lead to the targeting of dopaminergic nigral neurons for destruction [43]. Currently, whether or not these anti-αSyn AAbs are neurotoxic, or by contrast, they have a neuroprotective role as shown in a human αSyn transgenic mouse model of PD [100], remains unknown. Future studies aimed at clarifying a role for anti-αSyn AAbs, should evaluate their potential for diagnosis and therapy of PD [98].

Expression of αSyn in immunocompetent cells

It has been reported that αSyn is also expressed in astrocytes and that its level is increased by stimulation with the pro-inflammatory cytokine IL-1β [101]. Also, αSyn has been found to be expressed in cultured human macrophages [102]. In this case, αSyn protein (but not mRNA) levels were seen to be up-regulated by stimulation with LPS and IL-1β [102], further supporting a role for αSyn in the inflammatory process. Macrophages are known to participate in diverse biological processes, including the phagocytosis of pathogens and debris, antigen presentation, and regulation of the immune response through cytokine production.

It has been reported that αSyn expression in peripheral blood mononuclear cells of PD patients is significantly up-regulated, compared to healthy non-PD controls [10]. In addition, protein expression of αSyn in cultured human T cells, B cells, natural killer cells and in monocytes/macrophages, have been reported [103]. Currently, it is not known whether expression, or aggregation, of αSyn in T cells is regulated by ligand activation of these cells, an important issue as it could identify a key link between acquired immunity regulation and αSyn expression.

Prospects for αSyn- and immune-based therapeutic approaches in PD

αSyn is increasingly becoming a primary target for understanding and controlling the onset and pregression of PD. As misfolding and aggregation of αSyn into specific toxic morphologies are essential for the progression of the disease, prevention of aggregate accumulation is an important potential therapeutic strategy. Interactions with protein targets, lipid vesicles, transition metals and other small molecules have all been explored [104, 105] with a view towards developing strategies to control the aggregation of αSyn and its variants. Both β- and γSyn have been reported to be inhibitors of fibril formation by αSyn [106, 107], and short peptides directed at the central portion of αSyn have also been shown to inhibit aggregation and to reduce its toxicity [105]. Additionally, as mentioned, treatment with chaperone Hsp70 has been shown to inhibit αSyn fibril formation and/or to reduce the aggregates

toxicity, in animal models of PD [85, 86]. Another possible therapeutic strategy to combat protein-deposition disorders, including PD, could be to produce 'superproteins', or more soluble versions of the aggregating proteins [108]. Such added modified proteins would reduce the tendency of their natural counterparts to aggregate, while remaining compatible with their cellular environment and their function [108].

An interesting strategy is the generation of specific anti-αSyn single-chain Fv (scFv) antibody fragments that bind either to the monomeric [109] or oligomeric [110] protein, and inhibit its aggregation. These scFvs can be generated such that they only target the toxic oligomeric form of αSyn, allowing the monomer to perform its normal function freely [110], and they can also potentially be expressed intracellularly (intrabodies) to counteract aggregation and reduce neurodegeneration, as recently shown with a neural progenitor cell line [111], and in an animal model of Huntington's disease [112].

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 preventative) therapies for PD and related disorders. Epidemiological data has identified the non-steroidal anti-inflammatory drug ibuprofen as neuroprotective for PD [113]. A variety of other, both endogenous and synthetic compounds that might suppress neuroinflammation in PD by interacting with microglia, have been identified and proposed for therapeutic use (reviewed in [113]).

Along the same lines, compounds that block other signal pathways that are switched on as a consequence of microglial activation, which may ultimately lead to neuronal apoptosis or degeneration, might also represent new targets for pharmacotherapeutic intervention.

Concluding remarks

In the last few years, it has become accepted that abnormal aggregation of αSyn is likely to be one of the primary causes of the immunological abnormalities observed in PD. The implication of αSyn in PD is supported by observations that (*i*) fibrillar aggregates of αSyn are the main constituents of LB, (*ii*) certain missense mutations, as well as duplication or triplication of the αSyn gene, cause autosomal dominant PD and (*iii*) the principal molecular, cellular, immunological and pathophysiological aspects of PD can be recapitulated by expression of αSyn in neuronal cell lines or animal models. It is well established that onset and progression of PD are characterized by sustained activation of microglia, linked to significant dopaminergic neuron loss in the SN, and accumulated evidence has established that aggregated or modified αSyn can trigger the activation of microglia, inducing a lethal cascade of neuroinflammation and neuronal death. By releasing toxic factors, or by phagocytosing neighbouring cells, activated microglia and astrocytes may form a destructive cycle of self-perpetuating neuronal degeneration. In addition, recent findings suggest a possible link between αSyn, humoural-mediated mechanisms and the pathological events in PD. Prevention of αSyn aggregation and intervention in the mechanisms of microglial activation mechanisms appears therefore to be highly promising therapeutic targets for the treatment of PD and other synucleinopathies.

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References

1. **Fahn S, Clarence-Smith KE, Chase TN.** Parkinson's disease: neurodegenerative mechanisms and neuroprotective interventions – report of a workshop. *Mov Disord*. 1998; 13: 759–67.
2. **Mayeux R.** Epidemiology of neurodegeneration. *Annu Rev Neurosci*. 2003; 26: 81–104.
3. **Hornykiewicz O, Kish SJ.** Biochemical pathophysiology of Parkinson's disease. *Adv Neurol*. 1987; 45: 19–34.
4. **Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M.** alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci USA*. 1998; 95: 6469–73.
5. **Croisier E, Moran LB, Dexter DT, Pearce RK, Graeber MB.** Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation*. 2005; 2: 14.
6. **Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL.** Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. 1997; 276: 2045–7.
7. **Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O.** Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet*. 1998; 18: 106–8.
8. **Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B, Llorens V, Gomez Tortosa E, del Ser T, Munoz DG, de Yebenes JG.** The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol*. 2004; 55: 164–73.
9. **Gasser T.** Genetics of Parkinson's disease. *Curr Opin Neurol*. 2005; 18: 363–9.
10. **Kim S, Seo JH, Suh YH.** Alpha-synuclein, Parkinson's disease, and Alzheimer's disease. *Parkinsonism Relat Disord*. 2004; 10: S9–13.
11. **Richter-Landsberg C, Gorath M, Trojanowski JQ, Lee VM.** alpha-synuclein is developmentally expressed in cultured rat brain oligodendrocytes. *J Neurosci Res*. 2000; 62: 9–14.

12. **Mori F, Tanji K, Yoshimoto M, Takahashi H, Wakabayashi K.** Demonstration of alpha-synuclein immunoreactivity in neuronal and glial cytoplasm in normal human brain tissue using proteinase K and formic acid pretreatment. *Exp Neurol*. 2002; 176: 98–104.
13. **Jenco JM, Rawlingson A, Daniels B, Morris AJ.** Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by alpha- and beta-synucleins. *Biochemistry*. 1998; 37: 4901–9.
14. **Peng X, Tehranian R, Dietrich P, Stefanis L, Perez RG.** Alpha-synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells. *J Cell Sci*. 2005; 118: 3523–30.
15. **Jo E, McLaurin J, Yip CM, St George-Hyslop P, Fraser PE.** alpha-Synuclein membrane interactions and lipid specificity. *J Biol Chem*. 2000; 275: 34328–34.
16. **Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, Orrison B, Chen A, Ellis CE, Paylor R, Lu B, Nussbaum RL.** Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. *J Neurosci*. 2002; 22: 8797–807.
17. **Castagnet PI, Golovko MY, Barcelo-Coblijn GC, Nussbaum RL, Murphy EJ.** Fatty acid incorporation is decreased in astrocytes cultured from alpha-synuclein gene-ablated mice. *J Neurochem*. 2005; 94: 839–49.
18. **Bussell R Jr, Eliezer D.** Residual structure and dynamics in Parkinson's disease-associated mutants of alpha-synuclein. *J Biol Chem*. 2001; 276: 45996–6003.
19. **Dedmon MM, Lindorff-Larsen K, Christodoulou J, Vendruscolo M, Dobson CM.** Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations. *J Am Chem Soc*. 2005; 127: 476–7.
20. **Bertoncini CW, Jung YS, Fernandez CO, Hoyer W, Griesinger C, Jovin TM, Zweckstetter M.** Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein. *Proc Natl Acad Sci USA*. 2005; 102: 1430–5.
21. **Davidson WS, Jonas A, Clayton DF, George JM.** Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J Biol Chem*. 1998; 273: 9443–9.
22. **Eliezer D, Kutluay E, Bussell R Jr, Browne G.** Conformational properties of alpha-synuclein in its free and lipid-associated states. *J Mol Biol*. 2001; 307: 1061–73.
23. **Perez RG, Hastings TG.** Could a loss of alpha-synuclein function put dopaminergic neurons at risk? *J Neurochem*. 2004; 89: 1318–24.
24. **Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC.** Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell*. 2005; 123: 383–96.
25. **El-Agnaf OM, Salem SA, Paleologou KE, Cooper LJ, Fullwood NJ, Gibson MJ, Curran MD, Court JA, Mann DM, Ikeda S, Cookson MR, Hardy J, Allsop D.** Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J*. 2003; 17: 1945–7.
26. **Lee HJ, Patel S, Lee SJ.** Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci*. 2005; 25: 6016–24.
27. **Bennett MC.** The role of alpha-synuclein in neurodegenerative diseases. *Pharmacol Ther*. 2005; 105: 311–31.
28. **Zibaee S, Jakes R, Fraser G, Serpell LC, Crowther RA, Goedert M.** Sequence determinants for amyloid fibrillogenesis of human alpha-synuclein. *J Mol Biol*. 2007; 374: 454–64.
29. **Rivers RC, Kumita JR, Tartaglia GG, Dedmon MM, Pawar A, Vendruscolo M, Dobson CM, Christodoulou J.** Molecular determinants of the aggregation behavior of alpha- and beta-synuclein. *Protein Sci*. 2008; 17: 887–98.
30. **Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M.** Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature*. 2002; 416: 507–11.
31. **Stefani M, Dobson CM.** Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J Mol Med*. 2003; 81: 678–99.
32. **Bucciantini M, Calloni G, Chiti F, Formigli L, Nosi D, Dobson CM, Stefani M.** Prefibrillar amyloid protein aggregates share common features of cytotoxicity. *J Biol Chem*. 2004; 279: 31374–82.
33. **Baglioni S, Casamenti F, Bucciantini M, Luheshi LM, Taddei N, Chiti F, Dobson CM, Stefani M.** Prefibrillar amyloid aggregates could be generic toxins in higher organisms. *J Neurosci*. 2006; 26: 8160–7.
34. **Kim YS, Joh TH.** Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. *Exp Mol Med*. 2006; 38: 333–47.
35. **Aloisi F.** Immune function of microglia. *Glia*. 2001; 36: 165–79.
36. **Kreutzberg GW.** Microglia: a sensor for pathological events in the CNS. *Trends Neurosci*. 1996; 19: 312–8.
37. **Hayes GM, Woodroofe MN, Cuzner ML.** Characterisation of microglia isolated from adult human and rat brain. *J Neuroimmunol*. 1988; 19: 177–89.
38. **Hayes GM, Woodroofe MN, Cuzner ML.** Microglia are the major cell type expressing MHC class II in human white matter. *J Neurol Sci*. 1987; 80: 25–37.
39. **McGeer PL, McGeer EG.** Glial reactions in Parkinson's disease. *Mov Disord*. 2008; 23: 474–83.
40. **Chen H, Zhang SM, Hernan MA, Schwarzschild MA, Willett WC, Colditz GA, Speizer FE, Ascherio A.** Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. *Arch Neurol*. 2003; 60: 1059–64.
41. **Chen H, Jacobs E, Schwarzschild MA, McCullough ML, Calle EE, Thun MJ, Ascherio A.** Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease. *Ann Neurol*. 2005; 58: 963–7.
42. **Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, Torizuka T.** Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol*. 2005; 57: 168–75.
43. **Orr CF, Rowe DB, Mizuno Y, Mori H, Halliday GM.** A possible role for humoral immunity in the pathogenesis of Parkinson's disease. *Brain*. 2005; 128: 2665–74.
44. **Rockenstein E, Crews L, Masliah E.** Transgenic animal models of neurodegenerative diseases and their application to treatment development. *Adv Drug Deliv Rev*. 2007; 59: 1093–102.
45. **Fernagut PO, Chesselet MF.** Alpha-synuclein and transgenic mouse models. *Neurobiol Dis*. 2004; 17: 123–30.
46. **Castano A, Herrera AJ, Cano J, Machado A.** Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J Neurochem*. 1998; 70: 1584–92.
47. **Austin SA, Floden AM, Murphy EJ, Combs CK.** Alpha-synuclein expression modulates microglial activation phenotype. *J Neurosci*. 2006; 26: 10558–63.
48. **McGeer PL, Itagaki S, Boyes BE, McGeer EG.** Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology*. 1988; 38: 1285–91.

49. **Yamada T, McGeer PL, McGeer EG.** Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins. *Acta Neuropathol*. 1992; 84: 100–4.
50. **Griffin WS, Liu L, Li Y, Mrak RE, Barger SW.** Interleukin-1 mediates Alzheimer and Lewy body pathologies. *J Neuroinflammation*. 2006; 3: 5.
51. **Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM.** Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science*. 2000; 290: 985–9.
52. **Przedborski S, Chen Q, Vila M, Giasson BI, Djaldatti R, Vukosavic S, Souza JM, Jackson-Lewis V, Lee VM, Ischiropoulos H.** 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*. 2001; 76: 637–40.
53. **Mandel S, Grunblatt E, Riederer P, Amariglio N, Jacob-Hirsch J, Rechavi G, Youdim MB.** 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*. 2005; 1053: 356–75.
54. **Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong JS, Zhang J.** Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J*. 2005; 19: 533–42.
55. **Wersinger C, Sidhu A.** An inflammatory pathomechanism for Parkinson's disease? *Curr Med Chem*. 2006; 13: 591–602.
56. **Reynolds AD, Kadiu I, Garg SK, Glanzer JG, Nordgren T, Ciborowski P, Banerjee R, Gendelman HE.** Nitrated Alpha-Synuclein and Microglial Neuroregulatory Activities. *J Neuroimmune Pharmacol*. 2008; 3: 59–74.
57. **Klegeris A, Pelech S, Giasson BI, Maguire J, Zhang H, McGeer EG, McGeer PL.** Alpha-synuclein activates stress signaling protein kinases in THP-1 cells and microglia. *Neurobiol Aging*. 2008; 29: 739–52.
58. **Zhang W, Dallas S, Zhang D, Guo JP, Pang H, Wilson B, Miller DS, Chen B, Zhang W, McGeer PL, Hong JS, Zhang J.** Microglial PHOX and Mac-1 are essential to the enhanced dopaminergic neurodegeneration elicited by A30P and A53T mutant alpha-synuclein. *Glia*. 2007; 55: 1178–88.
59. **Zhou Y, Wang Y, Kovacs M, Jin J, Zhang J.** Microglial activation induced by neurodegeneration: a proteomic analysis. *Mol Cell Proteomics*. 2005; 4: 1471–9.
60. **Thomas MP, Chartrand K, Reynolds A, Vitvitsky V, Banerjee R, Gendelman HE.** Ion channel blockade attenuates aggregated alpha synuclein induction of microglial reactive oxygen species: relevance for the pathogenesis of Parkinson's disease. *J Neurochem*. 2007; 100: 503–19.
61. **Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, Cerny R, Gelman B, Thomas MP, Mosley RL, Gendelman HE.** Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem*. 2008; 104: 1504–25.
62. **Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H.** 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*. 2000; 275: 18344–9.
63. **Beyer K.** Alpha-synuclein structure, posttranslational modification and alternative splicing as aggregation enhancers. *Acta Neuropathol*. 2006; 112: 237–51.
64. **Croisier E, Graeber MB.** Glial degeneration and reactive gliosis in alpha-synucleinopathies: the emerging concept of primary gliodegeneration. *Acta Neuropathol*. 2006; 112: 517–30.
65. **Ruano D, Revilla E, Gavilan MP, Vizuete ML, Pintado C, Vitorica J, Castano A.** Role of p38 and inducible nitric oxide synthase in the *in vivo* dopaminergic cells' degeneration induced by inflammatory processes after lipopolysaccharide injection. *Neuroscience*. 2006; 140: 1157–68.
66. **Qin L, Liu Y, Wang T, Wei SJ, Block ML, Wilson B, Liu B, Hong JS.** NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. *J Biol Chem*. 2004; 279: 1415–21.
67. **Gao HM, Hong JS, Zhang W, Liu B.** Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. *J Neurosci*. 2003; 23: 1228–36.
68. **Kawai T, Akira S.** TLR signaling. *Semin Immunol*. 2007; 19: 24–32.
69. **Ko L, Mehta ND, Farrer M, Easson C, Hussey J, Yen S, Hardy J, Yen SH.** Sensitization of neuronal cells to oxidative stress with mutated human alpha-synuclein. *J Neurochem*. 2000; 75: 2546–54.
70. **Bosco DA, Fowler DM, Zhang Q, Nieva J, Powers ET, Wentworth P Jr, Lerner RA, Kelly JW.** Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. *Nat Chem Biol*. 2006; 2: 249–53.
71. **Klegeris A, Choi HB, McLarnon JG, McGeer PL.** Functional ryanodine receptors are expressed by human microglia and THP-1 cells: their possible involvement in modulation of neurotoxicity. *J Neurosci Res*. 2007; 85: 2207–15.
72. **Giorgi C, Romagnoli A, Pinton P, Rizzuto R.** Ca2+ signaling, mitochondria and cell death. *Curr Mol Med*. 2008; 8: 119–30.
73. **Lee D, Lee SY, Lee EN, Chang CS, Paik SR.** alpha-Synuclein exhibits competitive interaction between calmodulin and synthetic membranes. *J Neurochem*. 2002; 82: 1007–17.
74. **Martinez J, Moeller I, Erdjument-Bromage H, Tempst P, Lauring B.** Parkinson's disease-associated alpha-synuclein is a calmodulin substrate. *J Biol Chem*. 2003; 278: 17379–87.
75. **Hasegawa M, Fujiwara H, Nonaka T, Wakabayashi K, Takahashi H, Lee VM, Trojanowski JQ, Mann D, Iwatsubo T.** Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions. *J Biol Chem*. 2002; 277: 49071–6.
76. **Tofaris GK, Razzaq A, Ghetti B, Lilley KS, Spillantini MG.** Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. *J Biol Chem*. 2003; 278: 44405–11.
77. **Rott R, Szargel R, Haskin J, Shani V, Shainskaya A, Manov I, Liani E, Avraham E, Engelender S.** Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. *J Biol Chem*. 2008; 283: 3316–28.
78. **Lee JT, Wheeler TC, Li L, Chin LS.** Ubiquitination of alpha-synuclein by Siah-1 promotes alpha-synuclein aggregation and apoptotic cell death. *Hum Mol Genet*. 2008; 17: 906–17.
79. **Lim KL, Tan JM.** Role of the ubiquitin proteasome system in Parkinson's disease. *BMC Biochem*. 2007; 8: S13.
80. **Tanaka Y, Engelender S, Igarashi S, Rao RK, Wanner T, Tanzi RE, Sawa A, V LD, Dawson TM, Ross CA.** Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Hum Mol Genet*. 2001; 10: 919–26.

81. **Stefanis L, Larsen KE, Rideout HJ, Sulzer D, Greene LA.** Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. *J Neurosci*. 2001; 21: 9549–60.
82. **Chen L, Thiruchelvam MJ, Madura K, Richfield EK.** Proteasome dysfunction in aged human alpha-synuclein transgenic mice. *Neurobiol Dis*. 2006; 23: 120–6.
83. **Snyder H, Mensah K, Theisler C, Lee J, Matouschek A, Wolozin B.** Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. *J Biol Chem*. 2003; 278: 11753–9.
84. **Dedmon MM, Christodoulou J, Wilson MR, Dobson CM.** Heat shock protein 70 inhibits alpha-synuclein fibril formation *via* preferential binding to prefibrillar species. *J Biol Chem*. 2005; 280: 14733–40.
85. **Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ.** Hsp70 reduces alpha-synuclein aggregation and toxicity. *J Biol Chem*. 2004; 279: 25497–502.
86. **Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM.** Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science*. 2002; 295: 865–8.
87. **Czlonkowska A, Kurkowska-Jastrzebska I, Czlonkowski A, Peter D, Stefano GB.** Immune processes in the pathogenesis of Parkinson's disease – a potential role for microglia and nitric oxide. *Med Sci Monit*. 2002; 8: RA165–77.
88. **Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T.** Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett*. 1996; 211: 13–6.
89. **Nagatsu T, Mogi M, Ichinose H, Togari A.** Cytokines in Parkinson's disease. *J Neural Transm Suppl*. 2000: 143–51.
90. **Kim S, Jeon BS, Heo C, Im PS, Ahn TB, Seo JH, Kim HS, Park CH, Choi SH, Cho SH, Lee WJ, Suh YH.** Alpha-synuclein induces apoptosis by altered expression in human peripheral lymphocyte in Parkinson's disease. *FASEB J*. 2004; 18: 1615–7.
91. **Stefanova N, Schanda K, Klimaschewski L, Poewe W, Wenning GK, Reindl M.** Tumor necrosis factor-alpha-induced cell death in U373 cells overexpressing alpha-synuclein. *J Neurosci Res*. 2003; 73: 334–40.
92. **Miklossy J, Doudet DD, Schwab C, Yu S, McGeer EG, McGeer PL.** Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. *Exp Neurol*. 2006; 197: 275–83.
93. **Klegeris A, Giasson BI, Zhang H, Maguire J, Pelech S, McGeer PL.** Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. *FASEB J*. 2006; 20: 2000–8.
94. **Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H.** NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathol*. 2000; 99: 14–20.
95. **Campbell BC, McLean CA, Culvenor JG, Gai WP, Blumbergs PC, Jakala P, Beyreuther K, Masters CL, Li QX.** The solubility of alpha-synuclein in multiple system atrophy differs from that of dementia with Lewy bodies and Parkinson's disease. *J Neurochem*. 2001; 76: 87–96.
96. **He Y, Le WD, Appel SH.** Role of Fcgamma receptors in nigral cell injury induced by Parkinson disease immunoglobulin injection into mouse substantia nigra. *Exp Neurol*. 2002; 176: 322–7.
97. **Racke MK, Ratts RB, Arredondo L, Perrin PJ, Lovett-Racke A.** The role of costimulation in autoimmune demyelination. *J Neuroimmunol*. 2000; 107: 205–15.
98. **Papachroni KK, Ninkina N, Papapanagiotou A, Hadjigeorgiou GM, Xiromerisiou G, Papadimitriou A, Kalofoutis A, Buchman VL.** Autoantibodies to alpha-synuclein in inherited Parkinson's disease. *J Neurochem*. 2007; 101: 749–56.
99. **Chen S, Le WD, Xie WJ, Alexianu ME, Engelhardt JI, Siklos L, Appel SH.** Experimental destruction of substantia nigra initiated by Parkinson disease immunoglobulins. *Arch Neurol*. 1998; 55: 1075–80.
100. **Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Seubert P, Lee M, Goldstein J, Chilcote T, Games D, Schenk D.** Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron*. 2005; 46: 857–68.
101. **Tanji K, Imaizumi T, Yoshida H, Mori F, Yoshimoto M, Satoh K, Wakabayashi K.** Expression of alpha-synuclein in a human glioma cell line and its up-regulation by interleukin-1beta. *Neuroreport*. 2001; 12: 1909–12.
102. **Tanji K, Mori F, Imaizumi T, Yoshida H, Matsumiya T, Tamo W, Yoshimoto M, Odagiri H, Sasaki M, Takahashi H, Satoh K, Wakabayashi K.** Upregulation of alpha-synuclein by lipopolysaccharide and interleukin-1 in human macrophages. *Pathol Int*. 2002; 52: 572–7.
103. **Shin EC, Cho SE, Lee DK, Hur MW, Paik SR, Park JH, Kim J.** Expression patterns of alpha-synuclein in human hematopoietic cells and in *Drosophila* at different developmental stages. *Mol Cells*. 2000; 10: 65–70.
104. **Golts N, Snyder H, Frasier M, Theisler C, Choi P, Wolozin B.** Magnesium inhibits spontaneous and iron-induced aggregation of alpha-synuclein. *J Biol Chem*. 2002; 277: 16116–23.
105. **El-Agnaf OM, Paleologou KE, Greer B, Abogrein AM, King JE, Salem SA, Fullwood NJ, Benson FE, Hewitt R, Ford KJ, Martin FL, Harriott P, Cookson MR, Allsop D.** A strategy for designing inhibitors of alpha-synuclein aggregation and toxicity as a novel treatment for Parkinson's disease and related disorders. *FASEB J*. 2004; 18: 1315–7.
106. **Park JY, Lansbury PT Jr.** Beta-synuclein inhibits formation of alpha-synuclein protofibrils: a possible therapeutic strategy against Parkinson's disease. *Biochemistry*. 2003; 42: 3696–700.
107. **Hashimoto M, Rockenstein E, Mante M, Mallory M, Masliah E.** beta-Synuclein inhibits alpha-synuclein aggregation: a possible role as an anti-Parkinsonian factor. *Neuron*. 2001; 32: 213–23.
108. **Vendruscolo M, Dobson CM.** Chemical biology: more charges against aggregation. *Nature*. 2007; 449: 555-.
109. **Emadi S, Liu R, Yuan B, Schulz P, McAllister C, Lyubchenko Y, Messer A, Sierks MR.** Inhibiting aggregation of alpha-synuclein with human single chain antibody fragments. *Biochemistry*. 2004; 43: 2871–8.
110. **Emadi S, Barkhordarian H, Wang MS, Schulz P, Sierks MR.** Isolation of a human single chain antibody fragment against oligomeric alpha-synuclein that inhibits aggregation and prevents alpha-synuclein-induced toxicity. *J Mol Biol*. 2007; 368: 1132–44.
111. **Lynch SM, Zhou C, Messer A.** An scFv intrabody against the nonamyloid component of alpha-synuclein reduces intracellular aggregation and toxicity. *J Mol Biol*. 2008; 377: 136–47.
112. **Wolfgang WJ, Miller TW, Webster JM, Huston JS, Thompson LM, Marsh JL, Messer A.** Suppression of Huntington's disease pathology in *Drosophila* by human single-chain Fv antibodies. *Proc Natl Acad Sci USA*. 2005; 102: 11563–8.
113. **Klegeris A, McGeer EG, McGeer PL.** Therapeutic approaches to inflammation in neurodegenerative disease. *Curr Opin Neurol*. 2007; 20: 351–7.