Harnessing the immune system for the treatment of Parkinson’s disease

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ABSTRACT

Current treatment options for Parkinson’s disease (PD) typically aim to replace dopamine, and hence only provide symptomatic relief. However, in the long run, this approach alone loses its efficacy as it is associated with debilitating side effects. Hence there is an unmet clinical need for addressing levodopa resistant symptoms, and an urgency to develop therapies that can halt or prevent the course of PD. The premise that α-syn can transmit from cell-to-cell in a prion like manner has opened up the possibility for the use of immunotherapy in PD. There is evidence for inflammation in PD as is evidenced by microglial activation, as well as the involvement of the peripheral immune system in PD, and peripheral inflammation can exacerbate dopaminergic degeneration as seen in animal models of the disease. However, mechanisms that link the immune system with PD are not clear, and the sequence of immune responses with respect to PD are still unknown. Nevertheless, our present knowledge offers avenues for the development of immune-based therapies for PD. In order to successfully employ such strategies, we must comprehend the state of the peripheral immune system during the course of PD. This review describes the developments in the field of both active and passive immunotherapies in the treatment of PD, and highlights the crucial need for future research for clarifying the role of inflammation and immunity in this debilitating disease.

1. Introduction

Parkinson’s disease (PD) described originally by James Parkinson (Parkinson, 2002) is one of the most common neurodegenerative disorders. Over 6 million people were affected by PD worldwide in 2016, with this number only expected to increase with an increasingly aging population (Collaborators, 2018). PD is primarily characterized by classic motor symptoms including bradykinesia, rigidity and tremor (Parkinson, 2002), and is also associated with numerous cognitive (Hurtig et al., 2000; Harding et al., 2002; Zesiewicz and Hauser, 2002) and autonomic non-motor issues (Zhao and Yu, 2016; Fasano et al., 2015; Bonuccelli et al., 2003; Weiner, 2002). Pathologically, PD is characterized by intraneuronal and intra-axonal α-synuclein positive inclusions (Lewy bodies and Lewy neurites), and death of dopaminergic neurons in the substantia nigra resulting in decreased dopamine concentration in the striatum, which gives rise to the aforementioned classic motor symptoms (Hoehn and Yahr, 1967; Kalia and Lang, 2015). Aberrant immune functioning is recently gaining attention as a critical component of the susceptibility to, and progression of PD (Fiszer et al., 1991; McGeer et al., 1988b; Pouplard and Emile, 1984). Although immunological changes have been difficult to tease out, several independent lines of clinical evidence as well as preclinical evidence (Yamada et al., 1992; Tansey and Goldberg, 2010) support the engagement of the immune system in PD. This emergent field offers hope and direction on identifying potential targets within the immune system to slow or reverse neurodegeneration (Moehle and West, 2015). Due to the fact that the application of immunotherapy to PD is still relatively new, this review will discuss the (a) immune system, (b) brain engagement of the immune system in PD. This emergent field offers hope and direction on identifying potential targets within the immune system to slow or reverse neurodegeneration (Moehle and West, 2015). Due to the fact that the application of immunotherapy to PD is still relatively new, this review will discuss the (a) immune system, (b) brain immune system in the context of PD, (c) application of immunotherapy to PD and (c) considerations, risks and benefits.

2. The immune system

The immune system is an interactive network of cells, lymphoid organs, humoral factors and cytokines, together performing the essential function of host defense (Freund, 1930; Parkin and Cohen, 2001). Immunity is broadly divided into two components (innate and adaptive), and is determined by the time to mount a response, and specificity of the response (Janeway, 2001). Innate immunity (Metschnikoff, 1884) includes the body’s physical barriers (skin, hair, nasopharynx, GI tract),
secrections (saliva, mucous, bile, gastric acid, sweat) and immune cell responses (neutrophils, monocytes, macrophages, complement, cytokines) (Holmes et al., 1966; Wright et al., 1989; Silverstein, 1979). The innate response is typically rapid, but non-specific. Adaptive immunity consists of antigen-specific interactions mediated via lymphocytes (T and B cells). The adaptive response is precise, but develops over time. It also has memory, and future exposures are met with a more robust response (Delves and Roitt, 2000b, 2000a). There is accumulating evidence of the role of both innate and adaptive immunity in PD (Benner et al., 2004), and the comprehension of these systems is integral to the development of immune-mediated treatments for neurodegenerative diseases.

2.1. Innate immunity

Innate immunity mechanisms are encoded in the germ line, and work unspecifically and immediately against foreign agents (Beutler, 2004; Janeway, 1989, 2013). Some of the responses include the removal of foreign agents by phagocytes, recruitment of immune cells via cytokine and chemokine production to the infection site, complement cascade activation, and processing and presentation of antigens for activation of the adaptive immune response. Innate immunity functions by nonspecific, generic recognition of pathogen-associated molecular patterns (PAMPs), which are recognized by toll-like receptors (TLRs) (Hoffmann and Dutton, 1971). TLRs are expressed by microglia, astrocytes, oligodendrocytes, and neurons (Pfeiffer, 1892; Olson and Miller, 2004).

Innate immune cells are composed of a wide, expanding range of myeloid and lymphoid cell types (Mishell and Dutton, 1966). The origin of most of these cell types is the hematopoietic system. These cells do not have any somatically recombined antigen-receptors, or traditional immune memory capability (van der Meer et al., 2015), and possess anti-microbial, and/or tissue-protective capabilities (Gasteiger et al., 2017). The primary cells of the innate immune system include natural killer (NK) cells, mast cells, basophils, eosinophils, neutrophils, macrophages and dendritic cells (Jack et al., 2002; Janeway and Medzhitov, 2002). Neutrophils are one of the first innate immune cells recruited to the site of inflammation via chemokine gradients. They are followed by monocytes, and dendritic cells (DCs), which can then interact with the tissue-resident myeloid and lymphoid cells (Eberl et al., 2015; Gasteiger et al., 2017). These systemic cells travel throughout the body to eliminate pathogen entry and growth. The adaptive immune system kicks in when pathogens slip through these primary barriers (Netea et al., 2019).

2.2. Adaptive immunity

The adaptive immune system is more advanced but slower to react compared to the innate immune system (Dutton et al., 1970; Netea et al., 2019). Adaptive immunity takes one to two weeks for activation, and compared to the innate immune system (Dutton et al., 1970; Netea et al., 2019). Antigen-specific receptors in these cells results from random rearrangement and splicing together of DNA segments encoding peptide (Janeway et al., 1975). Both B- and T- lymphocytes can be cell-mediated and can locate circulating antigens from either the lymphatic system or circulatory system (Gowsan, 1975; Bonilla and Oettgen, 2010). Antigen-specific receptors in these cells results from random rearrangement and splicing together of DNA segments encoding for antigen-binding receptor areas (Parkin and Cohen, 2001). Effector response is a two-stage process: the first stage is antigen presentation and recognition by the antigen specific T- or B-cell, following which cell priming, activation and differentiation take place. The second stage is the effector response; activated T-cells either exit the lymphoid tissue and home to the site of disease, or, antibodies are released from activated B-cells into the blood stream (Parkin and Cohen, 2001; Ellis and Gowsan, 1975). There is significant T-cell heterogeneity (Cantor, 1972), and the two major types of effector T-cells are T-helper (Th, with CD4 surface receptor), and T-cytotoxic (Tc, with CD8 surface receptor) (Kruisbeek, 1999; Hare et al., 1999). CD4+ cells are capable of antigen recognition and activation of the cell-mediated response for pathogen clearing. They are crucial in B-cell activation. CD8+ cells are important in antiviral and antitumor activity (Parkin and Cohen, 2001). Lymphoid tissues also contain efficient antigen-presenting cells (APCs), which can produce the necessary cytokines for T- and B-lymphocyte maintenance. APCs are: dendritic cells (DCs), B cells and macrophages (Parkin and Cohen, 2001). Mature T-cells are activated when the T-cell receptor (TCR) recognizes an antigenic peptide, complexed with major histocompatibility complex (MHC) on an APC (Parkin and Cohen, 2001; Bonilla and Oettgen, 2010). Antibodies (Hektoen, 1909) produced by B-cells serve to neutralize toxins, activate complement, opsonize bacteria for phagocytosis and prevent adherence to mucosa, thereby enhancing aspects of the innate immune system (Carrel and Ebeling, 1922).

There is mounting evidence that indicates the presence of persistent inflammation, and immune involvement in PD (Fiszer et al., 1994; McGee et al., 1988a, 1988b; Cicchetti et al., 2002; Mogi et al., 1995; Kurkowska-Jastrzebska et al., 1999). Hence, it will be important to clarify the role of the immune system (as an etiological factor or propogator of inflammation) in PD, especially as immunotherapeutic strategies for reducing α-syn aggregation show promise in clinical trials. Some studies that have examined the composition of T-cell subsets in the peripheral blood of PD patients show an overall decrease in the total number of lymphocytes (Bas et al., 2001; Stevens et al., 2012) with a more activated, cytotoxic T-cell response (lower CD4+:CD8+ ratio, and higher IFNγ than IL-4 producing T-cells) (Baba et al., 2005) indicating an altered adaptive immune response. Another study has shown regulatory T-cells of PD patients to have more suppressive activity when compared to healthy controls, which likely promotes a chronic neuroinflammatory state thereby leading to the mounting of abnormal immune responses against CNS proteins normally recognized as “self” (Saunders et al., 2012). Peripheral T-cells can infiltrate brain parenchyma at the sites of neuronal injury has been demonstrated in post-mortem human brains, as well as animal models of PD (Brochard et al., 2009). This cell-mediated immune response results in dopaminergic cell degeneration via a CD4+ T-cell dependent mechanism (Brochard et al., 2009; Benner et al., 2008). Vaccinations and active antibody therapies utilize the adaptive immunity mechanism to provide enduring physiological clearance of targeted proteins (Pasteur and Chamberland, 2002). The benefit of active therapies is that they require minimal repeated dosing due to a prolonged period of protein clearance and decreased aggregation (Chatterjee and Kordower, 2019). Passive immunotherapy utilizes the adaptive immunity mechanism to allow for epitope specificity for target proteins (Chatterjee and Kordower, 2019). One of the drawbacks to the passive approach is the need for repetitive dosing. Ultimately, better comprehension of the role of the immune system in PD will be critical to the development of immune-based therapeutics.

3. Brain’s immune system

The CNS has been considered as immune privileged, given the absence of professional APCs, low levels of MHC I and II expression, and apparent absence of lymphatic drainage from the CNS (Louveau et al., 2015a). However, it is now known that CNS-derived antigen can result in an immune reaction in the cervical lymph nodes (Casier et al., 1992; Kida et al., 1998), and the CNS has a functional lymphatic system, which is able to drain cellular and soluble material from the CSF to the cervical lymph nodes (Aspelund et al., 2015; Andres et al., 1987). It has now become well accepted that microglia (Gomez Perdiguero et al., 2015) are the resident immune cells of the CNS (Torvik, 1975; Fujita and Kitamura, 1975; Wood et al., 1979; Louveau et al., 2015b). The primary function of microglia is to constantly survey the CNS environment,
which they achieve by movement of their fine processes (Nimmerjahn et al., 2005; Hines et al., 2009). Microglial processes are in direct contact with astrocytes, neuronal cell bodies and blood vessels in the cortex. This proximity implies close communication, which can aid in the microglial response to brain injuries (Davalos et al., 2005; Brockhaus et al., 1996). Microglia respond to several signals including bacterial and viral products, antibodies, cytokines and α-syn, and are crucial for recruiting peripheral immune cells by chemokine secretion (Croisier et al., 2005; Kim and de Vellis, 2005; Klegeris et al., 2008). Resting microglia have small cell bodies, with distinct, ramified processes. When microglia become activated in response to disturbances in the milieu, they respond by undergoing proliferation, morphogenesis and an increase in cell volume, in addition to extension of their processes (Akiyama and McGeer, 1989; Streit et al., 1988). While microglia are not considered dendritic cells per se, they are the primary MHC II-expressing APCs in the brain parenchyma (Wong et al., 1984; Suzumura et al., 1987; Hayes et al., 1987), and are able to express MHC I surface glycoproteins as well (Akiyama et al., 1988; Streit et al., 1989; Estrada et al., 2019). Microglia like macrophages and dendritic cells also use pattern recognition receptors (PRRs) to survey the environment, and act to prevent excessive neuronal damage (Perry et al., 2010). Antigen presentation of CNS-derived antigens results in T-cell activation and their concomitant infiltration into the brain, and it has been demonstrated that microglia are able to stimulate CD4+ and CD8+ T-cells in an MHC II dependent manner (Ford et al., 1996; Gottfried-Blackmore et al., 2009; Ebner et al., 2013). The crosstalk between microglia and T-cells indicates that therapies for neurodegenerative disease that affect T-cells can indirectly alter microglial phenotypes and specifically attenuate activated microglia during disease course (Kippis et al., 2000; Laurie et al., 2007; Farina et al., 2005).

3.1. Importance of microglia in PD

In PD, it is now known that microglia are activated as part of a neuroinflammatory response (McGeer and McGeer, 2008; Yamada et al., 1992; McGeer et al., 1988b, 1988c; Inamura et al., 2003), which implies that the immune system plays a role in the pathogenesis of PD. Hence it is likely that microglia play a role in successful immunotherapy for neurodegenerative diseases. Activated microglia are poised for inducing inflammation, demonstrate increased IgG reactivity (Oechmchen and Huber, 1976; Ulvestad et al., 1994) upregulate complement receptors (Pasinetti et al., 1992; Korotzer et al., 1995), and cell adhesion molecules (Miklossy et al., 2006), and can regulate T-cell responses through antigen presentation (Olson and Miller, 2004). Activated microglia produce toxic substances including reactive oxygen species (ROS; Colton and Gilbert, 1987) reactive nitrogen species (RNS) (Zielask et al., 1992), proinflammatory cytokines (Giulian and Lachman, 1985; Yao et al., 1992) and prostaglandins that together have the capacity to neutralize infectious agents, and can also cause neuronal injury and death (abd-el-Basset and Fedoroff, 1995; Suzumura et al., 1998). There is a high concentration of microglia in the substantia nigra (specifically ventral tier of the pars compacta), making neurons in this region likely susceptible to unchecked microglial activation (Lawson et al., 1990; Kim et al., 2000). Elevated levels of IL-1β, and TNF-α have been detected in the striatum and cerebrospinal fluid of patients with PD compared with control subjects, further supporting an inflammatory process in PD (Blum-Degen et al., 1995; Mogi et al., 1996) for review see Nagatsu and colleagues (Nagatsu et al., 2000). Due to the specificity in the increase in level of cytokines to the nigrostriatal pathway and not cortical regions, it is likely that microglia play a role in successful immunotherapy for neurodegenerative diseases. Activated microglia (Iseki et al., 2000; Waismann and Johann, 2018; Dufly et al., 2018). T-cells that infiltrate the brain in this manner are primarily CD4+ cells, which typically proliferate and release proinflammatory cytokines such as TNF, IL-1β and IFNγ, which can cause neurodegeneration (Harms et al., 2013; Waismann and Johann, 2018; Williams et al., 2018).

It is difficult to devise disease-modifying therapies for PD that can alter the course of the disease due to the fact that significant neurodegeneration has already occurred prior to clinical presentation. Hence, timely diagnosis and treatment are crucial to make an impact on the treatment of this chronic and progressive disease. Biomarker identification for early PD is a rapidly growing field, (Hartikainen et al., 1992; Simes et al., 2020; Tristan-Noguero et al., 2020; Quattrone et al., 2020) and it is therefore critical to continue identifying immunomodulatory therapies that can alter disease course.

4. Immunotherapy for PD

The immune privileged central nervous system (CNS) often hinders the entry of therapeutic drugs that are targeted at the brain via physical separation (meninges) and the blood brain barrier (BBB) (Hess, 1955; Roth and Barlow, 1961). The BBB is composed of brain microvascular endothelial cells, pericytes, astrocytes, neuronal processes, basal lamina and the perivascular microglia for what is known as the neurovascular unit or NVU (Hawkins and Davis, 2005; Iadecola, 2017), and the main functions for the BBB include regulation of paracellular permeability, ion balance, transport of nutrients, and provide protection from malignant pathogens or toxins (Aird, 1948; Obermeier et al., 2013; Aird and Becker, 1963; Abbott and Romero, 1996). It closely regulates which molecules are allowed through and which are not and when errors occur in regulation, it usually results in neuroinflammation (Carrano et al., 2011, 2012; Lakhani et al., 2009; Boutin et al., 2001; Yang et al., 1999a, 1999b). Therefore, an intact BBB is very important to ensure brain health.

While the BBB is restrictive, it does allow for the entry of certain molecules. Dopamine cannot cross the BBB, however the dopamine precursor oral levodopa, can pass the BBB into the brain (Celesia and Wanamaker, 1976), and here it is readily converted into dopamine (Hardebo et al., 1977). After almost 50 years, Levodopa remains the gold standard treatment for symptom alleviation of PD (Yahr et al., 1969). However, only 5–10% of the drug effectively crosses the barrier, and the remainder is metabolized to dopamine outside of the brain, where it can cause a number of sometimes severe side effects. These include motor response oscillations, and dyskinesias (Sacks et al., 1970; Weiss et al., 1971; Fahn, 1974). Therefore, there is an unmet need for alteration of disease course.

One of the cues for PD could be to tame the hyperactive autoimmune system via molecules that can induce an immune response (antigens) or, by using engineered immune cells that can train the autoimmune system to become tolerant (Chen et al., 1998). There are now several lines of evidence from preclinical studies as well as clinical trials that indicate that modulating the immune response can provide
neuroprotection in PD via several mechanisms including reduction of microglial activation, increasing neurotrophic support, dampening of pro-inflammatory T-cell responses as well as removal of aberrantly folded proteins (Benner et al., 2004; Delgado and Ganea, 2003; Reynolds et al., 2007; Viti jungle et al., 2006). Post-mortem studies have revealed that Lewy body pathology is found not only in the nigrostriatal dopaminergic system, but also in the peripheral autonomic system, such as neurons of the enteric plexus of the GI tract, and sympathetic nerve fibers in the adrenal gland, heart and cutaneous nerves. Later in the course of the disease, medulla, pons, midbrain, basal forebrain, olfactory bulb and higher order cortices can also be affected (Poewe et al., 2017; Braak et al., 2003). Extensive studies of normal and diseased human brains have provided evidence that α-syn inclusions occur in a relatively predictable sequential order in different parts of the brain. This made it possible to distinguish six stages of α-syn deposition, called Braak staging (Braak et al., 2003). Hence, α-syn pathology seems to continuously implicate further neuronal as well as non-neuronal cells, and progresses in a prion-like fashion (Desplats et al., 2009; Volpicelli-Daley et al., 2011; Rockenstein et al., 2014). Despite this aggressive mode of transmission, new therapeutic avenues are possible. One very promising approach is to eliminate excess and toxic forms of extracellular α-syn. The premise for the application of immunotherapy for PD therapy relies on the α-syn spreading from cell to cell via the prion-like mechanism, which makes it available to therapeutics in the extracellular space, (Hung and Schwarzschild, 2020; Uemura et al., 2020). This is also supported by the observation that there was host-to-graft spreading of Lewy body pathology in transplanted fetal cells in brains of patients with PD (Li et al., 2008; Kordower et al., 2008). Therefore, if sufficient levels of anti-α-syn antibodies are able to reach the brain, they should be able to trap α-syn aggregates when released into the extracellular synaptic space (Valera and Masliah, 2013; Hutter-Saunders et al., 2011).

It has been observed that circulating NK cells are increased in patients with PD when compared to controls (Niwa et al., 2012), and NK cells have been found in post-mortem human as well as mouse brains (Earls et al., 2020, 2019) in the substantia nigra. The relatively recent discovery of the ability of NK cells to internalize and degrade α-syn via endosomal/lysosomal pathways (Earls et al., 2020) and the increase in neuropathological burden of α-syn in a mouse model of synucleinopathy following depletion of NK cells (Earls et al., 2020) suggests the importance of these bone-marrow derived hematopoietic cells (Kessling et al., 1975; Hazenberg and Spits, 2014; Chee and Khakoo, 2009; Vivier et al., 2008; Shi et al., 2011) in the context of PD, and inflammation. Therefore, NK cells can also be a highly relevant cell type in the context of PD, and may play a crucial role in ameliorating a sustained, systemic immune response to α-syn, and in reducing α-syn burden.

Sulzer et al have shown that peptides derived from two specific regions of α-syn are capable of mounting a T-cell response in a patient cohort with PD (Sulzer et al., 2017). Furthermore, a recent study from this group shows a clear association of α-syn-specific T-cell responses in early disease stages in PD, which demonstrates the potential of these T-cells in the preclinical and early motor stages of the disease (Lindestam Arlehamn et al., 2020). Thus it is clear that inflammation in PD likely plays both a protective as well as detrimental role, and these findings support the fact that immunotherapy for PD can improve either by improving the immune system’s tolerance for α-syn, or by inhibiting the overactive response of the immune system (Garretti et al., 2019).

Despite progress in the comprehension of cellular pathways associated with pathogenesis of PD and several clinical trials over the last three decades, slowing the progression of PD has remained elusive. Here, we will review recent as well as ongoing clinical trials, specifically those employing active and passive methods which aim to modify the spread of α-syn in the brain.

### 4.1. Recent advances

Immunotherapies clearly have the potential for disease modification in PD. There are several approaches to this including delivery of anti-inflammatory drugs and immunosuppressants prior to disease onset to mitigate risk, restoration of lysosome functioning, hastening the process of α-syn clearing, neutralizing α-syn aggregates using an antibody-based approach, modulation of T-cell activity and inducing immune tolerance by modulating regulatory T-cell (Treg) activity. Here we will discuss the most promising recent advances in active and passive immunotherapies for PD, which primarily target extracellular α-syn and block cell-to-cell transmission (Fig. 1).

Using an active immunization approach, AFFiRiS has developed a novel immunotherapeutic (PD01A). This technology involves immunization with a short antigenic peptide, which mimics an epitope in the native C-terminal region of human α-synuclein (Mandler et al., 2014). PD01 consists of eight amino acids and is conjugated to a carrier protein (keyhole limpet haemocyanin/XLH) and absorbed to aluminium hydroxide. PD01A should induce antibodies that can selectively recognize α-syn aggregates and not the monomeric forms, and have no reactivity to β-synuclein (Mandler et al., 2014). The carrier protein should provide necessary T-helper epitopes such that there is an induction of long-lasting antibody response. The antigenic component is a B-cell epitope responsible for specificity of the humoral response, and preclinical trials demonstrated robust reduction of aggregated α-syn, with improved memory and motor defects (Mandler et al., 2014). These data catapulted PD01A into Phase I clinical testing. Results from the Phase 1 study indicate that PD01A is safe even with repeated administration, and is tolerated over long periods of time. A substantial increase in titers was observed against the antigenic peptide, and a humoral response was generated against α-syn target epitope and the safety profile was desirable (Volc et al., 2020).

The passive immunization approach typically comprises delivery of antibodies that can target a specific infectious disease agent (Winternitz and Kline, 1915). In the context of PD, the passive approach consists of...
the transfer of α-syn directed antibodies to the CNS (Zella et al., 2019; Shin et al., 2020; Hung and Schwarzschild, 2020). In one of the pioneering studies, monoclonal antibodies against the C-terminus of α-syn; 9E4 was administered to transgenic mice overexpressing human wild-type α-syn under the control of the PDGF-β promoter (Masliah et al., 2011). Weekly intravenous injections of the antibody for 6 months improved behavior in the water maze task. Further, there was a reduction of calpain-cleaved α-syn in axons and synapses of neurons in the cortex and hippocampus (Masliah et al., 2011).

Weekly intraperitoneal injections for 4 weeks using a different C-terminal antibody (Ab274) in the same mouse model as above provided an amelioration of behavioral deficits and neurodegeneration in the group that was passively immunized. Furthermore, they observed an increase in localization of α-syn and AB274 in microglia, which was mediated via the Fc receptor (Bae et al., 2012).

PRX002, a humanized IgG1 monoclonal version of 9E4 developed by Prothena was the first α-syn based therapy that entered into clinical trials in 2015. In healthy volunteers, a single ascending-dose intravenous infusion study showed safety and tolerability and a single intravenous infusion of 30 mg/kg showed a rise in antibody levels in serum to 578 μg/ml (Schenk et al., 2017). Data from a multiple ascending-dose trial of intravenous infusions of PRX002 in patients with PD of Hoehn and Yahr stages 1–3 showed favorable safety and tolerability data (Jankovic et al., 2018) as well as a significant reduction of α-syn. A Phase 2 study has been initiated in patients newly diagnosed with PD (PASA-DENA Study, ClinicalTrials.gov identifier NCT03100149).

In another passive strategy, immunization using three C-terminally directed α-syn antibodies, 1H7, SCI and SD12, weekly for 6 weeks in transgenic mice overexpressing α-syn under control of the mThy1 (Fleming et al., 2004; Rockenstein et al., 2002) promoter showed a significant reduction of cleaved α-syn in the cortex and striatum. This improved behavioral deficits, as well as making pathological burden (Games et al., 2014). It is possible that C-terminal antibodies can stabilize the C-terminal domain of α-syn, thus making it less likely to undergo truncation, which could reduce aggregate formation, and propagation via seeding (Games et al., 2014).

Antibodies directed against the N-terminal amino acids of α-syn have also been developed and have undergone preclinical testing (Shahaduzzaman et al., 2015). An animal model of PD was generated via injection of recombinant adeno-associated viral vector expressing human wild-type α-syn under control of the CBA promoter in the nigra. Antibody (AB1) was delivered intraperitoneally biweekly, for 3 months, and showed a moderate improvement of behavior, with protection of nigral dopaminergic neurons (Shahaduzzaman et al., 2015).

Another N-terminal α-syn antibody (Syn303) was developed by Tran et al targeting misfolded α-syn in a mouse model injected intrastriatally with preformed α-syn fibrils. Syn303 was administered intraperitoneally one a week for about six months. This treatment prevented α-syn aggregates from spreading, reduced dopaminergic cell loss and was also effective in improving behavior (Tran et al., 2014). The authors offer two possible routes by which the α-syn antibodies decreased pathological burden. (i) α-syn antibodies could reduce the amount of seeds for recruiting endogenous α-syn into aggregates by blocking the uptake of preformed α-syn fibrils into neurons. This has been demonstrated in both in vitro (Kloury et al., 2012) and in vivo models (Yamandura et al., 2013) for tau antibodies. It has been shown that binding to LAG3 is required for initiation of pathological α-syn transmission and toxicity, and the importance of pathological α-syn from cell-to-cell requires the endocytosis of exogenous α-syn fibrils via LAG3 engagement (Mao et al., 2016; Angelopoulou et al., 2020), (ii) α-syn antibodies could prevent cell to cell transmission of pathological α-syn. The authors suggest that the two mechanisms may not be necessarily mutually exclusive and likely occur simultaneously.

The most toxic effects of α-syn are when it is in its aggregated form, (Spillantini et al., 1998; Ostrerova et al., 1999) hence developing antibodies against α-syn oligomers and fibrils can reduce deleterious extracellular α-syn, and also protect physiological α-syn (Bae et al., 2012). A monoclonal antibody mAb47 was developed to specifically target aggregated forms of α-syn by Lindstrom et al (Lindstrom et al., 2014). When transgenic PD mice expressing the human α-syn A30P mutant under the Thy-1 promoter were immunized weekly with mAb47 for about 3 months, a decrease in pathological burden of α-syn was observed, and the monomeric form was not affected (Gustafsson et al., 2017).

Results from these preclinical passive immunization strategies targeting α-syn have shown promise and this approach is beginning to be translated into human clinical trials as discussed.

BIBI054 (Cinpanemab), developed by Neurimmune is an N-terminal directed α-syn antibody and is currently being evaluated in clinical trial. Isolated from B-cell lines from neurologically healthy individuals, it is highly selective for the aggregated form of α-syn (Weihofen et al., 2019). Results from a recently concluded Phase I trial indicate that BIBI054 is well tolerated up to 90 mg/kg (Brys et al., 2019; Weihofen et al., 2019). BIBI054 is currently being evaluated in a Phase II study in recently diagnosed PD patients (SPARK Study, ClinicalTrials.gov identifier NCT03318523).

There are other α-syn monoclonal antibodies currently in early stages of development. MED13414, developed collaboratively by AstraZeneca and Takeda is entering Phase I trials (ClinicalTrials.gov identifier NCT03272165). Another monoclonal antibody against α-syn oligomers is BAN0805, developed collaboratively by Bioarctic and Abbvie (https://www.bioarctic.se/en/section/media/press-releases/). UB-312, a peptide-based vaccine and LU- AFR2422, a humanized monoclonal IgG1 antibody are both in Phase I trials. An overview of therapies currently in human clinical trials is provided in Table 1.

Preclinical work in MPTP animals by Benner et al where lymphoid cells were adoptively transferred from Cop-1+ immunized mice resulted in an accumulation of T cells, production of GDNF and also a dampering of microglia (Benner et al., 2004). The Cop-1 activated CD4+ T cells were shown to be responsible for the neuroprotection (Laurie et al., 2007), as was evidenced by significantly higher numbers of surviving tyrosine hydroxylase positive neuronal cells bodies and striatal fibers in the substantia nigra pars compacta region (Benner et al., 2004). Additionally, it was observed that striatal dopamine levels were elevated in mice that received Cop-1 immune cells, suggesting that the Cop-1 immune cells trigger a T- cell dependent neuroprotective response in specific, affected brain regions (Benner et al., 2004) during the acute MPTP induced neurodegeneration phase (Jackson-Lewis et al., 1995).

Further studies from this group later demonstrated the role of Treg in inducing neuroprotection. When CD3 activated CD4CD25+ T’ cells were transferred into MPTP animals 12–18 h post MPTP injections (4 i.p injections of MPTP at 2 h intervals), protection of the nigrostriatal system comcomitant with TGFβ and CDNF production was observed (Laurie et al., 2007). They showed using vitro studies that detrimental redox reactions were modulated via Tregs, and microglia caused an activation of NF-Kb (Reynolds et al., 2009, 2008). Furthermore, analysis of mouse splenocytes demonstrated that immunization with N-4YSyn induced a Th17 cell response and resulted in Treg dysfunction in MPTP mice, however when Tregs were adoptively transferred from vasoactive intestinal peptide (VIP) immunized animals, attenuated Nu-syn caused inflammatory responses, and Tregs from VIP immunized mice resulted in neuroprotection of dopaminergic nigral neurons and striatal termini (Reynolds et al., 2010). Nu-syn is known to accumulate in dopaminergic neurons of the nigra in primates as part of the normal process of aging (McCormack et al., 2012) and hence could disrupt immune tolerance over time and ultimately cause neurodegeneration (Benner et al., 2008).

There is evidence for the role of Tregs in facilitating neuroprotection in MPTP animals from vaccination strategies such as BCg, which resulted in an increase in the number of Tregs, as well as a protection of dopaminergic neurons (Lacan et al., 2013). Bee venom (BV) has also been used as a potential vaccine against degeneration of dopaminergic neurons (Chung et al., 2012). Neuroprotection via BV was achieved by...
deactivation of microglia, and decreased CD4 T cell infiltration. The BV resulted in a substantial increase in the ratio of CD4+ T cells to CD8+ T cells, affecting Teff numbers. Patients treated with Sargramostim also showed an increase in Treg frequencies and function, without modulated to produce beneficial effects. An increase in Treg frequencies and function was observed, without affecting Treg numbers. Patients treated with Sargramostim also showed improved clinical scores. The safety and efficacy of Sargramostim is currently being evaluated in a Phase Ib trial in PD patients (NCT03790670).

It is important to mention here the ongoing preclinical studies investigating Lymphocyte-activation gene 3 (LAG3), an immune checkpoint receptor protein as a potential biomarker in PD (Angelo et al., 2020). Given its involvement in cell-to-cell transmission of α-syn, T-cell regulation and immune response in PD (Mao et al., 2016), LAG3 presents a promising approach for PD therapy.

Finally, ex vivo modification of autologous T-cells for targeting pathogenic T-cell populations in PD, or for producing anti-inflammatory molecules is a unique therapeutic approach. This would require targeting specific antigens or pathogenic T-cell subpopulations, which can be done using platforms that have been developed to harness the immune system in cancer immunotherapies (Park et al., 2011; Pohlmeyer et al., 2019). Recent work in our laboratory has shown promise in this area. Adoptive cellular transfer of autologous T-cells were able to mediate safe and effective immunoregulatory capacity in PD. While immunotherapies for PD are still rather unclear how microglial activation can affect α-synucleinopathy (unpublished). This therapeutic approach has the potential to become a personalized treatment targeting each patient’s unique antigen expression pattern.

Whatever the adopted approach, individualization of therapy and precision of treatment will be important, given the clinical heterogeneity of PD. While immunotherapies for PD are still in their early stages of development, it should become possible to overcome limitations in the field and genetically modify T cells to hone in on pathogenic/antigenic forms of α-syn. This will ideally modulate the neuroinflammation response via recruitment of specific T cell populations. Work in this area is underway in our lab, where we employ an adoptive cellular therapeutic approach to specifically target mutated forms of α-syn in a familial model of PD. Preliminary data from these studies suggests an improved survival of dopaminergic neurons as well as improvement in behavioral deficits. Further work in underway to comprehend the specific role that T cells play in this model, and also how microglia are modulated to produce beneficial effects.

5. Concluding remarks

Overall, preclinical studies evaluating active and passive immunization strategies for PD have shown promise in different models of α-synucleinopathies. Both active and passive immunization strategies are capable of reducing α-syn pathological burden and improving motor and cognitive behavior. Studies specifically targeting α-syn likely recruit microglia for extracellular α-syn degradation (Zhang et al., 2005). It is still rather unclear how microglial activation can affect α-syn clearing since it is antibody bound, and whether degradation of ingested α-syn is affected by microglial status. A more thorough comprehension of the role that microglia play will allow for assessing the functional type that can reduce the pathogenic spread of α-syn.

Since α-syn aggregates are the primary target of immunotherapy in...
PD, one possible detrimental side effect is inflammatory autoimmunity which can be mediated by Th17 cells since as α-syn is an endogenous protein. For effective immunity, the adaptive immune response must be considered, and the timing of immunotherapy to achieve a regulated response against α-syn will be crucial. There are very few studies that are focused on modulation of the adaptive immune response in PD. However, growing evidence suggests the involvement of the adaptive immune system in PD; the presence of T cells in postmortem PD human brains (Romero-Ramos et al., 2014; Brochard et al., 2009), observations in animal models of PD (Sanchez-Guajardo et al., 2013; Theodore et al., 2008; Reynolds et al., 2009) and data showing that the peripheral T cell compartment is altered in PD patients (Blum-Degen et al., 1995; Almeida et al., 2002; Baba et al., 2005; Bas et al., 2001; Fizser et al., 1994; Stevens et al., 2012). It appears as though in PD that the peripheral immune system does not return to homeostasis due to the loss of dopamine and serum increases of oxidative products as well as α-syn. The net result is for the adaptive immune system to mount an inflammatory response with microglia, and the normal protective T cell response now becomes harmful (Iba et al., 2020; Rentzos et al., 2007; Reynolds et al., 2009). Over time, this alters cytokine levels in the serum and cause damage to the T cell compartment. Hence, as put forth by Gendelman et al, immunomodulation for PD should address the adaptive immune system so as to restore natural tolerance to α-syn. Consequently, Tregs are able to dampen adaptive immune responses, and cause damage to the T cell compartment. Hence, as put forth by Gendelman et al, immunomodulation for PD should address the adaptive immune system so as to restore natural tolerance to α-syn. Moreover, growing evidence suggests the involvement of the adaptive immune system so as to restore natural tolerance to α-syn. However, the timing of delivery of immunomodulatory agents is also of vital importance (Yao et al., 2013), as they may provide significantly different effects if administered at the start of inflammation than if they were used to prime the system (Chatterjee and Kordower, 2019).

In other proteinopathies such as Alzheimer’s disease, where extracellular deposition of amyloid β (Aβ) is considered to be the central disease causing event (Hardy and Higgins, 1992; Gasiorowski and Leszek, 1997) immunotherapeutics targeting Aβ have shown mixed results (Penninkilampi et al., 2017; Loureiro et al., 2020; Ruthirakunah et al., 2016; Nimo et al., 2020). The development of therapies targeting intracerebral Aβ have faced several setbacks (Mathews and Nixon, 2003; Check, 2002), which has helped increase awareness to key aspects pertaining to disease modification for neurodegenerative diseases in general. The issues include clinical trial design, patient selection criteria, better definition of molecular targets, improved immunogenicity and delivery. Perhaps the biggest challenges in the field of AD immunotherapy have been an overall lack of clinical efficacy of the tested therapies and the large incidence of vascular adverse events (Imbimbo, 2002; Schenk, 2002; von Bernhardi, 2010). The results from AD immunotherapy while disappointing in the short term, provide optimism and promise for the future and guidance for the development of immune-based therapies for diseases such as PD for which the need to develop disease-modifying therapies remains ever so critical (Dorsey et al., 2018).

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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