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Review Article

Alpha synuclein in hematopoiesis and immunity

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative condition and intracellular deposition of Lewy bodies in the substantia nigra (SN), which can cause dopaminergic neuronal death, is the hallmark of this syndrome. α-synuclein (syn) is a small protein expressed mainly in neurons but can also be found in a number of tissues. It can be present as a soluble monomer under normal physiological conditions, but can be toxic in its oligomeric or fibrillary forms. Most of the available literature has focused on the effects of α-syn pathology in the mechanisms leading to PD. However, the normal functions of α-syn still remain to be fully elucidated. Notably, α-syn in the hematopoietic system seems to mediate important functions as indicated by anemia and incomplete cell maturation when this protein is absent. This review will summarize basic genetic and structural findings, and critical information that suggests an essential role of α-syn in the development and activation of the hematopoietic system and immunity.

1. Introduction

1.1. Genetic, biochemical and structural characteristics of α-syn

Alpha synuclein (α-syn) is a small protein consisting of 140 amino acid residues that is a major component of Lewy bodies present in PD [1, 2], and is found to accumulate in presynaptic terminals of dopaminergic neurons of the substantia nigra (SN). Since α-syn was first identified in the brain, most of its basic properties including structure, genetic mutations, pathological features, and functions have been described in the central nervous system (CNS). To begin this review, basic CNS properties of α-syn will be summarized.

α-syn belongs to the synuclein family, which also has two other members, β- and γ-syn [2]. Each member of the family has unique tissue distribution and functions in the body. α-syn is mostly concentrated in the brain but it is also ubiquitously expressed in small concentrations in a variety of tissues including heart, lung, kidney, skeletal muscle [3, 4]. Interestingly, it is well expressed and abundant in cells of the hematopoietic system [5]. Unlike α-syn, β-syn is mostly found in the brain, while γ-syn has been reported in a variety of neoplastic tissues, especially in invasive breast cancer and some types of sarcomas, and has been suggested as a potential prognostic biomarker in these settings [6]. Studies have shown that there appears to be some functional redundancy among

the three synucleins since α-syn knockout ($\alpha\text{-syn}^{-/-}$) mice are viable and only show minor neurological deficits [7]. Synucleins have also been shown to interact with each other so that β-syn has been reported to inhibit α-syn aggregation, and this provides neurons protection from pathological changes [8].

α-syn is encoded by the *SNCA* gene, and mutations and multiplications of the wild type gene are linked to the familial form of PD. Thus far, there are five *SNCA* mutations that have been described, including A30P, E46K, H50Q, G51D, and A53T [9, 10, 11, 12]. Each of these mutations leads to a different phenotype so that A53T, E46K, and H50Q promote higher rates of α-syn aggregation, while A30P has a slower fibrillary formation rate. Interestingly, G51D decreases α-syn aggregation rates, has a much earlier disease onset and patients with this mutation have α-syn inclusions in brain oligodendrocytes, a unique feature among α-syn mutations [12]. Additionally, each mutated form of α-syn shows different membrane affinity, and this binding affinity affects their potential to aggregate [13].

Structurally, α-syn is divided into 3 domains. The N-terminal domain contains 11-mer KTKEGV sequence repeats that are highly conserved among all 3 synucleins and across mammals. This domain forms apolipoprotein-like class A2 helical structures and is involved in membrane interaction and vesicle trafficking, especially in membranes with acidic phospholipid head groups [14]. Importantly, all of the afore-

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mentioned α -syn mutations are found in this domain. The NAC (non-amyloid-beta-component) middle domain is hydrophobic and may be important in synucleinopathies [14]. The C-terminus is acidic, and has been shown to prevent aggregation and be protective in PD. This terminus is the target of post-translational modifications, and has been shown to bind Ca^{2+} and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes. Additionally, the C-terminus has chaperone activity [15] and protects against oxidative stress [16].

α -syn has been described as an intrinsically-disordered protein due to its lack of an organized secondary structure [17]. Something that sets this molecule apart is that it can change its structure based on the surrounding microenvironment, and each conformation has characteristics leading to distinct functions. Under normal conditions, α -syn has been shown to be a soluble monomeric protein [18]; however, a helically folded α -syn tetramer has recently been identified in peripheral red blood cells (RBCs) [19] which has been suggested as the normal physiological form. This tetramer has greater lipid binding capacity than the monomeric form and is more resistant to aggregation. However, current research fails to fully explain the need for the tetramer to destabilize before changing into either oligomeric or fibrillary forms and more work is needed to resolve this mechanism. When the N-terminus of the protein interacts with lipid membranes [20] or high curvature ones [21], it changes to an α -helical conformation that allows it to interact and modulate synaptic vesicles. Oligomeric forms, different from the tetrameric form, have been observed prior to fibril formation of varying sizes and morphology [22], and appear to represent the most toxic forms of α -syn [23]. Of interest, the amyloid-like fibrillary aggregated α -syn is found in Lewy bodies that accumulate in dopaminergic neurons potentially resulting in cell death. α -syn forms different fibrillary structures in the presence or absence of bacterial endotoxin lipopolysaccharide (LPS). α -syn interacts with LPS via its N terminus and in turn different fibrillary conformations show unique interactions with microglia [24]. Interactions such as these with bacterial membranes may hint at potential mechanisms that explain the observed antimicrobial properties of α -syn [25]. Further research will be needed to solve these questions.

Since α -syn lacks signaling sequences needed to be transported extracellularly, it is usually identified as cytoplasmic protein yet also closely associated to membranes. Recently, α -syn has been identified in the nucleus of human cell lines and mouse neurons where it is thought to be involved in DNA repair [26]. Outside cells α -syn has been found in plasma and cerebrospinal fluid (CSF) of both PD patients and healthy controls [27, 28]. α -syn in CSF may result from neuronal secretion, either by degenerating neurons under stress due to the disease state [29] or by normal neurons [28, 30]. On the other hand, the origin of α -syn in plasma is less clear since peripheral blood mononuclear cells (PBMCs), RBCs, and platelets express α -syn in increasing concentrations from low to highest. Notably, increased concentrations of plasma α -syn are observed in PD patients secondary to neuronal degeneration that increases its concentration in CSF with subsequent efflux into plasma [29]. Interestingly, release of α -syn into plasma can also occur in a time-dependent manner secondary to aging of platelets as recently described in single donor platelet units during storage making it also a potential biomarker for studying biochemical changes during platelet storage [31].

Study of neurons has allowed for several mechanisms of α -syn secretion to be proposed. Unconventional exocytosis is one of these mechanisms [30]. In neuroblastoma cell lines overexpressing human α -syn, the protein selectively entered cytosolic vesicles and was secreted independently of the endoplasmic reticulum (ER)-Golgi classical vesicular pathway. This vesicular α -syn is more aggregation-prone compared to the cytosolic form and alternatively it can be secreted via exosomes [32, 33]. It has been shown that after radioactive α -syn injection into mice brains, their plasma contained radioactive α -syn in CNS-derived exosomes [32]. These α -syn-containing exosomes have been found in

both PD patients and healthy controls. This exosomal pathway was also seen in human neuroblastoma cell lines expressing wild type α -syn and appeared to be modulated by cytosolic Ca^{2+} concentration [33]. However, presence of free soluble α -syn suggests that exosomal secretion is just one of the mechanisms of α -syn secretion. α -syn-containing erythrocyte-derived extracellular vesicles (EEVs) can be another potential source of the protein found in plasma. These EEVs have been identified in plasma of both PD patients and healthy controls [34]. Notably, EEVs have been shown to cross the blood brain barrier (BBB) via adsorptive-mediated transcytosis during LPS-induced peripheral inflammation where they co-localize with microglia [35]. In addition to neuro-inflammation, microglia can also uptake plasma-derived α -syn containing exosomes and transmit them to neurons resulting in neuronal toxicity [36]. Finally, degradation of extracellular α -syn by proteases could provide another mechanism by which neuronal toxicity and neuro-inflammation occur once these protein fragments are uptaken by either neighboring cells or microglia [37].

Nevertheless, despite a greater understanding of this protein's conformational variants, pathological mechanisms leading to PD remain unclear especially if disease results from a gain of toxicity by α -syn aggregation or if it is secondary to loss of normal function. However, due to the bidirectional transport of α -syn across BBB, changes to protein structure/function in the periphery secondary to the distinct biochemical properties of blood and its cells or through interaction with pathogens could therefore affect CNS cells [38]. *In vivo* models expressing known α -syn mutations, overexpressing wild-type protein, and α -syn^{-/-} mice have shed light into its potential functions. At the cellular level, α -syn-protein interactions have been elucidated in yeast, *Drosophila*, and megakaryocytic/erythroid cell lines. Of these, at least in *Drosophila* α -syn expression is temporally and spatially regulated through development [5]. The functions for α -syn so far identified from these models include reduction of oxidative stress, regulation of neurotransmitter release, and modulation of SNARE protein complex assembly [39].

1.2. α -syn in microglia

In order to understand the potential functions that this protein has in the hematopoietic system it is worth to look at studies describing a cell population that resembles those in this system, i.e. the microglia. Even though these cells are not bone marrow-derived they share immunological properties with peripheral monocytes and macrophages. It is these similarities which make microglia studies important to understanding data presented later in this review relevant to hematopoiesis and immunity.

Microglia are constantly sensing the CNS microenvironment and once activated they can differentiate into either a classical M1 or an alternative M2 phenotype. Classical M1 microglia produce pro-inflammatory cytokines and promote T helper (h)1 CD4⁺ T cell activation, while activation of M2 microglia results in enhanced anti-inflammatory and tissue repair responses [40, 41]. α -syn modulates both microglia phenotypes and subsequent activation in response to stimuli.

Changes to microglia have been reported in PD patients including cell morphology, surface protein expression, antigen presentation, and phagocytosis [42]. Experimentally, α -syn can be overexpressed by microglia, exogenously assembled and added to microglia cell cultures, expressed on neighboring neurons, or secreted into culture media by dying neurons. In addition, the concentration and conformation of α -syn influence microglia phenotype. Murine-derived microglia cell lines transfected with human wild type, mutant A30P, and A53T α -syn forms showed increased levels of cyclooxygenase (Cox)-2, secretion of pro-inflammatory cytokine tumor necrosis factor (TNF)- α , interleukin (IL)-6, and nitric oxide production in the setting of impaired phagocytic function [43]. Moreover, microglia from transgenic mice overexpressing α -syn have shown decreased phagocytosis and cytokine release [44]. In particular, neuronal overexpression of α -syn led to increased microglia activation in mice. In this model, microglia were activated by α -syn from

SN neurons resulting in altered cell morphology including increased cytoplasm, swelling of cellular processes, and pro-inflammatory responses mediated by higher expression of intercellular adhesion molecule-1, IL-1 α , IL-6 and TNF- α in a time dependent manner [45]. Separately, it was shown that diffuse neuronal overexpression of wild type human α -syn leads to microglia activation in a temporally- and regionally-specific manner through increased expression of toll-like receptors (TLRs)-1, -4, and -8 restricted to the cerebrum's striatum and SN but not in the cerebral cortex or cerebellum [46]. Experimentally, viral vectors that lead to expression of different doses of human α -syn in rat brain can mimic both early and late PD stages. In the early stage, with limited neuronal α -syn fibrillary accumulation, microglia show responses characterized by higher major histocompatibility complex (MHC) II expression (higher antigen presentation), whereas in late stage when α -syn-induced neuronal pathology and cell death occur, microglia demonstrated slower responses with increased phagocytic marker CD68 expression and adaptive immune system infiltration [47]. In α -syn^{-/-} mice microglia showed a ramified vacuolar morphology, increased reactive marker expression, high pro-inflammatory cytokine production after LPS stimulation, and decreased phagocytosis [48]. Likewise, altered expression of key enzymes in lipid-mediated signaling pathways including cytosolic phospholipase, Cox-2, and phospholipase D2, which are essential to microglial cytokine secretion and phagocytosis, occur in microglia isolated from α -syn^{-/-} mice. This provides one plausible explanation for the altered microglia phenotype in these mice [49].

Receptors expressed on microglia appear partly regulated by α -syn. Among their functions are sensing the microenvironment, ligand recognition, and initiating downstream signaling pathways in response to pathogens or cell damage [50]. α -syn plays a role in the function of TLRs, scavenger receptors, complement receptors, Fc receptors and NADPH oxidase (NOX) activity among others. TLRs belong to the family of pattern-recognition receptors (PRRs) critical to innate immunity, triggering NF- κ B activation or interferon regulatory factor-3 signaling pathways after ligand binding [51]. α -syn functions as a danger/damage-associated molecular pattern molecule recognized by TLRs and thus modulates microglia function. For example, α -syn released from neuroblastoma cells activates murine primary microglia in culture through α -syn-TLR-2 interaction leading to cell proliferation and cytokine production [52]. Similarly, extracellular α -syn released from human neuroblastoma cells overexpressing it acts as an endogenous agonist of TLR-2 activation in a conformation-dependent manner. TLR-2 binds and internalizes α -syn leading to early activation of pro-inflammatory pathways [53]. As a stimulating agent, α -syn also appears to have profound effects even at low protein concentrations of its oligomeric form capable of sensitizing microglia *in vitro* and activating pro-inflammatory cascades through TLR-4-dependent signaling [54]. Altered TLR expression in response to α -syn has also been reported, as shown by increased expression of TLR-1, -2, -4, and -8 that mediate the temporally- and regionally-specific activation of microglia in a mouse neuronal model overexpressing human α -syn [46]. In addition, when murine microglia cell lines are treated with mixtures of recombinant human monomeric, oligomeric, and fibrillary forms of α -syn, changes to TLR expression are observed such as upregulation of TLR-2, TLR-3, and downregulation of TLR-7 [55].

Scavenger receptors expressed on microglia recognize modified lipoprotein and various polyanionic ligands, one of which is CD36 [50], that has been shown to mediate *in vitro* microglia activation by exogenous human α -syn [56]. This receptor, can be used by α -syn to enter microglia, prime the cell, and lead to inflammasome assembly [57]. Of note, higher CD36 mRNA expression in activated microglia has been reported in transgenic mice overexpressing double mutant α -syn A53T and A30P. Furthermore, microglia from CD36^{-/-} mice showed partially attenuated cell activation after treatment with exogenous double mutant human α -syn suggesting that activation is partially mediated by CD36 and its signaling pathway [58], and that likely additional receptors facilitate activation [56].

Macrophage antigen complex (MAC)-1 is a complement receptor consisting of CD11b (integrin α_M) and CD18 (integrin β_2) that functions as both adhesion molecule and PRR [50]. In mesencephalic cultures, both wild type and mutant forms of α -syn have to bind to and signal through MAC-1 receptors to activate microglia to produce reactive oxygen species (ROS), and mutant forms have shown enhanced neurotoxicity possibly via a MAC-1-dependent mechanism [59]. However, α -syn can also function as a chemo-attractant to direct microglia migration [60]. Specifically, binding of extracellular aggregated α -syn to CD11b on microglia is necessary for microglia directional migration mediated by H₂O₂ produced from activated NOX [60]. In addition, α -syn has been shown to interact with another integrin family receptor β -1 (CD29) that results in modulation of microglia migration and morphological changes [52].

Fc γ receptor (Fc γ R) proteins expressed on the surface of microglia bind IgG among other ligands. Fc γ R activation triggers downstream immunoreceptor tyrosine-based activation motif signaling pathways that result in sequential activation of Src and Syk kinases followed by activation of MAPK and NF- κ B [51]. α -syn activates microglia NF- κ B signaling and downstream pro-inflammatory mediator production via its interaction with Fc γ R. Along these lines, Fc γ R can mediate α -syn internalization and traffic to the autophagosome; and diffuse α -syn cellular localization and impaired NF- κ B activation have been reported in microglia of Fc γ R^{-/-} mice [61]. Likewise, selective overexpression of α -syn in SN results in activation of NF- κ B that is Fc γ R expression-dependent [62].

NOX on the microglia membrane generates ROS which are important to host defense and redox signaling [63]. NOX can be activated by microglia receptors PRRs and MAC-1 in response to stimuli, one of which is α -syn. After phagocytosis by microglia, extracellular aggregated human α -syn activates NOX increasing ROS production [64]. Furthermore, α -syn directs microglia migration via the NOX pathway [60]. Another receptor critical to microglia function that is regulated by α -syn is MHC-II. In mice, overexpression of human α -syn in SN resulted in increased MHC-II expression on activated microglia; while *in vitro* microglia showed increased antigen processing and presentation after treatment with aggregated α -syn [65].

α -syn, therefore, mediates the interaction between CNS and the periphery by modulating microglia function. Overexpression of human α -syn in mouse SN led to infiltrating B cells, T cells, and microglia activation in this region 4 weeks after viral vector injection; on the other hand by the 12th week while activated microglia declined, T and B cells infiltration persisted [45]. Late increases in CD4⁺ and CD8⁺ T cells have also been observed in the periphery after induced overexpression of human wild type α -syn in mouse brain [46]. It has also been shown in a similar mouse model that infiltrating T cells induced microglia and resident macrophages to differentiate into the M1 phenotype; while in the absence of lymphocytes, increased macrophage infiltration, decreased α -syn aggregation, and highly phagocytic microglia with the M2 phenotype were observed [66].

To summarize, microglia studies provide a foundation to understand the potential functions that α -syn may have in immune cells and in the hematopoietic system. Likewise, even though monocytes and macrophages reside in a different microenvironment from microglia they have similar protein receptors, signaling pathways, and interactions with T and B cells which suggest that the potential functions and/or mechanisms α -syn has in the hematopoietic system may resemble those described up to this point in the CNS.

2. Main text

2.1. α -syn in the hematopoietic system

Expression of α -syn in erythrocytes, T and B lymphocytes, monocytes, natural killer (NK) cells, and megakaryocytes has been known for quite some time [5], and this expression extends to hematopoietic neoplastic cells [67]. High α -syn expression under normal conditions and

dysfunctional hematopoietic cells in α -syn^{-/-} mouse models suggest that this protein plays an important role in maturation and normal function of this system [68, 69, 70]. More importantly, hematopoietic abnormalities have been reported in PD patients [71], suggesting that an association between α -syn expression/conformation and hematopoietic function exists. Therefore, the remainder of this review will present data in the context of this system.

PBMCs which include B lymphocytes, T lymphocytes, NK cells, and monocytes are essential to competent immunity. For almost twenty years it has been known that α -syn is expressed in PBMCs [5]; however, despite this knowledge there is no clear indication of those functions that α -syn mediates in these cells. PD patients show cell changes that include: quantitatively altered immune cell subpopulation distribution, modifications to cell morphology and function, and altered expression of both MHC-I and MHC-II molecules. This is further supported by recent findings describing that leukocytes from α -syn^{-/-} mice have abnormal ultrastructural morphology [72]. Since expression of α -syn is increased in PBMCs of PD patients, α -syn could affect and therefore be important functionally in these cells. One of these functions is sensitivity of PBMCs to apoptosis since overexpression of wild type α -syn as well as expression of its mutant forms induce PBMC apoptosis [73]. α -syn gene changes have also been identified in PBMCs of PD patients [74]. Among these, hypomethylation of CpG-2 sites may be associated with increased α -syn expression in leukocytes of PD patients. α -syn overexpression activates the JAK/STAT pathways promoting dysregulation of innate and adaptive immunity. This overexpression upregulates SN genes which are

important in adaptive immunity including antigen presentation, Ca²⁺-induced T lymphocyte apoptosis, B cell development, and graft-vs.-host disease [75]. Potentially, changes reported in PD patients' PBMCs could result from a loss of normal α -syn function, or PBMCs respond to it as foreign triggering an immune response. We will now discuss each cell type expressing α -syn separately.

2.1.1. α -syn in T cells

T cells depend on their cell surface protein expression and corresponding function to differentiate into either CD4⁺ helper T cells or CD8⁺ cytotoxic T cells. CD4⁺ T cells are further subdivided into regulatory T cells (Treg) [76] and effector T cells (Teff). Treg cells are CD4⁺CD25⁺FOXP3⁺ and mediate immune tolerance and suppression. On the other hand, Teff cells can be further divided into Th1, Th2, and Th17 subgroups based on their cytokine production profiles [77]. Th1 cells produce interferon- γ (IFN- γ) and mediate intracellular pathogen clearance; while Th2 cells produce IL-4, IL-5, and IL-13, mediate T helper function in humoral immunity, clearance of extracellular pathogens, and allergic responses. Th17 produces IL-17, mediate tissue inflammation and potentially autoimmunity [77]. Importantly, changes in T cells as well as changes in α -syn have been reported in PD patients. α -syn could be recognized as an intracellular or extracellular antigen, that depending on its origin and conformation, trigger immune responses exacerbated by different T cell subsets. Alternatively, changes in T cells could result from loss of normal α -syn function leading to defective or dysregulated T cell maturation and/or function (Fig. 1).

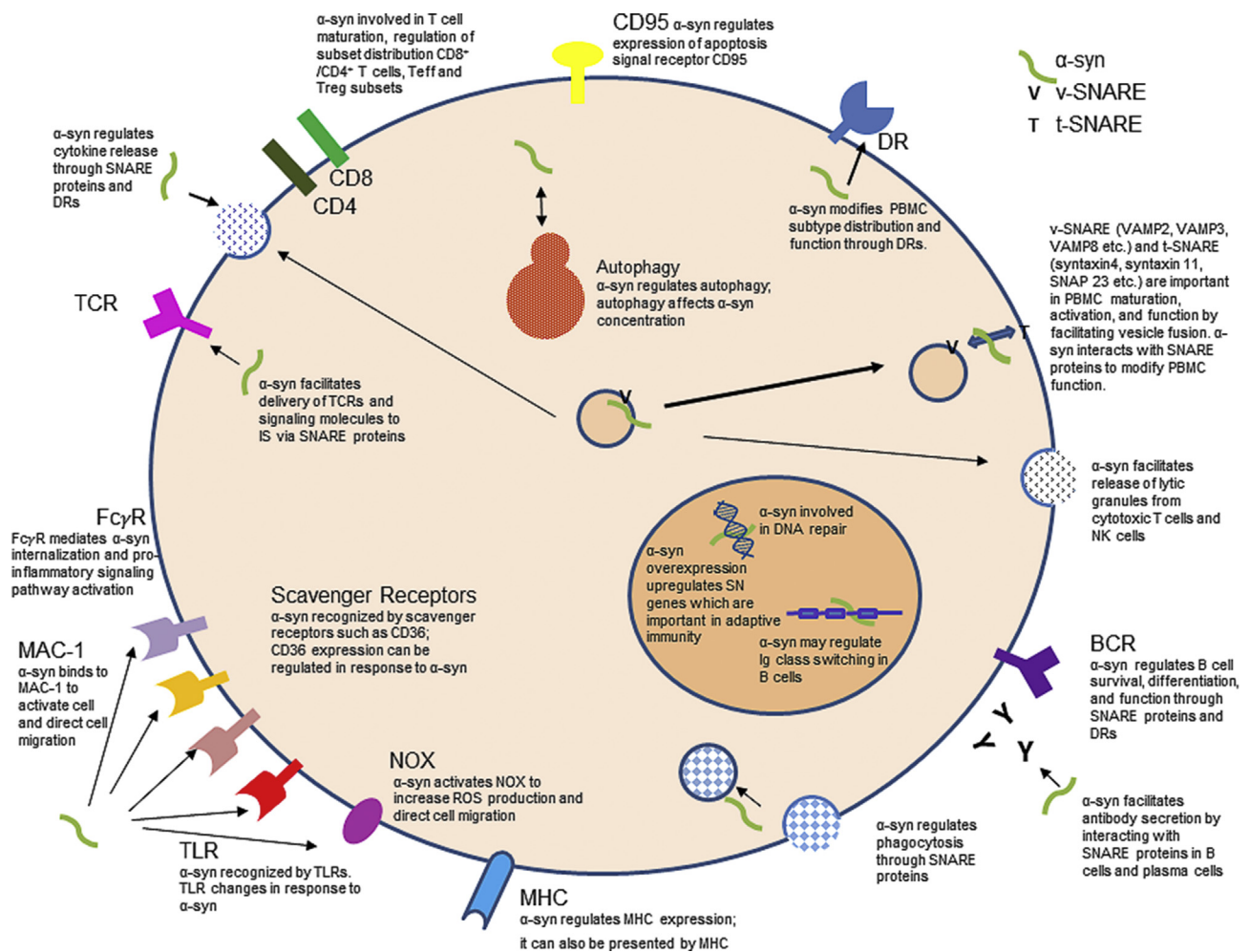


Fig. 1. Diagram of a generic white blood cell outlining some of the pathways that may be mediated or regulated by α -syn. Representation describes interaction with SNAREs in different cell types, receptors regulated or affected by α -syn expression and those cell processes that depend on regulated α -syn expression. BCR: B cell receptor, TCR: T cell receptor.

Studies have shown that in PD patients the percentage of T lymphocytes is decreased [78], there is a decreased CD4⁺/CD8⁺ ratio [79], fewer naïve helper CD4⁺CD45RA⁺ T cells [80], a shift toward Th1 cells [78, 79, 81] and Th17 cells [81], decreased percentage of Treg cells and Th2 cells [79, 81], and a selective increase in proportion of CD4⁺CD45RO⁺ memory T cells [82, 83]. These T cell subset changes suggest the presence of a chronic induction signal in PD stimulating T cell activation [83]. Furthermore, conflicting reports of either decreased percentage of Th1 cells [84] or increased number of activated CD4⁺CD25⁺ T cells [85] could be the result of analysis of different cell markers and/or inclusion of patients with different disease states.

Recent data using α -syn^{-/-} mice indicates that deficiency of this protein leads to arrest in T lymphocyte maturation including Treg cells [69], as well as abnormal T cell distribution in lymphoid organs including thymus, spleen and lymph nodes. In this model, the total number of thymocytes was reduced with significant decreases in both CD4⁺ and CD8⁺ cells and increases in the number of double negative thymocytes. Morphologically, thymi from these mice were smaller with a relatively large cortex and small medulla. T cell subset analysis indicated defective Th2 differentiation and a shift toward Th1 phenotype (as mentioned earlier in PD patients) after *in vitro* activation. Percentage of Treg was decreased in the thymus but increased in the spleen. Functionally, there was hyperactivity of remaining mature T cells in a background of altered cytokine production profile [69]. Notably, such T cell subset distribution abnormalities have also been described in an α -syn^{-/-} multiple sclerosis mouse model. These mice had early onset of autoimmune encephalomyelitis, increased frequency of Th1 cells and decreased frequency of Tregs in the spinal cord, and higher frequency of Th1 cells in the spleen. Taken together, these findings suggest that α -syn functions as a Th1 response regulator [86].

Vectors that increase α -syn expression change T cells subset composition. Mice subcutaneously immunized with α -syn 6 weeks and 10 weeks before overexpression of human α -syn in SN show microglia activation with upregulation of MHC-II expression, increase in Treg and B cell infiltration, and high titer of C-terminal- α -syn-specific IgG antibodies [87]. Additionally, mice injected with low dose α -syn on day 0 and day 7, and sacrificed on day 14, showed an increased percentage of Treg and reduced percentage of T_H1 in the spleen [88]. Post-transcriptional modifications of α -syn may modulate T cell function. T cells harvested from a mouse model immunized with nitrated- α -syn polarized to a Th1 and/or Th17 phenotype showed Treg with impaired inhibitory capacity [89]. Immunization with different conformations of α -syn also modulated peripheral T cells subset distribution in murine models. In this setting, monomeric α -syn reduced the percentage of CD4⁺ T cells, nitrated α -syn increased total number of T cells, and fibrillary α -syn increased the percentage of Treg. [90] These data indicate that α -syn is important to T cell development, response to stimulus and function, cell distribution, and cytokine production.

2.1.1.1. α -syn and SNARE protein complexes in T cells. The function of a protein is highly dependent on its structure. Because of the sequence and chemical properties of each functional domain, α -syn is able to interact with membranes and modulate synaptic vesicles through direct or indirect interactions with SNARE proteins. These proteins contain a conserved 60 - 70-amino acid domain called the SNARE motif. They are classified into vesicle-membrane SNAREs (v-SNAREs) and target-membrane SNAREs (t-SNAREs) based on cellular location and function. They can also be divided into Q-SNAREs and R-SNAREs based on structure. Q-SNAREs are subdivided into Qa-, Qb-, Qc-, and potentially Qb,c-SNAREs based on the amino acid sequence of the SNARE motif [91]. Formation of the SNARE protein complex, which is a tetrameric coiled-coil structure between appropriate set of v- and t-SNAREs, brings opposing membranes into close proximity to facilitate fusion [92]. SNARE proteins are critical in vesicular transport and have been shown to be important in neuronal synapses. SNARE proteins described in the

CNS include synaptic proteins syntaxin-1, synaptosomal nerve-associated protein (SNAP)-25, and vesicle-associated membrane protein (VAMP)2 (or synaptobrevin 2). Syntaxin-1 is a type 1 integral membrane protein found on the plasma membrane. SNAP-25 attaches to target membranes via its central region, leaving its amino- and carboxyl-termini to interact with syntaxin-1 and VAMP2 (associated with vesicle membranes). These proteins then coalesce to form a four-helix SNARE complex with one SNARE motif from each syntaxin-1 and VAMP2, and two motifs from SNAP-25 [93]. After docking and priming neurotransmitter-containing vesicles in the active zone [94], v- and t-SNAREs pull the membranes closer together leading to fusion and neurotransmitter release.

Pathological models expressing multiple copies of wild-type or mutant forms of α -syn have shown inhibition of SNARE protein function. Current evidence has identified α -syn as a protein chaperone that facilitates SNARE protein assembly. This function was identified in a murine model lacking cysteine-string protein- α (CSP α), a presynaptic protein chaperone important for complex assembly. In CSP α deficient mice, there is decreased SNAP-25, and chaperones Hsc 70 and Hsp 70 leading to impaired SNARE complex formation. Transgenic expression of human and murine wild type, as well as A53T mutant human α -syn partially compensate for loss of CSP α and rescue complex assembly [95]. Wild-type α -syn has been shown *in vitro* to promote SNARE protein complex assembly by clustering vesicles at the active zone, thus preventing loss of accessory proteins and increase in assembly rates. To achieve this, α -syn binds to both VAMP2 and anionic lipids [96]. For example, cytosolic monomeric α -syn changes to a multimer when binding to the membrane and VAMP2 which results in clustering and local increase of VAMP2 concentration [97]. α -syn can also directly promote complex assembly in a dose-dependent manner. Not surprisingly, in $\alpha\beta\gamma$ -triple KO mice, there is an age-dependent decrease in SNARE-complex assembly that is reversed with reintroduction of α -syn. In this model the C terminus of α -syn binds to VAMP2 while the N terminus binds to phospholipids [98]. α -syn also indirectly inhibits complex assembly by blocking arachidonic acid, a common lipid regulator released from membranes that promotes SNARE complex formation and exocytosis [99].

Notably, both regulatory and toxic effects of α -syn over SNARE proteins depend upon its concentration and conformation. High concentrations of non-aggregated α -syn inhibits vesicle docking by binding to negatively charged lipids without direct SNARE protein interaction [100]. Importantly, mutated forms of α -syn with higher lipid binding affinity have higher inhibitory potential [100]. On the other hand, large α -syn oligomers inhibit vesicle docking at lower concentrations that require direct binding to the N terminus of VAMP2 [101]. Therefore, both α -syn concentration and conformation must be maintained in a delicate balance to preserve normal function.

Analogous to the active zone in the neuronal synapse, the immunological synapse consists of a protein network formed at the cell surface in the area of contact between the T cell and antigen-presenting cell (APC) [102]. This synapse consists of three rings of membrane receptors, cytoskeletal elements, and signaling proteins. The first of these rings, the central supramolecular activation cluster (cSMAC) is made of the T cell receptor (TCR) and signaling molecules, the second or peripheral SMAC (pSMAC) consists of a ring of adhesion molecules, and the third (distal) ring dSMAC consists of membrane proteins such as CD45 [103]. This arrangement is analogous to protein complexes in neurons which express them in the presynaptic terminal while in T cells complexes occur anywhere on the cell surface [104]. SNARE protein-mediated vesicle trafficking is critical in both mechanisms. These proteins are ubiquitously expressed in T cells [102, 104], and are critical to cell maturation and function at the immunological synapse such as delivery of cell surface receptors or signaling molecules, and secretion of lytic granules and cytokines.

SNARE proteins modulate T cell maturation in the thymus. During T cell development, cells go through positive and/or negative selection in the thymus. These T cell developmental stages are tightly regulated by

the expression of distinct stage-specific cell surface molecules, and interaction between thymocytes and APCs such as thymic epithelial cells and dendritic cells that determines the fate of thymocytes [105]. This complex process is intimately modulated by SNARE proteins [106]. Among these, VAMP8 controls the organization of the thymic epithelium, thereby regulating thymocyte proliferation and apoptosis in thymic stroma. Along these lines, VAMP8-deficient mice have a smaller thymus with a disorganized thymic epithelium, and a decreased total number of thymocytes with double positive thymocytes most severely affected. This is similar to the phenotype seen in α -syn^{-/-} mice [69]. Likewise, reduced proliferative and increased apoptotic thymocytes have also been demonstrated.

SNARE proteins are important to T cell activation. Mechanistically, the TCR interaction with peptide antigens presented by MHC on APCs triggers downstream signaling pathways [107]. For this to work, delivery of the TCR and signaling molecules to the immunological synapse is essential to drive T cell activation [108]. Specifically, SNARE proteins assist polarized recycling of TCR at the immunological synapse via recycling endosomes [109]. t-SNARE proteins such as syntaxin-4 and SNAP-23 are necessary for TCR accumulation at the synapse. Notably, inactivation of v-SNARE proteins by tetanus toxoid inhibited TCR accumulation, and of these VAMP2 and VAMP3 may contribute to this polarized recycling process [109]. On the other hand, in cytotoxic T lymphocytes, syntaxin-7 mediates accumulation of recycled TCRs at the synapse. Reports indicated that syntaxin-7 deficiency blocked polarized recycling of TCRs from early to late endosomes leading to inefficient accumulation of TCRs and lytic granules at the synapse [110]. SNARE proteins also mediate the delivery of signaling molecules to the immunological synapse. Two of these molecules, lymphocyte-specific-protein tyrosine kinase and linker-for-activation of T cells are critical to T cell signaling and activation. Each has a unique vesicular compartment made of different Rab GTPases and SNARE proteins which temporally and spatially regulate signaling [111].

SNARE proteins also modulate Teff response. Th cells secrete cytokines that modulate immunological responses and two cytokine export pathways are essential in these cells. In one IL-2 and IFN- γ are secreted directly into the synapse while in the other TNF- α can be multidirectional. Both processes involve SNARE proteins such that syntaxin-6 colocalizes only with TNF- α and shows little localization with IL-2 and IFN- γ , while vesicle transport through interaction with t-SNAREs homolog 1b (Vti1b) partially overlaps with both types of cytokines. This provided evidence that SNARE proteins mediate cytokine secretion from Th cells, that certain SNARE proteins are pathway-specific, while others are shared by pathways [112].

Cytotoxic T cells recognize and destroy intracellular pathogens. One of the mechanisms used by these T cells is release of lytic granules at the synapse [113], and SNARE proteins mediate this release. For example, in the mouse model, VAMP2 is the v-SNARE controlling the final fusion step in cytotoxic T cell granule release [114]. Likewise, VAMP8 is expressed on lytic granules and cells lacking VAMP8 show normal granule polarization to the synapse but defective target cell killing. Importantly, this partial deficit observed in VAMP8 deficient mice indicates that additional v-SNARE proteins, possibly VAMP7, also mediate granule release [115]. Additionally, based on structural characteristics and previous studies, syntaxin-11 and SNAP-23 are predicted to be the t-SNARE proteins mediating the fusion step of lytic granule release [116]. This appears to be the case since VAMP7, syntaxin-11, and SNAP-23 have been identified in human cytotoxic T cells as facilitators of lytic granule exocytosis [117].

To maintain normal T cell maturation and function it is important to keep T cells and APCs in close proximity, to efficiently recycle and re-localize cell surface receptors and accessory proteins to the synapse, and to effectively produce and secrete mediators such as perforin and cytokines to execute immune functions. SNARE proteins are critical modulators of these processes. Therefore, interactions between SNARE proteins and α -syn could modulate T cells function and potentially affect hematopoiesis across lineages. This hypothesis could explain the altered

T cell phenotype observed in PD patients and α -syn^{-/-} mice. It would also explain how absence of α -syn changes leukocyte cell size, shape of secretory particles, increases smooth endoplasmic reticulum, number of secretory granules, and inclusion bodies [72]. This phenotype could result from defective SNARE protein complex assembly linking α -syn to leukocyte vesicular trafficking.

2.1.1.2. α -syn in T cell apoptosis. α -syn may modulate T cell apoptosis since changes to apoptosis receptor expression have been described in PD [80]. CD95 is an apoptosis signal receptor encoded by *FAS* gene [118] that is highly expressed in cytotoxic CD8⁺ compared to CD4⁺ cells of healthy subjects. In CD4⁺ T cells, this receptor is mainly expressed on memory cells and activated CD4⁺CD25⁺. PD patients have increased percentage of CD95/Fas⁺ CD4⁺ cells, mainly naive CD4⁺CD45RA⁺ T cells [80]. This increased CD95/Fas expression correlates with decreases in CD4⁺ T cells and of absolute counts of CD4⁺CD45RA⁺ T cells. These CD95/Fas⁺ naive CD4⁺ T cells may be more sensitive to apoptosis than memory CD4⁺ T cells which are CD95^{high} even in healthy controls. Cellular alterations in T cells of PD patients involve sensitivity changes to apoptosis which are partly mediated by α -syn [80]. It should be of interest, that overexpression of α -syn and mutant forms of α -syn upregulate CD95 gene expression in hematopoietic cell lines [73].

2.1.1.3. α -syn in T cell autophagy. Autophagy is a cellular process of protein/organelle degradation in response to either cell starvation or abnormal protein accumulation [119]. PBMCs from PD patients have high concentrations of nitrotyrosine-modified α -syn as well as enhanced autophagy [120]. The enhanced phenotype may result from this post-translational modification since α -syn is a negative regulator of autophagy. To support this, knocking-down α -syn resulted in higher T cell autophagy [121]; and expression of autophagy-related proteins correlates with α -syn concentration in PBMCs of PD patients [122]. α -syn modifications can also be a consequence of altered autophagy, since inducing autophagy led to decreased monomeric and aggregated α -syn in T cells while impaired autophagy increased α -syn concentration [121].

2.1.1.4. α -syn and dopaminergic receptors on T cells. α -syn interacts with dopaminergic receptors (DRs) expressed on CD4⁺ T cells to modulate their function. Five different dopaminergic receptors have been identified belonging to two distinct groups. D1 and D5 belong to the D1-like family and D2, D3, and D4 to the D2-like group. CD4⁺ T cells express all five DRs showing inter-individual variability [123]. D1-like DRs expressed on naive CD4⁺CD45RA⁺ T cells play a role in dendritic cell-induced naive T cell stimulation that results in Th2 polarization [124]. On the other hand, D3 promotes T cell differentiating into Th1 phenotype [125]. DRs are also involved in cytokine release by T cells. D3 mediates release of TNF- α and D2 triggers secretion of IL-10 [126]. Interestingly, there is higher DR expression in apoptotic cells [123]. PD patients show different expression of DRs on CD4⁺ T cells compared to healthy controls. These expression differences also vary among CD4⁺ T cell subsets [127]. Both monomeric and fibrillary α -syn have been shown *in vitro* to alter expression of DRs on naive and memory CD4⁺ T cells. The monomeric form increases D5 and D2 expression on naive T cells, and D2 on central memory T cells; on the other hand, fibrillary α -syn increases D1, D2, and D4 on central memory T cells, and D1, D4 on effector memory cells [127]. Separately, in mice DR expression on CD4⁺ T cell subsets changed in response to immunization with different forms and concentrations of α -syn. Moreover, immunization with α -syn modulates expression of homing marker CCR6 and tolerance marker CD103, and a relationship between both markers and DRs DR2 and DR3 has been observed [90, 128]. Therefore, α -syn can modify T cell function and subset distribution by changing DR expression on T cells.

2.1.2. α -syn in B cells

B cells produce antibody through mechanisms that depend on the

type of eliciting antigen and requirement for T cell help [129]. α -syn is expressed in B cells and changes to B cells of PD patients have been described [82, 84]. Autopsies of brains from PD patients have shown IgG deposition but not IgM against α -syn, mainly IgG1 co-localized with α -syn to surfaces of cell membranes and Lewy bodies in neurons, and the proportion of IgG positive neurons negatively correlated with the degree of cell loss [130]. Autoantibodies have also been detected in peripheral blood of familial PD patients with specificities to multiple epitopes across human α -syn [131]. When human α -syn transgenic mice were vaccinated with human α -syn, antibodies were detected in serum recognizing the C-terminus of α -syn [132]. In theory, changes to B cell numbers and humoral responses in PD could result from loss of normal α -syn function or stimulation of autoantibody responses.

Abnormalities in B lymphocytes have also been reported in α -syn^{-/-} mice [68]. This report indicated that absence of α -syn led to significant decreases in absolute B cell counts, specifically mature B lymphocytes in a background of smaller and disorganized lymphoid follicles, and decreased total serum IgG concentration without changes in IgM production indicative of defective immunoglobulin isotype class switching. Furthermore, IgG production was absent in response to challenges with both T-independent and T-dependent antigens. Taken together with findings reporting increases in the number of inclusions and secretory granules in PBMCs [72], it suggests that α -syn is essential to maturation and normal function of B cells (Fig. 1).

Similar to what we described for T cells, one potential mechanism by which α -syn regulates B cells is through interactions with SNARE protein complexes. After T cells and B cells come into contact at the synapse, which is regulated by SNARE proteins, a subgroup of B cells differentiates into plasma cells. These cells are critical to producing and secreting antibodies [129]. SNARE proteins regulate antibody secretion by plasma cells. All isoforms of VAMP family, except for VAMP1, have been identified in human plasma cells at different levels of expression. For example, VAMP2 is responsible for secretion of antibodies from plasma cells [133]. Likewise, SNAP-23 [134] and syntaxin-4 [135] have been identified as the corresponding t-SNARE proteins mediating antibody secretion.

Interaction with helper T cells, especially Th2, induces B cells to undergo clonal expansion, somatic hypermutation, immunoglobulin isotype class switching, and differentiation into memory B cells or plasma cells [136]. In α -syn deficiency, a defective immunological synapse could be caused by the inefficient delivery of receptors to the cell surface in both T cells and B cells or by impaired cytokine production by Th cells resulting in dysfunctional immunoglobulin class switching. This could partly explain the normal IgM concentration and low to absent of IgG production in α -syn^{-/-} mice [68]. Alternatively, as indicated by the accumulation of secretory vesicles and inclusions in PBMCs of these mice, a defective secretory pathway in plasma cells could also be responsible for the observed phenotype [72].

α -syn may also modulate B cells through its interactions with DRs [137]. By using flow cytometry, it was shown higher expression of DRs D2, D3 and D5, and lack of D1 expression on B cells from healthy donors compared to other types of PBMCs. This is important since as discussed earlier α -syn closely interacts with DRs and mechanistically it can also exert regulation of B cell function through this interaction.

2.1.3. α -syn in NK cells

NK cells recognize down-regulation of self MHC-I molecules on virally-infected cells or tumor cells that trigger their cytotoxicity. NK cells release lytic granules and secrete cytokines at the immunological synapse to kill target cells [138]. Changes in the percentage of NK cells of PD patients have been observed. Likewise, when NK cells from PD patients were stimulated with human α -syn peptides, there was a higher percentage of NK cells producing TNF- α [139]. Since α -syn is also expressed in NK cells, it is feasible that it may regulate NK cell function through interaction with SNARE protein complexes (Fig. 1). Similar to cytotoxic T cells, target killing by NK cells is facilitated by SNARE proteins. NK cells

express all seven VAMP family members. Of these VAMP4 and VAMP7 co-localize to lytic granules in activated NK cells and both are essential to NK cell degranulation and cytotoxicity; however, VAMP7 may have broader regulation over NK cell functions [140]. On the other hand, t-SNARE protein syntaxin-11 is expressed and regulated in a cell-type specific manner in different NK cell lines. Notably, in resting NK cells syntaxin-11 is found in distinct vesicular compartments different from those containing Rab27a or perforin, while all three co-localize at the synapse in activated cells [141].

2.1.4. α -syn in monocytes and macrophages

Monocytes can differentiate into macrophages or dendritic cells [142]. Dysregulation of monocytes and macrophages occur in PD patients and a mouse model overexpressing α -syn [44] (Fig. 1). Interestingly, monocytes from sporadic PD patients and a patient carrying a triplication of the α -syn gene have shown higher α -syn expression and impaired phagocytosis [44]. Monocytes from PD patients have an altered transcriptome and cell subset composition characteristic of a pro-inflammatory phenotype. These cells also show hyper-activation post-LPS stimulation and impaired phagocytosis [143]. Overexpression of human α -syn in murine brain can drive pro-inflammatory peripheral monocyte migration to the CNS that requires expression of the chemokine receptor CCR2 on monocytes [144]. α -syn can also serve as an inflammatory mediator that activates monocytes *in vitro*. When human monocyte cell lines were incubated with a combination of α -syn and IFN- γ , they produced pro-inflammatory cytokines IL-1 β and TNF- α via receptor-mediated activation of MAP kinase signaling pathways [145]. In this regard, both monomeric and fibrillary α -syn induce monocytes from healthy donors to express pro-IL-1 β through TLR-2. However, only fibrillary α -syn increases release of mature IL-1 β by activating the inflammasome [146].

α -syn expression in macrophages from healthy donors increases in response to LPS and IL-1 β stimulation suggesting that α -syn has roles in inflammation and macrophage response [147]. Along these lines, macrophages from transgenic mice overexpressing α -syn have shown impaired phagocytosis, disrupted cytokine production, and defective debris clearance [44]. Whereas exogenous α -syn leads to murine macrophage activation and TNF- α production in a dose dependent manner. In this setting, the N terminus or NAC region may facilitate macrophage entry by α -syn, while the C terminus is necessary for cell activation [148]. Just as previously mentioned, SNARE protein complexes also mediate macrophage function. Cytokine release and phagocytosis are important functions of activated macrophages that are regulated by SNARE proteins [142]. Of note, SNARE protein expression is upregulated in LPS-activated macrophages [149, 150]. Among t-SNAREs in macrophages, syntaxin-4 and SNAP-23 are involved in secretion of TNF- α [149]; while VAMP3 is responsible for TNF- α exocytosis [150]. Syntaxin-4, SNAP-23 [149], and VAMP3 [150,151] also facilitate fusion of recycling endosome membranes to phagosomes to assist phagocytosis. In addition, VAMP7 is involved in fusion of late endosome membranes to phagosomes to complete phagosome maturation [152].

2.1.5. α -syn and MHC molecules

MHC molecules on PBMCs facilitate cell-cell interactions, and mediate cell maturation and differentiation. α -syn correlates with MHC expression in PBMCs and aberrant MHC expression has been reported in PD patients. Both increased number of MHC-II positive microglia [153], and increased MHC-I light-chain β 2-microglobulin in the dopaminergic region of PD patients have been reported [154]. Furthermore, a gene variant in the noncoding single-nucleotide polymorphism region of the MHC-II locus alters its expression on B cells and monocytes in PD [155]. A recent study showed that α -syn is presented by specific MHC-I and MHC-II that activate cytotoxic and Th cells. Importantly, two antigenic regions in naïve and fibrillary α -syn, one near the C terminus and the other near the N terminus are presented in this manner [156].

2.1.6. α -syn in erythroid lineage

It has been reported that most of the α -syn in blood is found in erythrocytes since they are the most abundant cells; however, platelets have the highest concentration of α -syn per mg of cellular protein [157]. Of interest, α -syn^{-/-} mice manifest mild anemia [68, 72]. In humans, α -syn is found in both erythroid precursors in the marrow and erythrocytes in peripheral blood, and changes in its expression may differentiate erythroid from megakaryocytic neoplasms [67]. The expression of α -syn in erythrocytes is regulated during cell differentiation, specifically during the terminal steps of erythrocyte development. Thus erythroblasts have higher α -syn expression than reticulocytes and erythrocytes [158]. Moreover, increased α -syn mRNA at the erythroblast stage and decreased at the enucleation stage have been described, while protein concentration steadily increases late in erythroblast differentiation [159]. In regard to α -syn conformations, tetrameric α -syn was first identified in RBCs and this conformation is aggregation resistant [19]; notably, monomeric and high molecular bands of α -syn are detected in the erythroid proliferative stage while only the monomeric form is detected in the erythroblast stage. Localization of α -syn also changes during erythrocyte differentiation so that it is found in the nucleus, the cytoplasm, and the plasma membrane [159, 160]. This regulated expression and localization of α -syn suggests that this protein is important in the erythroid lineage (Fig. 2).

Several functions of α -syn in erythrocytes have been proposed based on its biochemical properties and structure. Due to its capacity to interact with lipids, proteins and cell membranes, α -syn is thought of as a tether in erythrocytes [161]. In this model, the N-terminus of α -syn binds to the cell membrane while the free C-terminus binds to other proteins as a

chaperone. This tethering function could allow cytosolic proteins to modulate those membrane-bound. Moreover, by binding to the inner leaflet of the plasma membrane, α -syn increases membrane mechanical strength thus regulating membrane fluidity, which might potentially extend erythrocyte lifespan [161]. Similarly, based on its different cellular localizations through RBC development α -syn may also be involved in erythroblast enucleation and stabilization of erythroid membrane [159].

α -syn may also be critical to maintaining iron homeostasis. Under normal conditions, iron is absorbed by intestinal enterocytes as ferrous, ferric, and heme iron, processed to ferric iron and bound to transferrin which then binds to the transferrin receptor 1 (TfR1) expressed on erythroblasts leading to its internalization by endocytosis [162]. Increased iron concentration in the brain has been described in PD suggesting disturbances in iron homeostasis [163]. Contrary to brain findings, serum iron, ferritin, and transferrin are decreased in PD patients [164]. Microarray studies have shown down-regulation of genes related to iron metabolism in PD patients. Gene analysis in PD indicated a connection between iron and hemoglobin (Hb) metabolism, and erythrocyte development with SNCA [165]. α -syn can also modulate trafficking of the transferrin/transferrin receptor (Tf/TfR) complex in erythrocytes, retina, and hematopoietic organs which utilize this pathway for iron uptake. This is demonstrated by α -syn^{-/-} mice showing iron deficiency, decreased ferritin and TfR, leading to lower Hb synthesis in RBCs and spleen. In this model, α -syn facilitates both endocytosis and exocytosis of Tf/TfR complexes [166]. α -syn is also a ferric reductase capable of binding to Fe(III) and converting it to Fe(II), making it available to cellular functions [167]. Likewise, α -syn binds to Fe(II) with

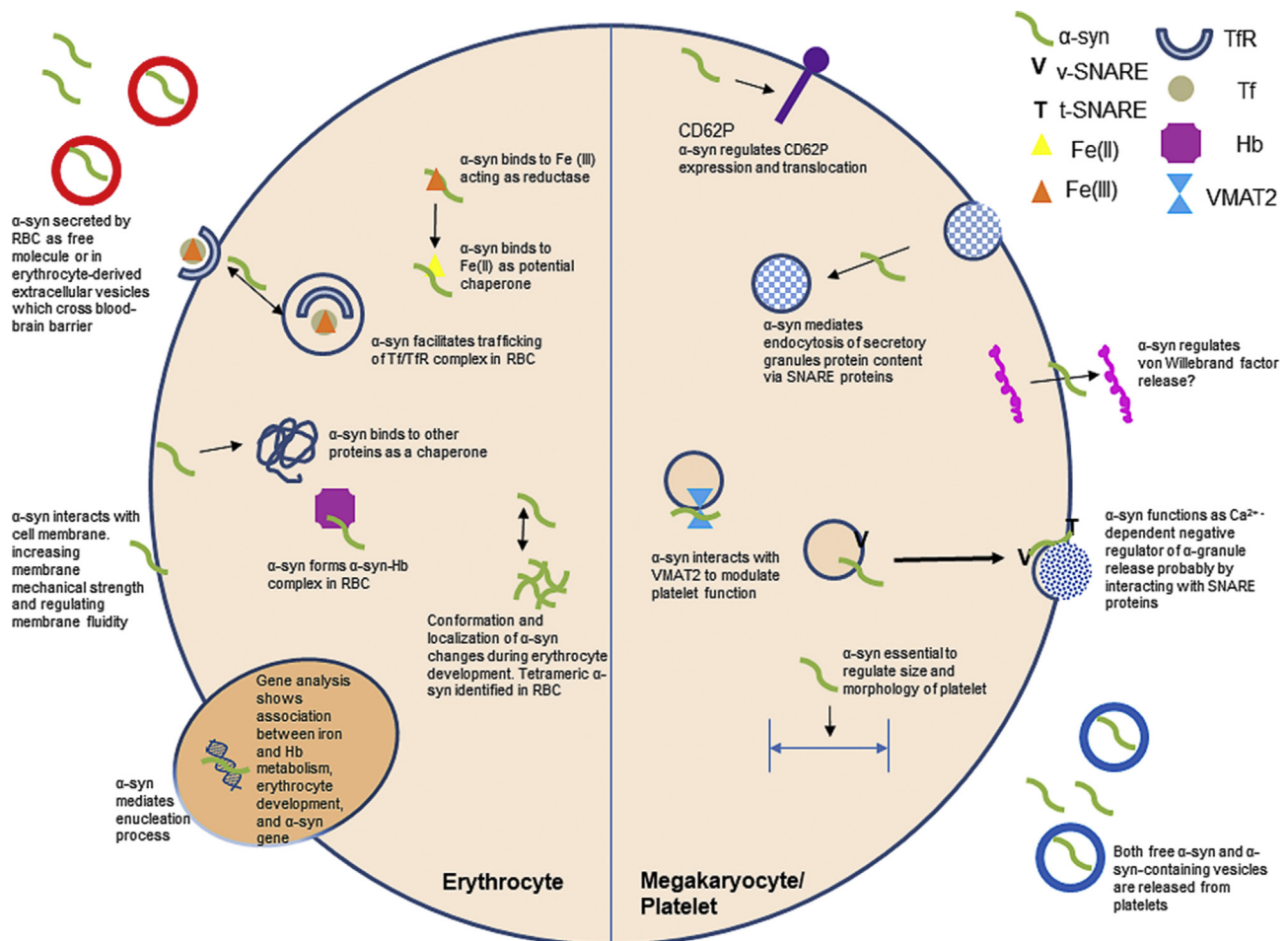


Fig. 2. Diagram representing both erythrocyte (left half) and platelet/megakaryocyte (right half). Processes mediated or regulated by α -syn expression are shown. Similarly, conformation changes of the protein in RBCs are indicated since this can mediate distinct functions.

a binding constant higher than that of transferrin binding to Fe(II) making α -syn a potential Fe(II) chaperone thus mediating iron transport [168]. In addition, α -syn regulates iron uptake and recycling by modulating phagocytes [162]. This may represent another potential pathway by which α -syn regulates iron homeostasis.

Beyond iron homeostasis regulation, α -syn interferes with both peripheral and neuronal Hb. It has been shown that both Hb and hematocrit positively correlate with serum α -syn concentration [169]. *SNCA* has also been linked to heme metabolism genes *ALAS2*, *FECH*, and *BLVRB* under GATA control, suggesting a role for α -syn in heme metabolism [170]. Microarray studies have indicated that down-regulation of genes related to Hb and erythrocyte-specific genes occur in PD patients [165]. α -syn-Hb complexes have also been identified in primates and humans. In the former, both RBC-derived α -syn-Hb complexes and neuro-striatal cytoplasmic-derived Hb- α -syn complexes show age-dependent increases [171]. On the other hand, in human RBCs, α -syn-Hb complexes concentration increases in acute ischemic stroke patients and is positively correlated with oligomerized and phosphorylated α -syn [172]. In summary, α -syn appears critical to the erythroid lineage through its regulation of iron and hemoglobin homeostasis.

2.1.7. α -syn in megakaryocytes and platelets

α -syn is well expressed in both megakaryocytes and platelets [173]. During megakaryocyte differentiation, α -syn is upregulated while β -syn is downregulated toward the end of megakaryocytic differentiation so that only α -syn is abundantly expressed in platelets suggesting that this protein plays an active role in the terminal differentiation of this lineage. External stimuli may also affect its expression in platelets since it changes with age and level of physical activity [174]. In platelets, α -syn is loosely associated with the plasma membrane, and membranes of organelles and secretory α -granules [173]; and it is found in platelet extracellular microvesicles [175]. Of interest, α -syn concentration increases in a time-dependent manner in plasma of single donor platelets during storage. This increase could result from platelets releasing their contents due to senescence or because of storage-dependent changes to platelets [31].

Abnormal platelet morphology characterized by larger size has been described in PD patients [176]; and platelet vacuoles, part of its open canalicular system important to aggregation, are also larger [177]. In contrast, studies in α -syn^{-/-} mice have reported smaller platelets with extensive degranulation and fragmentation [72, 178]. Abnormal platelet function has also been shown in PD patients and α -syn^{-/-} mice. In this regard, decreased platelet aggregation [179] and impaired platelet mitochondrial respiratory chain have been observed in PD patients [180]. Whilst higher CD62P expression in the absence of stimulation, as well as hypercoagulability has been shown in α -syn^{-/-} mice [72, 178].

These changes in platelet morphology and function in the setting of altered α -syn expression suggest that this protein is essential to megakaryocytes and platelets (Fig. 2). It has been shown that one function of α -syn is as a calcium-dependent negative regulator of α -granule release from platelets [181]. Notably, exogenous α -syn penetrates platelets via its NAC domain and inhibits α -granule release via both its N- and C-termini [181]. This inhibitory function of α -syn has been reported in endothelial cells. Endogenously overexpressed and exogenous α -syn inhibit von Willebrand factor release and CD62P translocation in endothelial cells; and both N terminus and NAC region are needed for this inhibition [182]. Higher CD62P expression and degranulation of platelets in α -syn^{-/-} mice lend support to this potential inhibitory role of α -syn [170].

Granule exocytosis, including vesicle docking, priming, triggering, and fusion is critical to platelet function. Several SNARE proteins are essential in these processes [183]. Syntaxins -2, -4, -7, and -11, and SNAP-23, -25, and -29 have been identified as t-SNARE proteins in platelets. VAMP8 is the critical v-SNARE for α -granule release, while VAMP2 and VAMP3 have subordinate roles [184]. However, without VAMP7 [185], there is partial dense granule and α -granule secretion deficit suggesting that this SNARE links exocytosis to cytoskeletal

reorganization during granule release. In the megakaryocyte-platelet lineage, endocytosis is as important as exocytosis. In megakaryocytes only part of the protein content of α -granules is synthesized by the cell and the remainder is actively endocytosed from blood. For example, fibrinogen in α -granules comes from plasma through endocytosis [186]. VAMP3 mediates endocytosis and membrane trafficking processes in platelets, which are important to fibrinogen uptake, platelet spreading, and clot retraction [187].

Alternatively, α -syn could interact with vesicular monoamine transporter 2 (VMAT2) to modulate platelet function. Under normal conditions, VMAT2 sequesters dopamine into synaptic vesicles for storage and release. Megakaryocytes express VMAT2 [188] and its mRNA is decreased in platelets of PD patients [189]. Along these lines, reduced expression of VMAT2 in mice leads to increased α -syn immunoreactivity and accumulation in SN [190].

The data presented suggests that α -syn is essential to megakaryocyte and platelet homeostasis and that SNARE protein modulation in both exocytosis and endocytosis are mechanistically important. Even though these underlying mechanisms remain to be elucidated, likely, lessons can be learned from what has been discovered in neurons since just as platelets neuronal granule release is paramount to normal cell function. Future research will determine these important functions mediated by α -syn in megakaryocytes and platelets.

2.1.8. α -synuclein and immunological diseases

The potential role of α -syn in diseases of the immune system is suggested by the available data, however, at the time of writing this manuscript no "syndrome/disease" has been associated to α -syn deficiency in the literature. Clearly, the preservation of this protein's sequence across species from rodents to humans indicates that this protein is important to maintain essential physiological/cellular responses. Studies using animal models have yielded information of the phenotype caused by α -syn deficiency. Defective development of mature T and B cells, regulatory T cells, disrupted cytokine profiles, lack of antibody class switching, abnormal lymphoid tissue morphology, abnormal leukocyte ultrastructure are all indicative that absence of this protein may lead to a substantial yet non-lethal phenotype. The latter is perhaps the reason for the lack of published literature due to residual immune function. However, as indicated earlier in this review there is growing literature indicating that mutations of the protein as those seen in some Parkinson's patients lead to abnormal T cell phenotypes, and therefore immune responses that could begin to shed light at potential immune sequelae caused by changes to this protein's sequence. As a result, additional research at potential disease mechanisms caused by these new findings is needed.

3. Conclusions

α -syn is a major pathological mediator in PD and is also ubiquitously expressed in the hematopoietic system under normal physiological conditions. Observations from PD patients and α -syn^{-/-} mice point that this protein is critical to this system. Available data suggests that due to these potentially important functions its expression and conformation may be tightly regulated. α -syn may prove to be essential to exocytosis/endocytosis, apoptosis, autophagy, maturation, and differentiation of hematopoietic cells. In addition, alterations of α -syn occurring in the hematopoietic system could affect CNS cells either through extracellular vesicles or infiltrating immune cells. Undoubtedly, future research will contribute to a greater understanding of those physiologic pathways modulated by this protein.

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