The MHC class II transactivator modulates seeded alpha-synuclein pathology and dopaminergic neurodegeneration in an in vivo rat model of Parkinson’s disease

Itzia Jimenez-Ferrer a, Filip Bäckström b, Alfredo Dueñas-Rey a, Michael Jewett a, Antonio Boza-Serrano b, Kelvin C. Luk c, Tomas Deierborg b, Maria Swanberg a, * a Translational Neurogenetics Unit, Wallenberg Neuroscience Centre, Lund University, Lund, Sweden
b Experimental Neuroinflammation Laboratory, Lund University, Lund, Sweden
c Department of Pathology and Laboratory Medicine, Institute on Aging and Centre for Neurodegenerative Disease Research, Philadelphia, PA, USA

ARTICLE INFO

Keywords:
Parkinson’s disease
MHCII
Dopaminergic neurons
Neurodegeneration
Microglia
Neuroinflammation
Alpha-synuclein
Pre-formed fibrils
PPF

ABSTRACT

Background: Abnormal folding, aggregation and spreading of alpha-synuclein (αsyn) is a mechanistic hypothesis for the progressive neuropathology in Parkinson’s disease (PD). Spread of αsyn between cells is supported by clinical, neuropathological and experimental evidence. It has been proposed that a pro-inflammatory microenvironment in response to αsyn can promote its aggregation. We have previously shown that allelic differences in the major histocompatibility complex class two transactivator (Mhc2ta) gene, located in the VRA4 locus, alter MHCII expression levels, microglial activation and antigen presentation capacity in rats upon human αsyn overexpression. In addition, Mhc2ta regulated dopaminergic neurodegeneration and the extent of motor impairment. The purpose of this study was to determine whether Mhc2ta regulates αsyn aggregation, propagation and dopaminergic pathology in an αsyn pre-formed fibril (PFF)-seeded in vivo model of PD.

Methods: The DA and DA.VRA4 congenic rat strains share background genome but display differential microglial antigen presenting capacity due to different Mhc2ta alleles in the VRA4 locus. PFFs of human αsyn or BSA solution were injected unilaterally to the striatum of DA and DA.VRA4 rats two weeks after ipsilateral administration of recombinant adeno-associated virus (rAAV) vectors carrying human αsyn or GFP to the substantia nigra pars compacta. Behavioural assessment was performed at 2, 5 and 8 weeks while histological evaluation of αsyn pathology, inflammation and neurodegeneration as well as determination of serum cytokine profiles were performed at 8 weeks.

Results: rAAV-mediated expression of human αsyn in nigral dopaminergic neurons combined with striatal PFF administration induced enhanced αsyn pathology in DA.VRA4 compared to DA rats. Mhc2ta thus significantly regulated the seeding, propagation and toxicity of αsyn in vivo. This was reflected in terms of wider extent and anatomical distribution of αsyn inclusions, ranging from striatum to the forebrain, midbrain, hindbrain and cerebellum in DA.VRA4. Compared to DA rats, DA.VRA4 also displayed enhanced motor impairment and dopaminergic neurodegeneration as well as higher levels of the proinflammatory cytokines IL-2 and TNFα in serum.

Conclusions: We conclude that the key regulator of MHCII expression, Mhc2ta, modulates neuroinflammation, αsyn-seeded Lewy-like pathology, dopaminergic neurodegeneration and motor impairment. This makes Mhc2ta and microglial antigen presentation promising therapeutic targets for reducing the progressive neuropathology and clinical manifestations in PD.

Abbreviations: ANOVA, analyses of variance; AP, anteroposterior; BSA, bovine serum albumin; CL, contralateral; DAB, 3,3’-diaminobenzidine; DV, dorsoventral; GFP, green fluorescent protein; HLA, human leukocyte antigen; Iba1, ionized calcium-binding adapter molecule 1; IL, ipsilateral; Mhc2ta, major histocompatibility complex class II transactivator; MHCII, major histocompatibility complex class II; ML, mediolateral; PD, Parkinson’s disease; PFA, paraformaldehyde; PFF, preformed fibrils; pαsyn, phosphorylated-ser129 α-synuclein; rAAV, recombinant adeno-associated virus; ROI, regions of interest; SEM, standard error of mean; SN, substantia nigra; SNpc, substantia nigra pars compacta; SNPs, single nucleotide polymorphisms; TEM, transmission electron microscopy; TH, tyrosine hydroxylase; αsyn, α-synuclein.

* Corresponding author.
E-mail address: maria.swanberg@med.lu.se (M. Swanberg).

https://doi.org/10.1016/j.bbi.2020.10.017
Received 12 June 2020; Received in revised form 24 September 2020; Accepted 19 October 2020
Available online 22 October 2020
0889-1591/© 2020 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
1. Background

Neuropathologically, PD is characterized by a progressive intraneuronal accumulation of α-synuclein (αsyn) in Lewy bodies and Lewy neurites, degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and neuroinflammation (Spillantini et al., 1998; Braak et al., 2003; McGeer et al., 1988).

The neuroinflammation observed in PD brains includes activation of microglia, up-regulation of major histocompatibility class II (MHCII) molecules, expression of inflammatory mediators and infiltration of T-lymphocytes (McGeer et al., 1988; Brochard et al., 2009; Imamura et al., 2003). These conditions allow activated microglia expressing MHCII to present antigens to CD4-positive (CD4+) T-lymphocytes, and the presence of circulating αsyn-reactive CD4+ T-lymphocytes was recently reported in PD patients (Sulzer et al., 2017). Interestingly, several studies have reported genetic risk variants for PD in the human leukocyte antigen (HLA) region encoding MHCII molecules (Wisemann et al., 2013; Lampe et al., 2003; Kannarkat et al., 2015; Hill-Burns et al., 2011; SDLta et al., 2010). These findings strongly support a key role for MHCII and antigen presentation capacity in PD aetiology.

From prodromal to early and late stages of PD, αsyn pathology has been described to start in the vagal nerve and brainstem and continue to the midbrain and cortical areas in a pattern that correlates with the progressive symptomatology (Braak et al., 2003). A prion-like seeding where misfolded αsyn acts as a template for naïve forms is suggested as a mechanism behind the spread of αsyn pathology to neighboring cells and brain regions, resulting in neuronal dysfunction and neurodegeneration (Brundin and Melki, 2017). This hypothesis is supported by the observed spread of αsyn from host cells to transplants in patients grafted with primary fetal ventral mesencephalic tissue (Li et al., 2008; Kordower et al., 2011, 2008) as well as in primary grafts in mice (Hansen et al., 2011). The prion-like hypothesis has been questioned (Surmeier et al., 2017) but has also led to the development of experimental models for progressive αsyn pathology and PD-like neurodegeneration. In vitro, administration of aggregated αsyn in the form of pre-formed fibrils (PFFs) to cells overexpressing αsyn has been shown to induce seeding and the formation of Lewy body-like insoluble inclusions (Luk et al., 2009). In vivo, models using aggregated forms of αsyn as seeds have been shown to induce a progressive PD-like pathology in rodents (Luk et al., 2012; Rey et al., 2016). Of note, PFF-induced microglial activation and MHCII expression have been shown to precede dopaminergic neurodegeneration and to significantly correlate with the load of phosphorylated-ser129 αsyn (pS129) inclusions in SNpc (Duffy et al., 2018). Recently, a rat model was developed combining viral vector-mediated expression of human αsyn, to act as substrate, with local administration of human PFFs, to act as seeds (Thakur et al., 2017). This model recapitulates many of the pathophysiological hallmarks observed in PD, including pS129 inclusions, microgliosis, dopaminergic cell death in SNpc and motor impairment. Compared to αsyn-based rat PD models using viral vectors alone (Decressac et al., 2012), models combining viral vectors with PFFs have the advantages of a more physiological level of transgene expression as well as a lower immune response to the viral vector. Compared to striatal administration of PFFs alone, the combined models allow for studies on the mechanisms behind seeded pathology on locally produced human αsyn. In addition, the combination enhances and accelerated formation of αsyn inclusions as well as degeneration of dopaminergic neurons and behavioural deficits (Paunier et al., 2015).

The activation pattern of microglia in rat PD models has been shown to be dependent on the degree of αsyn pathology and neurodegeneration (Sanchez-Guajardo et al., 2010) and we have previously shown that differential MHCII expression regulates αsyn-induced microglial activation and dopaminergic neurodegeneration (Jimenez-Ferrer et al., 2017). The extent of microglial activation, MHCII expression and αsyn pathology are thus mutually dependent. The MHCII transactivator (Mhc2ta) is the major regulator of MHCII expression on antigen presenting cells, including microglia and macrophages in the CNS. Variants in the rat Mhc2ta promoter region regulate MHCII expression upon nerve injury and the susceptibility to experimental autoimmune encephalomyelitis (Harnesk et al., 2008; Lidman et al., 2003). Interestingly, corresponding variants in the human MHC2TA gene are associated to the risk for multiple sclerosis, rheumatoid arthritis and myocardial infarction (Swanberg et al., 2005). With respect to PD, we have recently shown that Mhc2ta allelic variants significantly modify MHCII gene expression, microglial activation, dopaminergic neurodegeneration and motor deficits in an αsyn over-expression model in rats (Jimenez-Ferrer et al., 2017). However, if Mhc2ta and antigen presentation capacity influence the extent and propagation of αsyn pathology is not known. To fill this gap, we have used two congenic rat strains (DA and DA;VRA4) with allelic variants in the Mhc2ta gene leading to differential gene expression of Mhc2ta and its targets, mainly MHCII genes to determine whether Mhc2ta modulates αsyn aggregation, propagation and toxicity to dopaminergic neurons.

Our results from a combined model, with a low-titre recombinant adenoviral vector (rAAV) encoding human αsyn and administration of PFFs, show that lower Mhc2ta levels and reduced antigen presentation capacity is associated with Lewy-like αsyn pathology, enhanced αsyn anatomical spread, increased dopaminergic neurodegeneration, exacerbated motor impairment and a proinflammatory peripheral cytokine profile. These significant effects of Mhc2ta on PD-like pathology, together with previous findings that orthologous variants in the MHC2TA gene are associated to human inflammatory diseases, strongly support a role for antigen presentation in PD aetiology and future targeting of inflammatory responses as a promising strategy to prevent αsyn pathology and disease progression.

2. Materials and methods

2.1. Experimental design

In the current study we compared adult males of the DA and DA;VRA4 rat strains. These congenic strains are genetically identical with the exception of the VRA4 locus on chromosome 10 encoding the Mhc2ta gene, resulting in functional but lower MHCII expression levels in DA;VRA4 compared to DA rats (Fig. 1A). Our experimental design deviated from that of Thakur and colleagues (Thakur et al., 2017) in the site of PFF injection (striatum instead of SN) to reduce surgery-induced local inflammatory responses in SN and to study the interaction of substrate (nigrostrial human αsyn transgene expression) and seed (striatal PFF). A schematic of the experimental approach and timeline are given in Fig. 1C. In brief, rats were injected first with rAAV6 vectors carrying human αsyn (ASYN) or green fluorescent protein (GFP). Two weeks post-rAAV6 injection, GFP or bovine serum albumin (BSA) was injected into striatal terminal regions of the midbrain dopamine fibres. Midbrain injection of rAAV6-GFP was used to investigate whether virus-driven produced human αsyn is required for PFF-induced Lewy body-like inclusions (comparing GFP + PFF and ASYN + PFF groups) and striatal injection of BSA was used to investigate the effect of PFF as a seed for virus-driven produced human αsyn (comparing ASYN + BSA and ASYN + PFF groups). This approach resulted in the following groups (n = 10 each): DA ASYN + PFF, DA;VRA4 ASYN + PFF, DA GFP + PFF, DA;VRA4 GFP + PFF, DA ASYN + BSA, DA;VRA4 ASYN + BSA (Fig. 1C). Behavioural phenotypes were assessed at 2, 5, and 8 weeks post rAAV delivery (3- and 6-weeks post PFF/BSA injection). At 8 weeks after rAAV injection, serum and brains were collected.

2.2. Animals

All procedures were conducted in accordance with guidelines set by the Ethics Committee for the use of laboratory animals in the region Lund-Malmö Sweden (M188-14). All animals were housed 2–4 per cage and given ad libitum access to food and water during a 12:12 h light/dark
cycle. Sixty adult male rats were included (30 DA and 30 DA.VRA4). The DA.VRA4 congenic rats were generated as previously described (Harnesk et al., 2008) and genotyped accordingly (Jimenez-Ferrer et al., 2017) (founders kindly provided by Prof. Fredrik Piehl, Karolinska Institute, Stockholm, Sweden).

2.3. Viral vectors

Two titre-matched rAAV6 vectors were used to overexpress either human wild-type α-syn or GFP. Both rAAV6 vectors were injected at 1.02E+10 gc/μl in the midbrain of the experimental animals. The transgenes are under transcriptional control of the synpasin-1 promoter and enhanced using a woodchuck hepatitis virus posttranscriptional regulatory element (Decressac et al., 2012).

2.4. Preformed fibrils and BSA

Preformed fibrils were kindly provided by Prof. Virginia Lee and Dr. Kelvin C. Luk, University of Philadelphia, USA. Purification of recombinant full-length human α-syn was prepared as described previously (Luk et al., 2009) and injected 2 weeks after viral vector delivery (10 μg total, 2 μl per site). Before injections, the PFFs were thawed and sonicated at room temperature by probe sonication as has been reported before (Thakur et al., 2017; Volpicelli-Daley et al., 2011) in a sonicator (Qsonica sonicators). Transmission electron microscopy (TEM) pictures of PFFs after sonication are presented in Fig. 1B. BSA: Lyophilized BSA was purchased form Sigma (A9418), dissolved in DPBS at 5 mg/ml was used as a control for the PFF injection.

2.5. Surgical procedure

All surgical procedures were performed under 1–2% isoflurane anaesthesia (Isoflo vet, Orion Pharma AB Animal Health, Sweden) in a 2:1 O₂:NO mixture. Marcain (Apoteksbolaget, Stockholm, Sweden) was used as local anaesthetic. In brief, rats were placed in a stereotaxic frame (Kopf Instruments) and the vector solution was injected using a 10 μl Hamilton syringe fitted with a glass capillary (outer diameter of 250 μm). Intranigral injections (3 μl) were performed at the following coordinates given in mm relative to Bregma and dural surface (Paxinos et al., 1980) with the incisor bar set at −2.3 mm below the interaural line: anteroposterior (AP): −5.3; mediolateral (ML): −1.7; dorsoventral (DV): −7.2. Intrastriatal injections (2.5 μl per site) were made at the following coordinates; site 1: AP: +0.1; ML: −3.0; DV: −5.0 and site 2: AP: −0.4; ML: −3.0; DV: −4.5. All injections were performed at a constant flow rate of 0.2 μl per minute. The capillary was left in place for 2 min after injection to allow for diffusion before carefully retracting the...
for 3 trials per day. Steps for each forelimb were recorded and averaged.

2.6. Motor impairment assessment

To evaluate motor activity, forelimb akinesia (Olsson et al., 1995) was determined in the animals using the stepping test at 2, 5 and 8 weeks after vector injection. The same experimenter, blinded to the group-identity, performed each test in the same testing room, at the same interval during the day. The cages were tested in random order during the experiment days. At the initiation of the experiment, the animals were habituated and previously trained in the test for 3 days. Briefly, the experimenter moved the rat horizontally over a fixed distance (90 cm) for 3 trials per day. Steps for each forelimb were recorded and averaged across trials. Data are presented as the average number of adjusting steps made by the forepaw contralateral to the injection side. Before the first time point, the animals were habituated and pre-trained in the test for 3 days.

2.7. Serum and tissue preparation

Eight weeks after intrajugular injection peripheral blood was drawn by cardiac puncture in animals under 0.2 ml sodium pentobarbital intraperitoneal anaesthesia (200 mg/kg). After collection, blood was left undisturbed at room temperature for 30 min to allow the blood to coagulate. Serum was isolated by centrifugation (2,000 × g, 10 min, 4 °C), transfer into a new Eppendorf tube and stored at −80 °C until analysis. Immediately after cardiac puncture, animals were transcardially perfused with 50 ml 0.9% saline, followed by 250 ml ice-cold paraformaldehyde (4% pH 7.4). The brains were collected and post-fixed in 4% PFA overnight and then cryoprotected in 30% sucrose (in PBS, with 0.01% sodium azide).

2.8. Bright-field Immunohistochemistry, immunofluorescence, confocal microscopy

The brains were sectioned coronally on a freezing microtome (Microm HM 450, Thermo Scientific, Waltham, MA, USA) at 35 μm and stored them in antifreeze solution at 4 °C until immunostaining. Bright-field Immunohistochemical staining were performed on free-floating sections at room temperature using primary antibodies and secondary biotinylated antibodies listed in Table 1. A full description of the staining procedure can be found in (Jimenez-Ferrer et al., 2017). A standard peroxidase-based method was used (Vectastain ABC kit and DAB, Vector Laboratories). Initially, sections were quenched with 3% H2O2/10% MetOH for 30 min, blocked with 5% serum, accordingly with the secondary antibody before overnight incubation with primary antibody at RT. On the second day, sections were incubated with the corresponding biotinylated secondary antibody for 1 h at RT. Followed by a 30-min incubation with an avidin-biotin peroxidase solution (ABC Elite, Vector Laboratories). For the visualisation of the secondary antibody sections were developed using 3'-diaminobenzidine (DAB) and Peroxide (3%). After mounted on gelatine-coated slides, the sections were air-dried overnight, dehydrated in a series of ascending concentration of ethanol and xylene and cover slipped with DPX mounting medium (Sigma-Aldrich, Gillingham). Proteinase K digestion was performed on mounted sections. Briefly, sections were incubated for 1 h with Proteinase K (5 μg/ml, Thermo Scientific), diluted in 0.05 M TRIS-buffered saline at pH 7.6. Following Proteinase K digestion, the same procedure described above for free-floating sections was followed.

For fluorescent microscopy, stainings were performed on free-floating sections and were blocked with 5% Normal goat serum before overnight incubation with primary antibody (Table 1) at 4 °C. On the second day, sections were incubated with the corresponding fluorescent secondary antibody for 1 h at RT. Stained sections were mounted on gelatine-coated slides and secured with coverslips using PVA-DABCO (Sigma). Confocal images were acquired on a Leica SP8 confocal microscope under a 20× objective using the same settings for all sections.

2.9. Densitometry analysis of Dopaminergic Fibre Loss in the Striatum

Striatal TH+ fibre optical density was determined using the ImageJ software (Version 1.49, NIH, USA) at four coronal levels (1.60, 0.48, −0.26 and −0.40 mm relative to Bregma). The photomicrographs were obtained using a Light microscope (Olympus BX53, Tokyo, Japan) at 2× magnification. Each image was analysed using the optical density (O.D.) values obtained from the Rodbard calibration curve after being transformed into grey-scale image. The TH+ fibres density was determined by the mean grey and was normalized by measuring and subtracting background staining optical density from the corpus callosum for each animal. The data are presented as percentage of contralateral side density. From a total of 60 animals, an average of 3 animals were excluded from the analysis due to complications during surgery or with tissue processing, leaving 20 DA and 19 DA.VRA4 for analysis.

2.10. Axonal swelling quantification

Four sections from each animal at Bregma distance 1.60, 0.48, −0.26 and −0.40 were analysed. High-resolution z-stack 25× magnification images were taken using a Light microscope (Olympus BX53, Tokyo, Japan). Two pictures were taken of each section representing the dorsal and ventral part of the caudate putamen of striatum for every section. ImageJ software (Version 1.49, NIH, USA) was used to identify the TH+ swellings and calculate the total number of swellings, and their size. Each image was analysed after being transformed into grey-scale image. The number of TH+ swellings was determined by particle analysis function. The average number of TH+ swellings were calculated based on all the pictures from one animal. A genotype-blind operator performed image acquisition and quantification.

2.11. Alpha-synuclein aggregation / deposition / scoring of pathology

For histological mapping analysis immunoreactive p-syn+ inclusions/cells and neurites were mapped as previously described (Luk et al., 2012) in rostral to caudal levels. The presence of p-syn+-positive (p-syn+) accumulations was assessed in a blinded manner by two independent researchers screening every section at 20× in a Light microscope (Olympus BX53, Tokyo, Japan). For histological scoring of p-syn+-positive (p-syn+) pathology an adapted version of a previously described scoring scale (Rey et al., 2016) based on the presence of immunoreactivity for p-syn+ accumulations patterns was used: 0: no aggregates; 1: sparse (very few neurites, max one nuclear deposit, scattered dot-like inclusions); 2: mild (varicos and filiform neurites, with or without presence of nuclear deposits); 3: moderate (many

| Antigen/Secondary antibody | Company        | Host   | Dilution |
|----------------------------|----------------|--------|----------|
| αsyn (211)                 | Santa Cruz     | Mouse  | 1:1000   |
| GFIP                       | Abcam (ab13970) | Chicken| 1:1000   |
| p-syn (pS129)              | Abcam (EP1367) | Rabbit | 1:2000   |
| TH                         | Millipore (AB152) | Rabbit | 1:1000   |
| Iba1                       | Abcam (ab139590) | Chicken| 1:1000   |
| MHCII                      | Abcam (ab23990) | Mouse  | 1:500    |
| Biotinylated anti-rabbit   | Vector laboratories | Horse | 1:200    |
| Biotinylated anti-mouse    | Vector laboratories | Goat  | 1:200    |
| Alexa 647 anti-mouse       | Abcam (ab150119) | Goat  | 1:200    |
| Alexa 488 anti-rabbit      | Abcam (ab150081) | Goat  | 1:200    |
| Alexa 594 anti-chicken     | Abcam (ab150176) | Goat  | 1:200    |
nuclear and perinuclear, neurites, large areas with dot-like inclusions); 4: many nuclear, perinuclear, filiform neurites and Lewy neurite-like; 5: many perinuclear deposits, Lewy neurite-like and dot-like inclusions; 6: many Lewy bodies-like and Lewy neurites-like inclusions. Representative examples of the different aggregates are illustrated in Fig. 2. The analysis was carried out under 100× magnification using a Leica microscope connected to a digital camera (Leica MPS52) on the ipsilateral side of the brain by a genotype-blind operator using 12 sections/animal through the brain (AP + 4.68 to AP – 10.32) for each section at the levels mentioned above (Fig. 2D). Data is shown as average grades values at each level from each animal.

2.12. Stereological counts of dopaminergic cells in SNpc

Dopaminergic neuron loss in the SNpc was determined by unbiased stereological estimations of the TH+ cells in SNpc according to the optical fractionator principle as describe elsewhere (Kuroksa et al., 2016). An observer blinded to the genotype of the animal performed the counts every sixth section (section sampling fraction,ssf = 6) covering the full extent of the SNpc (4.04 to –7.56 from Bregma) yielding 8–10 sections per animal. Counts were performed employing MicroBrightfield stereological investigator software (Stereo Investigator, MBF Bioscience, V10, US) under 100× magnification using a Leica microscope connected to a digital camera (Leica MPS52). Tracing regions of interest (ROIs) was done using a SX/0.11 lens, and counting was performed with a 100X/1.30 lens. A series of counting frames (80 μm × 80 μm) was systematically and randomly distributed over grid (180 μm × 180 μm) placed over SNpc. The total population estimate was calculated using optical fractionator estimates. A maximal Gunderson coefficient error (CE) of 0.08 was accepted for cells to be counted. Sections with uneven, blurry, not penetrating staining were excluded from the analysis leaving 18 DA and 18 DA.VRA4 for quantification.

2.13. Semi-quantitative analysis of MHCII+ cells

To quantify MHCII+ cells, 4 equally z-stack images were acquired using a bright-field microscope (Olympus BX53, Tokyo, Japan). The regions of interest were digitalized at a 20x objective, an image matrix of 1024 × 1024 pixel and a pixel scaling of 0.2 × 0.2 μm. The number of MHCII+ cells in striatum or SN was obtained from each photomicrograph using ImageJ software (Version 1.49, NIH, USA) point tool. Photomicrographs were transformed into 8-bit images, with removal of the background and increment of the black and white contrast. The number of positive cells was registered. Immunopositive cells close to the site of injection or needle tract were excluded in order to analyse the response to αsyn rather than tissue damage. Data is shown as average values from 4 striatal and 4 nigral sections for each animal.

2.14. Cytokine analysis

Serum concentrations (pg/ml) of IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 and TNFα were determined using MSD multiplex assay platform (Meso Scale Diagnostics, Rockville, MD, USA). The assay was performed according to manufacturer’s protocol. Briefly, the plate was loaded with 50 μl per well of controls and unknown samples (diluted 1:2), incubated for 2 h at room temperature with shaking at 800 rpm, washed 3 times with PBS 1× , Tween 20 0.1%, 25 μl of detection antibody was added per well for 2 h with shaking at 800 rpm at room temperature, washed 3 times with PBS 1× , Tween 20 0.1%. The plate was developed using the 4-s reading buffer diluted 1 time with D2O and analysed using the QuickPlex Q120 reader from Mesoscale. The detection ranges of the different cytokines measured were as follows: IL1β (1670–0.408 pg/ml), IL4 (1660–0.405 pg/ml), IL12 (32200–7.86 pg/ml), IL10 (3410–0.833 pg/ml), IFNγ (938–0.229 pg/ml), IL2 (2630–0.642 pg/ml), IL5 (967–0.236 pg/ml), IL6 (5720–1.40), KC/GRO (homologue to human IL8, 1980–0.483 pg/ml) and TNFα (627–0.153 pg/ml).

2.15. Electron microscopy

The nature of fibrillar αsyn forms was assessed using transmission electron microscopy (TEM) analysis. The fibril sample (3 μl) was spotted onto a freshly glow-discharged carbon-coated electron microscopy grid. After 1 min, 6 μl uranyl acetate (2% in aqueous solution) was applied to the grid for 2 min. The excessive stain was removed by a filter paper. The samples were imaged using a FEI T12 Tecnai Spirit BioTWIN electron microscope.

2.16. Statistical analysis

Statistical tests were conducted using the GraphPad software (package INC. Version 8.0 La Jolla, USA). Data are expressed as mean ± standard error of the mean (SEM) and groups were compared using analyses of variance (ANOVA) and post hoc Tukeys test correction for multiple comparisons. P-values and F-ratios are given in the figure legends. Post-hoc pairwise comparisons are reported in both results and figure legends as being either significant or non-significant. A significance level of α ≤ 0.05 was chosen for all analyses.

3. Results

To investigate the impact of antigen presentation on the extent and consequences of αsyn pathology, we compared two congenic rat strains with naturally occurring variants in the Mhc2ta gene. These variants make the DA.VRA4 strain express lower transcripts levels of Mhc2ta and Mhcll (Jimenez-Ferrer et al., 2017) as well as lower MHCII protein levels compared to DA (Fig. 1A). To study aggregation, propagation and toxicity of αsyn, an in vivo seeding model combining rAAV6-αsyn and human αsyn PFFs was used. The PFFs were sonicated (Fig. 1B) and injected in the striatum two weeks after rAAV6-αsyn or rAAV6-GFP delivery to the SNpc (Fig. 1C). The rAAV6 vectors mediated strong αsyn or GFP transgene expression, with no differences observed between the treatment groups or between the two strains (Fig. 1D, Supplementary Fig. 1).

3.1. PFFs induce widespread propagation of αsyn pathology in rats expressing human αsyn

To assess the propagation of αsyn pathology we analysed the immunoreactivity to αsyn, the predominant posttranslational modification occurring in synucleinopathies (Fujiwara et al., 2002), throughout the brain at 8 weeks after rAAV6-αsyn delivery to the SN (6 weeks after PFF injection to the striatum). PFFs induced widespread αsyn immunoreactivity throughout the rostro-caudal axis, including motor cortex, insular cortices, thalamus, amygdala and cerebellum in rats expressing the human αsyn transgene (Fig. 2A). Despite the fact that PFFs were injected unilaterally, αsyn positive intraneuronal and neuritic accumulations were present bilaterally in the cortex but remained ipsilateral to the injection site in the SN (Fig. 2A).

We subsequently compared the effects of PFF alone (seeding effect) and the effect of αsyn-overexpression alone (substrate effect) on the induction and spreading of αsyn-pathology. In rats overexpressing GFP, thus lacking endogenously produced human αsyn, PFFs induced αsyn immunoreactivity in close proximity to the striatal injection site and in SN (Supplementary Fig. 2.1 A). In rats overexpressing human αsyn and receiving striatal BSA injections, strong αsyn immunoreactivity was observed in the SN but only minor staining in other brain regions (Supplementary Fig. 2.2 C). Thus, the presence of human αsyn substrate, in the form of transgene expression in SN dopaminergic neurons, enhances the propagation of αsyn pathology seeded by striatal administration of PFFs.
Fig. 2. Striatal injection of PFFs combined with nigral αsyn overexpression induces widespread αsyn pathology (pαsyn) in the brain. A. Map showing the distribution of pαsyn immunoreactivity (aggregates and neurites as red dots and lines, respectively) in coronal sections from animals sacrificed at 8 weeks post PFF injection. Representative plots are shown for DA and DA.VRA4 animals injected with rAAV-αsyn in substantia nigra and PFFs in striatum. B. Photomicrographs of positive pαsyn immunoreactivity of various CNS regions, 3 different striatal (B1-B6) and nigral levels (B7-B12), cerebellum (B13, B16), motor cortex (B14, B17), insular cortex (B15, B18), olfactory tubercle (B19, B22), amygdala (B20, B23) and thalamus (B21, B24) regions are shown. Scale bar = 25 μm. C. Scoring scheme illustrating the anatomical distribution of pαsyn pathology and aggregation pattern after nigral injection of rAAV-αsyn or rAAV-GFP followed by striatal injection of PFFs or BSA. Scoring was performed on 20 DA and 19 DA.VRA4 rats (n = 6–7 per group). Scale bar = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3.2. Lower MHCII expression in DA.VRA4 rats enhances αsyn propagation and inclusion formation

Formation of αsyn inclusions was detected in all experimental groups and were most abundant close to the sites of injection (Fig. 2A, B; Supplementary Figs. 2.1 and 2.2). In rats expressing human αsyn and seeded with PFFs (ASYN + PFF), the DA.VRA4 strain displayed more αsyn-positive inclusions and a larger spread of the pathology compared to DA (Fig. 2A, B). In addition to the localization and amount of aggregates, the deposition pattern of αsyn differed between the two strains. While DA rats displayed filiform neurites but no round inclusions, DA.VRA4 rats displayed Lewy-like aggregates and neurites located mainly in the striatum (Fig. 2B1 to B6) and midbrain (Fig. 2B7 to B12), but also in brain regions distal to injection sites (Fig. 2B13-B24).

DA and DA.VRA4 rats receiving GFP + PFF or ASYN + BSA, displayed fewer αsyn-immunoreactive cells but an anatomical distribution similar to rats receiving ASYN + PFF. More cells with inclusions were observed in the midbrain (Supplementary Fig. 2B7-B12 and D7-D12) compared to other brain regions (Supplementary Fig. 2B1-B24 and D1-D24).

The αsyn inclusions were insoluble as determined by Proteinase K digestion (Supplementary Fig. 3). To further characterize the type of αsyn inclusions they were characterized as dot-like, nuclear, perinuclear, filiform neurite, varicose neurite, Lewy neurite-like, and Lewy body-like structures and scored according to a modified rating scale (Rey et al., 2016) along the rostro-caudal axis (Fig. 2C). DA.VRA4 rats displayed enhanced inclusion formation compared to DA, both when receiving ASYN + PFF and ASYN + BSA (Fig. 2C), indicating that reduced antigen presenting capacity lead to enhanced seeding and aggregation of αsyn.

As expected, rats in the GFP + PFF or ASYN + BSA groups displayed smaller deposits, less broken somas and thinner neurites compared to the ASYN + PFF groups, and lower levels of αsyn pathology was observed distal to the injection sites in the control groups (Fig. 2C). These findings suggest that the model employed is suitable to study both biological effects and extent of αsyn pathology in vivo.

3.3. Seeding of human αsyn enhances dopaminergic neurodegeneration in rats with reduced Mhc2ta expression levels

Neurodegeneration in the form of reduced level of TH+ fibres in the striatum ipsilateral to injections related to the contralateral side was observed in all treatment groups. While similar levels of dopaminergic fibres remained in DA and DA.VRA4 rats treated with GFP + PFF (DA 71.9 ± 1.10%, DA.VRA4 79.6 ± 0.77%) or ASYN + BSA (DA 77.3 ± 8.13%, DA.VRA4 73.8 ± 6.25%), the combination of αsyn substrate and seed (ASYN + PFF) enhanced the loss of TH+ fibres (DA 58.7 ± 2.74%, DA.VRA4 49.3 ± 2.86%) (Fig. 3A, B, Supplementary Fig. 4A, B). The neurodegenerative effect of seeded αsyn was significantly enhanced compared to the control groups in DA.VRA4, but not DA rats (Fig. 3A, B), indicating that lower antigen presentation capacity mediated by Mhc2ta in the DA.VRA4 strain increases the susceptibility to αsyn-seeded degeneration of dopaminergic striatal projection fibres.

In line with the data on denervation, the DA.VRA4 strain was also more susceptible to dopaminergic neurodegeneration determined by stereological cell counts, with a significant cell loss in the ipsilateral vs. contralateral SN under all three experimental conditions (ASYN + BSA p < 0.05; GFP + PFFs p < 0.01; ASYN + PFF p < 0.01) (Fig. 3D). In contrast, only the combination of rAAV6-αsyn and PFFs resulted in significant cell loss in DA rats (ASYN + PFF p < 0.01, Fig. 3D). Thus, while dopaminergic nigral cell loss can be induced by viral overexpression of αsyn or by exposure to PFFs alone in DA.VRA4 rats, a combination resulting in enhanced αsyn pathology (see above) is required for DA rats.

Loss of nigrostriatal dopaminergic fibres and cell somas is considered to be preceded by axonal dysfunction. Therefore, TH+ axonal swellings were quantified in the striatum and related to the remaining dopaminergic cell somas to account for prior loss of cells and their axons (Fig. 3E). The load of axonal swellings was dependent on αsyn transgene expression, and were observed in rats receiving rAAV-αsyn in combination with either BSA or PFFs. Rats exposed to PFFs alone displayed little axonal swellings (GFP + PFF vs ASYN + PFF, p < 0.05) in both strains (Fig. 3E). The similar load of axonal swellings at 8 weeks after vector delivery despite increased neurodegeneration in DA.VRA4 compared to DA rats might reflect a Mhc2ta-mediated resilience of dopaminergic cells to withstand axonal dysfunction.

Taken together, three hallmarks of dopaminergic neurodegeneration; loss of striatal fibres, nigral cell loss and axonal swellings; showed an enhanced neurodegeneration in the combined model compared to rAAV6-αsyn or PFFs alone, which is in line with the observed seeding and propagation of αsyn described above. Of note, lower Mhc2ta expression in the DA.VRA4 strain was associated to an increased susceptibility to both striatal denervation and nigral cell loss.

3.4. Mhc2ta alleles are associated with differential severity of αsyn-induced motor deficits

To determine motor impairment, the use of ipsilateral and contralateral forelimb was assessed by the stepping test in animals at 2, 5 and 8 weeks after viral vector injections. DA.VRA4 rats displayed a progressive contralateral forelimb akinesia compared to sham controls (ASYN + PFF p < 0.01; GFP + PFF p < 0.05; ASYN + BSA p = 0.05), while no significant motor impairment was observed in DA rats (Fig. 3F, G).

Thus, dopaminergic neurodegeneration in terms of cell loss in the SN, reduced density of striatal fibres and presence of axonal swellings was reflected in significant motor impairment in DA.VRA4 rats with lower Mhc2ta expression compared to DA. In contrast, a combination of αsyn and PFFs was required to induce dopaminergic cell loss in DA, and despite the presence of axonal swellings, the density of dopaminergic striatal fibres and motor function was preserved. This suggests that Mhc2ta regulates the threshold for axonal dysfunction and motor impairment in response to αsyn aggregation and propagation.

3.5. Presence of MHCII+ cells in brain regions displaying αsyn-seeded pathology

In order to characterize the local neuroinflammatory response associated to αsyn inclusions, the anatomical distribution of MHCII+ cells in regions displaying αsyn immunoreactivity was determined. In both DA and DA.VRA4 rats with PFF-seeded αsyn pathology (ASYN + PFF), MHCII+ cells were abundant across the examined regions (Fig. 4A). The MHCII+ immunoreactivity matched the spatial distribution of αsyn pathology (comparing Figs. 2B and 4A), confirming previous reports (Duffy et al., 2018). The anatomical areas mostly affected in both strains were the SN and the medial striatum (Fig. 4A1 to A12). Although less compared to the sites of injection, MHCII+ cells were detected throughout the brain, including the cerebellum, motor cortex, insular cortex, amygdala, thalamus and olfactory tubercle (Fig. 4A13-A24). Based on morphology (Sanchez-Guajardo et al., 2010; Jimenez-Ferrer et al., 2017), the MHCII+ cells displayed a predominant highly activated phenotype (large, dark cell bodies and thick, short processes) in the ASYN + PFF groups but a surveilling-like phenotype (long and thin processes, with or without many branches) in the GFP + PFF and ASYN + BSA groups (Supplementary Fig. 5A, B). In rats receiving GFP + PFF or ASYN + BSA, MHCII+ cells were confined to the injection sites and immediate surrounding areas (Supplementary Fig. 5). Compared to αsyn or PFFs alone, the combined model thus induced a stronger microglial response with regard to abundance of MHCII+ cells and microglial morphology that colocalized with αsyn inclusions.
Brain Behavior and Immunity 91 (2021) 369–382

Fig. 3. PFF-seeded αsyn pathology induces fibre denervation and loss of cells in the dopaminergic system. A. Representative photomicrographs showing Tyrosine hydroxylase (TH) immunohistochemical staining throughout the brain of DA and DA.VRA4 rats after unilateral rAAV-αsyn and PFF injections (ASYN + PFF). B and C. DA.VRA4, but not DA rats, with nigral expression of αsyn and striatal injection of PFFs (ASYN + PFF) displayed significant TH + fibre loss in the striatum compared to (B) rats overexpressing GFP (GFP + PFF); Two-way ANOVA; Group: F(3,22) = 3.37, p = 0.04, Levels F(2,63,51.98) = 0.92, p = 0.42, Interaction F(9,66) = 0.37, p = 0.95; Two-way ANOVA; Group: F(3,22) = 3.87, p = 0.022, Levels F(2,63,49.52) = 5.12, p = 0.009, Interaction F(9,72) = 0.65, p = 0.75. Data are presented as % of optical density in the contralateral side and expressed as mean ± SEM. D. DA.VRA4 rats display enhanced susceptibility to nigral TH + cell loss compared to DA rats. Stereological cell counts performed at 8 weeks post PFF/BSA injections. Two-way ANOVA; Group: F(5,30) = 1.95, p = 0.02, Levels F(2,63,49.52) = 5.12, p = 0.009, Interaction F(9,72) = 0.65p = 0.75. E. Nigral overexpression of αsyn significantly enhances the load of axonal swellings in the striatum in both DA and DA.VRA4 rats receiving striatal injections of PFFs. One-way ANOVA; Group: F(5,29) = 4.48, p = 0.004. F and G. DA.VRA4, but not DA, rats display forelimb akinesia in the stepping test when subjected to the combined model (ASYN + PFF) as well as (F) PFFs alone (GFP + PFF) or (G) only αsyn overexpression (ASYN + BSA). Two-way ANOVA; Group: F(5,28) = 3.21, p = 0.02, Time F(1,241,54.35) = 3.11, p = 0.05, Interaction F(5,150) = 1.24p = 0.29. Results from post-hoc groups comparison (Tukey’s test) are reported as *p < 0.05, **p < 0.01.
3.6. Lower Mhc2ta levels is associated with increased number of MHCII+ cells and dopaminergic neurodegeneration

MHCII immunoreactive cells have been associated with accumulation of α-syn inclusions and to the degree of neurodegeneration (Duffy et al., 2018). Previously we have shown that DA.VRA4 rats with reduced antigen presentation capacity present an increased number of striatal MHCII+ cells in response to high levels of α-syn overexpression and striatal dopaminergic fibre loss (Jimenez-Ferrer et al., 2017). To determine the effect of reduced antigen presentation capacity in the number of MHCII+ cells in the presence of α-syn substrate and seed, we performed semi-quantitative analysis of MHCII+ cells in the striatum and SN. At 8 weeks post unilateral rAAV injection, MHCII+ cells were observed in the ipsilateral striatum of all groups (ASYN+ PFF, GFP + PFF and ASYN + BSA). The abundance of MHCII+ cells in striatum and SN was similar between rats receiving α-syn only as substrate (ASYN + BSA) or as seed (GFP + PFF). In the ASYN + PFF groups, more MHCII+ cells were observed in the ipsilateral striatum, and were also present in the contralateral striatum, with significantly higher numbers in DA.VRA4 rats receiving ASYN+PFF compared to DA rats (8.713 ± 1.520 vs 92.11 ± 15.20, p < 0.005) (Fig. 4B). These results show that seeded α-syn pathology enhances the inflammatory response with regard to MHCII+ cells in the striatum and SN and is associated to an increased number of MHCII+ cells in rats with lower Mhc2ta transcriptional activity. The higher number of MHCII+ cells in DA.VRA4 rats is paralleled by increased α-syn pathology and neurodegeneration compared to DA rats, which might reflect a compensatory mechanism where low Mhc2ta expression result in increased activation and recruitment of antigen-presenting cells contributing to neurodegeneration.

3.7. MHCII+ cells are associated with α-syn pathology

We have recently demonstrated that DA.VRA4 microglia express lower levels of MHCII relative to Iba1 due to lower Mhc2ta levels (Jimenez-Ferrer et al., 2017). To determine whether the levels of MHCII affect the interaction between Iba1+ cells and α-syn, we analysed MHCII immunoreactivity in the striatum and SN of DA and DA.VRA4 rats receiving ASYN + PFF (Fig. 5). We found that in both strains, MHCII+ cells were located in close proximity to α-syn immunoreactive cells and inclusions in the striatum as well as SN. In several instances MHCII immunoreactivity co-localized with α-syn (Fig. 5), indicative of MHCII+ cells being active in the local response to α-syn. Notably, MHCII+ cells co-localizing with α-syn had a lower Iba1 immunoreactivity, a hypotrophic cell body and an amoeboid morphology, suggesting that MHCII+ cells could be actively involved in the clearing and transfer of α-syn.

3.8. Lower expression of Mhc2ta is associated with a higher pro-inflammatory peripheral cytokine profile in response to α-syn

To establish the Mhc2ta-mediated effects on the peripheral immune response to PFF-seeded α-syn pathology, an inflammatory profile was determined using a cytokine- and chemokine panel (IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, TNFα) in serum at 8 weeks after vector injections. In the ASYN + PFF group, DA.VRA4 rats had higher levels of pro-inflammatory cytokines compared to DA (Fig. 4C). These included TNFα, a cytokine involved in systemic inflammation and implicated in multiple signalling pathways promoting exacerbation of the inflammatory response (0.65 ± 0.055 vs. 0.45 ± 0.044, p < 0.05) and IL-2, a cytokine involved in inducing tolerance and immunity primarily via direct effects on T-cells (0.81 ± 0.030 vs. 0.67 ± 0.009, p < 0.05) (Fig. 4C). After ASYN + BSA, IL-8, a proinflammatory mediator increased after oxidative stress, was significantly higher in DA.VRA4 compared to DA rats (0.77 ± 0.041 vs. 0.65 ± 0.020, p < 0.05) (Fig. 4C). These results indicate that lower expression of Mhc2ta and MHCII in the DA.VRA4 strain induces a more pro-inflammatory peripheral cytokine profile compared to DA in response to intracranial α-syn-seeded pathology.

4. Discussion

In this study, we show that allelic variants in the major regulator of MHCII expression, the Mhc2ta gene, are associated with different susceptibility to α-syn-seeded pathology in a rat PD model. The effects are seen on a range of pathological processes, including the extent of α-syn seeding and propagation, α-syn inclusion formation, local immune micro-environment, peripheral cytokine profile, dopaminergic neurodegeneration and motor dysfunction. Common variants in the human MHC2TA gene have been found to be associated to human complex inflammatory diseases (Swanberg et al., 2005). Taken together, these results make antigen presentation and the human MHC2TA gene highly relevant to investigate in relation to PD risk, as well as the progressive neuropathology and motor symptoms observed in PD patients.

Common variants in the HLA region, encoding MHCII molecules, are associated with PD risk (Wissemann et al., 2013; Lampe et al., 2003; Kannarkat et al., 2015; Hill-Burns et al., 2011; Hamza et al., 2010) and MHCII+ cells are present in the vicinity of α-syn in PD brains (Croiser et al., 2005) as well as in PD models based on α-syn-overexpression (Sanchez-Guijarro et al., 2010; Jimenez-Ferrer et al., 2017) and striatal injection of PFFs (Duffy et al., 2018). In addition, circulating CD4+ T-lymphocytes that react to α-syn presented on MHCII are found in PD patients (Sulzer et al., 2017). These findings give strong support to a functional link between α-syn pathology and MHCII-mediated antigen presentation. Despite the fact that Mhc2ta is the major regulator of MHCII in antigen-presenting cells, and has been shown to be required for α-syn- and PFF-induced activation of microglia and resulting neurodegeneration (Williams et al., 2018), the role of human MHC2TA in PD susceptibility remains elusive. MHC2TA loss-of-function mutations result in bare lymphocyte syndrome (BLS) and combined immunodeficiency (Steimle et al., 1993). In line with this, mhc2ta knock-out mouse models display an almost complete absence of MHCII and a dysfunctional adaptive immune system (Chang et al., 1996) which severely impacts studies on the role of antigen presentation in disease processes. Therefore, we have chosen to use the VRA4-congenic rat model where natural allelic variants in the Mhc2ta gene give rise to differential Mhc2ta and MHCII expression between DA.VRA4 and DA rats (Harnesk et al., 2018).
Fig. 5. Iba1+/MHCII+ cells with reactive morphology are associated to inclusions and deposits of pαsyn. Representative images of Iba1+/MHCII+ cells close to or overlapping with pαsyn immunoreactive cells and/or deposits in A. striatum (ST) and B. substantia nigra (SN). Cells with advanced pαsyn pathology were frequently seen to be completely enclosed or engulfed by one or several Iba1+/MHCII+ cells (stars). pαsyn dot-like inclusions were also observed inside Iba1+/MHCII+ cells (arrowheads).
Employing this model, we recently showed that Mhc2ta regulates α-syn-induced microglial activation, dopaminergic neurodegeneration and motor deficits in a rAAV-based PD model, with lower Mhc2ta expression levels being associated to increased neuroinflammation, neurodegeneration and motor impairment (Jimenez-Ferrer et al., 2017). The data presented here adds on to the previous findings and shows that natural genetic variants of Mhc2ta; in addition to regulating dopaminergic neurodegeneration and the subsequent development of motor deficits in response to α-syn, is promoting a proinflammatory micro- and peripheral environment and impacts another key aspect of PD; propagation of α-syn pathology.

In conjunction with α-syn pathology, there is increasing evidence of a coactive activation of microglia, and this activation is proposed to be α-syn-conformation dependent (Duffy et al., 2018; Kim et al., 2013). The interplay between α-syn and microglia could also be bidirectional, since altering the activation state of microglia using LPS or IL-4 was recently reported to affect the amount of α-syn in dopaminergic neurons (George et al., 2019). A subset of MHCII-expressing microglia has been reported to attenuate neurodegenerative diseases (Mittal et al., 2019; Mathys et al., 2017) and studies in α-syn overexpression models have shown that activation of microglia requires MHCII (Harms et al., 2013). Furthermore, DA.VRA4 rats, that display lower but physiological MHCII levels due to genetic variants in the Mhc2ta gene, have a highly activated microglial profile in striatum and SN in response to human WT α-syn overexpression in SN (Jimenez-Ferrer et al., 2017). The role of microglia and of Mhc2ta as a key factor regulating α-syn pathology is also supported by knockout and gene silencing models in mice (Williams et al., 2018). Since Mhc2ta levels that regulate antigen-presentation capacity have been shown to modulate immune responses and severity of α-syn pathology, Mhc2ta is a promising immunomodulator in PD.

To address the Mhc2ta-mediated effects on the inflammatory microenvironment as well as transfer and propagation of α-syn, we employed a new PD model in rats combining low α-syn transgene expression in SN with injections of PFFs in the striatum. The combination of low titre rAAV6 to overexpress human α-syn prior to the injection of human PFFs has previously been shown to result in a more efficient model for the spreading of α-syn pathology compared to transgene or PFFs alone (Thakur et al., 2017). However, in that study, the delivery of rAAV6 vectors and PFFs was done to the same brain region and therefore the local responses can be attributed to both the mechanical injury, viral vector and PFFs. Here we used a similar approach but instead injected the PFFs to dopaminergic terminals in striatum 2 weeks after the rAAV6 injection in the SN. At this time point, the transgene expression is established throughout the nigrostriatal pathway (Decressac et al., 2012) allowing assessment of PFF-seeded α-syn pathology from the striatum. As controls, we exchanged either the transgene (GFP instead of α-syn) or the seed (BSA instead of PFF).

While expression of human α-syn in the SN without addition of PFFs (ASYN + BSA) resulted mainly in local posyn inclusions, the addition of PFFs to the striatum resulted in widespread and bilateral propagation of phosphorylated forms of α-syn. This confirms the seeding capacity of PFFs on locally produced human α-syn in our combined model. In addition, the lower extent of posyn inclusions in rats lacking substrate in the form of a human α-syn transgene (GFP + PFF) confirms a more efficient recruitment of human compared to endogenous (rat) α-syn to inclusions (Rey et al., 2016; Volpicelli-Daley et al., 2011; Peelaerts et al., 2015). The endpoint at 6 weeks post-PFF administration in this study was reached quite short, it is possible that a more extended pathology would develop over time in the GFP + PFF groups. The combination of α-syn and PFFs not only led to enhanced α-syn propagation but also induced posyn inclusions similar to those described in PD patients. However, while the striatal posyn inclusion pattern was dominated by filiform and varicose neurites in DA rats, more Lewy neurite-like deposits were observed in DA.VRA4 rats with lower Mhc2ta levels. This highlights the importance of the local pro-inflammatory microenvironment, and specifically Mhc2ta, as a regulator or contributing factor to α-syn-induced pathology.

In addition to enhancing α-syn pathology, the combination of human α-syn substrate and seed (ASYN + PFF) led to a marked increase in MHCII+ cells in neurodegeneration-associated regions compared to only substrate (ASYN + BSA) or seed (GFP + PFF). These results suggest that the increase in MHCII+ cells is a response to seeded α-syn pathology and not to rAAV6 or PFFs injections alone. The observed spatial colocalization of Iba1+/MHCII+ cells displaying an amoeboid and hypertrophic morphology with posyn immunoreactive inclusions and intracellular punctuate posyn staining indicates that microglia can react to pathological forms of α-syn and induce a phagocytic response resulting in presentation of α-syn antigens on MHCII molecules. These results further suggest synucleinopathy-specific MHCII expression in Iba1 activated cells. Notably, DA.VRA4 rats displayed higher amounts of MHCII+ cells compared to DA, which might reflect a compensatory mechanism where a lower antigen-presenting capacity at the single cell level is coupled to an increased local population of MHCII+ cells.

One limitation of this study is the lack of discrimination between resident and infiltrating MHCII+ cells, and if these have different effects on α-syn-induced responses. To determine the Mhc2ta-mediated effects on peripheral immune responses, we characterized the cytokine profile in serum at 8 weeks. In general, DA.VRA4 animals presented a more pro-inflammatory peripheral cytokine profile compared to DA with increased serum levels of TNFα and IL-2. The observed pro-inflammatory cytokine profile in DA.VRA4 rats is particularly interesting in light of the recent identification of α-syn-reactive T lymphocytes in the blood of PD patients (Sulzer et al., 2017). It is important to note that this study did not address if the peripheral immune response is a consequence of the ongoing neurodegenerative process or a contributing factor to it. Nor did it assess local or peripheral T lymphocyte profiles, which requires analyses of lymphocytes in brain, cerebrospinal fluid and blood. The present study, however, shows that the more pro-inflammatory peripheral profile and local immune micro-environment with higher number of activated microglia and MHCII+ cells in DA. VRA4 rats are associated to an increased vulnerability of dopaminergic neurons to degeneration. Suggested mechanisms behind this include beneficial effects of degradation of cells with α-syn inclusions with respect to posyn pathology or spreading, but to a higher inflammatory cost resulting in neuronal damage and/or death. Alternatively, low expression of Mhc2ta could lead to insufficient levels of MHCII and an immunological imbalance favouring microglial activation and neurotoxic effects.

The important role of axonal degeneration in PD is reflected by a widespread axonal pathology in various regions of the brains in the early stages of the disease (Braak et al., 2003). The consequences of α-synseeded pathology in rats with normal (DA) or low (DA.VRA4) Mhc2ta levels were therefore evaluated by loss of dopaminergic terminals in the striatum as well as by neuronal loss in SN. DA.VRA4 rats with lower Mhc2ta levels showed to be more susceptible to both axonal neurodegeneration and nigral cell loss. Rats of both strains displayed a 20–30% dopaminergic fibre loss when injected with human α-syn only as seed (ASYN + BSA) or substrate (GFP + PFF). However, only DA.VRA4 rats displayed a significantly enhanced dopaminergic fibre loss by the combination of human α-syn substrate and seed (ASYN + PFF). An increased dopaminergic neurodegeneration in terms of TH+ cells in SN was also observed for DA.VRA4 rats, with a significant cell loss in all groups (ASYN + PFF, ASYN + BSA and GFP + PFF) while DA only displayed a significant cell loss in the ASYN + PFF group. Thus, the increased α-syn pathology seen in DA.VRA4 compared to DA rats was coupled to a greater extent of dopaminergic neurodegeneration in both the striatum and SN. Of note, a significant motor impairment in terms of contralateral forelimb akinesia was also observed in DA.VRA4, but not DA, rats. Taken together these results show increased α-syn pathology, dopaminergic neurodegeneration and subsequent motor impairment in DA.VRA4 rats with low Mhc2ta expression.

Further experiments are needed to characterize the impact of Mhc2ta
on cellular and soluble peripheral immune profiles over time in response to α-syn pathology, since both microglia and lymphocytes in DA.VRA4 mice might be primed by the lower expression levels of Mhc2ta and display altered inflammatory profiles prior to Transgene expression and injection of PFFs. However, the observed requirement of human α-syn as a substrate and PFFs as a seed for widespread α-syn pathology and dopaminergic neurodegeneration, in combination with increased dopaminergic susceptibility in the DA.VRA4 strain, strongly suggest Mhc2ta as a regulator of both inflammation and neurodegeneration in PD-like α-syn pathology.

5. Conclusions

By employing a seeding model recapitulating pathological hallmarks of PD including progressive and widespread propagation of α-syn pathology, we found an important interaction between Mhc2ta allelic variants, a pro-inflammatory response and an increased vulnerability to α-syn-induced PD-like pathology in rats. Common variants in the human MHC2TA gene are associated with MHCI expression, and in light of these findings we propose antigen presentation and microglial activation as promising targets in the quest to modify PD susceptibility and progression.

6. Availability of data and material

All original data are available for publication. All submitted figures are original files.

Funding

This study was supported by the Swedish Research Council, Åke Wibergs stiftelse, Kungliga Fysiografiska Sällskapet NMT, Magnus Bergvalls Stiftelse, Stiftelsen Sven-Olof Jansons livsförs, Apotekare Hedbergs Fond for Medicinsk forskning, Schyberg's stiftelse, and Bertil och Ebon Norlins Stiftelse for Medicinsk Forskning. I.-J.-F. acknowledges support from CONACYT through an international scholarship for PhD studies.

The funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Declaration of Competing Interest

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.

Acknowledgements

This study was supported by MultiPark - A Strategic Research Area at Lund University. We thank the MultiPark technical platforms (asAAV Vector Lab, CONFOCAL microscopy and MSEQ QuickPlex) and the Bioimaging Centre of Lund University for excellent technical assistance. We thank the Swedish Research Council, Åke Wibergs stiftelse, Kungliga Fysiografiska Sällskapet NMT, Magnus Bergvalls Stiftelse, Stiftelsen Sven-Olof Jansons livsförs, Apotekare Hedbergs Fond för Medicinsk forskning, Schyberg's stiftelse, and Bertil och Ebon Norlins Stiftelse for Medicinsk Forskning for funding. I.-J.-F. acknowledges support from CONACYT through an international scholarship for PhD studies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbi.2020.10.017.

References

Braak, H., Del Tredici, K., Rub, U., de Vos, R.A., Jansen Steur, E.N., Braak, E., 2003. Staging of brain pathology related to sporadic Parkinson’s disease. Neurobiol. Aging. 24 (2), 197–211.

Brodarch, V., Comedier, B., Prigent, A., Laouar, Y., Perrin, A., Beray-Berhat, V., et al., 2009. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J. Clin. Invest. 119 (1), 182–192.

Brundin, P., Melki, R., 2017. Priony into the Prion Hypothesis for Parkinson’s disease. J. Neurosci. 37 (41), 9808–9818.

Chang, C.H., Guerder, S., Hong, K.C., van Ewijk, W., Flavell, R.A., 1996. Mice lacking the MHC class II transactivator (CITA) show tissue-specific impairment of MHC class II expression. Immunity 4 (2), 167–178.

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

Decressac, M., Mattsson, B., Lundblad, M., Weikop, P., Bjorklund, A., 2012. Progressive neurodegenerative and behavioural changes induced by AAV-mediated overexpression of alpha-synuclein in midbrain dopamine neurons. Neurobiol. Dis. 45 (3), 939–953.

Duffy, M.F., Collier, T.J., Patterson, J.R., Kemp, C.J., Luk, K.C., Tansey, M.G., et al., 2018. Lewy body-like alpha-synuclein inclusion induction and trigger reactive microglialons prior to nigral degeneration. J. Neuroinflamm. 15 (1), 129.

Duffy, M.F., Collier, T.J., Patterson, J.R., Kemp, C.J., Luk, K.C., Tansey, M.G., et al., 2018. Correction to: Lewy body-like alpha-synuclein inclusion induction trigger reactive microglialons prior to nigral degeneration. J. Neuroinflamm. 15 (1), 169.

Fujiiwara, H., Hasegawa, M., Doehane, N., Kasahitma, A., Matihah, E., Goldberg, M.S., et al., 2002. Alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat. Cell Biol. 4 (2), 160–164.

Georgo, S., Rey, N.L., Tyson, T., Esquibel, C., Meyerd, L., Schulz, E., et al., 2019. Microglia affect alpha-synuclein cell-to-cell transfer in a mouse model of Parkinson’s disease. Mol. Neurodegener. 14 (1), 34.

Hamza, T.H., Zabetian, C.P., Tenesa, A., Laederach, A., Montimurro, J., Yearout, D., et al., 2010. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson’s disease. Nat. Genet. 42 (9), 781–785.

Hansen, C., Angot, E., Bergstrom, A.L., Steiner, A., Piersi, L., Paul, G., et al., 2011. alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J. Clin. Invest. 121 (2), 715–725.

Harms, A.S., Cao, S., Rowe, A.L., Thome, A.D., Li, X., Mangieri, L.R., et al., 2013. MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. J. Neurosci. 33 (23), 9592–9600.

Harnesk, K., Swanberg, M., Ockinger, J., Dize, M., Lidman, O., Wallstrom, E., et al., 2008. Va4 congeric rats with allelic differences in the class II transactivator gene display altered susceptibility to experimental autoimmune encephalomyelitis. J. Immunol. 180 (5), 3289–3296.

Hill-Burns, E.M., Factor, S.A., Zabetian, C.P., Thomson, G., Payami, H., 2011. Evidence for more than one Parkinson’s disease-associated variant within the HLA region. PloS One 6 (11), e27190.

Imamura, K., Hishikawa, M., Sawada, M., Nagatsu, Y., Yoshida, M., Hashizume, Y., 2003. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson’s disease brains. Acta Neuropathol. 106 (6), 518–526.

Jimenez-Ferrer, I., Jewett, M., Tostanahal, A., Romero-Ramos, M., Swanberg, M., 2017. Allelic difference in Mhc2ta and zeta confer altered microglial activation and susceptibility to alpha-synuclein-induced dopaminergic neurodegeneration. Neurobiol. Dis. 106, 279–290.

Kanzaki, T.T., Cook, D.A., Lee, J.K., Chang, J., Chung, J., Sandy, E., et al., 2015. Common genetic variant association with altered HLA expression, synergy with pyrethroid exposure, and risk for Parkinson’s Disease: an observational and case-control study. NJP Parkinsons Dis. 1.

Kim, C., Ho, D.H., Sok, J.E., You, S., Michael, S., Kang, J., et al., 2013. Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat. Commun. 4, 1562.

Kordower, J.H., Chu, Y., Hauser, R.A., Freeman, T.B., Olanow, C.W., 2008. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson’s disease. Nat. Med. 14 (5), 504–506.

Kordower, J.H., Dodla, H.R., Kordower, A.M., Terpstra, B., Paumier, K., Madshavan, L., et al., 2011. Transfer of host-derived alpha-synuclein to grafted dopaminergic neurons in rat. Neurobiol. Dis. 43 (3), 552–557.

Kurosawa, Z., Jewett, M., Brattas, P.I., Jimenez-Ferrer, I., Keneu, X., Bjorklund, T., et al., 2016. Identification of multiple QTLs linked to neuropathology in the engrailed-1 homozygous mouse model of Parkinson’s disease. Sci. Rep. 6, 31701.

Lampe, J.B., Gossrau, G., Herting, B., Kempe, A., Sonnerr, U., Fussel, M., et al., 2003. HLA typing and Parkinson’s disease. Eur. Neurol. 50 (2), 64–68.

Li, J.Y., Engholm, E., Holton, J.L., Soult, D., Haggel, P., Lees, A.J., et al., 2008. Lewy bodies in grafted neurons in subjects with Parkinson’s disease suggest host-to-graft disease propagation. Nat. Med. 14 (5), 501–503.

Lidman, O., Swanberg, M., Horvath, L., Broman, K.W., Olsson, T., Piehl, F., 2003. Discrete gene loci regulate neurodegeneration, lymphocyte infiltration, and major histocompatibility complex class II expression in the CNS. J. Neurosci. 23 (30), 9817–9823.

Luk, K.C., Song, C., O’Brien, P., Stieber, A., Branch, J.R., Brunden, K.R., et al., 2009. Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc. Natl. Acad. Sci. U.S.A. 106 (47), 20501–20506.
Luk, K.C., Kehm, V.M., Zhang, B., O'Brien, P., Trojanowski, J.Q., Lee, V.M., 2012. Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. J. Exp. Med. 209 (5), 975–986.

Mathys, H., Adaikkan, C., Gao, F., Young, J.Z., Manet, E., Hemberg, M., et al., 2017. Temporal tracking of microglia activation in neurodegeneration at single-cell resolution. Cell Rep. 21 (2), 366–380.

McGeer, P.L., Itagaki, S., Boyes, B.E., McGeer, E.G., 1988. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38 (8), 1285–1291.

McGeer, P.L., Itagaki, S., McGeer, E.G., 1988. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. Acta Neuropathol. 76 (6), 550–557.

Mittal, K., Eremenko, E., Berner, O., Elyahu, Y., Strominger, I., Apelblat, D., et al., 2019. CD4 T cells induce a subset of MHCII-expressing microglia that attenuates Alzheimer pathology. iScience 16, 298–311.

Olsson, M., Nikkhah, G., Bentlage, C., Bjorklund, A., 1995. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J. Neurosci. 15 (5 Pt 2), 3863–3875.

Paumier, K.L., Luk, K.C., Manfredsson, F.P., Kanaan, N.M., Lipton, J.W., Collier, T.J., et al., 2015. Intrastriatal injection of pre-formed mouse alpha-synuclein fibrils into rats triggers alpha-synucleinopathy and bilateral nigrostriatal degeneration. Neurobiol. Dis. 82, 185–199.

Peelaerts, W., Bousset, L., Van der Perren, A., Moskalyuk, A., Pulizzi, R., Giugliano, M., et al., 2015. Alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature 522 (7556), 340–344.

Rey, N.L., Steiner, J.A., Maroof, N., Luk, K.C., Madaj, Z., Trojanowski, J.Q., et al., 2016. Widespread transneuronal propagation of alpha-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson’s disease. J. Exp. Med. 213 (9), 1759–1778.

Sanchez-Guajardo, V., Febbraro, F., Kirik, D., Romero-Ramos, M., 2010. Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson’s disease. PLoS One 5 (1), e8784.

Spillantini, M.G., Crowther, R.A., Jakob, R., Hasegawa, M., Goedert, M., 1998. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson’s disease and dementia with Lewy bodies. Proc. Natl. Acad. Sci. U.S.A. 95 (11), 6469–6473.

Steinle, V., Otten, L.A., Zufferey, M., Mach, B., 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell 75 (1), 135–146.

Sulzer, D., Alclay, R.N., Garrett, F., Cote, L., Kanter, E., Agin-Liebes, J., et al., 2017. T cells from patients with Parkinson’s disease recognize alpha-synuclein peptides. Nature 546 (7660), 656–661.

Surmeier, D.J., Obeso, J.A., Halliday, G.M., 2017. Parkinson’s disease is not simply a prion disorder. J. Neurosci. 37 (41), 9799–9807.

Swanberg, M., Lidman, O., Padyukov, L., Eriksson, P., Akesson, E., Jagodic, M., et al., 2005. MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. Nat. Genet. 37 (5), 486–494.

Thakur, P., Breger, L.S., Lundblad, M., Wan, O.W., Mattsson, B., Luk, K.C., et al., 2017. Modeling Parkinson’s disease pathology by combination of fibril seeds and alpha-synuclein overexpression in the rat brain. Proc. Natl. Acad. Sci. U.S.A. 114 (39), E8284–E8293.

Volpicelli-Daley, L.A., Luk, K.C., Patel, T.P., Tanik, S.A., Riddle, D.M., Stieber, A., et al., 2011. Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron 72 (1), 57–71.

Williams, G.P., Schonhoff, A.M., Jurkvenaite, A., Thome, A.D., Standaert, D.G., Harms, A.S., 2018. Targeting of the class II transactivator attenuates inflammation and neurodegeneration in an alpha-synuclein model of Parkinson’s disease. J. Neuroinflamm. 15 (1), 244.

Wiseman, W.T., Hill-Burns, E.M., Zabetian, C.P., Factor, S.A., Patopsoulos, N., Hoglund, B., et al., 2013. Association of Parkinson disease with structural and regulatory variants in the HLA region. Am. J. Hum. Genet. 93 (5), 984–993.