Master's Theses

Theses and Dissertations

2013

Alpha-Synuclein Aggregates Activate the Nlrp3 Inflammasome Following Vesicle Rupture

Rudy Orlando Cedillos
Loyola University Chicago

Follow this and additional works at: https://ecommons.luc.edu/luc_theses

Part of the Neurosciences Commons

LOYOLA UNIVERSITY CHICAGO

ALPHA-SYNUCLEIN AGGREGATES ACTIVATE THE NLRP3 INFLAMMASOME

FOLLOWING VESICLE RUPTURE

A THESIS SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

IN CANDIDACY FOR THE DEGREE OF

MASTER OF SCIENCE

PROGRAM IN INTEGRATED CELL BIOLOGY

BY

RUDY ORLANDO CEDILLOS

CHICAGO, IL

DECEMBER 2013

ACKNOWLEDGEMENTS

There are many people who made this research and the creation of this document possible. Starting with my research advisor Dr. Campbell, I would like to thank him for his generous assistance with all facets of my work, and encouraging me to pursue scientific study. Drs. DonCarlons, Wiethoff, and Qiao are owed a debt of gratitude for the time that they spent on my thesis committee as well as for their insightful comments and suggestions. Finally, I would like to recognize my parents for helping me become the person I am today by providing me with good role models and great examples of how to live life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATP   Adenosine Triphosphate

Alum   Aluminum hydroxide

AD   Alzheimer's disease

APC   Antigen presenting cells

AA   Amino Acid

ASC   Apoptosis-associated speck-like protein containing a CARD

BIR   Baculovirus inhibitor of apoptosis repeat

CPPD   Calcium pyrophosphate dehydrate

CARD   Card activation and recruitment domain

CNS   Central Nervous System

CSF   Cerebral spinal fluid

CAPS   Cryopyrin-associated periodic syndrome

CLRs   C-type lectin receptors

DLB   Dementia with Lewy bodies

DA   Dopamine

DAMPs   Danger-associated molecular patterns
IFN-γ Interferon-γ

IL-1	Interleukin-1
LRRs	Leucine-rich repeat
LBs	Lewy bodies
LPS	Lipopolysaccharide
MSU	Monosodium urate
MSA	Multiple system atrophy
MDP	Muramyl dipeptide
NAC	Non-Aβ-component
NLRs	Nucleotide-binding domain leucine-rich repeat containing receptors
PD	Parkinson's disease
PRRs	Pathogen recognition receptors
PYD	Pyrin domain
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RLH	Rig-I-like RNA helicases
SNpc	Substantia nigra pars compacta
TLRs	Toll-like receptors
UPS	Ubiquitin-proteosome system

ABSTRACT

Parkinson's disease (PD) and related synucleinopathies are progressive neurodegenerative disorders that feature the accumulation of intracellular inclusions known as Lewy bodies (LBs) in the brain. The presynaptic protein α-synuclein is the primary constituent of LBs and has been documented to play a major role in the pathogenesis of synucleinopathies. Recently, aggregated α-synuclein has been implicated in prompting microglia-mediated inflammation. Neuroinflammation is a detrimental process when chronic and is associated with the progression of neuronal death in neurodegenerative disorders. Although the mechanisms surrounding the induction of neuroinflammation are not well understood, the recently discovered inflammasome-forming NLR proteins have emerged as important regulators of innate immunity and inflammation. In this study, we sought to understand the involvement of the inflammasome in response to aggregated α-synuclein in human microglia-like cells. We report that aggregated α-synuclein induces vesicle rupture in THP-1 cells that is sensed as a 'danger signal' resulting in the assembly of the NLRP3 inflammasome, activation of the inflammatory caspase-1, and the release of proinflammatory cytokines.

CHAPTER ONE

INTRODUCTION

Statement of the Problem

Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) belong to a family of age-related neurodegenerative disorders aptly named synucleinopathies that are becoming more prevalent in today's time due to the progressive aging of the population. These disorders are generally characterized by distinct neuronal loss, chronic neuroinflammation, and the abnormal deposition of highly organized α-synuclein fibrils termed Lewy bodies. There is an overwhelming amount of evidence pointing to α-synuclein as the culprit behind synucleinopathy pathogenesis. Although the exact mechanism by which α-synuclein promotes disease is not understood, growing evidence indicates that pathogenesis is fueled by α-synuclein's propensity to misfold into toxic oligomers/protofibrils, or intermediately sized aggregates. Furthermore, *in vitro* studies have suggested that aggregated α-synuclein may play a role in inducing neuroinflammation by directly activating glia cells. Reports of α-synuclein-induced microglia activation are in agreement with postmortem analysis of parkinsonian brain tissue that demonstrate α-synuclein depositions surrounded by activated microglia and a large assortment of pro-inflammatory mediators including IL-1β, caspase-1, and acute phase proteins.

Neuroinflammation has been implicated in the progression of neuronal death; however, recent reports label inflammation as a possible accomplice to the on-set of neurodegenerative diseases by propagating protein misfolding. In the central nervous system (CNS), inflammatory responses play a protective and restorative role that are kept under control by anti-inflammatory mechanisms; however, in a traumatized brain regulatory mechanisms prove insufficient and inflammation turns chronic, cultivating in secondary tissue damage. The mechanisms by which neuroinflammation occurs and the mediators involved have not been well characterized; however, the newly discovered inflammasome-forming NLR proteins have surfaced as important regulators of innate immunity and inflammation and may serve as potential therapeutic targets. These proteins are genetically linked to immunologic disorders and have a directive role in the secretion of the interleukin-1β (IL-1β) and interleukin-18 (IL-18), pro-inflammatory cytokines associated with autoinflammatory and neurodegenerative diseases.

This thesis describes the regulation of the multiprotein complex known as the inflammasome in human microglia-like cells and its response to α-synuclein. Although the molecular mechanisms by which α-synuclein activates microglia and mediates inflammation in the CNS are not fully understood, findings from our recent studies suggest that α-synuclein oligomers can lead to inflammasome activation by means of vesicle rupture. It is known that vesicle rupture by protein aggregates such as amyloid-β is sensed as a 'danger signal' in the target cell and can induce NLRP3 inflammasome activation and subsequent IL-1β production in microglia and macrophages. The goal of this project was to understand the involvement of the NLRP3 inflammasome and the

mechanisms underlying caspase-1 activation and subsequent production of IL-1β in response to α-synuclein oligomers in human macrophage THP-1 cells.

Synucleinopathies

Parkinson's disease (PD) is an age related neurodegenerative disorder that was first described in "An essay of the shaky palsy" by James Parkinson in 1817. His findings described individuals experiencing tremor, rigidity, and debilitated motor skills. In 1912, Frederick Lewy identified insoluble protein deposits in the brain of postmortem PD patients. The protein inclusions were termed Lewy bodies (LBs) and their presence is a hallmark of PD pathology (Holdoff 2002). It was not until recent studies by Spillantini and colleagues that the primary constituent of LB composition was identified as ubiquitinated and phosphorylated fibrillar forms of α-synuclein, a presynaptic protein (Uversky 2007). α-synuclein has been incriminated in several other neurodegenerative disorders (e.g., multiple system atrophy and dementia with Lewy bodies) collectively named synucleinopathies, all of which feature abnormal α-synuclein deposits in neurons or glia cells of the brain (Spillantini 1997).

Parkinson's disease (PD) is the second most common neurodegenerative disease, trailing Alzheimer's disease (AD). It is a chronic progressive disease characterized by the degeneration of dopaminergic neurons in the nigrostriatal system, predominantly those from the substantia nigra pars compacta (SNpc) with resultant depletion of dopamine (DA). The loss of DA creates irregular neurotransmissions that result in slowness of

movement (bradykinesia), varying degrees of rigidity, resting tremors, and postural volatility (Chinta 2005; Jankovic 2008).

Two forms of PD are recognized: early-onset also known as familial and idiopathic or late-onset PD, which are clinically indistinguishable from one another. PD is classified as a sporadic disease because more than 85% of PD cases are idiopathic and exhibit no inheritability (Simunovic 2009). Epidemiological studies suggest that idiopathic cases arise from exposure to environmental factors in the context of an aging brain. (Tan 2000; Benmoyal-Segal 2006). Less than 10% of all PD cases are familial and exhibit inheritability of an autosomal dominant or recessive form of the disease from a mutation in a specific gene (Simunovic 2009). Mutations in six genes have been identified to cause familial PD of which *SNCA, PRKN*, and *LRRK2* are the most prevalent. The α-synuclein gene (*SNCA*) was the first gene associated with PD after three point mutations (A53T, A30P, and E46K) in this gene were tied to early-onset or familial PD. (Polymeropoulos 1997; Kitada 1998; Zimprich 2004). Mutation A53T and A30P are both linked to early-onset autosomal dominant familial PD (Polymeropoulos 1997; Kruger 1998), while point mutation E46K is described to cause early onset of dementia with Lewy bodies (DLB) (Zarranz 2004). Furthermore, duplication and triplication of the *SNCA* gene are associated with autosomal dominant form of PD (Dekker 2003; Beyer 2009). Identification of α-synuclein as the main component of Lewy bodies (Spillantini 1997) and its genetic link to familial PD provide compelling evidence that α-synuclein holds a crucial role in the pathogenesis of PD.

Dementia with Lewy bodies (DLB) is a neurodegenerative disease characterized by the loss of a variety of neurons, including dopaminergic and acetylcholine neurons (Hanson 2009). DLB accounts for 20% of late onset dementia and symptoms include dementia, fluctuating cognition, and parkinsonism (Campbell 2001; Hanson 2009).

Multiple system atrophy (MSA) features neuronal loss in the cerebellum, pons, inferior olivary nuclei, basal ganglia, and spinal cord. MSA patients show signs of autonomic dysfunction and cellebellar ataxia (Gesine 2013).

Pathology

PD is associated with degeneration of dopaminergic neurons in the substantia nigra. This neurodegeneration is accompanied by intracellular inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs) in surviving neurons, whose presence in the substantia nigra of the midbrain is the signature neuropathological hallmarks of PD. Although dopaminergic cell loss is associated with accumulation of LBs in PD, the cause of these inclusions is not known. The neuropathological process of PD is first seen in the olfactory bulb and dorsal motor-nucleus of the vagus nerve. The process advances in an ascending order towards the locus coeruleus, and the substantia nigra until it reaches the neocortex (Braak 2003). Interestingly, pathological events in PD are suggested to occur in the enteric plexus of the gastrointestinal system as reports have identified increased permeability and α-synuclein inclusions in colonic biopsies from PD patients (Lebouvier 2010; Braak 2006). Gastrointestinal dysfunction is commonly seen all stages of PD and has driven the hypothesis that the intestines might serves as an early site of PD pathology

in response to an environmental toxin or pathogen (Forsyth 2011). DLB patients display LBs predominantly in the neocortex but pathology is also seen in the midbrain (Campbell 2001; Hanson 2009).

In addition to neuronal loss, and LB formation, microglia-mediated inflammation is another characteristic feature of PD and other synucleinopathies. An increase in activated microglia, resident immune cells of the CNS, has been identified in the SNpc of PD patients in postmortem studies (McGeer 1988). Moreover, Croisier and collegues demonstrated a relationship between activated microglia and α-synuclein deposition in the SNpc in PD patients (Croisier 2005). PD patients also demonstrate elevated levels of pro-inflammatory cytokines in the cerebral spinal fluid (CSF) along with influence from the adaptive immune system as CD4+ and CD8+ lymphocytes were shown to infiltrate the SNpc (McGeer 1988; Farkas 2000; Brochard 2009). Additionally, *in vitro* studies have demonstrated that accumulation of wild type, and mutated α-synuclein induces microglia activation (Zhang 2005; Zhang 2007; Klegeris 2006; Reynolds 2008; Su 2009; Sanchez-Guajardo 2010). Together, these studies suggest a significant role of α-synuclein-induced inflammation in the pathogenesis of PD.

Oxidative Stress

A common feature of neurodegenerative diseases is oxidative stress emanating from proteostatic dysfunction prompted by misfolded proteins. In the CNS, oxidative stress, induced when the cellular antioxidant response is overcome by reactive oxygen species (ROS) (e.g. superoxide), can lead to modifications of nucleic acids, lipids

peroxidation, and proteins nitration resulting in cellular damage or cell death. Neurons are vulnerable to oxidative damage due to their high respiratory turnover, high-energy demand dependent on oxidative phosphorylation, and the presence of catalytic transition metals. In PD, the dopaminergic neurons of the SNpc are further afflicted with oxidative stress deriving from dopamine (DA) metabolism, mitochondrial dysfunction, and microglial phagocytosis (Hald 2005).

The SNpc is a site particularly vulnerable to oxidative stress due to its already high basal level emanating from the dopaminergic neurons' autonomic pacemaking, and the tendency of abundant cytoplasmic DA to autoxidize into toxic intermediates. Furthermore, DA catabolism generates ROS and other highly reactive chemical species, which can cause oxidative stress and impair mitochondrial respiration. Mitochondrial dysfunction is a major source of oxidative stress. Dysfunction either from environmental or genetic factors leads to the excessive production of ROS and culminates in apoptotic cell death of neurons. Activated microglia have been shown to be a source of robust extracellular ROS in the CNS. Activated microglia are able to maintain homeostasis by removing debris or unwanted stressors through phagocytosis; however, clearance through phagocytosis is shadowed by a parallel activation of the phagocytic NADPH oxidase complex, a major source of ROS (Hald 2005; Jekabsone 2006; Brown 2007)

α-synuclein

Synucleins are a family of small, soluble presynaptic membrane binding proteins aptly termed synuclein for their localization to the nuclear envelope and presynaptic terminals of neurons. These proteins are structurally characterized by the bearing of an acidic C-terminal region and a repetitive, imperfect AA motif throughout their highly conserved N-terminal region. The synuclein family consists of three known proteins explicitly found in vertebrates: α, β, and γ-synuclein (Jakes 1994).

Synucleins attracted much attention after Ueda and colleagues correlated the central portion of α-synuclein to the non-Aβ-component (NAC) of amyloid plaques found in Alzheimer's disease (AD); making it the first study to couple α-synuclein and neurodegenerative diseases (Ueda 1993). α-synuclein is a 140 AA cytosolic protein (**Figure 1**) primarily localized to presynaptic nerve terminals. The exact physiological role of α-synuclein is unknown although studies suggest that it has a role in neurotransmitter regulation, neuronal differentiation, and synaptic plasticity (Spillantini 1997; Martin 2004).

α-synuclein features a N-terminal domain that houses the three point mutations associated with familial forms of PD. (**Figure 1**). Additionally, this domain shares a similar amino acid sequence to that of the class-A2 lipid-binding domains of apolipoproteins; amino acid repeats bearing imperfect motifs that constitute amphipathic helices, which are features that mediate membrane and lipid interactions with phospholipid vesicles (Perrin 2000). Recent studies demonstrated that association of
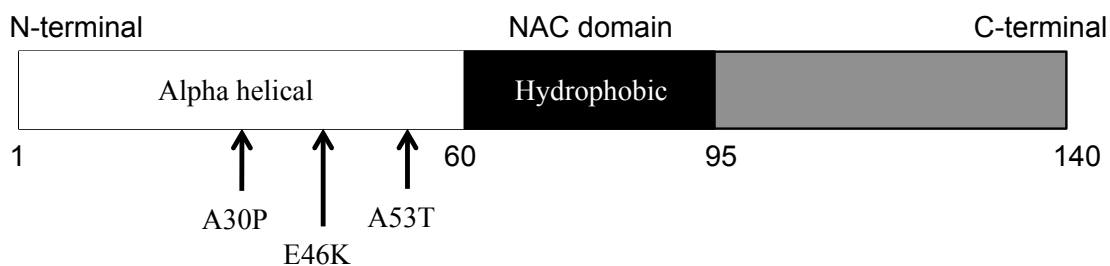
Figure 1. Schematic Structure of α-synuclein. α-synuclein carries a highly conserved amphipathic N-terminal domain (AA 1-60; associated with lipid binding), a hydrophobic NAC central domain (AA 61-95; unique to α-synuclein; facilitates aggregation), and an acidic C-terminal domain (96-140). The figure is a modified version from Dickson *et al.* 2001.

α- synuclein aggregates with lipid bilayers result in the rupture of lysosomal vesicles in neuronal cell lines (Freeman 2013).

Synucleins are natively unfolded proteins due to their low hydropathy and the highly negative charge of its C-terminus. The central hydrophobic region (NAC) is involved in protein aggregation, a feature unique to α-synuclein.

α-synuclein Aggregation

There is overwhelming evidence that α-synuclein plays a role in PD and synucleinopathy pathology. As previously stated α-synuclein is the main component of LBs, the key feature in synucleinopathies. Although the underlying mechanisms of LB formation have yet to be mapped, α-synuclein assembly into amyloid-like fibrils is known to be a fundamental event in its development.

α-synuclein aggregation is dependent on the α-synuclein protein concentration derived from its synthesisv and degradation equilibrium. Moreover, aggregation is augmented by a number of factors. Point mutations and overexpression due to increased gene dosage accelerates the aggregation process (Conway 2000). Chemical modifications by small amines, pH, oxidative and nitrative stresses, and a variety of environmental insults affects aggregation propensity by influencing α-synuclein's conformation (Conway 2000; Singleton 2003; Bennett 2005; Hoyer 2002).

It has been established that purified recombinant α-synuclein can assemble into amyloid-like fibrils and filaments similar to the LBs affecting PD patients (Conway 1998; Uversky 2007). Fibrillization of α-synuclein has been proposed to occur in a two-step

process. Aggregation begins with the induction of the natively unfolded monomer to a partially folded conformation that self-assembles and formulates oligomers or protofibrils. Moreover, protofibrils may act as seeds for the recruitment of additional monomers to form insoluble fibrils that escape the ubiquitin-proteosome system (UPS) and accumulate in the cytoplasm (Jarrett 1993; Conway 2000; Uversky 2001; Luk 2009).

It is believed that protein misfolding and oligomeric aggregation leads to α-synuclein toxic-gain-of-function. Although fibrillar species are found in LBs, growing evidence points to soluble protofibrils, or intermediate sized oligomers as instigators of disease due to their toxic effect on cells. It has been difficult to test this hypothesis *in vitro* because oligomeric species only exist for a brief period of time before continuing the fibrillization process. Additionally, there is no current method to selectively remove the soluble protofibrils. (el-Agnaf 2002; Winner 2011).

Protofibrils are thought to be responsible for pore formation, ER trafficking deficit, mitochondrial damage, and inhibition of protein turnover all of which result in cellular dysfunction (Volles 2001; Lashuel 2002; Smith 2005; Stefanis 2001; Tanaka 2001; Petrucelli 2002).

Microglia Mediated Inflammation

The inflammatory response in the CNS is a complex, multi-component process driven by microglia with assistance from infiltrating macrophages and T-cells when requested. Neurons and astrocytes are also known to influence and modulate the complex milieu of an immune response in the CNS. Both injured and healthy cells release

inflammatory mediators in the attempt to build an environment favoring the removal of infectious or sterile stressors, and to assist in tissue repair. When the damage is too great or the stimulus sustained too long, it leads to chronic neuroinflammation.

Microglia: Microglia are the resident immune cells of the CNS, comprising 12% of total glia population. These resident macrophages are the first line of defense and are therefore mapped in all quarters of the CNS, although certain areas are known to house a higher density of microglia, including the hippocampus and the substantia nigra (Lawson 1990). Microglia are of mesodermal origin, as opposed to neuroectoderm derived neurons, astrocytes, and oligodendrocytes; however, the cellular origin of microglia has been an issue of debate. Recent studies by Kierdof *et al.* (2013) show that microglia stem from erythromyeloid progenitors that are limited to erythrocyte or macrophage lineages. Early committed macrophages that migrate from the yolk sac into the neuroepithelium during embryonic hematopoiesis differentiate into the microglia population (Neumann 2013; Ginhoux 2013; Kierdof 2013).

Microglia are commonly generalized as resting or activated. Resting microglia are characterized with a small soma and ramified processes. Contradicting the term, 'resting microglia' are in constant motion using their elaborated processes to surveying the environment. An activated state is depicted by the production of context-specific cytokines and chemokines, and an amoeboid morphology where processes shorten, and the cell body enlarges (Kohman 2013). Microglia can obtain an 'actived' phenotype in response to chemical or cellular cues. The simplified classifications, M1 and M2, are drive inflammation and phagocytosis of debris, respectively. The classically activated M1

phenotype expressing pro-inflammatory cytokines is induced via toll-like receptors (TLRs) or interferon-γ (IFN-γ). The M2 phenotype is subdivided into two stages: the alternatively activated M2 (anti-inflammatory) stage and the M2-deactivating (tissue repair) stage, induced by IL-4 and IL-10, respectively (Olah 2011; Sanchez-Guajardo 2013).

Microglia cells express most known toll-like receptors. Toll-like receptors are important in properly mounting an innate immune response and will be further discussed in the next section. Under sterile conditions, endogenous proteins or molecules released from damaged or stressed cells have been shown to stimulate TLRs. TLR endogenous ligands include but are not limited to sialic acid-containing glycosphingolipids concentrated on neuronal membranes, HSP60, HSP70, HMGB1, biglycan, hyaluronic acid, and host DNA. (Ohashi 200; Park 2004; Schaefer 2005; Termeer 2002). Certain species of oligomerized amyloid-β and α-synuclein have also been proposed to stimulate TLR2 and TLR4 by binding to the accessory protein CD14 (Udan 2008).

Studies have denoted a balance between M2 and M1 phenotype in early stages of α-synuclein induced neurodegeneration. It has proposed that as the disease progresses there is a change in the local milieu (e.g., accumulation of protein aggregates, neuronal dysfunction, infiltrating immune cells) that disrupts the balance of microglia to a M1 domineering phenotype that features cytotoxic pro-inflammatory cytokines (**Figure** 2) (Varnum 2012; Sanchez-Guajardo 2013). *In vitro* studies also suggest a M1 phenotype with the progression of PD as it was observed that after not being able to phagocytose the activating stimuli, certain aggregated species of α-synuclein, microglia were driven to a
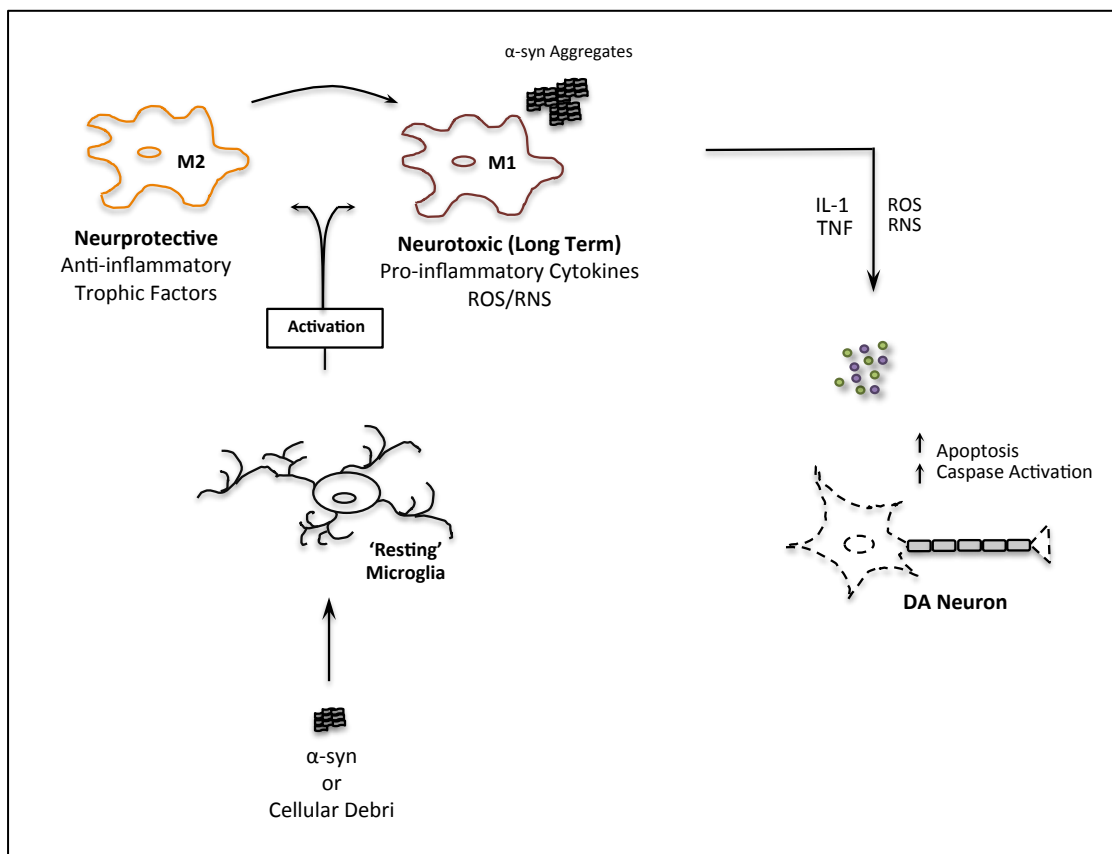
Figure 2. **Illustration of Microglia Activation and their Dichotomic Role in Neuroinflammation.** Depending on the stimuli, microglia can display a phagocytic or neurotoxic phenotype. There are solid indications that α-synuclein polarizes microglia towards a neuroprotective role (M2) in early stages of disease; however, as the disease progresses, the accumulation of insoluble aggregates shift microglia towards a chronic inflammatory and cytotoxic state (M1). The cytotoxic phenotype results in the release of pro-inflammatory cytokines, chemokines, ROS, and RNS that contribute to the oxidative stress of nigral neurons.

M1 state described as an inflammatory phenotype with less phagocytic abilities (Park 2008). The overproduction of inflammatory molecules such as IL-1β, and TNFα induce cell death, therefore, an understanding of the mechanism by which α-synuclein activates microglia deserves further investigation. The NLR family of protein receptors has been identified as important regulators of immunity and inflammation; therefore, the next section will review current knowledge regarding NLR dependent cellular pathways in inflammation and the possibility of NLRs as therapeutic targets.

Innate Immune Response

The innate immune system relies on germline-line encoded receptors known as pattern recognition receptors (PRRs) for host cell recognition of pathogens (Beutler 2010). PRRs include Toll-like receptors (TLRs), Rig-I-like RNA helicases (RLH), C-type lectin receptors (CLRs), and nucleotide-binding domain leucine-rich repeat containing receptors (NLRs) (Medzhitov 2009). These receptors recognize conserved microbial motifs on pathogens known as pathogen-associated molecular patterns (PAMPs) that include bacterial and viral nucleic acids, and pathogen associated cell wall components from a variety of microbes.

Additionally, it was known that endogenous danger signals or danger-associated molecular patterns (DAMPs) could promote an immune response, however, the mechanism was unclear. In 1994, Matzinger and colleagues proposed the 'danger model,' where it was hypothesized that host antigen presenting cells could be activated by danger signals released from host cells undergoing cellular or mechanical stress prompted by

pathogens or sterile insults (Matzinger 2002). It has been reported that danger signals can induce downstream production of pro-inflammatory mediators by exogenously signaling through PRRs such as TLRs or NLRs. NLR proteins, another class of PRRs, are cytoplasmic receptors with a regulatory role in inflammation and the ability to sense both intracellular PAMPs and DAMPs (Matzinger 2002).

The NLR Family

NLRs or Nucleotide-binding-domain-and-leucine-rich-repeat-containing-gene-family-of-receptors are cytoplasmic protein receptors with an active role in innate immune sensing, apoptosis, and reproductive biology (Martinon 2002). The NLR family contains 23 human members composed of a tripartite domain structure consisting of a N-terminal effector domain, a central NACHT domain for oligomerization, and a C-terminal leucine-rich repeat (LRR) domain. NLR protein receptors are designated into subfamilies categorized by their N-terminal effector domain. **Table 1** illustrates the human NLR members and their accredited subfamilies (Ting 2008).

In 2002, Martinon and colleagues discovered that a subset of NLRs, able to detect both PAMPs and DAMPs, were capable of forming a protein complex termed the inflammasome. Assembly of the inflammasome resulted in the activation of the inflammatory caspase-1 and the secretion of the pyrogenic cytokines IL-1β and IL-18 (Martinon 2002).

Table1. Human NLR Family

NLR Family	Symbol in Humans	Domain Organization	
		N-terminus	**C-terminus**
NLRA	*CIITA*	CARD — AD --- NACHT --- LRR	
NLRB	*NAIP*	BIR3X --- NACHT --- LRR	
NLRC	*NOD1, NLRC3-5*	CARD --- NACHT --- LRR	
	NOD2	CARD 2x --- NACHT --- LRR	
NLRP	*NLRP1*	PYD --- NACHT --- LRR FIIND CARD	
	NLRP2-9	PYD --- NACHT --- LRR	
	NLRP10	PYD --- NACHT	
	NLRP11-14	PYD --- NACHT --- LRR	

Table1. Abbreviations: CARD, Card activation and recruitment domain; AD, acidic activation domain; BIR, baculovirus inhibitor of apoptosis repeat (BIR) domain; LRR, leucine rich repeats; PYD, pyrin domain

The Inflammasome

Recognition of DAMPs or PAMPs by NLRs can result in the assembly of the inflammasome, a multi-protein complex responsible for caspase-1 activation. This molecular complex is composed of inactive protein monomers, typically NLR receptors, an apoptosis-associated speck-like protein containing a CARD (ASC) adaptor protein, and caspase-1. When exposed to DAMPs or PAMPs the cell can signal for the protein monomers to activate and oligomerize; however, the order of events leading to inflammasome assembly and activation are unclear. The inflammasome acts as a platform for caspase-1 activation by recruiting and facilitating the cleavage of the inactive zymogen, pro-caspase-1, via ASC or NLR association.

Caspase-1 is an inflammatory caspase and the effector molecule of the inflammasome. Its activation by the inflammasome leads to the processing and cleavage of pro-IL-1β and pro-IL-18, into their biologically active forms, IL-1β and IL-18, respectively. Although caspase-1 is mostly known for its role in pro-IL-1β processing, studies by Shao *et al.* (2007) and Keller *et al.* (2008) reported that caspase-1 may have over 70 additional cleavage targets, and a role in the regulation of glycolysis, and unconventional protein secretion of targets lacking a leader sequence.

IL-1β and IL-18 are potent pyrogenic cytokines crucial for proper immune responses that impede pathogenic assaults. Deregulation of their production can be harmful to the host and lead to autoinflammatory disorders; therefore, regulation of inflammasome activation leading to the secretion of IL-1β and IL-18 requires two signals. Signal one upregulates the transcription and translation of pro-IL-1β, pro-IL-18,

and NLR receptors typically through TLR priming or signaling. Signal two, produced by a number of diverse stimuli, triggers the assembly and activation of the inflammasome (Cassel 2010).

In recent years, three inflammasomes complexes, NLRP1, NLRP3, and NLRC4 inflammasome complexes have been well studied and shown to have a physiological role (Pedra 2009). **Figure 3** illustrates the domain organization of the NLRP1, NLRP3, and NLRC4 receptors and that of the proteins associated with their multifaceted assembly. **Table 4** presents published findings regarding their expression profiles in human tissue. Recent studies have demonstrated that NLR proteins are expressed ubiquitously throughout the human body in a non-overlapping manner. This proposes different roles in distinctive cell types that allows for optimal detection of pathogens (Kummer 2007).

The NLRC4 holds a N-terminal CARD domain, a central NACHT domain, and a C-terminal LRR domain (**Figure 3**). The N-terminal CARD domain allows NLCR4 to directly bind to pro-caspase-1 via a homotypic CARD-CARD interaction leading to an autocatalytic activation of caspase-1 (Eitel 2010). NLRC4 is a cytosolic sensor of flagellin and flagellated pathogens such as *S. typhimurium* The NLRC4 can initiate an inflammatory cell death, pyroptosis, under constant activation (Miao 2006).
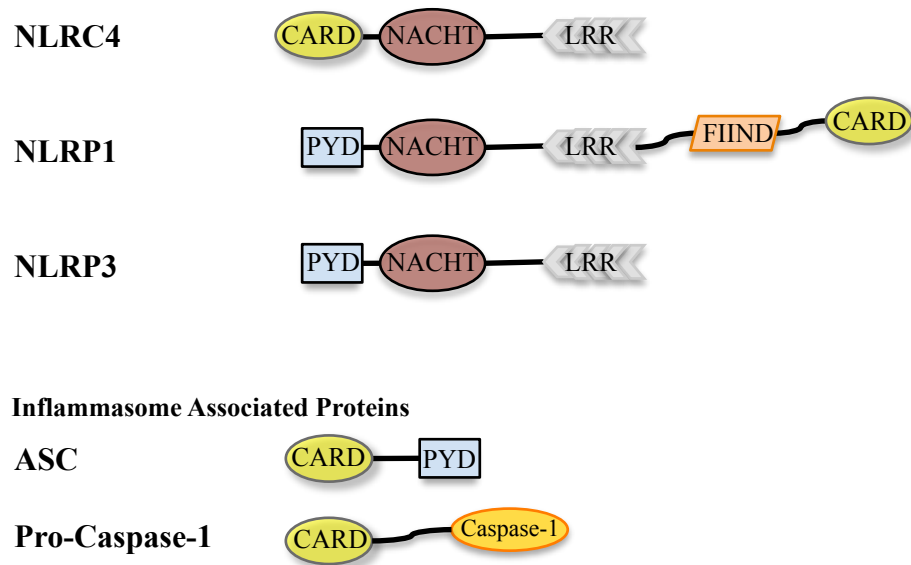
Figure 3. Domain Organization of NLRP1, NLRP3, NLRC4, and Proteins Associated with Inflammasome Assembly. ASC is an adaptor protein that binds NLRP3 to recruit caspase-1. NLRP1 and NLRC4 can bind directly to caspase-1 via the CARD domain or recruit caspase-1 through ASC. Caspase-1 is cleaved into its active subunits.

Table 2. Expression Profiles of Human NLR Proteins

NLR	Expression	Reference
NLRP1	Heart, Spleen, Thymus, Kidney, Liver Pyramidal Neurons, Oligodendrocytes, Gastrointestinal/ Respiratory Tract Epithelial Cell lining, Primary Immune Cells	Kummer, 2007 de Rivero Vaccari 2008
NLRP3	Esophagus and ectocervix (Stratified Non-keratinizing squamous epithelium) Primary Immune Cells (Including Microglia)	Kummer 2007
NLRC4	Bone Marrow, Lungs (Highly Expressed) Lymph Nodes, Placenta, Spleen Brain Cellular Localization Unknown	Kummer 2007 Poyet 2001 Cai 2012

Structurally, the NLRP1 protein receptor sets itself apart due to its unique C-terminal tail, which carries a CARD domain, and a function-to-find domain (FIIND) (**Figure 3**). The NLRP1 inflammasome was the first complex identified as a caspase-1 activation platform involved in the inflammatory response; it is composed of the NLRP1 receptor, ASC, and caspase-1. The involvement of ASC is believed to be cell-type dependent as a study by Faustin *et al.* (2007) demonstrated that the NLRP1 inflammasome was able to assemble with only the NLRP1 receptor and pro-caspase-1 (Faustin 2007; Broz 2010). The NLRP1 inflammasome activates in response to anthrax lethal toxin and muramyl dipeptide (MDP) (Levinsohn 2012; Faustin 2009).

The NLRP3 inflammasome is the most well studied and characterized inflammasome complex. Activation of the NLRP3 receptor leads to its oligomerization, via NACHT-NACHT domain interactions, and proceeds with the recruitment and association of ASC via the Pyrin domain. Subsequently, ASC recruits pro-caspase-1 via a CARD-CARD contact, constituting the NLRP3 inflammasome (**Figure 4**) (Agostini 2004).

The NLRP3 Inflammasome

NLRP3 first received attention when mutations in the *NLRP3* gene were implicated in the autoinflammatory syndromes collectively known as cryopyrin-associated periodic syndrome (CAPS), characterized by an increase in IL-1β production in the absence of infection (Hoffman 2011). Following the discovery that NLRP3 formed

Caspase-1　　　　**ASC**　　　　**NLRP3**
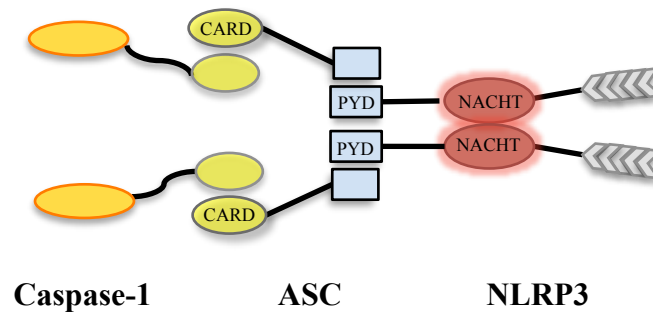
Figure 4. Schematic Representation of the NLRP3 Inflammasome. The NLRP3 inflammasome is comprised of the NLRP3 protein, ASC, and caspase-1. NLRP3 oligomerizes via the NACHT domain upon recognition of activating stimuli and recruits ASC via PYD-PYD binding. Association of NLRP3 with ASC is required for recruitment of pro-caspase-1. The adaptor protein ASC recruits pro-caspase-1 via the CARD domain, leading to the processing of pro-caspase-1 to active caspase-1.

an inflammasome, mutations in the *NLRP3* gene were proven to have a gain-of-function that produced an unregulated amount of IL-1β, which resulted in chronic inflammation (Martinon 2002; Agostini 2004).

In the CNS, NLRP3 is predominantly expressed by microglia and infiltrating macrophages. As previous stated, NLRP3 inflammasome activation leading to the secretion of IL-1β and IL-18 requires two signals:

Signal One: Signal one upregulates inflammatory gene expression typically through TLR priming. As previously stated 'resting' microglia cells express pathogen recognition receptors (PRRs) including TLRs that bind a range of endogenous and exogenous substrates that can result in the downstream activation of the transcription factor, NFkB, a key regulator of the inflammatory response. NFkB shifts microglia from a 'resting' to an 'active' phenotype and triggers the expression of many inflammatory relevant proteins such as pro-IL-1β, and pro-IL-18; however, many inflammatory proteins are expressed as zymogens and require cleavage or a biochemical change, events that result from Signal two.

Signal Two: Signal two leads to the activation of the NLRP3 protein. A variety of stimuli, including microbial PAMPs and host-derived DAMPs, are sensed by NLRP3 and promote inflammasome activation. Due to the number and divergent characteristics of the stimuli, it is unlikely that they are bound directly by the NLRP3 receptor. Rather, it has been proposed that the NLRP3 activators converge on a common pathway that leads to the generation of a common endogenous NLRP3 ligand. These pathways differ in the upstream recognition of PAMPs and DAMPs but all induce potassium efflux and

generate mitochondrial-derived ROS believed to culminate in the exposure or production of a common cytosolic product that serves as the NLRP3 ligand and activator. Furthermore, Ca^{2+} mobilization has been has been found to hold a role in the regulation of NLRP3. (Sutterwala 2007; Latz 2013).

Pore-formation by bacterial toxins or ATP stimulation of P2X7 receptors incites the activation of the cytoplasmic NLRP3 receptor (Kanneganti 2007). Additionally, crystalline substances activators are known to lead to inflammasome assembly following phagocytosis through the destabilization of lysosomal membranes leading to the release of the protease, cathepsin-B, resulting in NLRP3 activation. (Halle 2008; Hornung 2008). Although it is unclear of how Ca^{2+} influences NLRP3 activation, Ca^{2+} influx has been implicated in inflammasome activation induced by particulate or crystalline substances. It has been reported that the production of intracellular ROS induced by crystalline substances is sensed by TRPM2, a receptor that facilitates Ca^{2+} influx into the cell (Latz 2013). Ca^{2+} mobilization has also been implicated in other pathways that regulate the NLRP3 inflammasome such as the response of C/EPB-homologous protein (CHOP) to unfolded protein (Latz 2013). NLRP3 inflammasome activation is diagrammed in **Figure 5.**

Inflammasome-forming NLRs, particularly NLRP3, have received much attention do to their ability to sense pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). NLRP3 responds to an extensive and diverse list of exogenous/endogenous stressors. **Figure 6** displays a number of those activating factors. NLRP3 responds to pathogens including bacteria like *Staphylococcus aureus,*

Listeria monocytogenes, and Streptococcus pneumonia (Mariathasan 2006; Munoz-Planillo 2009; Bauernfeind 2011). Viruses including *Influenza A virus, Adenovirus, and Sendai virus* are also known to activate NLRP3 (Kanneganti 2006; Bauernfeind 2011). Host-derived DAMPs are implicated in initiating and perpetuating sterile inflammatory responses and can lead to cell damage or cell death. NLRP3 recognizes: Adenosine triphosphate (ATP), Monosodium urate (MSU), calcium pyrophosphate dehydrate (CPPD), aluminum hydroxide (alum), asbestos, silica, hyaluronan, amyloid-β, and glucose (Sutterwala 2007; Bauernfeind 2011; Park 2007).

ATP is a danger signal released from damaged and dying cells that alerts the environment to tissue damage and prompts inflammasome activation by binding to the P2X7 receptor triggering a pannexin-1 hemichannel. The internalization of environmental irritants such as silica, alum, and asbestos or host derived factors such as uric acid, cholesterol crystals, and amyloid-β have been shown to disrupt lysosomal membranes, triggering NLRP3 (Sutterwala 2007).
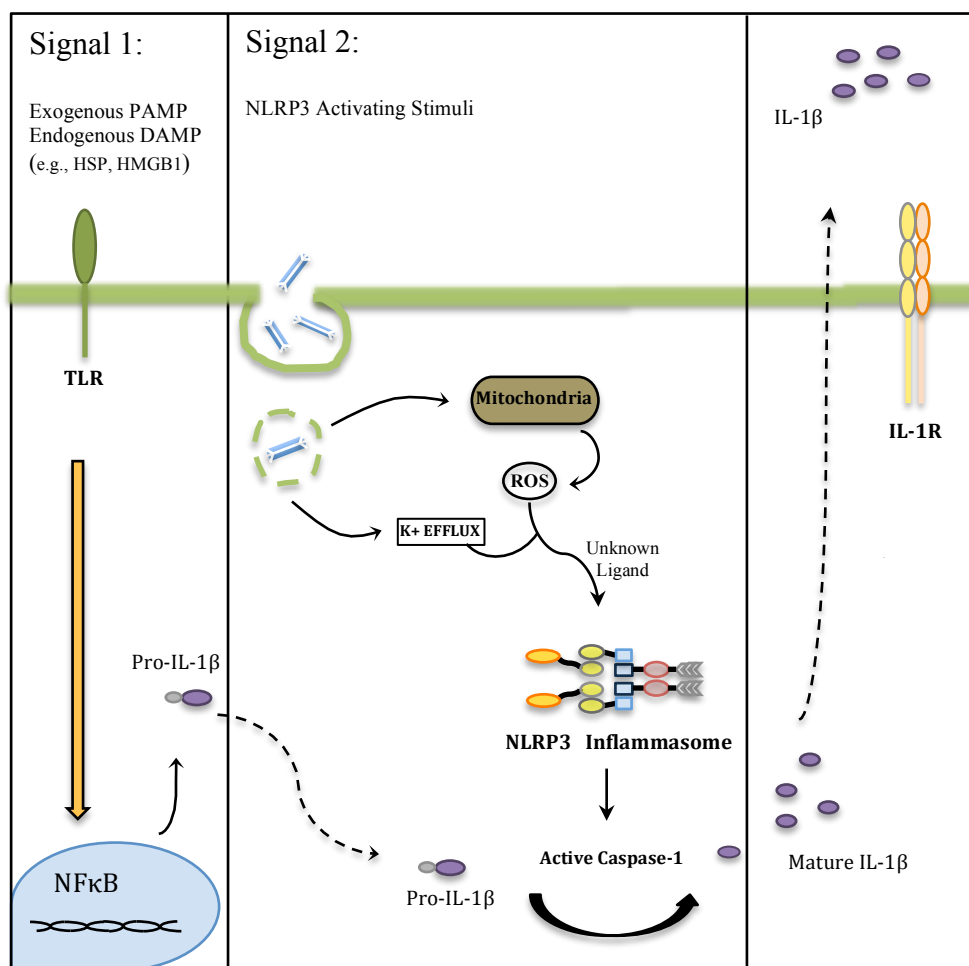
Figure 5. **Model for NLRP3 Inflammasome Activation**. The release of IL-1β and IL-18 requires two signals for production and secretion. **Signal 1:** The first signal occurs upon PAMP/DAMPs binding to TLRs to initiate the transcription and translation of inactive cytokine precursors. **Signal 2:** The second signal involves activation of the NLRP3 protein that leads to inflammasome assembly, caspase-1 activation and subsequent cleavage of cytokines. The inflammasome is activated through stimuli that induce vesicle disruption (illustrated above). The figure is a modified version from Ciraci *et al.* 2012

DAMPs

Host Derived
Cytoplasmic DNA
ATP
Heparan sulfate
Amyloid-β
Hyaluronan
Cholesterol

Environmental
UV Radiation
Asbestos
Silica

PAMPs

Bacteria
Staphylococcus aureus
Listeria monocytogenes
Streptococcus pneumonia

Virus
Influenza A.
Adenovirus

**NLRP3
Inflammasome**

Figure 6. Known NLRP3 Activators.

IL-1 and Inflammation

The interleukin family of cytokines consists of 11 members that serve as agonist, antagonist, or anti-inflammatory ligands. The interleukin-1 receptor (IL-1R) family includes 10 members that serve as ligand binding, decoy, or antagonist receptors (Boraschi 2006; Dinarello 2009).

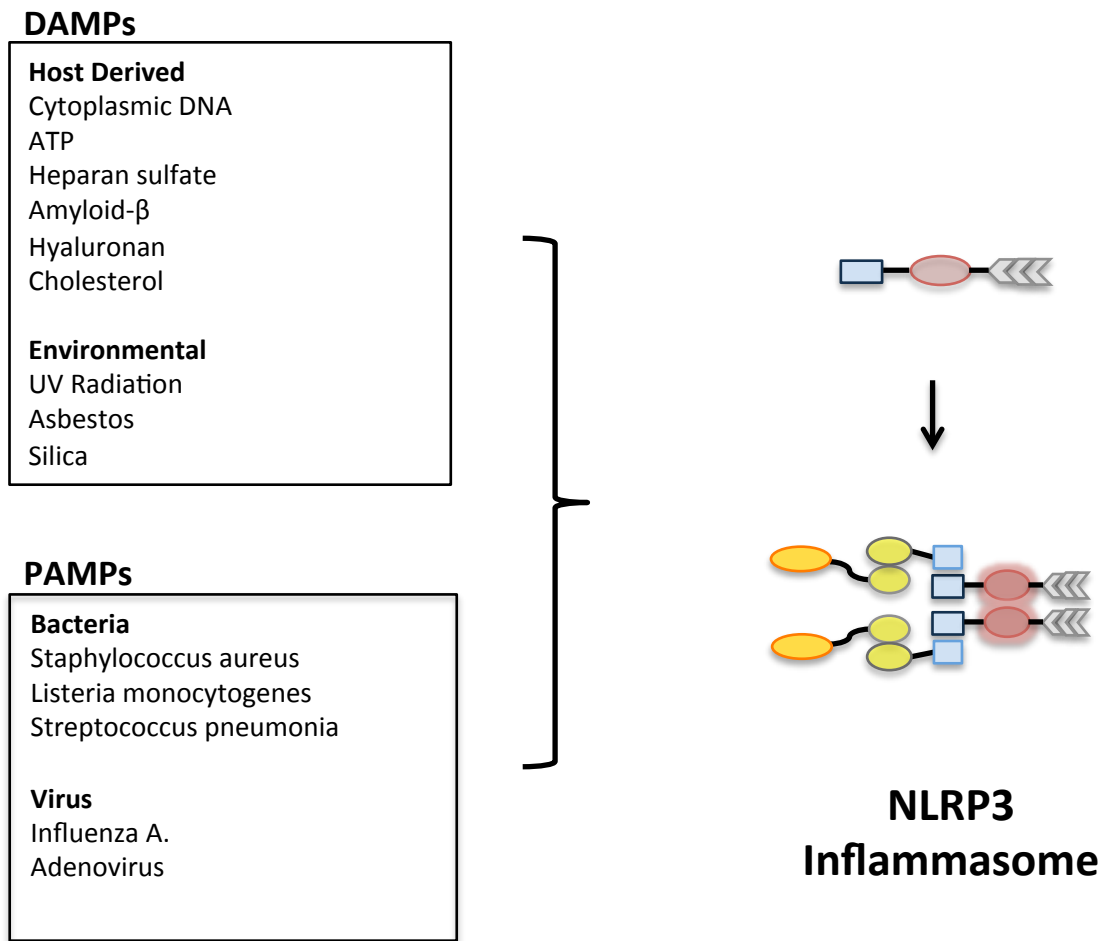IL-1β and IL-18 execute their signaling through MyD88, IL-1 receptor-associated kinases (IRAKs), and TNF receptor associated factor 6 (TRAF-6) that results in downstream activation of transcription factors, mitogen-activated protein kinase (MAPK), and NF-κB (Shaftel 2007; Muroi 2008; Dinarello 2009).

IL-1β: IL-1β is a potent pyrogenic cytokine vital for innate immune confrontations against pathogenic assault but it also participates in several crucial physiological processes including CNS injury, and hypothalamic temperature regulation (Dinarello 2009). IL-1β signaling fosters the production of IL-18, TNF, and additional IL-1β by binding to IL-1R in an autocrine and paracrine manner. It also upregulates the expression of adhesion molecules in endothelial and mesenchymal cells that lead to the infiltration of leukocytes that aid in the resolution of infection and tissue restoration. In the CNS, IL-1β is primarily produced by microglia and macrophages and promotes astrocyte activation and proliferation as well as disruption of the BBB. (Koziorowski 2012; Dinarello 2009).

IL-18: IL-18 is synthesized by monocytes, macrophages, splenocytes, and keratinocytes and stimulates the production of IFN-γ from T-helper cells (Th1). In the CNS, IL-18 is produced by microglia, astrocytes, and neurons. It stimulates microglia

proliferation and emulates IL-1β in the production of IL-1β, TNFα, and adhesion molecules leading to the infiltration extravasation of inflammatory cells (Conti 1999; Prinz 1999; Sugama 2002).

Chronic IL-1β and IL-18 production can be detrimental to host tissue, and is therefore regulated at the level of transcription, translation, and secretion to prevent over activation of the innate immune response. Patients with autoinflammatory diseases are afflicted with chronic inflammation due to disorders in IL-1β secretion, NF-κB activation, protein folding, complement cytokine signaling, and macrophage activation circumventing the regulatory mechanisms (Master 2009).

CHAPTER TWO

EXPERIMENTAL METHODS AND RATIONALE

Cell lines and reagents

The human monocyte cell line THP-1 was obtained from the American Type

Culture Collection (ATCC). ATP and phorbol-12-myristate-13-acetate (PMA) were

purchased from Sigma-Aldrich. The human IL-1β enzyme-linked-immunosorbent assay

(ELISA) Kit Ready-SET-Go! Kit was obtained from eBioscience (catalog no. 88-7010-

88).

Cell Culture

The human monocyte cell line THP-1 was obtained from the American Type

Culture Collection (ATCC). The THP-1 cells were maintained in Roswell Park Memorial

Institute (RPMI) 1640 media, supplemented with 10% FBS, 100 IU/ml penicillin,

1mg/ml streptomycin, 0.25 µg/ml amphotericin B, non-essential amino acids, 1mM

sodium pyruvate, 10mM HEPES buffer and 2 mM glutamine. THP-1 cells stably

expressing short hairpin RNAs for NLRP3 and control shRNAs were a kind gift from Dr.

Christopher Wiethoff.

α-synuclein

Full length α-synuclein was purchased (rPeptide) and the lyophilized protein was rehydrated to a concentration of 1mg/ml. Alpha-synuclein was incubated for 3 days at 37 C in PBS with 100 mM NaCl under constant agitation followed by aliquoting and storage at -80 C.

Immunofluorescence microscopy

Cells were allowed to adhere to Fibronectin (Sigma-Aldrich) treated glass coverslips and fixed with 3.7% formaldehyde (Polyscience) in 0.1 M piperazine-N, N'bis (2-ethanesulfonic acid) PIPES buffer at pH 6.8 for 15 min. Images were collected with a DeltaVision microscope (Applied Precision) equipped with a digital camera (CoolSNAP HQ; photometrics), using a 1,4-numerical aperture (NA) 100X objective lens, and were deconvolved with SoftWoRx deconvolution software (Applied Precision). Tiff images were generated using Imaris software (Bitplane).

Quantification of IL-β secretion by ELISA

THP-1 cells were plated at $2x10^5$ cells per well in a black 96-well plate with 5 ng/ml PMA for 48 hours to induce macrophage differentiation. Differentiated macrophages were washed, and then left untreated or pretreated with α-synuclein aggregates before being serum starved for 2 hours. A subset of cells were then treated with ultrapure LPS (10 ng/ml) for 2 hours, washed and then left untreated, or treated for 1 hour with 5 mM ATP, as a positive control. Supernatants from each sample were collected the morning after, and an ELISA was performed.

Caspase-1 Assay

Diffentiated THP-1 cells were incubated in the presence or absence of α-synuclein (2.4 μg/ml) for 48 hours before being serum starved for 2 hours, and then left untreated or treated with LPS for 2 hours. Cells were then washed in PBS. Caspase-1 activity in THP-1 cells was assessed with a caspase-1 FLICA kit (Immunochemistry Technologies) according to the manufacturer's instructions. The maximum fluorescent intensity of 50 cells in each treatment group was assessed microscopically on a DeltaVision wide field fluorescent microscope (Applied Precision).

CFP-ASC expressing THP-1 cells

The CFP-ASC plasmid was obtained from Dr. Douglas T. Golenbock (University of Massachusetts Medical School). Lentivirus was produced in human 293T cells transfected with FUGW-based expression vector encoding CFP-ASC with the packaging plasmid and the envelope plasmid (VSV-G). Supernatants collected after 48 hours were passed through 0.2 μm filters and used to transduce THP-1 cells by spinoculation.

For experiments, CFP-ASC expressing THP-1 cells (THP-1cASC) were seeded at $3x10^5$ cells per group sample with 5 ng/ml PMA for 48 hours to induce macrophage differentiation and then stimulated. Quantification of ASC complexes per group was assessed microscopically on a DeltaVision wide field fluorescent microscope (Applied Precision).

ROS Assay

THP-1 cells were plated at $2x10^5$ cells per well in a black 96-well (Costar) plate with 5 ng/ml PMA for 48 hours, washed, and rested for 1 day in RPMI 1640 plus 10% FBS. Cells were then loaded with the ROS-sensitive fluorescent dye 2,7-dichlorofluorescein diacetate (DCFDA; Invitrogen) for 30 min by following the manufacturer's protocol, and washed with phosphate-buffered saline (PBS) to remove unincorporated dye. α-synuclein aggregates were added at a concentration of 2.4 μg/ml. Fluorescence intensity was measured over the course of 72 hours at an excitation wavelength of 485 nm and an emission wavelength of 485 nm and an emission wavelength of 520 nm on a fluorescent plate reader (Biotek). Results are presented as background subtracted values (background was defined as cells that were not loaded with DCF).

CHAPTER THREE

RESULTS

Aggregated α-synuclein induces vesicle damage in THP-1 cells

Galectin-3 is a sugar binding protein that recognizes beta-galactosides found on the outer leaflet of the plasma membrane and the interior leaflet of intracellular vesicles, as so, studies have used Galectin-3 relocalization as an assay to identify vesicle rupture (Di Lella 2011; Maier 2012; Ray 2010). By using a retroviral vector expressing mCherry-Galectin3 our recent studies identified that α-synuclein can rupture intracellular vesicles following endocytosis in human neuroblastomas cells (Freeman 2013). Therefore, we wanted to investigate whether α-synuclein could induce vesicle rupture in differentiated THP-1 cells transduced with the retroviral vector expressing mCherry-Galectin3 (THP-1chGal3). We treated differentiated THP-1chGal3 cells with α-synuclein for a series of time points and found that α-synuclein induced vesicle rupture in cells treated for 48 hours. Untreated THP-1chGal3 cells displayed a diffuse cytoplasmic localization of mcherry-Galectin3, while cells treated with aggregates displayed intracellular punctate structures, suggesting vesicle rupture, starting at 24 hours with prominent relocalization at 48 hours (**Figure 7**).
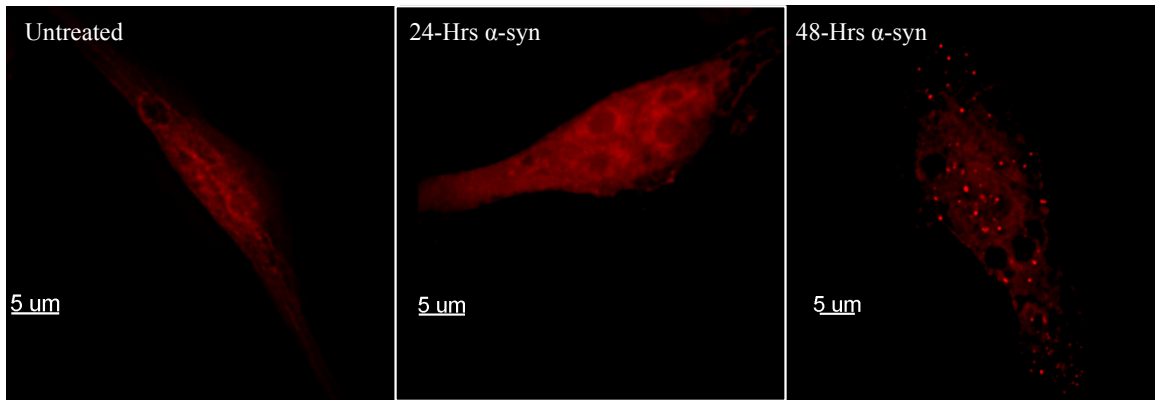
Figure 7. α-synuclein aggregates induce vesicular rupture in THP-1 cells.
THP-1 cells stably expressing mcherry-Galectin3 were treated with α-synuclein aggregates for a series of time points. Galectin-3 relocalization at 48 hours is indicative of vesicle rupture.

Aggregated α-synuclein induces IL-1β secretion from THP-1 cells

As previously stated, activated microglia and microglia-derived IL-1β has been identified surrounding Lewy body deposits as wells in the CSF of PD patients (McGeer 1988; Mogi 1996). It is known that several crystals and aggregated proteins activate the NLRP3 inflammasome by means of vesicle rupture in activated microglia, leading to the maturation and release of IL-1β. We investigated whether aggregated α-synuclein leads to the activation and assembly of the NLRP3 inflammasome and subsequent release of IL-1β following vesicle rupture. We primed differentiated THP-1 cells with ultrapure lipopolysaccharide (LPS) to induce production of pro-IL-1β since it is not constitutively expressed and thus requires transcriptional stimulation. Activated microglia have an upregulation of pro-IL-1β whose maturation and secretion is achieved upon NLRP3 inflammasome activation.

It is important to note that in this study, activation of the NLRP3 inflammasome and subsequent release of IL-1β derailed from the traditional model that sees cell priming or signal one come before treatment with an NLRP3 activator or signal two. There was little to no difference in IL-1β release between cells simply primed with LPS and those first primed with LPS followed by α-synuclein treatment. We had noted that vesicle rupture was induced by α-synuclein 48 hours after initial treatment, therefore, we hypothesized that pro-IL-1β levels induced by LPS priming had diminished by the time α-synuclein had prompted vesicle rupture and NLRP3 inflammasome activation. We therefore treated with α-synuclein for 48 hours then increased production of pro-IL-1β with LPS treatment.

We found that aggregated α-synuclein led to a time-dependent and dose-dependent release of IL-1β into the supernatant with prominent secretion 48 hours after treatment, which correlated with the time dependent vesicle rupture observed seen in THP-1chGal3 ($p<0.01$)(**Figure 8A, B**). In all subsequent experiments, THP-1 cells were treated with α-synuclein aggregates for 48 hours.

In order for IL-1β to be secreted it requires processing from caspase-1. Caspase-1 is found as the zymogen, pro-caspase-1, that is activated upon inflammasome assembly. As such, we tested whether α-synuclein led to the activation of caspase-1 by using a fluorescent cell-permeable probe that covalently binds to only activate caspase-1 (FLICA). The fluorescent intensity of individual cells was assessed microscopically for each treatment group. We noted the highest FLICA fluorescence in primed THP-1 cells treated with α-synuclein ($p<0.01$)(**Figure 8C**). These data collective demonstrate that α-synuclein vesicle rupture induced activation of the inflammasome and subsequent release of IL-1β from THP-1 cells.

A

B

C
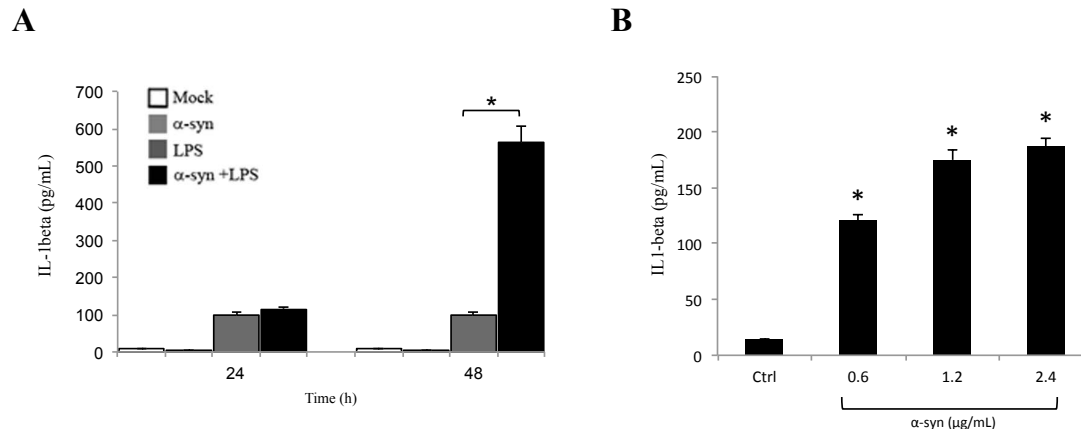
Figure 8. α-synuclein induces caspase-1 dependent IL-1β release from THP-1 cells.
(**A**). ELISA of the time dependent release of IL-1β by differentiated THP-1 cells left untreated (Mock) or stimulated with α-synuclein (2.4 μg/ml) and/or LPS. (**B**). ELISA of the dose dependent release of IL-1β into the supernatant of LPS-primed THP-1 cells left unstimulated (Ctrl) or stimulated with an increasing amount of α-synuclein. (**C**). Quantification of caspase-1 activation of THP-1 cells. Caspase-1 activation was visualized by incubation with fluorescent cell-permeable probe that binds only activated caspase-1 (FLICA). Data represents the mean and standard errors of 3 replicates. * indicates a P value <0.01.
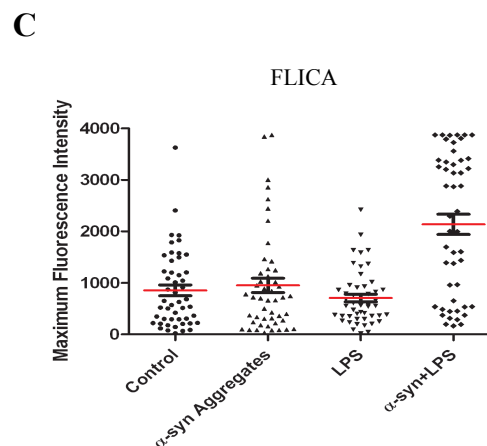
α-synuclein activates the NLRP3 inflammasome

Next we investigated whether the adapter protein ASC and the NLRP3 inflammasome were involved in the activation of caspase-1 and subsequent IL-1β release. We stably transduced a fusion protein ASC and cyan fluorescent protein (CSF-ASC) into THP-1 cells. The adaptor protein ASC is found diffused throughout the cytoplasm, but inflammasome assembly leads to ASC protein oligomerization, changing the cytoplasmic fluorescence of CFP-ASC from a diffuse pattern to a punctate formation, a relocalization event indicative of ASC activation (**Figure 9A; white arrowhead**). In agreement with the IL-1β assay, the α-synuclein and LPS treatment group supported more cells with oligomerized CFP-ASC protein than α-synuclein or LPS alone. (**Figure 9A**).

Next, we confirmed that α-synuclein aggregates specifically activated the NLRP3 inflammasome by obtaining stably transduced THP-1 cells with a lentivirus expressing shRNA against NLRP3 or control shRNA. We observed that LPS primed cells expressing shRNA knockdown of NRLP3 (THP-1N3KD) released significantly less IL-1β compared to LPS primed cells expressing control shRNA (THP-1cntrl) after being treated with α-synuclein (**Figure 9C**) (p = 0.0117) To test whether THP-1N3KD cells were competent for IL-1β production and release we treated cells with transfected DNA. A different inflammasome complex, the AIM2 inflammasome, recognizes transfected DNA and is able to mediate casapase-1 activation leading to the secretion of IL-1β in primed cells. Both control and NLRP3 knockdown cells produced a robust release of IL-1β (**Figure 9B**). Taken together, these results demonstrate that α-synuclein activates the NLRP3 inflammasome, resulting in caspase-1 activation and the release of IL-1β.

A
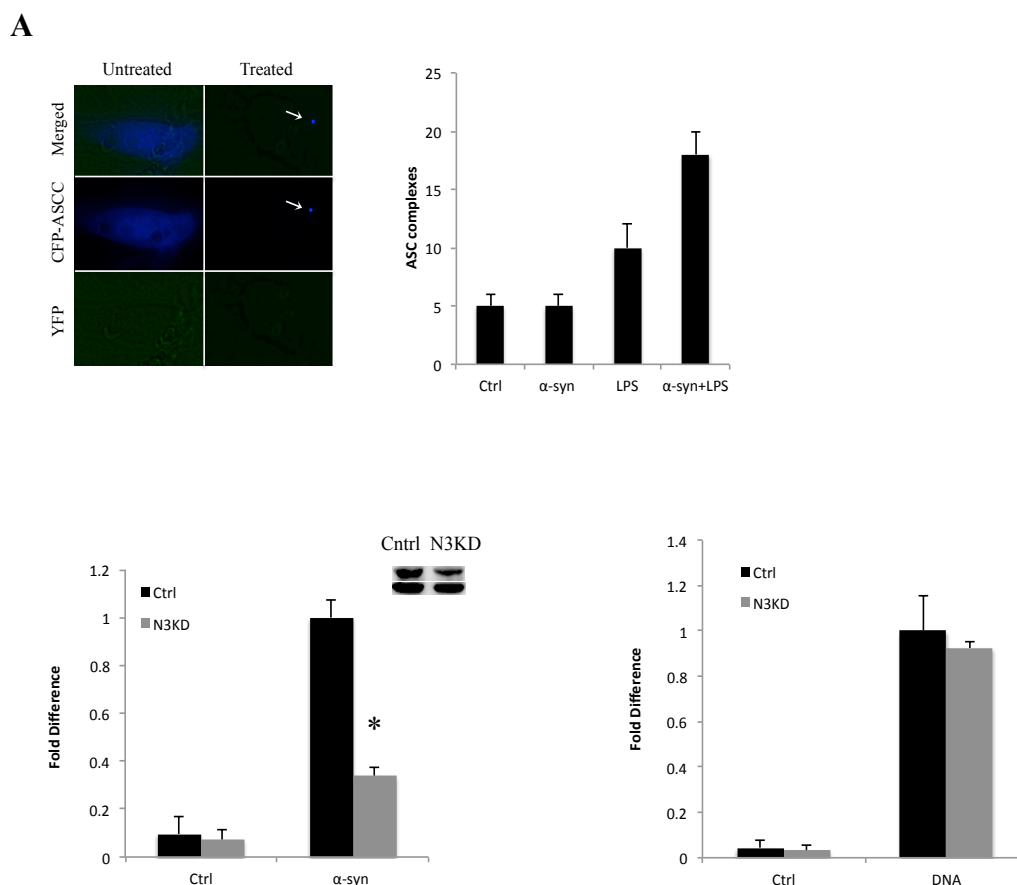
Figure 9. α-synuclein aggregates activate the NLRP3 inflammasome.
(**A**). THP-1 cells stably transduced with CFP-ASC. Cellular
outlines/boundaries were captured using the YFP filter. Under baseline
conditions CFP-ASC fluorescence is distributed throughout the cell;
however, once the inflammasome is assembled and the adapter protein ASC
is activated it will proceed to form oligomers that change the fluorescent
distribution into a punctate structure (white arrowhead). This grouping of
CFP-ASC protein serves as a marker of ASC activation. We noted a greater
number of cells with CFP-ASC clusters in THP-1 cells treated with α-
synuclein and primed with LPS than in cells treated with α-synuclein or LPS
alone. (**B, C**). ELISA of IL-1β released into supernatants of LPS primed
THP-1N3KD and THP-1cntrl left unstimulated (Ctrl) or treated with
α-synuclein (1.2 μg/ml). Data represents the mean and standard errors of 3
replicates. * indicates a P value = 0.0117.

α-syn-induced inflammasome activation involves K$^+$ efflux and ROS production

We further elucidated the mechanism of IL-1β secretion induced by α-synuclein by investigating potassium efflux, and ROS production, two events known to be involved in NLRP3 inflammasome. *In vitro* studies have inhibited the efflux process in cells with the addition of extracellular KCl to growth medium, and observed an inhibition of NLRP3 inflammasome activation. **Figure 10A** demonstrates that treatment with extracellular KCl arrests α-synuclein-induced IL-1β secretion in a dose dependent manner. The exact role of potassium efflux in NLRP3 inflammasome activation remains elusive; however, it is believed that a low potassium environment might a prerequisite for NLRP3 inflammasome assembly (Ciraci 2012).

Although it is not clear how ROS is involved in the activation of NLRP3, inflammasome activation is dependent on the production of mitochondrial-derived ROS. It is hypothesized that the mitochondria-derived ROS may be a common event upstream of NLRP3 activation that may prompt events leading to the generation or exposure of the endogenous NLRP3 ligand that too may be mitochondrial-derived (Ciraci 2012). Particulate NLRP3 activators such as uric acid, and amyoid- β have been shown to induce vesicular damage, an event shown to drive the production and increase of intracellular ROS (Suzanne 2010; Ciraci 2012); therefore, we investigated whether α-synuclein-induced vesicle rupture also led to the increased production of ROS. We noted the production of ROS in THP-1 cells treated with α-synuclein aggregates using the

A

B
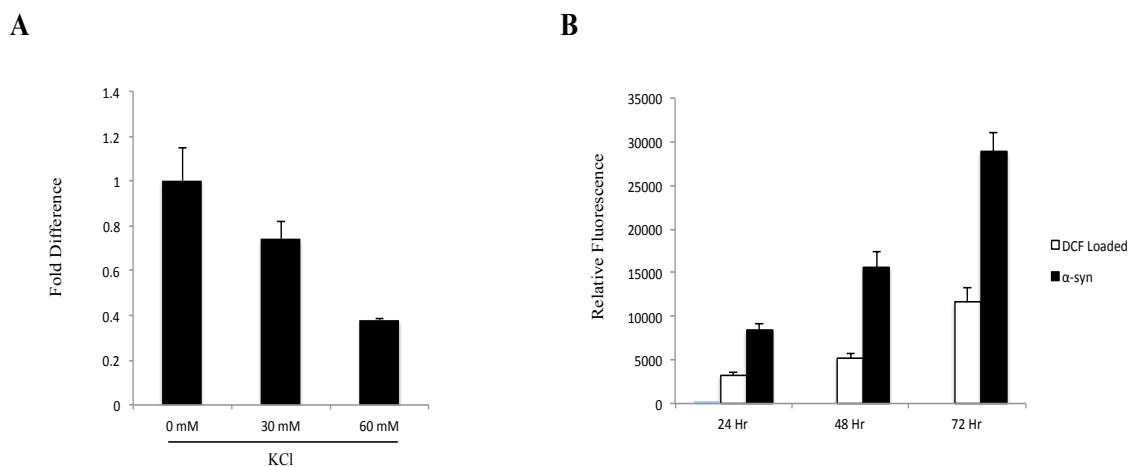
Figure 10. α-synuclein aggregates induce both IL-1β release dependent on K$^+$ efflux and the production of ROS in THP-1 cells. (A). ELISA of IL-1β release from differentiated wild type THP-1 cells treated with α-synuclein for 48 hr, rested in serum-free media for 2 hr, and primed with 10 ng/ml of LPS, while simultaneously treating with medium alone or with KCl (potassium efflux inhibitor; 30 mM or 60 mM) for two hours. **(B)**. Differentiated wild type THP-1 cells loaded with the fluorescent substrate H$_2$DCFDA reagent and incubated in the presence of or absence of α-synuclein aggregates.

fluorophore H_2DCFDA that fluoresces after being oxidized by ROS. Differentiated

THP-1 cells treated with α-synuclein aggregates responded with the generation of ROS as

a significant increase in fluorescence was observed in a time dependent manner (**Figure**

10B).

CHAPTER FOUR

DISCUSSION

An inflammatory response is built to promote homeostasis; however, in the

context of an aging or traumatized brain, inflammation can bring about detrimental

effects. In the CNS, neuroinflammation is mediated by activated microglia and is a

neuropathological hallmark of Parkinson's disease and related synucleinopathies. It is not

clear whether neuroinflammation feeds the on-set of disease or whether it is a feature that

drives the progression of neurodegeneration as α-synuclein deposition increases. It is

important to define the signaling pathways and the mediators through which α-synuclein

influences microglia secretion of proinflammatory cytokines in order to develop anti-

inflammatory treatments. In this thesis, we demonstrated that α-synuclein oligomers, or

intermediate sized aggregates induce NLRP3 inflammasome activation in human THP-1

cells. The NLRP3 inflammasome is a multiprotein complex that serves as a platform for

caspase-1 activation and results in the release of proinflammatory cytokines IL-1β and

IL-18. Our experimental results show that aggregated α-synuclein can be added to the

long list of NLRP3 activators as it is sensed as an endogenous 'danger signal' following

vesicle rupture. Furthermore, we noted that α-synuclein led to the release of IL-1β in a

time and dose dependent manner that involved ASC oligomerization, caspase-1

activation, and was dependent on the NLRP3 protein.

The central nervous system (CNS) has been perceived to be a site of immune privilege due to its physical separation from the rest of the body by the blood brain barrier (BBB) and the lack of a lymphatic drainage; however, it is now well established that insults to the CNS by injury or disease results in an immune response by resident immune cells, microglia and astrocytes. The resident immune cells are also able to recruit circulating macrophages and T cells to facilitate robust inflammatory responses. Due to the complexity of an immune response, it is believed that treatments against inflammation in progressive diseases such as PD might involve a combination of targets. Although we have identified that α-synuclein leads to NLRP3 activation, it is important to note that the cells used in these experiments are THP-1 cells. THP-1 cells belong to a human monocytic cell line that can be differentiated into macrophage like cell. Although a number of studies have used THP-1 cells as a microglia-like model, it would be beneficial to see the level of NLRP3 activation in an immortalized or primary microglia cell line.

For future experiment, it would be of importance to dissect each step in inflammasome activation. In this study we used LPS treatment to activate cells through TLR4 signaling for Signal one. It would be of value to use a number of endogenous proteins such as HMGB-1 to induce cell priming. Further investigations should also target what different surface receptors on microglia lead to downstream signaling and production of pro-inflammatory cytokines.

Signal two leads to inflammasome assembly. It would be wise to further investigate whether mutant forms of α-synuclein would also induce inflammasome assembly. Studies by Freeman *et al.* (2013) found that neuronal cells could not

endocytose aggregated forms of the mutant E46K. It would be noteworthy to investigate whether this mutant form of α-synuclein could be phagocytosed by microglia cells and if not, whether or not it could activate the inflammasome through frustrated phagocytosis.

The newly discovered inflammasome-forming NLRs have been under extensive study due to their role in sensing pathogens and endogenous danger molecules, however, their role in neuroinflammation has yet to be studied. It is necessary to investigate what other NLRs are found in the CNS and their cellular localization. Recent studies by Minkiewicz *et al.* (2013) found that astrocytes, the most abundant glia cell in the CNS, express and are able to assemble the NLRP2 inflammasome in response to extracellular ATP.

REFERENCES

1. Agostini, L., F. Martinon, K. Burns, M. F. McDermott, P. N. Hawkins and J. Tschopp (2004). "NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle- Wells autoinflammatory disorder." Immunity **20**(3): 319-325.

2. Bauernfeind, F., Bartok, E., Rieger, A., Franchi, L., Nu ́ ñ ez, G., and Hornung, V. (2011). Cutting edge: reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 inflammasome. J. Immunol. *187*, 613–617.

3. Benmoyal-Segal, L. & Soreq, H. (2006) Gene-environment interactions in sporadic Parkinson's disease. J Neurochem, 97, 1740-1755.

4. Bennett MC. The role of alpha-synuclein in neurodegenerative diseases. Pharmacol Ther. 2005; 105: 311–31.

5. Beutler, B., and. Blasius, A.L (2010). Intracellular toll-like receptors. Immunity *32*, 305- 315.

6. Beyer K, M Domingo-Sàbat and A Ariza. 2009. Molecular Pathology of Lewy Body Diseases. Int J Mol Sci 10: 724-745.

7. Boraschi D & Tagliabue A. (2006). The interleukin-1 receptor family. *Vitam Horm* **74,** 229-254.

8. Braak, H., et al., Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett, 2006. 396(1): p. 67- 72.

9. Braak, H., et al., Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging, 2003. 24(2): p. 197-211.

10. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay JM, Duyckaerts C, Flavell RA, Hirsch EC, Hunot S (2009) Infiltration of CD4 + lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest 119:182–192.

11. Brown GC. Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase. Biochem Soc Trans 35: 1119–1121, 2007.

12. Broz, P., von Moltke, J., Jones, J.W., Vance, R.E., and Monack, D.M. (2010). Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. Cell Host Microbe 8, 471-483.

13. Campbell, S., S. Stephens, and C. Ballard, Dementia with Lewy bodies: clinical features and treatment. Drugs Aging, 2001. 18(6): p. 397-407.

14. Cassel SL, Sutterwala FS (2010) Sterile inflammatory responses mediated by the NLRP3 inflammasome. Eur J Immunol 40:607–611.

15. C. Ceraci, et al, Control of innate and adaptive immunity by the inflammasome, Microbes and Infection (2012), http//dx.doi.org/10.1016/j.micinf.2012.07.007

16. Conti B, Park LC, Calingasan NY, Kim Y, Kim H, Bae Y, Gibson GE, Joh TH (1999) Culturesofastrocytesandmicrogliaexpressinterleukin18.Brain Res Mol Brain Res 67:46 –52.

17. Conway KA, Harper JD, Lansbury PT (1998) Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. *Nat Med* 4:1318 –1320.

18. Conway, K.A., J.D. Harper, and P.T. Lansbury, Jr., Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. Biochemistry, 2000. 39(10): p. 2552-63.

19. Conway, K.A., et al., Accelerated oligomerization by Parkinson's disease linked alpha-synuclein mutants. Ann N Y Acad Sci, 2000. 920: p. 42-5

20. Croisier, E., Moran, L.B., Dexter, D.T., Pearce, R.K. & Graeber, M.B. (2005) Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. J Neuroinflammation, 2, 14.

21. Chinta, S.J. & Andersen, J.K. (2005) Dopaminergic neurons. Int J Biochem Cell Biol, 37, 942-946.

22. Dekker M C J, V Bonifati and C M van Duijn. Parkinson's disease: piecing together a genetic jigsaw. Brain 2003; 126: 1722-1733

23. Dickson, D.W., Fujishiro, H., Orr, C., DelleDonne, A., Josephs, K.A., Frigerio, R., Burnett, M., Parisi, J.E., Klos, K.J., Ahlskog, J.E., 2009. Neuropathology of non-motor features of Parkinson disease. Parkinsonism Relat. Disord. 15 (Suppl. 3), S1–S5.

24. Di Lella S, Sundblad V, Cerliani JP, Guardia CM, Estrin DA, et al. (2011) When galectins recognize glycans: from biochemistry to physiology and back again. Biochemistry 50: 7842–7857.

25. Dinarello CA. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* **27,** 519-550.

26. el-Agnaf, O.M. and G.B. Irvine, Aggregation and neurotoxicity of alpha-synuclein and related peptides. Biochem Soc Trans, 2002. 30(4): p. 559-65.

27. Eitel J, Suttorp N, Opitz B. 2010. Innate immune recognition and inflammasome activation in listeria monocytogenes infection. Front Microbiol 1:149.

28. Faustin B, Lartigue L, Bruey JM, Luciano F, Sergienko E, Bailly-Maitre B, Volkmann N, Hanein D, Rouiller I, Reed JC (2007) Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activa- tion. Mol Cell 25:713–724.

29. Farkas E, De Jong GI, de Vos RA, Jansen Steur EN, Luiten PG (2000) Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease. Acta Neuropathol (Berl) 100:395–402.

30. Forsyth CB, Shannon KM, Kordower JH, et al. Increased intestinal permeability correlates with sigmoid mucosa   -synuclein staining and endotoxin exposure markers in early Parkinson's disease. PloS One 2011;6:e28032.

31. Freeman D, Cedillos R, Choyke S, Lukic Z, McGuire K, Marvin S, Burrage AM, Sudholt S, Rana A, O'Connor C, Wiethoff CM, Campbell EM. Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. *PLoS One*. 2013;8:e62143

32. G. Paul, S.V. Anisimov, The secretome of mesenchymal stem cells: Potential implications for neuroregeneration, Biochimie (2013)

33. Hald A, Lotharius J (2005) Oxidative stress and inflammation in Parkinson's disease: is there a causal link? Exp Neurol 193:279–290.

34. Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT. 2008. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol 9:857-65.

35. Hanson, J.C. and C.F. Lippa, Lewy body dementia. Int Rev Neurobiol, 2009. 84: p. 215-28.

36. Harald Neumann and Hartmut Wekerle. (2013). Brain microglia: watchdogs with pedigree. Nature Neuroscience. doi:10.1038/nn.3338.

37. Hoffman HM, Brydges SD. 2011. Genetic and molecular basis of inflammasome-mediated disease. J Biol Chem 286:10889-96.

38. Holdorff, B. (2002) Friedrich Heinrich Lewy (1885-1950) and his work. J Hist Neurosci, 11, 19-28.

39. Hornung, V., Bauernfeind, F., Halle, A., Samstad, E.O., Kono, H., Rock, K.L., Fitzgerald, K.A., and Latz, E. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol 9, 847- 856.

40. Jakes, R., M.G. Spillantini, and M. Goedert, Identification of two distinct synucleins from human brain. FEBS Lett, 1994. 345(1): p. 27- 32.

41. Jankovic, J., Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry, 2008. 79(4): p. 368-76.

42. Jarrett, J.T. and P.T. Lansbury, Jr., Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell, 1993. 73(6): p. 1055-8.

43. A Jekabsone, PK Mander, A Tickler, M Sharpe, GC Brown Fibrillar beta-amyloid peptide Abeta1-40 activates microglial proliferation via stimulating TNF-alpha release and H2O2 derived from NADPH oxidase: a cell culture study J Neuroinflammation, 3 (2006), p. 24.

44. Kanneganti, T.D., Ozo¨ren, N., Body-Malapel, M., Amer, A., Park, J.H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., et al. (2006). Bacterial RNA and small antiviral compounds activate caspase-1 through cry-opyrin/Nalp3. Nature *440*, 233–236.

45. Kanneganti TD, Lamkanfi M, Kim YG, Chen G, Park JH, Franchi L, Vandenabeele P, Nunez G. 2007. Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. Immunity 26:433-43.

46. Keller M, Ruegg A,Werner S,Beer HD (2008) Activecaspase-1isaregulator of unconventional protein secretion. Cell 132:818 – 831.

47. Kierdorf, K. *et al. Nat. Neurosci.* **16**, 273–280 (2013).

48. Kitada, T., et al., Mutations in the parkin gene cause autosomal recessive juvenile

parkinsonism. Nature, 1998. 392(6676): p. 605-8.

49. Klegeris A, Glasson BI, Zhang H, Maguire J, Pelech S, McGeer PL (2006). Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells.

50. Koziorowski D, Tomasiuk R, Szlufik S, Friedman A (2012) Inflammatory cytokines and NT-proCNP in Parkinson's disease patients. Cytokine 60:762–766.

51. Kruger, R., et al., Ala30Pro mutation in the gene encoding alpha- synuclein in Parkinson's disease. Nat Genet, 1998. 18(2): p. 106-8.

52. Kummer, J.A. et al. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. J. Histochem. Cytochem. 55, 443–452 (2007).

53. Lashuel, H.A., et al., Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J Mol Biol, 2002. 322(5): p. 1089-102.

54. Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39: 151-70

55. Lebouvier, T., et al., Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One, 2010. 5(9): p. e12728.

56. Levinsohn JL, Newman ZL, Hellmich KA, Fattah R, Getz MA, Liu S, Sastalla I, Leppla SH, Moayeri M. 2012. Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. PLoS Pathog 8:e1002638.

57. Lewy, F.H., Paralysis agitans, in Handbuch der Neurologie. 1912. p. 920-933.

58. Luk KC, Song C, O'Brien P, Stieber A, Branch JR, et al. (2009) Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci U S A 106: 20051–20056.

59. Maier O, Marvin S, Wodrich H, Campbell EM, Wiethoff CM (2012) Spatiotemporal Dynamics of Adenovirus Membrane Rupture and Endosomal Escape. Journal of Virology 86: 10821.

60. Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W.P., Weinrauch, Y., Monack, D.M., and Dixit, V.M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. Nature

440, 228–232.

61. Martinon, F., K. Burns and J. Tschopp (2002). "The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta." Mol Cell **10**(2): 417-426.

62. Martin F L, et al. 2004. α-Synuclein and the pathogenesis of Parkinson's disease. Protein and Peptide Letters 11: 229-237.

63. Masters SL, Simon A, Aksentijevich I & Kastner DL. (2009). Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. *Annu Rev Immunol* **27,** 621- 668.

64. Matzinger P. (2002). The Danger Model: A Renewed Sense of Self. *Science* 296, 301-305.

65. McGeer PL, Itagaki S, Boyes BE, McGeer EG (1988). Reactive microglia are positive for HLA- DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38(8): 1285-1291

66. McGeer, P.L., Hong, J.S. & Zhang, J. (2007) Microglial PHOX and Mac-1 are essential to the enhanced dopaminergic neurodegeneration elicited by A30P and A53T mutant alpha-synuclein. Glia, 55, 1178-1188.

67. Medzhitov, R. (2009). Approaching the asymptote: 20 years later. Immunity 30, 766-775.

68. Miao EA, Alpuche-Aranda CM, Dors M, Clark AE, Bader MW, Miller SI, Aderem A. 2006. Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. Nat Immunol 7:569-75.

69. Middeldorp J, van den Berge SA, Aronica E, Speijer D, Hol EM. 2009. Specific human astrocyte subtype revealed by affinity purified GFAP antibody; unpurified serum cross-reacts with neurofilament-L in Alzheimer. PLoS One 4:e7663.

70. Minkiewicz J, de Rivero Vaccari JP, Keane RW. Human astrocytes express a novel NLRP2 inflammasome. Glia 2013; 61: 1113–1121.

71. Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T (1996) 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 211: 13–16.

72. Munoz-Planillo, R., Franchi, L., Miller, L.S., and Nunez, G. (2009). A critical role for hemolysins and bacterial lipoproteins in Staphylococcus aureus-induced activation of the Nlrp3 inflammasome. J Immunol 183, 3942-3948.

73. Olah, M., Biber, K., Vinet, J., and Boddeke, H. W. (2011). Microglia phenotype diversity. *CNS Neurol. Disord. Drug Targets* 10, 108–118.

74. Ohashi, K., Burkart, V., Flohe, S., and Kolb, H. (2000). Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol 164, 558-561.

75. Park JS, Svetkauskaite D, He Q, et al. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem. 2004; 279: 7370–7.

76. Park JY, Paik SR, Jou I, Park SM (2008) Microglial phagocytosis is enhanced by monomeric alpha-synuclein, not aggregated alpha-synuclein: implications for Parkinson's disease. Glia 56:1215–1223.

77. Parkinson, J., An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci, 2002. 14(2): p. 223-36; discussion 222.

78. Pedra JH, Cassel SL & Sutterwala FS. (2009). Sensing pathogens and danger signals by the inflammasome. *Curr Opin Immunol* **21,** 10-16.

79. Perrin, R.J., et al., Interaction of human alpha-Synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis. J Biol Chem, 2000. 275(44): p. 34393-8.

80. Petrucelli, L., et al., Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. Neuron, 2002. 36(6): p. 1007-19.

81. Polymeropoulos, M.H., et al., Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science, 1997. 276(5321): p. 2045-7.

82. R.A. Kohman, J.S. Rhodes / Brain, Behavior, and Immunity 27 (2013) 22–32

83. Ray K, Bobard A, Danckaert A, Paz-Haftel I, Clair C, et al. (2010) Tracking the dynamic interplay between bacterial and host factors during pathogen-induced vacuole rupture in real time. Cell Microbiol 12: 545–556.

84. Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, Cerny R, Gelman B, Thomas MP, Mosley RL, Gendelman HE (2008). Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. Jounral of Neurochemistry 104(6): 1504-1525.

85. Sanchez-Guajardo V, Febbraro F, Kirik D, Romero-Ramos M (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): e8784.

86. Sanchez-Guajardo V, Barnum CJ, Tansey MG and Romero-Ramos M (2013) Neuroimmunological processes in Parkinson's disease and their relation to α-synuclein: microglia as the referee between neuronalprocesses and peripheral immunity. ASN NEURO 5(2):art:e00112

87. Schaefer L, Babelova A, Kiss E, et al. The matrix component biglycan is proinflam- matory and signals through toll-like recep- tors 4 and 2 in macrophages. J Clin Invest. 2005; 115: 2223–33.

88. Shao W, Yeretssian G, Doiron K, Hussain SN, Saleh M (2007) The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. J Biol Chem 282:36321–36329.

89. Shaftel, S.S. et al. Sustained hippocampal IL-1b overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. J. Clin. Invest. 117,1595–1604 (2007).

90. Simunovic F, et al.2009. Gene expression profiling of substantia nigra dopamine neurons: further insights into Parkinson's disease pathology Brain 132: 1795-1809.

91. Singleton, A.B., et al., alpha-Synuclein locus triplication causes Parkinson's disease. Science, 2003. 302(5646): p. 841.

92. Smith, W.W., et al., Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity. Hum Mol Genet, 2005. 14(24): p. 3801-11.

93. Spillantini, M.G., et al., Alpha-synuclein in Lewy bodies. Nature, 1997. 388(6645): p. 839-40.

94. Stefanis, L., et al., 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(24): p. 9549-60.

95. Su X, Federoff GH, Maguire-Zeiss KA (2009). Mutant alpha-synuclein overexpression mediates early proinflammatory activity. Neurotoxicity Research 16(3): 238-254.

96. Sugama S, Cho BP, Baker H, Joh TH, Lucero J, Conti B (2002) Neurons of the

superior nucleus of the medial habenula and ependymal cells express IL-18 in rat CNS. Brain Res 958:1–9.

97. Sutterwala, F.S., Mijares, L.A., Li, L., Ogura, Y., Kazmierczak, B.I., and Flavell, R.A. (2007). Immune recognition of Pseudomonas aeruginosa mediated by the IPAF/NLRC4 inflammasome. J Exp Med 204, 3235-3245.

98. Tan, E.K., Khajavi, M., Thornby, J.I., Nagamitsu, S., Jankovic, J. & Ashizawa, T.(2000) Variability and validity of polymorphism association studies innParkinson's disease. Neurology, 55, 533-538.

99. Tanaka, Y., et al., Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. Hum Mol Genet, 2001. 10(9): p. 919-26.

100. Termeer C, Benedix F, Sleeman J, et al. Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. J Exp Med. 2002; 195: 99–111.

101. Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, et al. The NLR gene family: a standard nomenclature. Immunity 2008;28:285–87. [PubMed: 18341998]

102. Udan ML, Ajit D, Crouse NR, Nichols MR (2008) Toll-like receptors 2 and 4 mediate Abeta(1-42) activation of the innate immune response in a human monocytic cell line. J Neurochem 104: 524-33.

103. Ueda, K., et al., Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci U S A, 1993. 90(23): p. 11282-6.

104. Uversky, V.N., J. Li, and A.L. Fink, Evidence for a partially folded intermediate in alpha-synuclein fibril formation. J Biol Chem, 2001. 276(14): p. 10737-44.

105. Uversky, V.N., Neuropathology, biochemistry, and biophysics of alpha-synuclein aggregation. J Neurochem, 2007. 103(1): p. 17-37.

106. Varnum MM, Ikezu T (2012) The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer's disease brain. Arch Immunol Ther Exp (Warsz) 60:251–266.

107. Volles, M.J., et al., Vesicle permeabilization by protofibrillar alpha- synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry, 2001. 40(26): p. 7812-9.

108. Volles 2001, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ (2008). Synuclein activates microglia in a model of Parkinson's disease. Neurobiology of Aging 29(11): 1690- 1701.

109. W. Hoyer, T. Antony, D. Cherny, G. Heim, T.M. Jovin, V. Subramaniam, J. Mol. Biol. 322 (2002) 383–393.

110. Winner, B., et al., In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A, 2011. 108(10): p. 4194-9.

111. Zarranz, J.J., et al., The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol, 2004. 55(2): p. 164-73.

112. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong JS, Zhang J (2005). Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB Journal 19(6): 533-542.

113. Zhang W, Dallas S, Zhang D, Guo JP, Pang H, Wilson B, Miller DS, Chen B, Zhang W, McGeer PL, Hong JS, Zhang J (2007). Microglial PHOX and Mac-1 are essential to the enhanced dopaminergic neurodegeneration elicited by A30P and A53T mutant alpha-synuclein. Glia 55(11): 1178-1188.

114. Zimprich, A., et al., Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron, 2004. 44(4): p. 601-7.

VITA

The author, Rudy Cedillos was born in San Salvador, El Salvador on September 5, 1988 to Maritza and Rudy Cedillos. He received a Bachelor of Science degree in Biology from Texas Christian University (Fort Worth, TX) in 2011.

In June 2011, Rudy joined the Department of Cell Biology, Neurobiology, and Anatomy at Loyola University Medical Center (Maywood, IL). Shortly thereafter, he joined the laboratory of Dr. Edward M. Campbell, where he studied the role of alpha-synuclein as a danger associated molecular pattern and its ability to activate of the NLRP3 inflammasome.