Journal of Clinical Neuroscience xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical study

The possible role of an autoimmune mechanism in the etiopathogenesis of Parkinson's disease

Savvas Dalitis, Natalia Filippidou, George Krashias, Christina Christodoulou, Marios Pantzaris, Anastasia Lambrianides *

Cyprus School of Molecular Medicine, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

ARTICLE INFO

Article history:
Received 6 December 2017
Accepted 3 June 2018
Available online xxxx

Keywords:
Parkinson's disease
Neurodegeneration
Autoantibodies
Oxidative stress

ABSTRACT

The clinical diagnosis of Parkinson's disease (PD) is established through clinical signs such as bradykinesia, rigidity, and resting tremor. Recently, immune system involvement has been implicated as a major pathogenic factor in the onset and progression of PD. We examine the presence of autoantibodies against phosphatidylserine (PS), cardiolipin (CL) and dsDNA in 45 PD patients and 38 healthy controls and provide evidence to the possible connection to oxidative stress. We report higher frequency of IgG anti-PS and anti-dsDNA in PD patients (24.4% and 15.6%), compared to controls (2.6% in both cases, $p < 0.05$). Moreover, the presence of these autoantibodies is not analogous with increased levels of oxidative stress in PD. A great need exists for improved understanding of the pathogenesis and identification of relevant biomarkers and future studies in clarifying the role of autoantibodies in PD are required to address its role as a potential risk factor.

1. Introduction

Parkinson's disease is a progressive neurodegenerative disease characterized by a large number of motor and non-motor impairments that can impact on function to a variable degree. The cardinal features of PD generally consist of resting tremor, bradykinesia, muscular rigidity and impaired postural reflexes [1]. Nonmotor symptoms accompany the disease, which involve autonomic, cognitive and mood alteration symptoms [2]. Although the etiopathogenesis of PD still remains unclear, various hypotheses have emerged including genetic vulnerability, environmental factors and immunological conditions for the resulting death of dopaminergic neurons in the substantia nigra pars compacta and the presence of Lewy bodies containing a high concentration of α-synuclein [3,4]. Mitochondrial dysfunction caused by mutations in DJ-1, PINK-1, SNCA and parkin-encoding genes such as PRKN has been also implicated in the pathogenesis [5].

Inflammation in the central nervous system is associated with neurodegenerative diseases such as PD, and activation of cell signaling pathways contributes to pathogenic inflammation [6]. Neuroinflammation is a feature of PD pathology but it has yet to be established whether neuroinflammation protects or promotes neurodegeneration. Although previously it was believed that PD and autoimmunity are not associated, evidence accumulated over the past decade concerning immune alterations in PD increased the relevance in pursuing a possible role of the immune system. Alterations in the activity of the cellular or the humoral components, an increase in the level of innate immune components including complement and cytokines, or defects in immune regulation as well as increased apoptosis are all biological consequences of PD [6]. Increased populations of proinflammatory cytokines have been found in the circulation of elderly individuals, suggesting a low-grade inflammatory state in these individuals [7,8].

Given that during apoptosis, intracellular components including the nucleus often make up the spectrum of target autoantigens, defective clearance of apoptotic fragments has already generated much interest in autoimmunity [9]. Therefore, impaired cell death/autophagy pathways, both in the brain and peripheral blood of patients with PD [10] may predispose individuals to an exposure of immunodominant autoantigens and hence the development of antibodies. A cluster of autoantibodies have been associated with neurological diseases, which includes anti-ribosomal phosphoprotein, brain-reactive autoantibodies, antibodies targeting neuronal structures, anti-dsDNA antibodies and antiphospholipid antibodies [11–13], which further points out the role of autoimmunity in neurodegeneration.

Moreover, a line of evidence provides support that oxidative stress and apoptosis are closely linked physiological phenomena

* Corresponding author.
E-mail address: nancyl@cing.ac.cy (A. Lambrianides).

https://doi.org/10.1016/j.jocn.2018.06.004
0967-5868/

Please cite this article in press as: Dalitis S et al. The possible role of an autoimmune mechanism in the etiopathogenesis of Parkinson's disease. J Clin Neurosci (2018), https://doi.org/10.1016/j.jocn.2018.06.004

[14]. Oxidative stress causes peroxidation of cardiolipin and other oxidation-derived epitopes that are generated on self-antigens, which are important immunodominant targets of natural antibodies. Subsequently, it has been suggested that these antibodies play an important role in the host response to the effects of oxidative stress during oxidative events that follow when cells undergo apoptosis [15]. Similarly, the fact that the enhanced post-translational modification of proteins with advancing age and neurodegenerative disease arises under physiological conditions suggests the existence of an association between the formation of oxidation-specific epitopes and immune disorders [16]. More recently, oxidative stress has been also shown to target proteins to the adaptive immune system via antigen modification, providing an additional mechanism for oxidative stress-induced antibody production [17].

We enrolled a well-defined cohort of PD patients and controls to determine the presence of specific autoantibodies; given the implication of the immune system there are only a handful of studies examining autoantibodies as potential targets that may be exploited in the future for the prognosis and even prevention of PD. In response to these autoantibodies, here we explore another avenue and attempt to address whether the levels of oxidative stress are linked to the presence of these antibodies, as an opportunity for identification of biomarkers, potential screening tests and disease-modifying therapies for PD.

2. Patients and methods

2.1. Patient population

Forty-five patients diagnosed with PD and followed at the Cyprus Institute of Neurology and Genetics donated blood for the purposes of this study. The diagnosis was made based on the clinical presentation (resting tremor, bradykinesia, rigidity, postural imbalance) of the patients at the time of consultation and following the established guidelines for diagnosis of PD. In parallel, 38 samples were collected from healthy controls. None of the participants had any underlying autoimmune disease. Demographics, medical history and laboratory data from all the participants were collected. Written informed consent was obtained from each participant enrolled in the study and all sera collected had been stored at −20 °C. The study was approved by the Cyprus National Bioethics Committee.

2.2. Detection of IgG and IgM anti-CL antibodies in human serum

A previously described method by Giles et al. [18] was followed. In brief, one half of a 96-well Polysorp ELISA plate was coated with 50 μg/ml cardiolipin and the other half, the control side, was coated with ethanol alone. Plates were incubated overnight at 4 °C and then blocked for 1 h at room temperature using 10% FBS/PBS. Human serum was diluted 1:50 with 10% FBS/PBS and tested in triplicate. The plates were incubated at room temperature for 90 min. Bound IgG and IgM was detected by addition of goat anti-human horseradish peroxidase-conjugated IgG or IgM (Sigma, UK) in 10% FBS/PBS for 1 h followed by addition of substrate. Absorbance was measured at 450 nm and background binding to control wells lacking CL was subtracted from binding to CL-coated wells in each ELISA plate. Inter and intra plate variations were evaluated using an appropriate control on each ELISA plate.

2.3. Detection of IgG and IgM anti-PS antibodies in human serum

Binding of anti-PS in human serum was detected using Polysorp (Nunc) microtiter plates, coated with 50 μg/ml in methanol:chloroform (4:1) or methanol:chloroform alone overnight uncovered at 4 °C. After washing with 0.05% PBS/Tween, plates were blocked with 10% FBS/PBS for 1 h at 37 °C. Serum was diluted 1:50 in 10% FBS/PBS and incubated for 90 min at 37 °C, followed by the addition of horseradish peroxidase-conjugated goat anti-human IgG or IgM and substrate and absorbace read at 450 nm.

2.4. Detection of IgG and IgM anti-dsDNA antibodies in human serum

Calf-thymus DNA (Sigma, UK) was further purified with phenol/chloroform and sonicated to ensure reproducible coating on a 96-well Maxisorp ELISA plate. Single-stranded was removed by passing the sample through a 0.45 mg filter (Millipore, UK). Concentration and purity were confirmed and the anti-DNA ELISA was carried out as previously described [19].

2.5. Dimension of thiobarbituric acid reactive substances (TBARS)

The TBARS assay kit (Cayman Chemical) was used for assaying lipid peroxidation in serum as follows. Serum (100 μl) received 4 ml of 1% TBA/TBA acetic acid solution, incubated for 1 h at 90 °C, followed by 10 min incubation on ice to stop the reaction. The samples were then clarified by centrifugation (1600×*g* for 10 min). The resulting supernatants were quantified colorimetrically at 540 nm by comparison with a standard curve of TBA Malondialdehyde standard in water.

2.6. Statistical analysis

Graphical representations were constructed using GraphPad Prism Version 5 for Windows, La Jolla, California USA. All statistical tests were conducted using the statistical software STATA 14® (StataCorp., TX, USA). Independent sample's *T*-test was used to compare the mean and distribution of age among PD patient group and healthy control group, whereas gender match between the two groups was carried out using the Pearson's chi-square test. Autoantibodies were considered seropositive when their levels exceeded the value of the mean titer plus two standard deviations of the healthy control group. The Fisher's Exact test was used to compare the prevalence of positive antibodies between cases and controls.

Table 1
Summary of clinical data on patients with Parkinson's disease and healthy controls.

	PD patients (n = 45)	HC (n = 38)
Gender		
Male/Female*	28/17	18/20
Mean age (±SD)	68.7 ± 9.8 (44–91)	69.0 ± 9.6 (42–83)
Age of onset (mean ± S.D.; (range))	56.6 ± 11.2 (30–77)	N/A
Age of diagnosis (mean ± S.D.; (range))	58.3 ± 11.4 (31–77)	N/A
PD duration (mean ± S.D.; (range))	11.8 ± 6.9 (1–27)	N/A

N/A; Not Applicable, PD; Parkinson's disease, HC; Healthy controls.
* Values indicated represent number of subjects.

Please cite this article in press as: Dalitis S et al. The possible role of an autoimmune mechanism in the etiopathogenesis of Parkinson's disease. J Clin Neurosci (2018), https://doi.org/10.1016/j.jocn.2018.06.004