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Differential effects of immunotherapy with antibodies targeting α-synuclein oligomers and fibrils in a transgenic model of synucleinopathy

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ABSTRACT

Disorders with progressive accumulation of α-synuclein (α-syn) are a common cause of dementia and parkinsonism in the aging population. Accumulation and propagation of α-syn play a role in the pathogenesis of these disorders. Previous studies have shown that immunization with antibodies that recognize C-terminus of α-syn reduces the intra-neuronal accumulation of α-syn and related deficits in transgenic models of synucleinopathy. These studies employed antibodies that recognize epitopes within monomeric and aggregated α-syn that were generated through active immunization or administered via passive immunization. However, it is possible that more specific effects might be achieved with antibodies recognizing selective species of the α-syn aggregates. In this respect we recently developed antibodies that differentially recognized various oligomers (Syn-O1, -O2, and -O4) and fibrilar (Syn-F1 and -F2) forms of α-syn. For this purpose wild-type α-syn transgenic (line 61) mice were immunized with these 5 different antibodies and neuropathologically and biochemically analyzed to determine which was most effective at reducing α-syn accumulation and related deficits. We found that Syn-O1, -O4 and -F1 antibodies were most effective at reducing accumulation of α-syn oligomers in multiple brain regions and at preventing neurodegeneration. Together this study supports the notion that selective antibodies against α-syn might be suitable for development new treatments for synucleinopathies such as PD and DLB.

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1. Introduction

Synucleinopathies (Galvin et al., 2001) of the aging population, including dementia with Lewy bodies (DLB) (McKeith et al., 2004), Parkinson disease (PD), and multiple system atrophy (MSA), are characterized by behavioral alterations, cognitive impairment, sleep disorders, olfactory deficits, and gastrointestinal dysfunction (Savica et al., 2013). α-Synuclein (α-syn) is a 140 amino acid synaptic protein (Iwai et al., 1995) involved in neurotransmitter release (Liu et al., 2004; Nemani et al., 2010) that accumulates in synaptic terminals (Bellucci et al., 2012; Kramer and Schulz-Schaeffer, 2007; Roy et al., 2007), axons (Dickson et al., 1994; Games et al., 2013), neuronal soma (Spillantini et al., 1997; Takeda et al., 1998), and oligodendrocytes (Papp and Lantos, 1992; Wakabayashi et al., 2000). Under physiological conditions, α-syn is a relatively unstructured monomer (Lashuel et al., 2013; Tsigelny et al., 2007; Tsigelny et al., 2008; Tsigelny et al., 2012) that adopts a β-helical structure when associated with membranes (Ulmer et al., 2005). α-Syn can also adopt a tetramer conformation (Bartels et al., 2011) that is important for vesicular function (Wang et

Abbreviations: AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride; α-syn, α-synuclein; BiFC, bimolecular fluorescence complementation; CT, C-terminal; DLB, dementia with Lewy bodies; ER, endoplasmic reticulum; mAbs, monoclonal antibodies; MSA, multiple system atrophy; NAC, non-amyloid β component; PD, Parkinson disease; PK, proteinase K; pS129, phosphorylated Ser129; tg, transgenic; t-α-syn, total-α-syn.

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al., 2014) and accumulates as aggregated species (oligomers, protofibrils and fibrils) (Conway et al., 1998; Hashimoto and Masliah, 1999; Iwatsubo et al., 1996; Lansbury, 1999; Lashuel et al., 2013; Oueslati et al., 2010; Taschenberger et al., 2012; Trojanowski et al., 1998; Tsigelny et al., 2008; Winner et al., 2011). Aggregated species trigger neurodegeneration and can propagate from neuron-to-neuron and neuron-to-glial cells via prion-like fashion (Lee et al., 2010; Prusiner et al., 2015). The mechanisms through which α-syn might trigger degeneration are complex and might include autophagy, endoplasmic reticulum (ER) stress, and mitochondrial alterations (Nakamura et al., 2011; Oliveira et al., 2011; Plotegher et al., 2014; Song et al., 2004) among others.

Currently, there are no effective treatments available for these disorders that affect over 1 million in the US alone (NIA, 2015) and probably over 10 million worldwide (NIA, 2015). Experimental treatment strategies include reducing α-syn expression with anti-sense or miRNA; decreasing α-syn aggregation with small molecules, increasing the clearance of α-syn with drugs that promote autophagy and preventing the seeding and prion-like spreading of α-syn (Lashuel et al., 2013; Valera and Masliah, 2016). Alternatively, previous immunotherapy studies have demonstrated that vaccination against α-syn protected against neurodegeneration and reduced α-syn accumulation by triggering clearance via autophagy (Mandler et al., 2014; Masliah et al., 2005; Masliah et al., 2011) and microglial pathways (Mandler et al., 2015). Similarly, immunization with monoclonal antibodies that recognize epitopes in the non-amyloid β component (NAC) and C-terminus of α-syn ameliorated behavioral deficits, reduced neurodegeneration, and α-syn accumulation in neurons (Masliah et al., 2011) and glial cells reducing inflammation (Bae et al., 2012). Moreover, these antibodies reduced α-syn dissemination (Bae et al., 2012; Valera and Masliah, 2013), by blocking α-syn C-terminal (CT) truncation (Games et al., 2014; Valera and Masliah, 2013) and prion-like propagation (Tran et al., 2014).

However, most of these studies utilized antibodies recognizing monomeric and aggregated α-syn. Therefore, we developed single chain antibodies targeting α-syn oligomers and fibrils expressed from lentiviral vectors (Price et al., 2016; Spencer et al., 2014; Spencer et al., 2016). Although promising, there were some limitations, which led to the development of highly specific monoclonal antibodies differentially targeting oligomers versus fibrils. These antibodies demonstrated high specificity utilizing inhibition ELISA, as well as preabsorption of the monoclonal antibodies with α-syn fibrils or monomers, which was further confirmed using surface plasmon resonance (Vaikath et al., 2015). None of the antibodies cross-reacted with monomeric or fibrillar forms of β- or γ-syn, or with other amyloidogenic proteins and peptides (Vaikath et al., 2015) Further characterization, indicated that monoclonal antibodies (mAbs) Syn-O1, -O2, and -O4 recognized both early "oligomers" and late aggregates "amyloid fibrils", whereas Syn-F1 and -F2 preferentially recognized late aggregates (Vaikath et al., 2015). In this context, for the present study, we immunized mThy1-α-syn mice that mimic some aspects of DLB and PD with these 5 different antibodies (Syn-O1, -O2, -O4, -F1, and -F2) against oligomers and fibrils. We found that the Syn-O1, Syn-O4 and Syn-F1 antibodies were most effective at ameliorating neurodegeneration by preventing of α-syn accumulation and related mitochondrial alterations. Together, this study supports the notion that selective antibodies against α-syn oligomers might be suitable for development of new treatments for synucleinopathies such as DLB and PD.

2. Materials and methods

2.1. Mouse model of α-syn accumulation and passive immunization

We utilized 6 month old female mice over-expressing human α-syn under the mThy1 promoter (mThy1-α-syn, Line 61) (Rockenstein et al., 2002) and female non-transgenic (tg) mouse littermates. The line 61 α-syn tg model was selected because these mice develop behavioral motor deficits (Fleming et al., 2004), axonal pathology and accumulation of CT-cleaved α-syn and aggregates in neocortex, limbic system and subcortical regions (Games et al., 2013). In this model, accumulation of α-syn in the hippocampus results in Lewy body neurites and degeneration of CA3 neurons similar to DLB (Overk et al., 2014; Rockenstein et al., 2007; Rockenstein et al., 2002). Female mice were utilized since the transgene integration site is on the X chromosome; therefore, female mice display higher levels of α-syn and pathology that is more consistent.

For the immunotherapy experiments, 6 non-tg and 36 α-syn tg mice were included in this randomized and double-blind study. Groups of 6 mice each were immunized with either vehicle or Syn-O1, -O2, -O4, -F1 and -F2 antibodies. Mice received weekly intraperitoneal injections at 30 mg/kg for a period of 3 months (Fig. 1A). The antibodies were generated with recombinant α-syn where Syn-O1, -O2, and -O4 antibodies preferentially recognize early "oligomers" and late aggregates "amyloid fibrils", while Syn-F1 and -F2 antibodies preferentially recognizes late aggregates (Vaikath et al., 2015). We have previously shown that comparable FITC-tagged monoclonal antibodies traffic into the CNS and recognize α-syn aggregates (Masliah et al., 2011).

2.2. Behavioral analysis

Mice were tested in the open field (25.5 × 25.5 cm) for 15 min using an automated system (TruScan system for mice; Coulbourn Instruments, Allentown, PA). Time spent in-motion (total activity) was automatically collected for 15 min using the TruScan software. This test reflects adaptation of the animals to the novel environment, as well as anxiety behavior.

Upon sacrifice, the whole brain was removed and split in the sagittal plane. The right hemisphere was post-fixed in phosphate-buffered 4% paraformaldehyde (pH 7.4) at 4 °C for 48 h for neuropathological analysis, and the right hemisphere was flash-frozen and stored at −80 °C for ELISA assays. All experiments described were approved by the animal use and care committee of the University of California San Diego (UCSD) and were performed according to NIH guidelines for animal use.

2.3. Antibodies for immunotherapy and in vitro cell based characterization

The detailed generation and characterization of the antibodies were reported previously, (Vaikath et al., 2015). In brief, for the generation of the antibodies, mice were immunized with α-syn fibrils. Following booster immunization, mice with high antibody titers were sacrificed, and the spleen cells isolated and fused with myeloma cells. Positive hybridomas were selected by ELISA and stable clones selected by limiting dilution. The antibody was purified by protein G affinity and characterized extensively. The specificity of the antibodies was detected by dot blot, affinity of the antibodies was determined by inhibition ELISA and surface plasmon resonance. The epitope mapping was carried out by pepscan.

To further characterize the differential ability of the 5 monoclonal antibodies at reducing α-syn oligomerization, a cell based α-syn assay based on bimolecular fluorescence complementation (BiFC) was utilized (Bae et al., 2014). The assay consists with two different SH-SY5Y cell lines expressing α-syn fused to either the N-terminus (V1S) or C-terminus (SV2) fragment of Venus, a variant of yellow fluorescence protein (Fig. 1A). For co-culture, V1S and SV2 stable cells (125,000 cells each) were mixed and seeded on to poly-L-lysine coated coverslips. After a 3-day co-culture, the interaction of V1S and SV2 fused α-synuclein was visualized and analyzed by venus protein complementation analysis (Fig. 1A).

2.4. ELISA for total, human, oligomers and p-α-synuclein

As previously described (Vaikath et al., 2015), frozen hemi-brains from mice passively immunized using one of five different monoclonal