Human Vaccines & Immunotherapeutics 10:4, 852–867; April 2014; © 2014 Landes Bioscience

Vaccination strategies for Parkinson disease

Induction of a swift attack or raising tolerance?

Marina Romero-Ramos[1,2], Marianne von Euler Chelpin[1,2,3], and Vanesa Sanchez-Guajardo[2,3,*]

[1]CNS disease modeling group; Department of Biomedicine; Aarhus University; Aarhus, Denmark; [2]NEURODIN; Department of Biomedicine; Aarhus University; Aarhus, Denmark; [3]Neuroimmunology of Degenerative Diseases group; Department of Biomedicine; Aarhus University; Aarhus, Denmark

Keywords: Treg, dopamine, antibodies, T cells, immunomodulation, cytokines, tolerance, humoral response

Abbreviations: AAV, adeno-associated virus; AD, Alzheimer disease; α-syn, alpha-synuclein; BCG, *Bacillus* Calmette–Guerin; CDNF, Glia cell derived neurotrophic factor; CFA, Complete Freund's adjuvant; Cop-1, Copolymer 1, also knows as Galatiramer acetate, Copaxone; CSF, cerebrospinal fluid; DA, dopamine; DC, dendritic cell; EGF, epithelial growth factor; Foxp3, forkhead transcription factor; GDNF, Glia derived neurotrophic factor; GM-CSF, granulocyte macrophage colony stimulating factor; ICAM, intracellular adhesion molecule; IL, interleukine; iTreg, induced regulatory T cells; LB, Lewy body; LPS, lipopolysaccharide; MOG, myelin oligodendrocyte glycoprotein ; NO, nitric oxide; PBMC, peripheral blood mononuclear cells; PBL, Peripheral blood leucocytes; PD, Parkinson's disease; PHA, phytohaemagglutinin; RORγt, RAR-related orphan receptor; ROS, reactive oxygen species; TGFβ, tumor growth factor beta; TNFα, tumor necrosis factor alpha; Treg, regulatory T cells ($CD25^+foxp3^+$); Th, $CD4^+$ T helper cell; VCAM, vascular cell adhesion molecule; Vip, vasoactive intestinal peptide; VLA, very late antigen integrin dimers (CD49a-f = ITGA1-6); WT, wild type

Parkinson disease is the second most common neurodegenerative disease in the world, but there is currently no available cure for it. Current treatments only alleviate some of the symptoms for a few years, but they become ineffective in the long run and do not stop the disease. Therefore it is of outmost importance to develop therapeutic strategies that can prevent, stop, or cure Parkinson disease. A very promising target for these therapies is the peripheral immune system due to its probable involvement in the disease and its potential as a tool to modulate neuroinflammation. But for such strategies to be successful, we need to understand the particular state of the peripheral immune system during Parkinson disease in order to avoid its weaknesses. In this review we examine the available data regarding how dopamine regulates the peripheral immune system and how this regulation is affected in Parkinson disease; the specific cytokine profiles observed during disease progression and the alterations documented to date in patients' peripheral blood mononuclear cells. We also review the different strategies used in Parkinson disease animal models to modulate the adaptive immune response to salvage dopaminergic neurons from cell death. After analyzing the evidence, we hypothesize the need to prime the immune system to restore natural tolerance against α-synuclein in Parkinson disease, including at the same time B and T cells, so that T cells can reprogram microglia activation to a beneficial pattern and B cell/IgG can help neurons cope with the pathological forms of α-synuclein.

*Correspondence to: Vanesa Sanchez-Guajardo; Email: vsg@biokemi.au.dk
Submitted: 12/02/2013; Revised: 03/10/2014; Accepted: 03/18/2014;
Published Online: 03/26/2014 ; http://dx.doi.org/10.4161/hv.28578

Introduction

Parkinson disease (PD) is mainly characterized by loss of dopaminergic neurons in substantia nigra (SN), which leads to severe and progressive motor impairment.[1] These symptoms comprise slowness, stiffness, postural imbalanced and tremor, and 80% of cases eventually develop dementia. PD goes beyond the movement disorder, and non-motor symptoms, known to be part of the disease manifestation, have been proposed to precede the classical motor defects.[2] During the last decade α-synuclein (α-syn), normally expressed in neuronal terminals, has been implicated in the etiology of the disease; multiplication and/or mutation of the α-syn gene is related to PD[3-7]; aggregation and/or modification of the protein has been shown to contribute to the disease[8-12]; and prion-like spreading of α-syn has been proposed to occur and it was shown to induce PD-like neurodegeneration in animals.[13-17] Indeed, surviving neurons not only in SN but also in other areas of the CNS, are characterized by the presence of protein aggregates known as Lewy bodies (LB), which are mainly composed of fibrilar α-syn.[18]

Another important factor in PD pathology is neuroinflammation, the chronic activation of microglia that ensures a constant production of IL-1, IL-6, TNF, reactive oxygen species (ROS) and NO in the brain, resulting in a persistent detrimental insult to neurons trying to deal with α-syn pathology (reviewed in ref. 19). Additionally, it is becoming evident that microglia interact with the peripheral immune system, and thus probably, instrument a detrimental peripheral immune response from the infiltrating immune cells.[20] The fact that the peripheral immune system is involved in PD makes it an ideal target for the modulation of the pathology, as priming it through vaccination could result in harnessing of microglia-induced neuroinflammation. This would allow neurons to better handle the pathological

processes induced by α-syn dysfunction and help them to survive by changing the cytokine/growth factor microenvironment, removing pathological α-syn, and re-establishing beneficial microglia-neuron cell-cell interactions. Therefore immunotherapies in PD are extensively studied and debated (reviewed in refs. 21 and 22).

When considering vaccination strategies for curing/modifying disease progression in PD, one has to take into account that the peripheral immune system is affected during the disease, and thus, it will not necessarily react as expected. Independently of whether the vaccination is done to prevent or modulate the disease, T and B cells will react differently when under the PD-induced "environment." It should also be precisely considered what kind of immune response one aims to achieve: a prophylactic one that will prepare the system to destroy the pathologic entity, i.e., α-syn; or a therapeutic one that will modulate the response and render the system tolerant. This will be greatly influenced by the type of adjuvant used, the dose of antigenic entity, the target and the type of the reaction induced (for example Th17 vs. Treg).

In this review we will discuss the particular immune environment observed in PD (cytokines, T cells compartment, DA regulation, humoral response) and the work done so far in PD animal models to design an immunotherapy for PD. We would like to note that there are also current immunological therapy studies concerning targeted gene delivery of protective molecules such as cytokines, antioxidants, and growth factors through monocytes/liposome, but due to the scope of this review they will not be discussed here.

The Immune Environment in PD

The cytokine environment in PD

Numerous laboratories have shown changes in different cytokines, not only in brain and CSF but also in serum from PD patients, confirming the systemic involvement of the immune system in the disease. These studies suggest a role for pro-inflammatory cytokines in PD progression, but increased levels of other cytokines with anti-inflammatory or repair functions such as IL-10 have also been reported (**Table 1**). In addition to the changes in patients, a recent meta-analysis reported that the TNFα-1031 gene polymorphism increases the risk of suffering from PD, while the IL-6–174 polymorphism and the variable number tandem repeat (VNTR) polymorphism in intron 2 of the IL-1 receptor antagonist (IL-1RA) may decrease such a risk; overall further supporting a role for cytokines in PD.[23]

Pioneering work by Mogi et al., showed cytokine changes in PD relevant areas of the brain, with increased IL-1β, IL-2, IL-6, EGF, and TGFα and β1 in striatum (for a review see ref. 24). In addition, Hirsch and coworkers showed microglial TNFα upregulation in SN of parkinsonian patients.[25] These seminal studies, together with the previously observed microgliosis by McGeer,[26] gave rise to the hypothesis that excess of pro-inflammatory cytokines such as TNFα and IL-1 could sustain the activation of microglia and in turn contribute to cell death of dopaminergic neurons in PD (for a review see ref. 27). IL-1 is of particular interest with respect to the interaction CNS-periphery because it primes the epithelium of capillary vessels to express integrins (such as ICAM, VCAM) that allow leucocytes to extravasate to the inflammatory site.[28,29]

It is normally accepted that TNFα is elevated in patients (**Table 1**)[a] and that it has a deleterious impact on neurons. Indeed there are many experimental therapies aiming to halt TNFα-signaling and release.[31-33] Interestingly, TNFα levels have recently been correlated with the non-motor symptoms, such as cognition, depression, stress, fatigue and disability in PD, highlighting an early role of the immune system in the disease.[34,35] Notably TNFα is also known immunologically for abrogating regulatory T cell (Treg) function[36-40] and driving an M1 (phagocytic/pro-inflammatory) macrophage phenotype, thus reducing IL-10 secretion by immune cells.[39,40]

Depression and fatigue were also associated with soluble IL-2R levels,[35] however, when excluding depressed PD patients, the correlation with fatigue was lost, suggesting that IL-2R has a role in depression.[41] Although IL-2 was shown to be elevated in serum, CSF and brain in PD patients,[42-44] it was not increased in de novo PD patients,[45] suggesting that IL-2 elevation is associated to later stages of the disease, rather than being an early event. This may influence the late response of T-cells in PD, as IL-2 is required for T cell activation.[46,47] This could explain why there is an overly activation of $CD4^+$ T cells in PD patients.[48-50] IL-2 is also required for Treg survival[46,47] and low IL-2 concentrations are required for naive T cells in order to differentiate into Treg in the periphery upon self-antigen encounter (inducible Treg, iTreg).[51,52]

IL-6 has been correlated with disease severity[53,54] and high levels of IL-6 have been observed in the early stage PD patients suffering from depression.[55] Moreover, although IL-6 concentrations were always higher in PD patients than in controls, the highest IL-6 levels were described in idiopathic PD patients with cardiovascular risk factor, suggesting a role for the immune system in the non-motor problems of PD.[43] Indeed, fatigue has been correlated with changes in IL-6.[56] The increment in IL-6 is of particular interest when considering vaccination strategies, because it signals through Stat3, inducing its phosphorylation and then changing the balance between RORγt and Foxp3 expression, the transcription factors responsible for inducing Th17 (autoimmune inflammation) or Treg (tolerance) cells respectively (reviewed in ref. 57).

Chemokines also seem involved in PD, although they may be differently regulated in the periphery than in the nigro-striatal system, as is the case of RANTES, which is increased in serum but decreased in SN.[58,59] Since this was directly correlated with disease severity, this may suggest that the increase in RANTES in serum is a compensatory mechanism to processes happening in the brain.

Other research groups have approached the study of cytokines differently, by isolating, culturing and activating peripheral immune cells from PD patients or controls. This approach allows for a more direct study of the differentiation state of the immune system and its capacity to react to antigenic challenge. This approach with whole blood or PBMC has shown a decrease

Table 1. Cytokine profiles in PD

Cytokine	Changes	Notes	References
TNF	serum ↑CSF ↑Brain	Correlates with depression[34,35] TNFR1 in serum and it is directly correlated with disease onset[30]	25,186-188
IL-1β	↑CSF ↑Brain		42,45
IL-2	↑CSF serum ↑Brain	No change in CSF or serum in de novo PD[45]	42-44
IL-4	↑CSF serum		42,43
IL-6	serum ↑CSF	Correlates with fatigue[56] Negative correlation with ADL scale[54] Directly correlated with severity of disease[53] and higher in early stages with depression[55]	35,42,43,45,53,56,188
IL-10	serum	IL-10 directly correlated with IL-12[187,189]	43,189
IL-12	No change, serum		189
IL-15	No change, serum		58
IFNγ	serum		43
TGFβ1	↑CSF ↑Brain		190,191
TGFβ2	↑CSF		191
RANTES (CCL5)	serum ↓SN	Positive correlation serum RANTES and disease severity[58]	58 59
CCL3 CCL11 CCL24 CXCL8 CXCL10	No change, serum		192
MIF	serum		193
CXCL12	↑Brain	Its receptor CXCR4 is elevated in SN and striatum	59

in IL2,[60,61] TNFα, IL1α, IL1β, IL6,[62] and in IFNγ production[63] when un-stimulated. However, other groups report no significant change in basal production of IL1β and TNFα,[61] nor increased production in IL6,[61] TNFα, IL-1β, IL-8, and IFNγ.[64] Ex-vivo stimulation with LPS of PBMC also resulted in variable results: some see reduced expression of TNFα, IL-1α, IL-1β, and IL-6[62] while others report increased levels of TNFα, IL-1β, IL-8, IFNγ, MCP-1, RANTES, and MIP-1α.[64]

Thus, due to the few studies concerning cytokine profiles in PD, the different approaches to determine their concentrations and the contradictory results, further studies are required to exactly determine what particular environment will exist in the patient when an immunotherapy is approached. It has to be noted that both levodopa and amantadine, drugs commonly used in PD, can affect cytokine production by blood immune cells, suggesting that not only the disease, but also the therapeutic approach can modify the immune system.[61,63] This may be important to remember as dopamine (DA) regulates T cell function as discussed below.

Dopamine regulates the adaptive immune system

During the last decade it has become evident and well documented that DA and other neurotransmitters regulate CD4 T cell differentiation, altering the Th1/Th2/Th17/Treg response (reviewed in refs. 65 and 66). Human T cells express 2 types of functional DA receptors: type I (DR1/DR5) receptors, which are coupled to Gαe protein receptors, increase cAMP production, and are thus considered stimulatory; type II (DR2/DR3/DR4) receptors, which are coupled to Gαi protein receptors, decrease cAMP production, and are therefore inhibitory. From ex vivo studies it appears that DA helps to direct the outcome of Th differentiation by regulating the availability of cAMP in cells and thus helps to determine which kinases will be active upon TCR stimulation (**Table 2**).

How and where does DA act on T cells?

T cells can encounter DA at different stages of their activation pathway: (1) As naive cells in secondary lymphoid organs due to sympathetic nerve innervation,[67] (2) as resting cells in the circulation, where DA is normally found in plasma at a concentration of 10 pg/mL,[68] and (3) during activation by dendritic cells (DC, specialized antigen presenting cells). DCs produce DA and release it upon MHCII-TCR engagement, thus greatly increasing the micro-concentration of available DA.[70,71] Additionally, immune cells produce catecholamines by an autocrine regulatory mechanism, so that the estimated cellular content of DA in T cells is 3×10^{-4} pg/cell, 1.1×10^{-5} pg/cell in B cells and 3.1×10^{-3} pg/cell in macrophages.[72,73] Thus, interaction between the different immune cells will also increase the relative concentration of DA in a particular microenvironment. Additionally it is also documented that under disease/stress DA increases to 80 pg/mL, but not during neurodegenerative diseases.[68,69] One could thus

Table 2. Effects of dopamine on CD4+ T cell differentiation

	DA affinity (K_i mM)	Human	
		Naive/resting (no TCR stimulation)	Activated (TCR stimulation)
DR1	2340	Th17 induction,[96] induces TNFα/IL-10 [194]	Impairs proliferation[68]
DR5	228	IFNγ secretion, induces MMP-9[195]	Impairs proliferation[68]
DR2	1705	Enhanced production of IL-10/VLA-4&5[83,194]	Inhibition IL-2/IL-4/IFNγ [196]
DR3	27	TNFα/IFNγ /VLA-4&5 production[83,194]	Inhibition IL-2/IL-4/IFNγ[196,197]
DR4	450		Quiescence[198]
		Mouse	
DR1	2340	Inhibit Treg suppression and IL-10/TGFβ production / Induce Th2[36,70]	
DR5	228	Inhibit Treg[36]	
DR2	1705	Induce Treg (reviewed in[65])	
DR3	27	IFNγ production (Th1)[197]	
DR4	450	Induction Th17?/Quiescence[65,198]	

DA, dopamine; DR, dopamine receptor; K_i, dissociation constant; Treg, regulatory T cells; Th, helper T cells; MMP, matrix metaloproteinase; VLA, very late antigen, integrin α4β1 (CD49d/CD29) or α5β1 (CD49e/CD29); IL, interleukine.

expect that depending on the activation state of the T cell, DA synthesis and tyrosine hydroxylase (TH) expression will vary, as is the case in MS where ex vivo stimulation of T cells with PHA resulted in lower DA production if the T cells were active, and lower TH expression if they were inactive.[74] Dopamine's effect on T cells is of particular interest when considering vaccination strategies for PD. CNS DA signaling is decreased during PD disease progression, thus the available amount of DA in secondary lymphoid organs will be much lower than normally encountered by T cells upon priming/differentiation and the available DA will bind to the receptor with the lowest K_i (**Table 2**). This will lead to a shift in the CD4 T cell's response toward Th1 (IFNγ producing cell) and will induce their extravasation through the VLA integrins. Additionally, tolerance will be compromised, as IL-10 production will be suppressed.

At the same time, activated/circulating T cells would become inactive if the levels of DA were increased in serum as a consequence of the DA replacement treatment in patients. Indeed, there appears to be a higher proportion of activated T cells in PD (reviewed in refs. 75 and 76). During PD, due to L-DOPA treatment, a precursor of DA, the amount of available DA in serum is elevated to levels normally not encountered by resting T cells ($1.2 \pm 0.5 \times 10^6$ pg/mL)[77]. T cells express the DA transporter (DAT) and are able to uptake L-DOPA,[78-80] potentially modifying their immune response as shown by different studies. In vitro incubation of T cells with L-DOPA increases the cellular content of DA, leading to suppression of proliferation and cytokine production.[72] When L-DOPA was administrated in vivo to mice, the number of T cells producing IFNγ was decreased and an IL-2 independent proliferation induced.[81,82] The seeming discrepancy between these 2 studies may be due to the stage of activation at which the T cell encountered DA. But in PD patients this outcome is not straightforward because together with L-DOPA, patients may be taking additional compounds to block conversion of L-DOPA to DA in the periphery (DOPA decarboxylase inhibitors), or to inhibit DA degradation (such as MAO or COMT inhibitors). Additionally L-DOPA may accumulate in T cells and bind to cellular components impairing T cell function. Indeed, despite the decarboxylase inhibitors, DA levels in PD patient sera are elevated ($1.2 \pm 0.5 \times 10^6$ pg/mL)[77] to a concentration above the optimal required for T cell signaling (1.5×10^3 pg/mL), as shown by in vitro kinetic studies. These same studies have shown that high DA doses inhibit proliferation and cytotoxicity.[83,84]

Why is dopamine signaling relevant for T cell immune reactions in PD?

Any therapy aimed at modulating the immune response has to take into account how the patient's unique environment, and the drug cocktail used to treat the disease, will modify the patients' immunological status and therefore the outcome of such therapy. This is indeed an important factor to consider since it has been shown in human PD patients that DR3 (one of the inhibitory receptors described above) is significantly reduced in PBMC,[63,85,b] which could imply a compensatory mechanism by which plasma T cells deal with excess DA signaling. It has also been observed that L-DOPA modulated the T cell proteome in PD patients.[86] Additional studies using MPTP intoxication in DR3 knockout mice, showed that there is less microgliosis when CD4 T cells lack DR3. This correlates with significant decrease in dopaminergic cell death in SN.[87] Whether lack of DR3 expression determined a protective CD4 T cell phenotype in the periphery, or modified T cell-microglia interactions in brain that resulted in an alternative neuroprotective microglia activation pattern, remains yet to be determined. Another possibility is that lack of DR3 protected T cells from the immunotoxic effect of MPTP and thus altered the immune response normally observed in this PD-like model. Studies from the late 80s and 90s showed that MPTP reduced the absolute number of PBLs,[88] the percentage of T cells in spleen and their proliferative capacity when stimulated with ConA (but

Table 3. Immunotherapeutical strategies used in PD animal models

Model	Immunization therapy	Immunogen	Results
Active Immunization			
MPTP 10 mg/kg 4 i.p. injections with 1 h intervals	Flank injections of MOG 35–55 peptide (150 μg) in CFA 6 d before treatment.	MOG 35–55 peptide	MOG 35–55 peptide immunization enhanced neuronal survival. CFA alone prevented neuronal cell death but in a smaller magnitude. Better MOG neuroprotection in 10 mo vs. 2 mo mice[168]
Heterozygous tg α-syn mice under the PDGF promoter	1st injection recombinant hα-syn (80 μg/mL, 100 μL) in CFA. Two weeks later injection with the same dose hα-syn in IFA followed by re-injection every month for the subsequent 7 mo with hα-syn (80 μg/mL, 100 μL) in PBS.	Recombinant hα-syn	Vaccine-induced hα-syn antibodies reduced α-syn abnormal accumulation in neurons and ameliorated the synaptic loss. Vaccination reduced hα-syn accumulation in the membrane. Mouse IgG and hα-syn co-localized to the outer membrane of neurons.[158]
Unilateral stereotaxic injection (2 μL) of rAAV2/5-α-syn into SN of rats	Immunization with hα-syn, s.c. 10 and 6 wk before stereotaxic surgery. 1st: 150 μg hα-syn/200 μL + 150 μl CFA) 2nd: 4 wk later (100 μg hα-syn/200 μL + 150 μl IFA)	Recombinant hα-syn	The vaccination strategy resulted in: - High-titer anti-α-syn antibody response upon α-syn overexpression. - The accumulation of CD4+/MHC II+ ramified microglia in SN. Long lasting infiltration and accumulation of CD4+/FoxP3-+ cells in striatum - Fewer pathologic TH^+ aggregates in the striatum - GDNF induction in striatum - Modification of cytokine patterns in serum - High anti-α-syn antibody titer and deposition[98]
MPTP (20 mg/kg) Daily injection for 5 consecutive days starting 10 d after vaccination.	Mouse TH cDNA was subcloned into a bacterial expression vector (pET-15b). Mice received 0.10 mL s.c. of the pET-15b (100 μg) in CFA and other mice were vaccinated with live BCG (2 x 10^7 cfu) i.p.	TH BCG	CFA was the major beneficial component and promoted neuronal survival BCG vaccination partially preserved striatal DA and DAT expression BCG vaccination prevented MPTP-induced microglia activation in SN[169]
MPTP (18 mg/kg) Daily i.p. injections for 5 consecutive d starting 10 d after vaccination.	I.p. injections 6 x 10^6cfu BCG or with saline (control)	BCG	BCG vaccination partially protected the striatum for DA and DAT loss in a dose dependent manner. BCG vaccination increased the number and frequency of splenic Tregs, which were positively correlated with stratial DA and DAT levels.[161]
Intrastriatal 6-OHDA or saline 10 d after immunization treatment.	S.c. injections (0.10 mL/flank) of CFA or vehicle (PBS).	CFA	CFA pretreatment markedly reduced the SN neuronal loss and associated microglial activation. The neuroprotective effects of CFA pretreatment were due to transient increases in nigrostratial levels of GDNF and pro-inflammatory cytokines associated with the peripheral inflammation elicited by CFA.[162]
MPTP (20 mg/kg) 4 i.p. injections with 2 h intervals.	Daily bee venom (BV), 1 mg/kg, or PBS i.p. for 6 d starting 12 h after the last MPTP injection. For Treg depletion: 1 mg/kg of anti-CD25 rat IgG1 (clone PC61) or normal anti-rat IgG1 for 3 d before first MPTP injection.	BV	BV prevented nigral dopaminergic degeneration BV-neuroprotection was associated with microglial deactivation and reduction of CD4 T cell infiltration. BV treatment increased the proportion of $CD4^+CD25^+Foxp3^+$ Tregs in vivo and in vitro.[163]
Passive Immunization: antibody based therapies			
Tg α-syn mice under the PDGF-β promoter	Stereotaxic injection of 3 μL of either non-immune IgG control or the antibody against α-syn (clone 274, 1 mg/mL) into the hippocampus. Mice survived for 4 wk after Ab injection. *Passive immunization*: Non-immune IgG control or the mouse monoclonal antibody against α-syn (100 μL of 1 mg/mL /wk for 4 wk).	α-syn monoclonal antibody	Ab against α-syn specifically targeted and aided clearance of extracellular α-syn by microglia through FcγR (not by neurons or astrocytes). Stereotaxic administration of Abs into the brains of tg mice prevented neuron-to-astroglia transmission of α-syn. Passive immunization with α-syn Ab reduced neuronal and glial accumulation of α-syn and ameliorated neurodegeneration.[146]

Table 3. Immunotherapeutical strategies used in PD animal models (continued)

Model	Immunization therapy	Immunogen	Results
Tg α-syn mice under the PDGF-β promoter	Weekly i.p. injections of the CT-α-syn Ab (9E4) and IgG1 control (10 mg/kg) for 6 mo.	9E4 C Terminus-α-syn antibody	Passive immunization with an Ab against the C-terminus of α-syn reduced memory and learning deficits and promoted α-syn clearance. Passive immunization reduced the accumulation and formation of CT fragments of α-syn[167]
PDGF- and mThy1-α-syn tg mice	Bi-weekly and monthly transfer of the antibody AFF1-AF488 or AF488 for 6 mo. This antibody recognizes specifically C-terminal human α-syn AlOH is used as adjuvant.	AFFITOPE PD01® (AFF1-AF488)	- Reduced oligomeric α-syn aggregates - Reduced astroglia (GFAP) and microglia (Iba1) immunostaining - Increased IL-2, IL-27, IL-1Ra and Fraktalkine - AFF1-AF488 co-localizes with α-syn and microglia[180]
Adoptive transfer of T cells: modulation of the adaptive immune response			
MPTP (20 mg/kg) 4 i.p. injections with 2 h intervals.	Splenocytes from WT or D3R ko mice were transferred i.v. (2×10^7 cells/mouse) into RAG1KO-recipient mice 21 d before MPTP-intoxication.	N.A.	D3R-deficiency protected against MPTP-induced dopaminergic cell loss and microglial activation. D3R-deficient mice become susceptible to MPTP upon transfer of wt $CD4^+$ T cells. D3R favored both T cell activation and acquisition of Th1 inflammatory phenotype.[87]
MPTP (18 mg/kg) 4 i.p. injections with 2 h intervals or PBS (vehicle ; 10 mL/kg).	*Immunization:* Cop-1 (200 μg) in CFA s.c. Animals were boosted twice every 14 d with an equivalent amount of Cop-1 in IFA. *Adoptive transfer:* Lymphoid cells in 250 μL Hank's solution were adoptively transferred i.v to separate groups of MPTP-intoxicated mice 12 to 18 h after last MPTP-injection.	Cop-1	Adoptive transfer of T cells from Cop-1 immunized mice into MPTP intoxicated mice lead to: - T cell accumulation in SN - Reduced dopaminergic neuron cell death - CD4 subset was responsible for protection.[165]
MPTP (18 mg/kg) 4 i.p. injections with 2 h intervals or PBS (vehicle ; 10 mL/kg). Twelve hrs after the last MPTP injection random mice received adoptive transfer.	*Immunization:* 200 μg of either Cop-1 or OVA in CFA. *Adoptive transfer:* MPTP-intoxicated mice received i.v. injection of $5x10^7$ splenocytes in 0.25 mL Hanks'solution.	Cop-1 OVA	Transfer of spleenocytes from Cop-1 Immunization animals into MPTP intoxicated host resulted in:. - Accumulation of T cells in SN - Lower microglia activation. - GDNF production Cop-1 immune cells stimulate the local production of GDNF by astrocytes.[170]
MPTP	Transfer of CD3 activated CD4+CD25+ T cells (Treg) into MPTP intoxicated mice	CD4CD25+ T cells	Adoptive transfer of CD4CD25+ T cells resulted in: - Dose dependent neuroprotection of DA neurons - Reduced microglia activation - Induction of CDNF and TGFb[166]
MPTP (16 mg/kg) 4 i.p. injections with 2 h intervals, or vehicle PBS (10 mL/k) 12 h after the last MPTP injection SPCs or Tregs were adoptively transferred.	*Immunization:* S.c. injection of N-4YSyn in CFA and boosted s.c. with N-4YSyn in IFA 2 wk after. *Adoptive transfer:* MPTP-intoxicated mice received an i.v. injection of $5x10^7$ SPCs or $1x10^6$ Tregs in 0.25 mL HBSS.	Nitrated-4YSyn VIP	Nitrated α-syn-induced neurotoxicity was Th17 cell-mediated, with $CD4^+CD25^+$ Treg dysfunction. VIP induced natural Tregs and reversed Nα-syn T cell nigrostriatal degeneration. Combinations of adoptively transferred Nα-syn and VIP immunocytes or natural Tregs administered to MPTP mice attenuated microglial inflammatory responses and led to nigrostrial protection.[101]
MPTP (16 mg/Kg) 2 s.c. injection at 2hrs intervals. Twelve hrs after the last MPTP injection received T cells or Treg.	*Immunization:* I.p. injection of recombinant GM-CSF (50 mg/Kg) daily for 5 d. *Adoptive transfer:* MPTP-intoxicated mice received purified $CD4^+$ (10^7 cells) or CD4CD25Foxp3+ cells (10^6 cells) i.v.	GM-CSF	Transfer of CD4CD25foxp3+ cells: - Reduced TH^+Nissl+ cells in SN - Had a very small effect on terminal survival in striatum - Reduced microglial density in SN - Laser capture followed by qRT-PCR showed that Treg transfer increased IL-27 15.42-fold.[182]

Abbreviations: DA, dopamine; α-syn, α-synuclein; DAT, dopamine transporter; SN, Substantia nigra; Tg, transgenic; Ab, antibodies; ko, knock out; VIP, vasoactive intestinal peptide; GM-CSF, Granulocyte macrophage colony stimulating factor; TH, Thyrosin Hydroxylasa; N.A., not applicable.

not PHA).[84,89,90] MPTP also reduced the LPS induced proliferation and antibody production in B cells.[84,90] MPTP-induced immuno-toxicity could be reversed by administrating DR agonists in the periphery,[84] such as sodium diethyldithiocarbonate (antioxidant acting on the monooxygenase signaling pathway),[89] indicating that the adverse effects of MPTP in adaptive immunity are related to DA metabolism and not due to an immune response to deal with DA cell death in brain. One should keep in mind that low DA doses (15.4–769 pg/mL) reduce ROS production and lower T cell propensity to oxidative stress-related apoptosis, while high doses (15 380–76 900 pg/mL) induce ROS production and increase the apoptotic propensity.[91]

Why lack of DR3 on lymphocytes protects from MPTP induced neurodegeneration may be an important question to resolve, since DA production by DCs determine the fate of T cell differentiation. In absence of DC-produced DA during T cell activation, T cells become Th1, while in its presence they become Th2.[70] However, with excess of DA (as in the case of generalized anxiety disorder) a Th17 phenotype is induced.[92] The polarization to the Th17 is probably mediated via DR5 (stimulatory receptor), as another study using the experimental autoimmune encephalitis model showed that mice were resistant to induction of pathology when DCs lacked DR5.[71] Furthermore, as mentioned above, not only DCs but also Tregs produce DA and this in turn can abrogate their suppression activity.[36,93] Another lesson to learn from MS is that IFNβ therapy blocks the inhibitory effect of DA on Tregs so they can suppress again and this correlated with decreased DR5 and TH expression.[94] The use of DA has actually already been proposed in the MS field to modulate immune responses (reviewed in ref. 95).

Altogether if DA signaling on T cells is an important T cell differentiation factor that modulates neuroinflammation in PD, we need to elucidate if this is taking place in the periphery, due to increased DA in plasma (by L-DOPA), or in lymph nodes where we anticipate that catecholamine innervation could be diminished. This is important because in lymph nodes most of the T cells will be naive, whereas in serum an important proportion will be activated/differentiated T cells. A recent study has shown that PD patients have ineffective Tregs[96] and Kipnis et al.[36] showed previously that DA abrogated CCR4 and CD44 expression in Treg but not effector cells. In the context of designing a vaccine for PD, this is of relevance because CCR4 is involved in tissue homing and CD44 is a marker for effector/memory activity, suggesting that DA affects the ability of Tregs to become active. If this proves to be the case during PD, this could lead to a loss of tolerance. However, comparative studies in the MPTP and 6-OHDA model, have shown that only MPTP (but not 6-OHDA) is able to alter the number of activated T cells, thus implying that other mechanisms before DA imbalance due to dopaminergic cell death are also at play during PD[49] (or at least in this PD-model). Thus if we are to therapeutically target the immune system to halt/prevent PD progression we need to understand the unique immune characteristics of the PD patient. Especially since we have observed that immunization of WT mice with WT α-syn and disease associated modified (nitrated) or fibrilar α-syn modulates dopamine receptor expression and induces specific migration/tolerance related molecules in a type and dose specific manner and this correlates with changes of microglia activation profiles (Sanchez-Guajardo et al. manuscript in preparation).

T cells are impaired in PD

The possible involvement of the adaptive immune system in PD and the fact that it is affected during PD has been a source of debate and much disbelief. However, there is mounting evidence showing the involvement of the adaptive immune system in PD: the observation of T cells in postmortem PD human brains,[26,97] findings in animal PD models[97-102] and data suggesting that the peripheral T cell compartment is altered in PD patients.[48-50,96,103-105] We will here concentrate on the last aspect, as we believe it to be crucial to evaluate the strengths and weaknesses of the PD patient immune system if we are to harness it to modulate processes in the brain. When studying the T cell compartment one should consider the changes in absolute cell counts, the distribution between the different stages of T cell function (naive, activated, effector/memory), the type of CD4 cells (TCRαβ vs. TCRγδ, $CD4^+$ vs. $CD4^+CD8^{lo}$, CD4:CD8 ratio), and the type of effector T cells (Th1/Th2/Th17/Treg, etc). We will address each aspect separately.

Is it the T cell number or the relative percentage of their different activation states that are affected in PD?

Regarding absolute cell numbers, there appears to be consensus of a net reduction in $CD4^+$ T cells in PD, although one study also reported decreased $CD8^+$ T cells.[49,50,105] This CD4 reduction is supported by the altered CD4:CD8 ratio described.[48,49,104] It is still unclear though, if this net reduction is due to the contraction of the CD4 T cell compartment as a whole or a change in balance between different activation stages.

The data regarding the activation state of $CD4^+$ T cells in PD, i.e., the proportion of naive ($CD45RA^+$), activated ($CD25^+$), and effector/memory ($CD45RO^+$) cells in PD seems conflicting. Especially if it is not considered as the percentage within the $CD4^+$ T cell population, but as absolute numbers (cell counts in plasma). Both the absolute numbers of naive $CD4^+$ cells and the percentage of $CD45RA^+$ cells were shown to be reduced.[49,96] Stevens et al. reported a decrease in total counts of effector/memory cells, but when expressed as the % within the $CD4^+$ cells it appeared to be increased, which is in accordance with other groups.[96,105,106]

The strongest discrepancy concerns the activated fractions: The percentage of cells expressing CD25 within the $CD4^+$ population has been reported to be equal[48,105,107] or increased.[49,50] Tregs are characterized by a high expression of CD25, higher than activated T cells. The detailed analysis of CD25 by Baba et al. showed that although the overall CD25 expression in PD patients was not altered, the $CD25^{hi}$ fraction was reduced.[48] This Treg specific alteration in PD is supported by Saunders at al., who showed ex vivo that PD-derived Tregs ($CD4^+CD25^+CD127^-$) have less suppressive activity than those from healthy controls. They, however, did not observe any changes in the percentage of Tregs compared with healthy controls.[96] Comparing both studies, Baba et al.

focused only on the $CD25^{hi}$ fraction within the $CD25^{+}$, so this may explain the different findings and further studies will have to resolve this matter. Akt kinase phosphorylation, another activation marker, is significantly increased in PD,[108] which is relevant since phosphorylated Akt prevents the induction of Foxp3 (reviewed in ref. 109). However, aging studies have reported that Treg absolute numbers increase with age, even in PD.[110,111]

Taken together, there seems to be an increase in the CD45RA population (effector/memory) to the apparent detriment of the CD45RO population (naive). Furthermore, it appears that the Treg compartment may be altered in PD, but further studies are needed to elucidate in which way.

Are there changes in the TCR subtypes of CD4 T cells during PD?

Not many studies have addressed the CD4 T cell compartment in PD from the TCR subtype point of view. Nevertheless, it was reported that there is an increase in the TCRγδ [103] and a decrease in the TRCαβ population,[105] thus increasing the net proportion of the TCRδγ subtype. This is interesting because: (1) TCRδγ cells can be activated in situ, i.e., they do not need to migrate to a lymph node or spleen, of relevance when thinking about activation processes in brain parenchyma[112]; (2) women, who have a lower incidence of PD, appear to have twice as much of this T cell subtype,[103] so this could be a reason for the observed gender bias in PD; and (3) an increase in activated TCRδγ in PD patients' serum and CFS has been observed,[103] pointing to a role for them in the disease.

Another subpopulation that has been studied but given conflicting results is the $CD4^{+}CD8^{lo}$: Hisanaga et al. have reported it increased, while Stevens et al. unchanged.[104,105] So further studies could possibly elucidate if these populations could be beneficial in PD and whether they should be targeted.

Are T cells in PD more sensitive to oxidative stress and prone to apoptosis?

The T cell compartment in PD has also been assessed in terms of its apoptotic propensity (CD95 = Fas receptor expression) and resistance to oxidative stress. PBMC from PD patients are more vulnerable to ex vivo induced oxidative stress and this vulnerability was reduced in patients treated with L-DOPA, suggesting that DA protects lymphocytes from oxidative stress.[113-115] Oxidative stress leads often to apoptosis, and there are several studies indicating that $CD4^{+}$ T cells in PD patients have an increased potential to become apoptotic, as they have a markedly higher percentage of CD95 expression,[50]. In particular this increase was noted in the CD4+CD25-CD45RA- population, which suggest that the main population affected may be the memory/effector.[116] Thus the increased effector/memory pool appears to have a higher susceptibility to apoptosis, suggesting that the oxidative damage observed in PD that leads to cell loss, is not restricted to the brain, but it is affecting T cells also.

The humoral response: Do autoantibodies play a role in PD?

B cell numbers are also decreased in PD[49,105] and their proliferation is also regulated by DA,[117] but little is known about the role of B cells and humoral responses during PD progression.

There is a comparable amount of anti-neuronal antibodies both in idiopathic and genetic parkinsonism,[118] but during aging the presence of IgG autoantibodies in serum is increased,[119] and the presence of autoantibodies may be related to debris clearance, as brain-reactive autoantibodies are found in all humans. Actually, there are many autoantibodies that are neither disease-inducing nor protective. Nevertheless, the elevated load of anti-neuronal antibodies found in PD directly correlated with depressive and dyskinetic symptoms.[120] Accordingly it has been proposed to use a panel based on autoantibodies for PD diagnose. The panel includes IgG antigens such as FRMD8, a diagnostic marker also proposed in AD, supporting a common process in neurodegenerative diseases related to protein aggregation.[121]

IgG deposited in neurons has been observed in brains from PD patients, while IgG receptors, FcγRI and II, were expressed on nearby activated microglia or lymphocyte-like cells respectively.[122] It has been suggested that IgG infiltration is due to BBB damage/leakage and brain disease.[123] Auto-antibodies are also found in CSF from PD patients and they are able to react with DAergic neurons in SN.[124] A deleterious effect of these autoantibodies is suggested by the cytotoxic effect of PD derived IgG on DAergic nigral neurons in mice,[125] which appears to be mediated by FcR,[126] and also by the observation that antibodies from PD patients react with proteins oxidized by DA.[127] However, the presence of antibodies against neuromelanin in PD and the fact that these levels were inversely correlated with disease progresion, suggest a role for IgG in the clearance of cellular components upon cell death.[128] Thus the humoral response may also contribute to help the brain to cope with pathology, and accordingly IgG deposition correlates with neuronal survival.[122] Interestingly in MS, B cells and their MS related antibodies (reviewed in ref. 129-133) can be both beneficial and detrimental depending on the subtype of B cell,[134,135] (reviewed in ref. 136) and if they are infected by the Epstein–Barr virus (reviewed in ref. 137). This aspect should be kept in mind, as for example in MS, therapeutic use of rituximab (anti CD20 antibody) shows variable results, as it depletes both the pathological B cells and the ones that are protective.[138,139]

There is evidence that anti-α-syn antibodies are present in serum, albeit the levels and how they relate to disease progression in PD is still unclear. One research group described elevated anti-α-syn in serum from inherited PD but not in sporadic.[140] Elevated autoantibodies against α-syn, myelin related antigens and S100B have been found in LB-associated dementia, both in serum and CSF.[141] Furthermore, antibodies recognizing WT α-syn and its fibrilar aggregated form are found early in the disease to later decrease during PD progression,[142] suggesting a protective role for IgG during the early stages of PD progression. Another study suggests on the contrary that anti-α-syn antibodies were decreased in PD serum vs. controls or AD patients.[143] A detailed study of the specificity of the anti-α-syn antibodies as regards its aggregated/modified forms, titer, and its relation to disease progression is needed to clarify the possible beneficial effect of anti-α-syn IgG in PD. One could nevertheless hypothesize that the presence of specific antibodies against α-syn may be protective, while other anti-neuronal antibodies that could appear in the later stages of the disease, as it was described for antibodies against S100, GAFP, NK,P and MP-65,[144] could be deleterious. For example the presence of anti-heat shock proteins 65 and 70 found in CSF of PD patients, could be contributing

to the disease,[145] while it has been shown that antibodies help clearing extracellular α-syn and prevent aggregate transmission to other neighboring cells.[146]

Antibody mediated autoimmune diseases might develop because of a failure of Tregs to control antibody production (reviewed in ref. 147). In fact, the ability of mature B cells to generate high affinity self-reactive antigen receptors through somatic hypermutation is a constant threat, and it is believed that $CD4^+$ T-cell mediated tolerance is the dominant factor preventing autoreactive B cells.[148] For this reason, it is important to assess the role that B cells play in the etiology of PD: Does the activation of B cells promote pathology or might B cells have a regulatory/beneficial role at the early stages of the disease? Is the type of B cell response dependent on the sub-type/activation state of the cells, as seen in Experimental Autoimmune Encephalitis (EAE), or do all B cells mount the same type of response in PD? As we discussed above, there are many types of auto-antibodies produced during PD and not all of them correlate with disease severity. Additionally, B cell stimulation via TLR-signaling suppresses inflammatory T cell responses (both Th1 and Th17) resulting in recovery from EAE.[149] Indeed, it is known that B cells can be used to induce tolerance, program T cell responses, activate the Treg response via IgG and act as immune regulators directly.[150-153]

Immunotherapeutic Strategies For PD

During the last decade the study of the access of immune cells, such T-cells, into the CNS has been extensively developed (for a review see ref. 154). Pioneering work from several researchers is now being exploited to design novel therapies aiming at T-cells for the treatment of neurodegenerative diseases (for a review see refs. 21,22,155-157)

The current PD immunoregulatory therapies based on vaccine design can be divided into 2 strategies: One is based on generating antibodies against α-syn,[98,158,159] and the other one aims at the induction of a particular T cell response to modulate the neuroinflammatory response.[87,98,101,102,160-166] The first strategy has as primary goal the removal of α-syn aggregates, as it is has been shown that this will modify the course of the disease.[146,158,167] The second strategy targets microglia, as neuroinflammation has long been recognized to exacerbate the disease (reviewed in 19 and 21). A summary of all the recently designed immunotherapies used in experimental animal PD models is presented in **Table 3**.

Modulating inflammatory processes by inducing Treg

The effect of modulating the adaptive immune response, in particular the T cell response, to change the microglia response in brain, has been studied by: (1) Vaccination strategies with a broad series of antigens using the MPTP, 6-OHDA, rAAV-α-syn and α-syn transgenic PD models,[98,158,162,168,169] and (2) the adoptive transfer of previously in vitro activated T cells or purified from immunized mice into MTPT intoxicated animals.[101,102,165,166,170] All these approaches have in common the reduction of neuronal cell death and modulation of the microglia response. This was achieved whether a non-PD-related antigen was used as immunogen (VIP, CFS, MOG, BCG, COP-1) or α-syn.

Benner, et al., showed that adoptive transfer of lymphoid cells from Cop-1^c immunized mice into MPTP intoxicated animals leads to T cell accumulation, GDNF induction and a modulation of microglial responses,[170] and that the Cop-1 activated $CD4^+$ T cells were responsible for the neuroprotection.[165] Further studies from this group later showed for the first time the key role of Treg in inducing neuroprotection in the MPTP model. Reynolds, et al., transferred CD3 activated $CD4CD25^+$ T cells into MPTP intoxicated animals and observed protection of the nigrostriatal system correlating with TGFβ and CDNF production.[166] Using in vitro studies they demonstrated that Treg modulated detrimental redox reactions and NF-Kb activation by microglia,[102] as CD4CD25+ T cells can modify microglia's protein expression profile.[171] They further showed in the MPTP model that N-4YSyn immunization induced a Th17 cell response and resulted in Treg dysfunction, but adoptive transfer of Treg from VIP immunized animals attenuated Nα-syn induced microglial inflammatory responses and led to nigrostratial protection in the same model.[101] Nα-syn is known to induce cell death in SH-SY5Y cells[172,173] and to have a pathological effect on α-syn influencing its aggregation,[174-176] but it is also accumulated in DAergic neurons of the SN of monkeys as a normal result of aging.[177] Thus Nα-syn could help break immune tolerance as it accumulates with age and ultimately induce neurodegeneration. This principle was elegantly shown by the adoptive transfer of T cells from an Nα-syn immunized mice into a lymphocyte deficient MPTP intoxicated mice, where the transfer induced dopaminergic cell death in the otherwise resistant MPTP intoxicated mice.[178]

Support for the role of Treg in mediating neuroprotection in the MPTP-PD animal model has also come from different vaccination strategies by other research groups. BCG vaccination resulted in increased number and frequency of splenic Tregs, increased DA levels and restoration of DAergic neurons.[161] BCG is closely related to the active biological component in CFA, *Mycobacterium tuberculosis*, so probably the reported neuroprotective effect of CFA was also Treg mediated.[162,169] Bee venom (BV) has also been used as a vaccine to prevent degeneration of dopaminergic neurons.[163] The neuroprotective effect of BV was associated with microglial deactivation and the significant increase in the proportion of $CD4^+CD25^+Foxp3^+$ Tregs.[163] Treg are also protective during α-syn induced pathology, as vaccination with human recombinant α-syn 10 wk prior to the unilateral induction of α-syn overexpression in the nigrostriatal pathway also led to the infiltration of $CD4^+Foxp3^+$ cells and their enrichment in striatum through time, as well as a strong IgG titration and deposition. This correlated with 66% reduction of striatal α-syn-related terminal pathology and GDNF induction, as well as changes of cytokines and other soluble products in serum.[98]

Inducing a protective humoral response to clear α-syn aggregates

Immune protection is also mediated by the humoral response, and the induction of therapeutic humoral responses against α-syn has been achieved through 2 different approaches: (1) Active immunization where one induces the production of antigen specific antibodies,[158] and (2) passive immunization

which involves administration of anti-α-syn antibodies to the PD-animal model.[146,167] Active immunization consists of generating an immune response involving both T and B cells toward the immunizing agent, as part of this response antibodies against the immunizing agent will be produced. This response can be directed towards a specific type of immune response by carefully choosing the type and dose of the antigen as well as the adjuvant used. Given the fact that the α-syn aggregates (the main target of humoral therapies in PD) are composed of self-proteins, these immunotherapies need to consider the possible induction of inflammatory autoimmunity mediated by Th17 cells, which have been shown to be involved in the neuroinflammatory process.[101,179] However, in none of the studies using antibodies to induce protection has the T cell compartment been studied.

Passive immunization is achieved by the administration of antibodies against a specific protein, so as to target the protein in question to its removal through complement and/or FcR. Passive immunization strategies where α-syn specific antibodies are directly transferred to the host, transgenically expressing α-syn, is protective.[146,167] Bae, et al., showed that antibodies against α-syn targets and aids clearance of the extracellular protein by microglia.[146] Masliah, et al., used an antibody against the C-terminal fraction of α-syn, which was able to promote clearance of α-syn aggregates and reduce the accumulation and formation of C-terminal fragments of α-syn.[167] Recently, a new vaccination strategy using an antibody that recognizes a peptide sequence small enough to activate B cells but not T cells has been used in 2 α-syn transgenic animals to successfully clear oligomeric α-syn in brain. This approach modified glial activity and cytokine profiles.[180] There is currently an ongoing clinical trial using this antibody in early PD patients (ClinicalTrials.gov/show/NCT01568099). The authors further showed that this vaccination strategy did not generate Th1/Th2 cells upon ex vivo stimulation of immunized WT mice nor induced T cell infiltration in parenchyma. Unfortunately they did not test for Treg/Th17 generation nor did they test their vaccination strategy in an animal overexpressing the protein for which the vaccine was specific (e.i. human C-terminal α-syn). The AFFITOPE® used was shown to not recognize mouse α-syn, and thus the antigen that would induce brain homing and T cell activation was not present in the animals where they checked for T cell migration.

We believe that a complementary strategy to triggering Treg is to induce IgG that will help clearing excess α-syn before it becomes pathological. Indeed B cells have been described to be involved in tolerance induction by acting on DCs.[149] Modulation of DC may well be a critical factor in reestablishing tolerance as seen in a recent article where granulocyte macrophage colony stimulating factor (GM-CSF), which is able to induce tolerogenic DCs that expand and/or induce Treg[181], was neuroprotective in the MPTP PD model[182]; although in vitro studies suggests GM-CSF could have a protective effect directly over dopaminergic neurons.[183] Notably, this approach is now on clinical trials for PD patients since GM-CSF was already approved for human use in cases where enhancement of bone marrow cell production was needed (ClinicalTrials.gov/ct2/show/record/NCT01882010). IgGs are also able to induce Treg, as shown by the intravenous delivery of unspecific Ig (IVIg) that increase the number of splenic Tregs.[184] Unfortunately, the treatment was not effective in the MPTP model; whether this was due to lack of specificity of the Treg induced by IVIg (thus did not expand when challenged by the antigens induced by MPTP) or the immune response was neutralized by MPTP's immunotoxic effect cannot be discerned. So it appears that in order to ensure a good vaccination strategy, we have to fine tune the exact T cell activation needed.

What is required to induce a good immunomodulating therapy?

We know that $CD4^+CD25^+Foxp3$ regulatory T cells inhibit autoimmunity and protect against tissue injury,[179] in the case of PD, against neuronal death caused by the aggregation of α-syn. Thus the best therapy for the treatment of PD appears to be the one that approaches enhancing Treg cell functions to restore tolerance to α-syn and thereby protect the neurons against the detrimental chronic inflammatory response mounted by the immune system as a response to α-syn toxicity. Tolerance still remains a fundamental concept of modern immunology[147] and one has to consider that the induction of Treg could be coupled to therapeutic IgG production. B cells can also influence the T cell response indirectly by modulating DCs[148] and both effects are probably mediated by IL-10 production.[148] Alternatively, B cells could be directly involved in the generation of Tregs: Mann, et al., found that B cell deficiency resulted in a delay in the emergence of Foxp3 expressing Treg cells and IL-10 in the CNS during EAE, but not in the periphery, and that reconstitution with wild type B cells resulted in disease recovery and normalized IL-10 and Foxp3 expression.[185]

In summary, the need for an adequate vaccine for PD where the natural tolerance to α-syn is boosted without inducing autoimmunity is of extreme importance. To achieve this we need to understand the interplay between T and B cells, and how we can profit from it to generate a response that will harness the neuroinflammatory process and help clear α-syn aggregates.

Conclusion

Almost 10 years ago M Schwartz and J Kipnis hypothesized that during neurodegeneration DA or another brain compound would peripherally suppress Tregs and give rise to T cells with specificity to brain self-protein. These cells would then migrate to brain, activate/modulate microglia and once the insult was controlled, Treg would again suppress these protective autoimmune cells, as the periphery would return to normal.[157] We now bring forward the hypothesis that in PD the periphery never normalizes, due to loss of DA and increase of oxidation products and α-syn in serum. This results in the adaptive immune response to engage in a vicious circle of autoimmune inflammation with microglia, where the originally protective T cell response becomes detrimental, each potentiating the inflammatory reaction of the other population. With time this will alter the cytokine levels in serum and further damage the T cell compartment. At the same time, what appears to be a beneficial humoral response, aimed at removing excess α-syn at the beginning of the disease

would become detrimental as the number of autoreactive antibodies increases in repertoire. Thus, adding to the pioneering work of Mosley and Gendelman in the field, we suggest that any immunoregulatory therapy based on manipulation of the adaptive immune system should be addressed to restore natural tolerance against α-syn, so that Treg can suppress the autoreactive T cells in brain and induce a benign microglia reaction. We further believe that for the vaccination strategy to be persistently protective, and as suggested by the work of Masliah and coworkers, IgG against α-syn has to be concomitantly generated to aid neurons deal with the accumulation of α-syn before the formation of its pathological α-syn forms. Boosting only one of these 2 arms of the adaptive immunity, in our opinion, would not be beneficial in the long run because: either α-syn will continue accumulating, or the chronic neuroinflammation will persist. After all, for a good B cell response you need T cells, and B cells provide help to T cells to maintain their effector function.

Disclosure of Potential Conflicts of Interest

The authors declare that they do not have any conflicting interest.

Acknowledgments

M.vE.C. is recipient of a PhD grant from CONACyT (Mexico), V.S.G. and M.R.R. have had their work herein cited supported by the Lundbeck Foundation (VSG/MRR), the MJF Foundation (VSG/MRR), the Parkinsons Forening (MRR), and the Familie Hede Nielsens fond (VSG).

Endnotes

[a]There is one single study that did not find such increase.
[b]In the study in ref. 87 the patients were all taking amantidine and in ref. 85 they were under L-dopa treatment.
[c]Cop-1 is a TCR agonist that blocks MHCII function and induces Treg.

References

1. Martí MJ, Tolosa E, Campdelacreu J. Clinical overview of the synucleinopathies. Mov Disord 2003; 18(Suppl 6):S21-7; PMID:14502652; http://dx.doi.org/10.1002/mds.10559
2. Gaig C, Tolosa E. When does Parkinson's disease begin? Mov Disord 2009; 24(Suppl 2):S656-64; PMID:19877243; http://dx.doi.org/10.1002/mds.22672
3. Farrer M, Kachergus J, Forno L, Lincoln S, Wang DS, Hulihan M, Maraganore D, Gwinn-Hardy K, Wszolek Z, Dickson D, et al. Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. Ann Neurol 2004; 55:174-9; PMID:14755720; http://dx.doi.org/10.1002/ana.10846
4. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schöls L, Riess O. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 1998; 18:106-8; PMID:9462735; http://dx.doi.org/10.1038/ng0298-106
5. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997; 276:2045-7; PMID:9197268; http://dx.doi.org/10.1126/science.276.5321.2045
6. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science 2003; 302:841; PMID:14593171; http://dx.doi.org/10.1126/science.1090278
7. Zarranz JJ, Alegre J, Gómez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atarés B, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 2004; 55:164-73; PMID:14755719; http://dx.doi.org/10.1002/ana.10795
8. Trojanowski JQ, Lee VM. Aggregation of neurofilament and alpha-synuclein proteins in Lewy bodies: implications for the pathogenesis of Parkinson disease and Lewy body dementia. Arch Neurol 1998; 55:151-2; PMID:9482355; http://dx.doi.org/10.1001/archneur.55.2.151
9. Gao HM, Kotzbauer PT, Uryu K, Leight S, Trojanowski JQ, Lee VM. Neuroinflammation and oxidation/nitration of alpha-synuclein linked to dopaminergic neurodegeneration. J Neurosci 2008; 28:7687-98; PMID:18650345; http://dx.doi.org/10.1523/JNEUROSCI.0143-07.2008
10. Liu CW, Giasson BI, Lewis KA, Lee VM, Demartino GN, Thomas PJ. A precipitating role for truncated alpha-synuclein and the proteasome in alpha-synuclein aggregation: implications for pathogenesis of Parkinson disease. J Biol Chem 2005; 280:22670-8; PMID:15840579; http://dx.doi.org/10.1074/jbc.M501508200
11. Lee JT, Wheeler TC, Li L, Chin LS. Ubiquitination of alpha-synuclein by Siah-1 promotes alpha-synuclein aggregation and apoptotic cell death. Hum Mol Genet 2008; 17:906-17; PMID:18065497; http://dx.doi.org/10.1093/hmg/ddm363
12. Lo Bianco C, Shorter J, Régulier E, Lashuel H, Iwatsubo T, Lindquist S, Aebischer P. Hsp104 antagonizes alpha-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease. J Clin Invest 2008; 118:3087-97; PMID:18704197; http://dx.doi.org/10.1172/JCI35781
13. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 2012; 338:949-53; PMID:23161999; http://dx.doi.org/10.1126/science.1227157
14. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. J Exp Med 2012; 209:975-86; PMID:22508839; http://dx.doi.org/10.1084/jem.20112457
15. Bae EJ, Ho DH, Park E, Jung JW, Cho K, Hong JH, Lee HJ, Kim KP, Lee SJ. Lipid peroxidation product 4-hydroxy-2-nonenal promotes seeding-capable oligomer formation and cell-to-cell transfer of α-synuclein. Antioxid Redox Signal 2013; 18:770-83; PMID:22867050; http://dx.doi.org/10.1089/ars.2011.4429
16. Volpicelli-Daley LA, Luk KC, Patel TP, Tanik SA, Riddle DM, Stieber A, Meaney DF, Trojanowski JQ, Lee VM. Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron 2011; 72:57-71; PMID:21982369; http://dx.doi.org/10.1016/j.neuron.2011.08.033
17. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003; 24:197-211; PMID:12498954; http://dx.doi.org/10.1016/S0197-4580(02)00065-9
18. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 1998; 95:6469-73; PMID:9600990; http://dx.doi.org/10.1073/pnas.95.11.6469
19. Sanchez-Guajardo V, Barnum CJ, Tansey MG, Romero-Ramos M. Neuroimmunological processes in Parkinson's disease and their relation to α-synuclein: microglia as the referee between neuronal processes and peripheral immunity. ASN Neuro 2013; 5:113-39; PMID:23506036; http://dx.doi.org/10.1042/AN20120066
20. Stone DK, Reynolds AD, Mosley RL, Gendelman HE. Innate and adaptive immunity for the pathobiology of Parkinson's disease. Antioxid Redox Signal 2009; 11:2151-66; PMID:19243239; http://dx.doi.org/10.1089/ars.2009.2460
21. Valera E, Masliah E. Immunotherapy for neurodegenerative diseases: focus on α-synucleinopathies. Pharmacol Ther 2013; 138:311-22; PMID:23384597; http://dx.doi.org/10.1016/j.pharmthera.2013.01.013
22. Hutter-Saunders JA, Mosley RL, Gendelman HE. Pathways towards an effective immunotherapy for Parkinson's disease. Expert Rev Neurother 2011; 11:1703-15; PMID:22091596; http://dx.doi.org/10.1586/ern.11.163
23. Chu K, Zhou X, Luo BY. Cytokine gene polymorphisms and Parkinson's disease: a meta-analysis. Can J Neurol Sci 2012; 39:58-64; PMID:22384497
24. Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. J Neural Transm Suppl 2000; 58:143-51; PMID:11128604
25. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. Neurosci Lett 1994; 172:151-4; PMID:8084523; http://dx.doi.org/10.1016/0304-3940(94)90684-X
26. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 1988; 38:1285-91; PMID:3399080; http://dx.doi.org/10.1212/WNL.38.8.1285
27. Tansey MG, McCoy MK, Frank-Cannon TC. Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention. Exp Neurol 2007; 208:1-25; PMID:17720159; http://dx.doi.org/10.1016/j.expneurol.2007.07.004

28. Myers CL, Wertheimer SJ, Schembri-King J, Parks T, Wallace RW. Induction of ICAM-1 by TNF-alpha, IL-1 beta, and LPS in human endothelial cells after downregulation of PKC. Am J Physiol 1992; 263:C767-72; PMID:1357985
29. Mbow ML, Rutti B, Brossard M. Infiltration of CD4+ CD8+ T cells, and expression of ICAM-1, Ia antigens, IL-1 alpha and TNF-alpha in the skin lesion of BALB/c mice undergoing repeated infestations with nymphal Ixodes ricinus ticks. Immunology 1994; 82:596-602; PMID:7835923
30. Scalzo P, Kümmer A, Cardoso F, Teixeira AL. Increased serum levels of soluble tumor necrosis factor-alpha receptor-1 in patients with Parkinson's disease. J Neuroimmunol 2009; 216:122-5; PMID:19732964; http://dx.doi.org/10.1016/j.jneuroim.2009.08.001
31. McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J Neuroinflammation 2008; 5:45; PMID:18925972; http://dx.doi.org/10.1186/1742-2094-5-45
32. Tansey MG, Szymkowski DE. The TNF superfamily in 2009: new pathways, new indications, and new drugs. Drug Discov Today 2009; 14:1082-8; PMID:19837186; http://dx.doi.org/10.1016/j.drudis.2009.10.002
33. Barnum CJ, Tansey MG. The duality of TNF signaling outcomes in the brain: potential mechanisms? Exp Neurol 2011; 229:198-200; PMID:21377463; http://dx.doi.org/10.1016/j.expneurol.2011.02.016
34. Menza M, Dobkin RD, Marin H, Mark MH, Gara M, Bienfait K, Dicke A, Kusnekov A. The role of inflammatory cytokines in cognition and other non-motor symptoms of Parkinson's disease. Psychosomatics 2010; 51:474-9; PMID:21051678
35. Lindqvist D, Kaufman E, Brundin L, Hall S, Surova Y, Hansson O. Non-motor symptoms in patients with Parkinson's disease - correlations with inflammatory cytokines in serum. PLoS One 2012; 7:e47387; PMID:23082161; http://dx.doi.org/10.1371/journal.pone.0047387
36. Kipnis J, Cardon M, Avidan H, Lewitus GM, Mordechay S, Rolls A, Shani Y, Schwartz M. Dopamine, through the extracellular signal-regulated kinase pathway, downregulates CD4+CD25+ regulatory T-cell activity: implications for neurodegeneration. J Neurosci 2004; 24:6133-43; PMID:15240805; http://dx.doi.org/10.1523/JNEUROSCI.0600-04.2004
37. Boschetti G, Nancey S, Sardi F, Roblin X, Flourié B, Kaiserlian D. Therapy with anti-TNFα antibody enhances number and function of Foxp3(+) regulatory T cells in inflammatory bowel diseases. Inflamm Bowel Dis 2011; 17:160-70; PMID:20848510; http://dx.doi.org/10.1002/ibd.21308
38. Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. J Exp Med 2004; 200:277-85; PMID:15280421; http://dx.doi.org/10.1084/jem.20040165
39. Harms AS, Barnum CJ, Ruhn KA, Varghese S, Treviño I, Blesch A, Tansey MG. Delayed dominant-negative TNF gene therapy halts progressive loss of nigral dopaminergic neurons in a rat model of Parkinson's disease. Mol Ther 2011; 19:46-52; PMID:20959812; http://dx.doi.org/10.1038/mt.2010.217
40. McCoy MK, Ruhn KA, Martinez TN, McAlpine FE, Blesch A, Tansey MG. Intranigral lentiviral delivery of dominant-negative TNF attenuates neurodegeneration and behavioral deficits in hemiparkinsonian rats. Mol Ther 2008; 16:1572-9; PMID:18628756; http://dx.doi.org/10.1038/mt.2008.146
41. Katsarou Z, Bostantjopoulou S, Hatzizisi O, Giza E, Soler-Cardona A, Kyriazis G. [Immune factors or depression? Fatigue correlates in Parkinson's disease]. Rev Neurol 2007; 45:725-8; PMID:18075986
42. Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. Neurosci Lett 1996; 211:13-6; PMID:8809836; http://dx.doi.org/10.1016/0304-3940(96)12706-3
43. Brodacki B, Staszewski J, Toczyłowska B, Kozłowska E, Drela N, Chalimoniuk M, Stepien A. Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFalpha, and INFgamma concentrations are elevated in patients with atypical and idiopathic parkinsonism. Neurosci Lett 2008; 441:158-62; PMID:18582534; http://dx.doi.org/10.1016/j.neulet.2008.06.040
44. Mogi M, Harada M, Kondo T, Riederer P, Nagatsu T. Interleukin-2 but not basic fibroblast growth factor is elevated in parkinsonian brain. Short communication. J Neural Transm 1996; 103:1077-81; PMID:9013395; http://dx.doi.org/10.1007/BF01291792
45. Blum-Degen D, Müller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 1995; 202:17-20; PMID:8787820; http://dx.doi.org/10.1016/0304-3940(95)12192-7
46. Almeida AR, Legrand N, Papiernik M, Freitas AA. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. J Immunol 2002; 169:4850-60; PMID:12391195
47. Almeida AR, Zaragoza B, Freitas AA. Indexation as a novel mechanism of lymphocyte homeostasis: the number of CD4+CD25+ regulatory T cells is indexed to the number of IL-2-producing cells. J Immunol 2006; 177:192-200; PMID:16785514
48. Baba Y, Kuroiwa A, Uitti RJ, Wszolek ZK, Yamada T. Alterations of T-lymphocyte populations in Parkinson disease. Parkinsonism Relat Disord 2005; 11:493-8; PMID:16154792; http://dx.doi.org/10.1016/j.parkreldis.2005.07.005
49. Bas J, Calopa M, Mestre M, Molleví DG, Cutillas B, Ambrosio S, Buendia E. Lymphocyte populations in Parkinson's disease and in rat models of parkinsonism. J Neuroimmunol 2001; 113:146-52; PMID:11137586; http://dx.doi.org/10.1016/S0165-5728(00)00422-7
50. Gruden MA, Sewell RD, Yanamandra K, Davidova TV, Kucheryanu VG, Bocharov EV, Bocharova OR, Polyschuk VV, Sherstnev VV, Morozova-Roche LA. Immunoprotection against toxic biomarkers is retained during Parkinson's disease progression. J Neuroimmunol 2011; 233:221-7; PMID:21239064; http://dx.doi.org/10.1016/j.jneuroim.2010.12.001
51. Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 2005; 6:1219-27; PMID:16244650; http://dx.doi.org/10.1038/ni1265
52. Zheng SG, Wang J, Wang P, Gray JD, Horwitz DA. IL-2 is essential for TGF-beta to convert naive CD4+CD25- cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. J Immunol 2007; 178:2018-27; PMID:17277105
53. Müller T, Blum-Degen D, Przuntek H, Kuhn W. Interleukin-6 levels in cerebrospinal fluid inversely correlate to severity of Parkinson's disease. Acta Neurol Scand 1998; 98:142-4; PMID:9724016; http://dx.doi.org/10.1111/j.1600-0404.1998.tb01736.x
54. Hofmann KW, Schuh AF, Saute J, Townsend R, Fricke D, Leke R, Souza DO, Portela LV, Chaves ML, Rieder CR. Interleukin-6 serum levels in patients with Parkinson's disease. Neurochem Res 2009; 34:1401-4; PMID:19214748; http://dx.doi.org/10.1007/s11064-009-9921-z
55. Selikhova MV, Kushlinskii NE, Lyubimova NV, Gusev EI. Impaired production of plasma interleukin-6 in patients with Parkinson's disease. Bull Exp Biol Med 2002; 133:81-3; PMID:12170314; http://dx.doi.org/10.1023/A:1015120930920
56. Scalzo P, Kümmer A, Cardoso F, Teixeira AL. Serum levels of interleukin-6 are elevated in patients with Parkinson's disease and correlate with physical performance. Neurosci Lett 2010; 468:56-8; PMID:19857551; http://dx.doi.org/10.1016/j.neulet.2009.10.062
57. Zhou L, Littman DR. Transcriptional regulatory networks in Th17 cell differentiation. Curr Opin Immunol 2009; 21:146-52; PMID:19328669; http://dx.doi.org/10.1016/j.coi.2009.03.001
58. Rentzos M, Nikolaou C, Andreadou E, Paraskevas GP, Rombos A, Zoga M, Tsoutsou A, Boufidou F, Kapaki E, Vassilopoulos D. Circulating interleukin-15 and RANTES chemokine in Parkinson's disease. Acta Neurol Scand 2007; 116:374-9; PMID:17986095; http://dx.doi.org/10.1111/j.1600-0404.2007.00894.x
59. Shimoji M, Pagan F, Healton EB, Mocchetti I. CXCR4 and CXCL12 expression is increased in the nigro-striatal system of Parkinson's disease. Neurotox Res 2009; 16:318-28; PMID:19551455; http://dx.doi.org/10.1007/s12640-009-9076-3
60. Klüter H, Vieregge P, Stolze H, Kirchner H. Defective production of interleukin-2 in patients with idiopathic Parkinson's disease. J Neurol Sci 1995; 133:134-9; PMID:8583216; http://dx.doi.org/10.1016/0022-510X(95)00180-A
61. Bessler H, Djaldetti R, Salman H, Bergman M, Djaldetti M. IL-1 beta, IL-2, IL-6 and TNF-alpha production by peripheral blood mononuclear cells from patients with Parkinson's disease. Biomed Pharmacother 1999; 53:141-5; PMID:10349502; http://dx.doi.org/10.1016/S0753-3322(99)80079-1
62. Hasegawa Y, Inagaki T, Sawada M, Suzumura A. Impaired cytokine production by peripheral blood mononuclear cells and monocytes/macrophages in Parkinson's disease. Acta Neurol Scand 2000; 101:159-64; PMID:10705937; http://dx.doi.org/10.1034/j.1600-0404.2000.101003159.x
63. Wandinger KP, Hagenah JM, Klüter H, Rothermundt M, Peters M, Vieregge P. Effects of amantadine treatment on in vitro production of interleukin-2 in de-novo patients with idiopathic Parkinson's disease. J Neuroimmunol 1999; 98:214-20; PMID:10430055; http://dx.doi.org/10.1016/S0165-5728(99)00093-4
64. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M. Peripheral cytokines profile in Parkinson's disease. Brain Behav Immun 2009; 23:55-63; PMID:18678243; http://dx.doi.org/10.1016/j.bbi.2008.07.003
65. Pacheco R, Riquelme E, Kalergis AM. Emerging evidence for the role of neurotransmitters in the modulation of T cell responses to cognate ligands. Cent Nerv Syst Agents Med Chem 2010; 10:65-83; PMID:20236043; http://dx.doi.org/10.2174/187152410790780154
66. Pacheco R, Prado CE, Barrientos MJ, Bernales S. Role of dopamine in the physiology of T-cells and dendritic cells. J Neuroimmunol 2009; 216:8-19; PMID:19732962; http://dx.doi.org/10.1016/j.jneuroim.2009.07.018
67. Mignini F, Streccioni V, Amenta F. Autonomic innervation of immune organs and neuroimmune modulation. Auton Autacoid Pharmacol 2003; 23:1-25; PMID:14565534; http://dx.doi.org/10.1046/j.1474-8673.2003.00280.x
68. Saha B, Mondal AC, Majumder J, Basu S, Dasgupta PS. Physiological concentrations of dopamine inhibit the proliferation and cytotoxicity of human CD4+ and CD8+ T cells in vitro: a receptor-mediated mechanism. Neuroimmunomodulation 2001; 9:23-33; PMID:11435749; http://dx.doi.org/10.1159/000049004

69. Saha B, Mondal AC, Basu S, Dasgupta PS. Circulating dopamine level, in lung carcinoma patients, inhibits proliferation and cytotoxicity of CD4+ and CD8+ T cells by D1 dopamine receptors: an in vitro analysis. Int Immunopharmacol 2001; 1:1363-74; PMID:11460316; http://dx.doi.org/10.1016/S1567-5769(01)00068-6
70. Nakano K, Higashi T, Takagi R, Hashimoto K, Tanaka Y, Matsushita S. Dopamine released by dendritic cells polarizes Th2 differentiation. Int Immunol 2009; 21:645-54; PMID:19332443; http://dx.doi.org/10.1093/intimm/dxp033
71. Prado C, Contreras F, González H, Díaz P, Elgueta D, Barrientos M, Herrada AA, Lladser A, Bernales S, Pacheco R. Stimulation of dopamine receptor D5 expressed on dendritic cells potentiates Th17-mediated immunity. J Immunol 2012; 188:3062-70; PMID:22379034; http://dx.doi.org/10.4049/jimmunol.1103096
72. Josefsson E, Bergquist J, Ekman R, Tarkowski A. Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. Immunology 1996; 88:140-6; PMID:8707341; http://dx.doi.org/10.1046/j.1365-2567.1996.d01-653.x
73. Bergquist J, Tarkowski A, Ekman R, Ewing A. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc Natl Acad Sci U S A 1994; 91:12912-6; PMID:7809145; http://dx.doi.org/10.1073/pnas.91.26.12912
74. Cosentino M, Zaffaroni M, Marino F, Bombelli R, Ferrari M, Rasini E, Lecchini S, Ghezzi A, Frigo G. Catecholamine production and tyrosine hydroxylase expression in peripheral blood mononuclear cells from multiple sclerosis patients: effect of cell stimulation and possible relevance for activation-induced apoptosis. J Neuroimmunol 2002; 133:233-40; PMID:12446028; http://dx.doi.org/10.1016/S0165-5728(02)00372-7
75. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S. The immunoregulatory role of dopamine: an update. Brain Behav Immun 2010; 24:525-8; PMID:19896530; http://dx.doi.org/10.1016/j.bbi.2009.10.015
76. Levite M. Neurotransmitters activate T-cells and elicit crucial functions via neurotransmitter receptors. Curr Opin Pharmacol 2008; 8:460-71; PMID:18579442; http://dx.doi.org/10.1016/j.coph.2008.05.001
77. Dethy S, Laute MA, Van Blercom N, Damhaut P, Goldman S, Hildebrand J. Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients. Clin Chem 1997; 43:740-4; PMID:9166225
78. Amenta F, Bronzetti E, Cantalamessa F, El-Assouad D, Felici L, Ricci A, Tayebati SK. Identification of dopamine plasma membrane and vesicular transporters in human peripheral blood lymphocytes. J Neuroimmunol 2001; 117:133-42; PMID:11431013; http://dx.doi.org/10.1016/S0165-5728(01)00317-4
79. Mill J, Asherson P, Browes C, D'Souza U, Craig I. Expression of the dopamine transporter gene is regulated by the 3′ UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet 2002; 114:975-9; PMID:12457396; http://dx.doi.org/10.1002/ajmg.b.10948
80. Gordon J, Barnes NM. Lymphocytes transport serotonin and dopamine: agony or ecstasy? Trends Immunol 2003; 24:438-43; PMID:12909457; http://dx.doi.org/10.1016/S1471-4906(03)00176-5
81. Carr L, Tucker A, Fernandez-Botran R. In vivo administration of L-dopa or dopamine decreases the number of splenic IFN gamma-producing cells. J Neuroimmunol 2003; 137:87-93; PMID:12667651; http://dx.doi.org/10.1016/S0165-5728(03)00047-X
82. Carr L, Tucker A, Fernandez-Botran R. The enhancement of T cell proliferation by L-dopa is mediated peripherally and does not involve interleukin-2. J Neuroimmunol 2003; 142:166-9; PMID:14512176; http://dx.doi.org/10.1016/S0165-5728(03)00270-4
83. Levite M, Chowers Y, Ganor Y, Besser M, Hershkovits R, Cahalon L. Dopamine interacts directly with its D3 and D2 receptors on normal human T cells, and activates beta1 integrin function. Eur J Immunol 2001; 31:3504-12; PMID:11745370; http://dx.doi.org/10.1002/1521-4141(200112)31:12<3504::AID-IMMU3504>3.0.CO;2-F
84. Tsao CW, Lin YS, Cheng JT. Effect of dopamine on immune cell proliferation in mice. Life Sci 1997; 61:361-71; PMID:9399637; http://dx.doi.org/10.1016/S0024-3205(97)00962-4
85. Nagai Y, Ueno S, Saeki Y, Soga F, Hirano M, Yanagihara T. Decrease of the D3 dopamine receptor mRNA expression in lymphocytes from patients with Parkinson's disease. Neurology 1996; 46:791-5; PMID:8618685; http://dx.doi.org/10.1212/WNL.46.3.791
86. Alberio T, Pippione AC, Comi C, Olgiati S, Cecconi D, Zibetti M, Lopiano L, Fasano M. Dopaminergic therapies modulate the T-CELL proteome of patients with Parkinson's disease. IUBMB Life 2012; 64:846-52; PMID:22815142; http://dx.doi.org/10.1002/iub.1073
87. González H, Contreras F, Prado C, Elgueta D, Franz D, Bernales S, Pacheco R. Dopamine receptor D3 expressed on CD4+ T cells favors neurodegeneration of dopaminergic neurons during Parkinson's disease. J Immunol 2013; 190:5048-56; PMID:23589621; http://dx.doi.org/10.4049/jimmunol.1203121
88. Chi DS, Gong L, Daigneault EA, Kostrzewa RM. Effects of MPTP and vitamin E treatments on immune function in mice. Int J Immunopharmacol 1992; 14:739-46; PMID:1512070; http://dx.doi.org/10.1016/0192-0561(92)90070-2
89. Renoux G, Bizière K, Renoux M, Steinberg R, Kan JP, Guillaumin JM. Sodium diethyldithiocarbamate protects against the MPTP-induced inhibition of immune responses in mice. Life Sci 1989; 44:771-7; PMID:2539542; http://dx.doi.org/10.1016/0024-3205(89)90374-3
90. Bieganowska K, Członkowska A, Bidziński A, Mierzewska H, Korlak J. Immunological changes in the MPTP-induced Parkinson's disease mouse model. J Neuroimmunol 1993; 42:33-7; PMID:8423206; http://dx.doi.org/10.1016/0165-5728(93)90209-H
91. Cosentino M, Rasini E, Colombo C, Marino F, Blandini F, Ferrari M, Samuele A, Lecchini S, Nappi G, Frigo G. Dopaminergic modulation of oxidative stress and apoptosis in human peripheral blood lymphocytes: evidence for a D1-like receptor-dependent protective effect. Free Radic Biol Med 2004; 36:1233-40; PMID:15110388; http://dx.doi.org/10.1016/j.freeradbiomed.2004.02.065
92. Ferreira TB, Kasahara TM, Barros PO, Vieira MM, Bittencourt VC, Hygino J, Andrade RM, Linhares UC, Andrade AF, Bento CA. Dopamine up-regulates Th17 phenotype from individuals with generalized anxiety disorder. J Neuroimmunol 2011; 238:58-66; PMID:21872345; http://dx.doi.org/10.1016/j.jneuroim.2011.06.009
93. Cosentino M, Fietta AM, Ferrari M, Rasini E, Bombelli R, Carcano E, Saporiti F, Meloni F, Marino F, Lecchini S. Human CD4+CD25+ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. Blood 2007; 109:632-42; PMID:16985181; http://dx.doi.org/10.1182/blood-2006-01-028423
94. Cosentino M, Zaffaroni M, Trojano M, Giorelli M, Pica C, Rasini E, Bombelli R, Ferrari M, Ghezzi A, Comi G, et al. Dopaminergic modulation of CD4+CD25(high) regulatory T lymphocytes in multiple sclerosis patients during interferon-β therapy. Neuroimmunomodulation 2012; 19:283-92; PMID:22472872; http://dx.doi.org/10.1159/000336981
95. Cosentino M, Marino F. Adrenergic and dopaminergic modulation of immunity in multiple sclerosis: teaching old drugs new tricks? J Neuroimmune Pharmacol 2013; 8:163-79; PMID:23074017; http://dx.doi.org/10.1007/s11481-012-9410-z
96. Saunders JA, Estes KA, Kosloski LM, Allen HE, Dempsey KM, Torres-Russotto DR, Meza JL, Santamaria PM, Bertoni JM, Murman DL, et al. CD4+ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson's disease. J Neuroimmune Pharmacol 2012; 7:927-38; PMID:23054369; http://dx.doi.org/10.1007/s11481-012-9402-z
97. Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay JM, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest 2009; 119:182-92; PMID:19104149
98. Sanchez-Guajardo V, Annibali A, Jensen PH, Romero-Ramos M. α-Synuclein vaccination prevents the accumulation of parkinson disease-like pathologic inclusions in striatum in association with regulatory T cell recruitment in a rat model. J Neuropathol Exp Neurol 2013; 72:624-45; PMID:23771222; http://dx.doi.org/10.1097/NEN.0b013e31829768d2
99. Sanchez-Guajardo V, Febbraro F, Kirik D, Romero-Ramos M. Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. PLoS One 2010; 5:e8784; PMID:20098715; http://dx.doi.org/10.1371/journal.pone.0008784
100. Theodore S, Cao S, McLean PJ, Standaert DG. Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. J Neuropathol Exp Neurol 2008; 67:1149-58; PMID:19018246; http://dx.doi.org/10.1097/NEN.0b013e31818e5e99
101. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. J Immunol 2010; 184:2261-71; PMID:20118279; http://dx.doi.org/10.4049/jimmunol.0901852
102. Reynolds AD, Stone DK, Mosley RL, Gendelman HE. Nitrated alpha-synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets. J Immunol 2009; 182:4137-49; PMID:19299711; http://dx.doi.org/10.4049/jimmunol.0803982
103. Fiszer U, Mix E, Fredrikson S, Kostulas V, Olsson T, Link H. gamma delta+ T cells are increased in patients with Parkinson's disease. J Neurol Sci 1994; 121:39-45; PMID:8133310; http://dx.doi.org/10.1016/0022-510X(94)90154-6
104. Hisanaga K, Asagi M, Itoyama Y, Iwasaki Y. Increase in peripheral CD4 bright+ CD8 dull+ T cells in Parkinson disease. Arch Neurol 2001; 58:1580-3; PMID:11594915; http://dx.doi.org/10.1001/archneur.58.10.1580
105. Stevens CH, Rowe D, Morel-Kopp MC, Orr C, Russell T, Ranola M, Ward C, Halliday GM. Reduced T helper and B lymphocytes in Parkinson's disease. J Neuroimmunol 2012; 252:95-9; PMID:22910543; http://dx.doi.org/10.1016/j.jneuroim.2012.07.015

106. Fiszer U, Mix E, Fredrikson S, Kostulas V, Link H. Parkinson's disease and immunological abnormalities: increase of HLA-DR expression on monocytes in cerebrospinal fluid and of CD45RO+ T cells in peripheral blood. Acta Neurol Scand 1994; 90:160-6; PMID:7847055; http://dx.doi.org/10.1111/j.1600-0404.1994.tb02699.x
107. Niwa F, Kuriyama N, Nakagawa M, Imanishi J. Effects of peripheral lymphocyte subpopulations and the clinical correlation with Parkinson's disease. Geriatr Gerontol Int 2012; 12:102-7; PMID:21929737; http://dx.doi.org/10.1111/j.1447-0594.2011.00740.x
108. Armentero MT, Sinforiani E, Ghezzi C, Bazzini E, Levandis G, Ambrosi G, Zangaglia R, Pacchetti C, Cereda C, Cova E, et al. Peripheral expression of key regulatory kinases in Alzheimer's disease and Parkinson's disease. Neurobiol Aging 2011; 32:2142-51; PMID:20106550; http://dx.doi.org/10.1016/j.neurobiolaging.2010.01.004
109. Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. Cell 2010; 140:845-58; PMID:20303875; http://dx.doi.org/10.1016/j.cell.2010.02.021
110. Rosenkranz D, Weyer S, Tolosa E, Gaenslen A, Berg D, Leyhe T, Gasser T, Stoltze L. Higher frequency of regulatory T cells in the elderly and increased suppressive activity in neurodegeneration. J Neuroimmunol 2007; 188:117-27; PMID:17582512; http://dx.doi.org/10.1016/j.jneuroim.2007.05.011
111. Wang L, Xie Y, Zhu LJ, Chang TT, Mao YQ, Li J. An association between immunosenescence and CD4(+)CD25(+) regulatory T cells: a systematic review. Biomed Environ Sci 2010; 23:327-32; PMID:20934123; http://dx.doi.org/10.1016/S0895-3988(10)60072-4
112. Huber H, Descossy P, van Brandwijk R, Knop J. Activation of murine epidermal TCR-gamma delta+ T cells by keratinocytes treated with contact sensitizers. J Immunol 1995; 155:2888-94; PMID:7673705
113. Prigione A, Begni B, Galbussera A, Beretta S, Brighina L, Garofalo R, Andreoni S, Piolti R, Ferrarese C. Oxidative stress in peripheral blood mononuclear cells from patients with Parkinson's disease: negative correlation with levodopa dosage. Neurobiol Dis 2006; 23:36-43; PMID:16563783; http://dx.doi.org/10.1016/j.nbd.2006.01.013
114. Prigione A, Isaias IU, Galbussera A, Brighina L, Begni B, Andreoni S, Pezzoli G, Antonini A, Ferrarese C. Increased oxidative stress in lymphocytes from untreated Parkinson's disease patients. Parkinsonism Relat Disord 2009; 15:327-8; PMID:18640066; http://dx.doi.org/10.1016/j.parkreldis.2008.05.013
115. Battisti C, Formichi P, Radi E, Federico A. Oxidative-stress-induced apoptosis in PBLs of two patients with Parkinson disease secondary to alpha-synuclein mutation. J Neurol Sci 2008; 267:120-4; PMID:18061619; http://dx.doi.org/10.1016/j.jns.2007.10.012
116. Calopa M, Bas J, Callén A, Mestre M. Apoptosis of peripheral blood lymphocytes in Parkinson patients. Neurobiol Dis 2010; 38:1-7; PMID:20044003; http://dx.doi.org/10.1016/j.nbd.2009.12.017
117. Meredith EJ, Holder MJ, Rosén A, Lee AD, Dyer MJ, Barnes NM, Gordon J. Dopamine targets cycling B cells independent of receptors/transporter for oxidative attack: Implications for non-Hodgkin's lymphoma. Proc Natl Acad Sci U S A 2006; 103:13485-90; PMID:16938864; http://dx.doi.org/10.1073/pnas.0605993103
118. van de Warrenburg BP, Church AJ, Martino D, Candler PM, Bhatia KP, Giovannoni G, Quinn NP. Antineuronal antibodies in Parkinson's disease. Mov Disord 2008; 23:958-63; PMID:18383532; http://dx.doi.org/10.1002/mds.21929
119. Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, Nagele RG. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. PLoS One 2013; 8:e60726; PMID:23589757; http://dx.doi.org/10.1371/journal.pone.0060726
120. Benkler M, Agmon-Levin N, Hassin-Baer S, Cohen OS, Ortega-Hernandez OD, Levy A, Moscavitch SD, Szyper-Kravitz M, Damianovich M, Blank M, et al. Immunology, autoimmunity, and autoantibodies in Parkinson's disease. Clin Rev Allergy Immunol 2012; 42:164-71; PMID:21234712; http://dx.doi.org/10.1007/s12016-010-8242-y
121. Nagele E, Han M, Demarshall C, Belinka B, Nagele R. Diagnosis of Alzheimer's disease based on disease-specific autoantibody profiles in human sera. PLoS One 2011; 6:e23112; PMID:21826230; http://dx.doi.org/10.1371/journal.pone.0023112
122. Orr CF, Rowe DB, Mizuno Y, Mori H, Halliday GM. A possible role for humoral immunity in the pathogenesis of Parkinson's disease. Brain 2005; 128:2665-74; PMID:16219675; http://dx.doi.org/10.1093/brain/awh625
123. Levin EC, Acharya NK, Han M, Zavareh SB, Sedeyn JC, Venkataraman V, Nagele RG. Brain-reactive autoantibodies are nearly ubiquitous in human sera and may be linked to pathology in the context of blood-brain barrier breakdown. Brain Res 2010; 1345:221-32; PMID:20546711; http://dx.doi.org/10.1016/j.brainres.2010.05.038
124. Carvey PM, McRae A, Lint TF, Ptak LR, Lo ES, Goetz CG, Klawans HL. The potential use of a dopamine neuron antibody and a striatal-derived neurotrophic factor as diagnostic markers in Parkinson's disease. Neurology 1991; 41(Suppl 2):53-8, discussion 59-60; PMID:2041594; http://dx.doi.org/10.1212/WNL.41.5_Suppl_2.53
125. Chen S, Le WD, Xie WJ, Alexianu ME, Engelhardt JI, Siklós L, Appel SH. Experimental destruction of substantia nigra initiated by Parkinson disease immunoglobulins. Arch Neurol 1998; 55:1075-80; PMID:9708957; http://dx.doi.org/10.1001/archneur.55.8.1075
126. He Y, Le WD, Appel SH. Role of Fcgamma receptors in nigral cell injury induced by Parkinson disease immunoglobulin injection into mouse substantia nigra. Exp Neurol 2002; 176:322-7; PMID:12359173; http://dx.doi.org/10.1006/exnr.2002.7946
127. Rowe DB, Le W, Smith RG, Appel SH. Antibodies from patients with Parkinson's disease react with protein modified by dopamine oxidation. J Neurosci Res 1998; 53:551-8; PMID:9726426; http://dx.doi.org/10.1002/(SICI)1097-4547(19980901)53:5<551::AID-JNR5>3.0.CO;2-8
128. Double KL, Rowe DB, Carew-Jones FM, Hayes M, Chan DK, Blackie J, Corbett A, Joffe R, Fung VS, Morris J, et al. Anti-melanin antibodies are increased in sera in Parkinson's disease. Exp Neurol 2009; 217:297-301; PMID:19289120; http://dx.doi.org/10.1016/j.expneurol.2009.03.002
129. Weber MS, Hemmer B, Cepok S. The role of antibodies in multiple sclerosis. Biochim Biophys Acta 2011; 1812:239-45; PMID:20600871; http://dx.doi.org/10.1016/j.bbadis.2010.06.009
130. Berer K, Wekerle H, Krishnamoorthy G. B cells in spontaneous autoimmune diseases of the central nervous system. Mol Immunol 2011; 48:1332-7; PMID:21146219; http://dx.doi.org/10.1016/j.molimm.2010.10.025
131. Franciotta D, Salvetti M, Lolli F, Serafini B, Aloisi F. B cells and multiple sclerosis. Lancet Neurol 2008; 7:852-8; PMID:18703007; http://dx.doi.org/10.1016/S1474-4422(08)70192-3
132. Wilson HL. B cells contribute to MS pathogenesis through antibody-dependent and antibody-independent mechanisms. Biologics 2012; 6:117-23; PMID:22690126
133. Fontoura P. Roles of B lymphocytes in multiple sclerosis: diversifying beyond the antibody response. Immunotherapy 2009; 1:181-5; PMID:20635939; http://dx.doi.org/10.2217/1750743X.1.2.181
134. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest 2008; 118:3420-30; PMID:18802481
135. Begum-Haque S, Christy M, Ochoa-Reparaz J, Nowak EC, Mielcarz D, Haque A, Kasper LH. Augmentation of regulatory B cell activity in experimental allergic encephalomyelitis by glatiramer acetate. J Neuroimmunol 2011; 232:136-44; PMID:21111489; http://dx.doi.org/10.1016/j.jneuroim.2010.10.031
136. Ray A, Mann MK, Basu S, Dittel BN. A case for regulatory B cells in controlling the severity of autoimmune-mediated inflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. J Neuroimmunol 2011; 230:1-9; PMID:21145597; http://dx.doi.org/10.1016/j.jneuroim.2010.10.037
137. 't Hart BA, Jagessar SA, Haanstra K, Verschoor E, Laman JD, Kap YS. The Primate EAE Model Points at EBV-Infected B Cells as a Preferential Therapy Target in Multiple Sclerosis. Front Immunol 2013; 4:145; PMID:23781220
138. Anwar Jagessar S, Fagrouch Z, Heijmans N, Bauer J, Laman JD, Oh L, Migone T, Verschoor EJ, 't Hart BA. The different clinical effects of anti-BLyS, anti-APRIL and anti-CD20 antibodies point at a critical pathogenic role of γ-herpesvirus infected B cells in the marmoset EAE model. J Neuroimmune Pharmacol 2013; 8:727-38; PMID:23508625; http://dx.doi.org/10.1007/s11481-013-9448-6
139. Barr TA, Shen P, Brown S, Lampropoulou V, Roch T, Lawrie S, Fan B, O'Connor RA, Anderton SM, Bar-Or A, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. J Exp Med 2012; 209:1001-10; PMID:22547654; http://dx.doi.org/10.1084/jem.20111675
140. Papachroni KK, Ninkina N, Papapanagiotou A, Hadjigeorgiou GM, Xiromerisiou G, Papadimitriou A, Kalofoutis A, Buchman VL. Autoantibodies to alpha-synuclein in inherited Parkinson's disease. J Neurochem 2007; 101:749-56; PMID:17448146; http://dx.doi.org/10.1111/j.1471-4159.2006.04365.x
141. Maetzler W, Berg D, Synofzik M, Brockmann K, Godau J, Melms A, Gasser T, Hörnig S, Langkamp M. Autoantibodies against amyloid and glial-derived antigens are increased in serum and cerebrospinal fluid of Lewy body-associated dementias. J Alzheimers Dis 2011; 26:171-9; PMID:21593566
142. Yanamandra K, Gruden MA, Casaite V, Meskys R, Forsgren L, Morozova-Roche LA. α-synuclein reactive antibodies as diagnostic biomarkers in blood sera of Parkinson's disease patients. PLoS One 2011; 6:e18513; PMID:21541339; http://dx.doi.org/10.1371/journal.pone.0018513
143. Besong-Agbo D, Wolf E, Jessen F, Oechsner M, Hametner E, Poewe W, Reindl M, Oertel WH, Noelker C, Bacher M, et al. Naturally occurring α-synuclein autoantibody levels are lower in patients with Parkinson disease. Neurology 2013; 80:169-75; PMID:23255825; http://dx.doi.org/10.1212/WNL.0b013e31827b90d1
144. Morozov SG, Ivanova-Smolenskaia IA, Markova ED, Piradov MA, Poleshchuk VV, Labunskiĭ DA, Gnedenko BB. [Immunochemical correlates of the severity of Parkinson's disease]. Vopr Med Khim 1997; 43:34-8; PMID:9281224
145. Fiszer U, Fredrikson S, Członkowska A. Humoral response to hsp 65 and hsp 70 in cerebrospinal fluid in Parkinson's disease. J Neurol Sci 1996; 139:66-70; PMID:8836974; http://dx.doi.org/10.1016/0022-510X(96)00002-0
146. Bae EJ, Lee HJ, Rockenstein E, Ho DH, Park EB, Yang NY, Desplats P, Masliah E, Lee SJ. Antibody-aided clearance of extracellular α-synuclein prevents cell-to-cell aggregate transmission. J Neurosci 2012; 32:13454-69; PMID:23015436; http://dx.doi.org/10.1523/JNEUROSCI.1292-12.2012

147. Shevach EM. Regulatory T cells in autoimmmunity. Annu Rev Immunol 2000; 18:423-49; PMID:10837065; http://dx.doi.org/10.1146/annurev.immunol.18.1.423
148. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. Nat Immunol 2002; 3:944-50; PMID:12244307; http://dx.doi.org/10.1038/ni833
149. Lampropoulou V, Hoehlig K, Roch T, Neves P, Calderón Gómez E, Sweenie CH, Hao Y, Freitas AA, Steinhoff U, Anderton SM, et al. TLR-activated B cells suppress T cell-mediated autoimmunity. J Immunol 2008; 180:4763-73; PMID:18354200
150. Wang R, Han G, Wang J, Song L, Chen G, Xu R, Yu M, Qian J, Shen B, Li Y. The role of STAT3 in antigen-IgG inducing regulatory CD4(+)Foxp3(+)T cells. Cell Immunol 2007; 246:103-9; PMID:17697673; http://dx.doi.org/10.1016/j.cellimm.2007.07.001
151. Cousens LP, Su Y, McClaine E, Li X, Terry F, Smith R, Lee J, Martin W, Scott DW, De Groot AS. Application of IgG-derived natural Treg epitopes (IgG Tregitopes) to antigen-specific tolerance induction in a murine model of type 1 diabetes. J Diabetes Res. 2013; 2013:621693
152. Mauri C. Regulation of immunity and autoimmunity by B cells. Curr Opin Immunol 2010; 22:761-7; PMID:21087847; http://dx.doi.org/10.1016/j.coi.2010.10.009
153. Barr TA, Gray M, Gray D. B cells: programmers of CD4 T cell responses. Infect Disord Drug Targets 2012; 12:222-31; PMID:22394172; http://dx.doi.org/10.2174/187152612800564446
154. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. Nat Rev Immunol 2012; 12:623-35; PMID:22903150; http://dx.doi.org/10.1038/nri3265
155. Mosley RL, Hutter-Saunders JA, Stone DK, Gendelman HE. Inflammation and adaptive immunity in Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2:a009381; PMID:22315722; http://dx.doi.org/10.1101/cshperspect.a009381
156. Appel SH, Beers DR, Henkel JS. T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening? Trends Immunol 2010; 31:7-17; PMID:19879804; http://dx.doi.org/10.1016/j.it.2009.09.003
157. Schwartz M, Kipnis J. Therapeutic T cell-based vaccination for neurodegenerative disorders: the role of CD4+CD25+ regulatory T cells. Ann N Y Acad Sci 2005; 1051:701-8; PMID:16127010; http://dx.doi.org/10.1196/annals.1361.114
158. Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Seubert P, Lee M, Goldstein J, Chilcote T, et al. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. Neuron 2005; 46:857-68; PMID:15953415; http://dx.doi.org/10.1016/j.neuron.2005.05.010
159. Schneeberger A, Mandler M, Mattner F, Schmidt W. Vaccination for Parkinson's disease. Parkinsonism Relat Disord 2012; 18(Suppl 1):S11-3; PMID:22166404; http://dx.doi.org/10.1016/S1353-8020(11)70006-2
160. Avidan H, Kipnis J, Butovsky O, Caspi RR, Schwartz M. Vaccination with autoantigen protects against aggregated beta-amyloid and glutamate toxicity by controlling microglia: effect of CD4+CD25+ T cells. Eur J Immunol 2004; 34:3434-45; PMID:15549735; http://dx.doi.org/10.1002/eji.200424883
161. Laćan G, Dang H, Middleton B, Horwitz MA, Tian J, Melega WP, Kaufman DL. Bacillus Calmette-Guerin vaccine-mediated neuroprotection is associated with regulatory T-cell induction in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. J Neurosci Res 2013; 91:1292-302; PMID:23907992; http://dx.doi.org/10.1002/jnr.23253
162. Armentero MT, Levandis G, Nappi G, Bazzini E, Blandini F. Peripheral inflammation and neuroprotection: systemic pretreatment with complete Freund's adjuvant reduces 6-hydroxydopamine toxicity in a rodent model of Parkinson's disease. Neurobiol Dis 2006; 24:492-505; PMID:17023164; http://dx.doi.org/10.1016/j.nbd.2006.08.016
163. Chung ES, Kim H, Lee G, Park S, Kim H, Bae H. Neuro-protective effects of bee venom by suppression of neuroinflammatory responses in a mouse model of Parkinson's disease: role of regulatory T cells. Brain Behav Immun 2012; 26:1322-30; PMID:22974722; http://dx.doi.org/10.1016/j.bbi.2012.08.013
164. Shinoda M, Hudson JL, Strömberg I, Hoffer BJ, Moorhead JW, Olson L. Allogeneic grafts of fetal dopamine neurons: immunological reactions following active and adoptive immunizations. Brain Res 1995; 680:180-95; PMID:7663976; http://dx.doi.org/10.1016/0006-8993(95)00260-W
165. Laurie C, Reynolds A, Coskun O, Bowman E, Gendelman HE, Mosley RL. CD4+ T cells from Copolymer-1 immunized mice protect dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. J Neuroimmunol 2007; 183:60-8; PMID:17196666; http://dx.doi.org/10.1016/j.jneuroim.2006.11.009
166. Reynolds AD, Banerjee R, Liu J, Gendelman HE, Mosley RL. Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. J Leukoc Biol 2007; 82:1083-94; PMID:17675560; http://dx.doi.org/10.1189/jlb.0507296
167. Masliah E, Rockenstein E, Mante M, Crews L, Spencer B, Adame A, Patrick C, Trejo M, Ubhi K, Rohn TT, et al. Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. PLoS One 2011; 6:e19338; PMID:21559417; http://dx.doi.org/10.1371/journal.pone.0019338
168. Kurkowska-Jastrzebska I, Bałkowiec-Iskra E, Joniec I, Litwin T, Członkowski A, Członkowska A. Immunization with myelin oligodendrocyte glycoprotein and complete Freund adjuvant partially protects dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced damage in mouse model of Parkinson's disease. Neuroscience 2005; 131:247-54; PMID:15680707; http://dx.doi.org/10.1016/j.neuroscience.2004.10.027
169. Yong J, Lacan G, Dang H, Hsieh T, Middleton B, Wasserfall C, Tian J, Melega WP, Kaufman DL. BCG vaccine-induced neuroprotection in a mouse model of Parkinson's disease. PLoS One 2011; 6:e16610; PMID:21304945; http://dx.doi.org/10.1371/journal.pone.0016610
170. Benner EJ, Mosley RL, Destache CJ, Lewis TB, Jackson-Lewis V, Gorantla S, Nemachek C, Green SR, Przedborski S, Gendelman HE. Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. Proc Natl Acad Sci U S A 2004; 101:9435-40; PMID:15197276; http://dx.doi.org/10.1073/pnas.0400569101
171. Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, Cerny R, Gelman B, Thomas MP, Mosley RL, et al. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. J Neurochem 2008; 104:1504-25; PMID:18036154; http://dx.doi.org/10.1111/j.1471-4159.2007.05087.x
172. Liu Y, Qiang M, Wei Y, He R. A novel molecular mechanism for nitrated alpha-synuclein-induced cell death. J Mol Cell Biol 2011; 3:239-49; PMID:21733982; http://dx.doi.org/10.1093/jmcb/mjr011
173. Yu Z, Xu X, Xiang Z, Zhou J, Zhang Z, Hu C, He C. Nitrated alpha-synuclein induces the loss of dopaminergic neurons in the substantia nigra of rats. PLoS One 2010; 5:e9956; PMID:20386702; http://dx.doi.org/10.1371/journal.pone.0009956
174. Uversky VN, Yamin G, Munishkina LA, Karymov MA, Millett IS, Doniach S, Lyubchenko YL, Fink AL. Effects of nitration on the structure and aggregation of alpha-synuclein. Brain Res Mol Brain Res 2005; 134:84-102; PMID:15790533; http://dx.doi.org/10.1016/j.molbrainres.2004.11.014
175. Hodara R, Norris EH, Giasson BI, Mishizen-Eberz AJ, Lynch DR, Lee VM, Ischiropoulos H. Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. J Biol Chem 2004; 279:47746-53; PMID:15364911; http://dx.doi.org/10.1074/jbc.M408906200
176. Yamin G, Uversky VN, Fink AL. Nitration inhibits fibrillation of human alpha-synuclein in vitro by formation of soluble oligomers. FEBS Lett 2003; 542:147-52; PMID:12729915; http://dx.doi.org/10.1016/S0014-5793(03)00367-3
177. McCormack AL, Mak SK, Di Monte DA. Increased α-synuclein phosphorylation and nitration in the aging primate substantia nigra. Cell Death Dis 2012; 3:e315; PMID:22647852; http://dx.doi.org/10.1038/cddis.2012.50
178. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, Nemachek C, Ciborowski P, Przedborski S, Mosley RL, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. PLoS One 2008; 3:e1376; PMID:18167537; http://dx.doi.org/10.1371/journal.pone.0001376
179. Bettelli E, Carrier Y, Gao W, Korn T, Strom T, Oukka M, Weiner H, Kuchroo V. Reciprocal depvelomental pathways for the generation of pathogenic effector Th17 and regulatory T cells. Nature Letters 2006; 441:235-8; http://dx.doi.org/10.1038/nature04753
180. Mandler M, Valera E, Rockenstein E, Weninger H, Patrick C, Adame A, Santic R, Meindl S, Vigl B, Smrzka O, et al. Next-generation active immunization approach for synucleinopathies: implications for Parkinson's disease clinical trials. Acta Neuropathol 2014; PMID:24525765; http://dx.doi.org/10.1007/s00401-014-1256-4
181. Bhattacharya P, Gopisetty A, Ganesh BB, Sheng JR, Prabhakar BS. GM-CSF-induced, bone-marrow-derived dendritic cells can expand natural Tregs and induce adaptive Tregs by different mechanisms. J Leukoc Biol 2011; 89:235-49; PMID:21048215; http://dx.doi.org/10.1189/jlb.0310154
182. Kosloski LM, Kosmacek EA, Olson KE, Mosley RL, Gendelman HE. GM-CSF induces neuroprotective and anti-inflammatory responses in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxicated mice. J Neuroimmunol 2013; 265:1-10; PMID:24210793; http://dx.doi.org/10.1016/j.jneuroim.2013.10.009
183. Kim NK, Choi BH, Huang X, Snyder BJ, Bukhari S, Kong TH, Park H, Park HC, Park SR, Ha Y. Granulocyte-macrophage colony-stimulating factor promotes survival of dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced murine Parkinson's disease model. Eur J Neurosci 2009; 29:891-900; PMID:19245369; http://dx.doi.org/10.1111/j.1460-9568.2009.06653.x
184. St-Amour I, Bousquet M, Paré I, Drouin-Ouellet J, Cicchetti F, Bazin R, Calon F. Impact of intravenous immunoglobulin on the dopaminergic system and immune response in the acute MPTP mouse model of Parkinson's disease. J Neuroinflammation 2012; 9:234; PMID:23046563; http://dx.doi.org/10.1186/1742-2094-9-234
185. Mann MK, Maresz K, Shriver LP, Tan Y, Dittel BN. B cell regulation of CD4+CD25+ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis. J Immunol 2007; 178:3447-56; PMID:17339439

186. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci Lett 1994; 165:208-10; PMID:8015728; http://dx.doi.org/10.1016/0304-3940(94)90746-3
187. Koziorowski D, Tomasiuk R, Szlufik S, Friedman A. Inflammatory cytokines and NT-proCNP in Parkinson's disease patients. Cytokine 2012; 60:762-6; PMID:22910321; http://dx.doi.org/10.1016/j.cyto.2012.07.030
188. Dobbs RJ, Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson DW. Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. Acta Neurol Scand 1999; 100:34-41; PMID:10416510; http://dx.doi.org/10.1111/j.1600-0404.1999.tb00721.x
189. Rentzos M, Nikolaou C, Andreadou E, Paraskevas GP, Rombos A, Zoga M, Tsoutsou A, Boufidou F, Kapaki E, Vassilopoulos D. Circulating interleukin-10 and interleukin-12 in Parkinson's disease. Acta Neurol Scand 2009; 119:332-7; PMID:18976327; http://dx.doi.org/10.1111/j.1600-0404.2008.01103.x
190. Mogi M, Harada M, Kondo T, Narabayashi H, Riederer P, Nagatsu T. Transforming growth factor-beta 1 levels are elevated in the striatum and in ventricular cerebrospinal fluid in Parkinson's disease. Neurosci Lett 1995; 193:129-32; PMID:7478158; http://dx.doi.org/10.1016/0304-3940(95)11686-Q
191. Vawter MP, Dillon-Carter O, Tourtellotte WW, Carvey P, Freed WJ. TGFbeta1 and TGFbeta2 concentrations are elevated in Parkinson's disease in ventricular cerebrospinal fluid. Exp Neurol 1996; 142:313-22; PMID:8934562; http://dx.doi.org/10.1006/exnr.1996.0200
192. Scalzo P, de Miranda AS, Guerra Amaral DC, de Carvalho Vilela M, Cardoso F, Teixeira AL. Serum levels of chemokines in Parkinson's disease. Neuroimmunomodulation 2011; 18:240-4; PMID:21430395; http://dx.doi.org/10.1159/000323779
193. Nicoletti A, Fagone P, Donzuso G, Mangano K, Dibilio V, Caponnetto S, Bendtzen K, Zappia M, Nicoletti F. Parkinson's disease is associated with increased serum levels of macrophage migration inhibitory factor. Cytokine 2011; 55:165-7; PMID:21550814; http://dx.doi.org/10.1016/j.cyto.2011.03.027
194. Besser MJ, Ganor Y, Levite M. Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers the selective secretion of either IL-10, TNFalpha or both. J Neuroimmunol 2005; 169:161-71; PMID:16150496; http://dx.doi.org/10.1016/j.jneuroim.2005.07.013
195. Giorelli M, Livrea P, Trojano M. Dopamine fails to regulate activation of peripheral blood lymphocytes from multiple sclerosis patients: effects of IFN-beta. J Interferon Cytokine Res 2005; 25:395-406; PMID:16022584; http://dx.doi.org/10.1089/jir.2005.25.395
196. Ghosh MC, Mondal AC, Basu S, Banerjee S, Majumder J, Bhattacharya D, Dasgupta PS. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. Int Immunopharmacol 2003; 3:1019-26; PMID:12810359; http://dx.doi.org/10.1016/S1567-5769(03)00100-0
197. Ilani T, Strous RD, Fuchs S. Dopaminergic regulation of immune cells via D3 dopamine receptor: a pathway mediated by activated T cells. FASEB J 2004; 18:1600-2; PMID:15319371
198. Sarkar C, Das S, Chakroborty D, Chowdhury UR, Basu B, Dasgupta PS, Basu S. Cutting Edge: Stimulation of dopamine D4 receptors induce T cell quiescence by up-regulating Kruppel-like factor-2 expression through inhibition of ERK1/ERK2 phosphorylation. J Immunol 2006; 177:7525-9; PMID:17114421