Journal of Neuroimmunology 313 (2017) 16–24

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Characterization of isolated tau-reactive antibodies from the IVIG product, plasma of patients with Alzheimer's disease and cognitively normal individuals

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ARTICLE INFO

Keywords:
Alzheimer's disease
Brain homogenates
Plasma antibodies
Reactivity profile
Tau protein

ABSTRACT

The presence of natural tau-reactive antibodies was reported in human blood. In this study, we isolated and characterized natural tau-reactive antibodies occurring in IVIG product Flebogamma, plasma of patients with Alzheimer's disease (AD) and older cognitively normal persons (controls). Using blotting immunoassays and ELISA, we showed reactivity of antibodies obtained from IVIG and controls against a recombinant fragment of tau (155–421 aa) and aggregates present in brains of AD patients. In contrast, antibodies isolated from plasma of AD patients reacted mainly with the recombinant full-length tau form and tau monomeric forms in brain tissue.

1. Introduction

Nowadays, the therapeutic potential of antibodies in neurodegenerative diseases is being studied quite intensively (Schroeder et al., 2016). Several research groups have focused on the treatment of Alzheimer's disease (AD) using specific monoclonal antibodies, polyclonal intravenous immunoglobulins (IVIG) and vaccines (Castillo-Carranza et al., 2015; Davtyan et al., 2016; Dodel et al., 2004; Gu et al., 2013; Kayed, 2010; Knight and Gandy, 2014; Kontsekova et al., 2014; Sigurdsson, 2009; Steinitz, 2009). The IVIG products attracted a lot of attention as a possible treatment of neurological disorders (Fuchs et al., 2008) and even AD (Dodel et al., 2010; Kile et al., 2015; Neff et al., 2008; Relkin, 2014). However, the clinical trials for treatment of AD failed for two of the IVIG products, Octagam and Gammagard (Baxter U.S., 2013a, 2013b; Dodel et al., 2013). Another two clinical trials with Flebogamma® from Grifols (AMBAR, phase III, NCT01561053) and Sutter Health's IVIG NewGam™ (phase II, NCT01300728) are still under investigations (Grifols Biologicals Inc., 2015; Kile et al., 2015). However, the NewGam™ IVIG trial reported that a short course of IVIG administered in the MCI stage of AD reduces brain atrophy, prevents cognitive decline in late stage of MCI and delays conversion to AD dementia for at least 1 year (Kile et al., 2015). In the light of these findings, the characterization of reactivity of specific antibodies against either amyloid beta or tau protein present in these products (Balakrishnan et al., 2010; Dodel et al., 2004; Klaver et al., 2013; Smith et al., 2014, 2013) could clarify their contribution to the effect of these treatments. Notably, when we consider the use of AD-specific IVIG preparations enriched by antibodies specific for tau, amyloid-beta, complement, cytokines and other factors as was discussed by (Loeffler, 2014).

In the previous work, we showed specificity of plasma antibodies obtained from Flebogamma IVIG product against fragment 155–421 aa of tau protein (Hromadkova et al., 2015). The fragment 155–421 aa is cleaved at the Asp421 which is an early event in the pathological assembly of truncated tau in the neurofibrillary tangles (NFTs) evolution (Basurto-Islas et al., 2008). Moreover, tau comprises thrombin cleavage site at Arg155 and the products of thrombin proteolysis are potentially pathogenic (Chesser et al., 2013; Hanger and Wray, 2010) and both thrombin and its precursor prothrombin, are expressed by neurons and glia, and accumulate in NFTs in AD (Arai et al., 2006). Thus, our findings point towards the involvement of immune system in controlling the occurrence of pathological proteins under physiological conditions. However, an investigation of the characteristics and reactivity of naturally occurring antibodies in plasma against native physiological and pathological forms of tau protein is needed. In this study, we used homogenates of brain tissue from AD patients and control subject to reflect the reactivity of naturally occurring antibodies against native forms of tau protein. Moreover, in spite of the effort, little is known

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http://dx.doi.org/10.1016/j.jneuroim.2017.09.011
Received 13 April 2017; Received in revised form 20 June 2017; Accepted 27 September 2017

about the naturally occurring tau-reactive antibody profile in serum of patients with diagnosed AD and old, but cognitively normal individuals. Therefore, we looked into the reactivity profiles of antibodies found in the pool of plasma samples from patients with diagnosed AD and age-matched cognitively normal individuals with non-inflammatory neurological diseases. We isolated three fractions of natural tau-reactive antibodies from IVIG (nTau-IVIG), plasma of AD patients (nTau-AD) and cognitively normal control subjects (nTau-Ctrl). Their reactivity was investigated by ELISA and blotting techniques against recombinant non-phosphorylated/phosphorylated full-length (1–441 aa) form and fragment (155–421 aa) of tau protein and native tau proteins present in the homogenates of brain tissue.

2. Materials and methods

2.1. Participants

The research was approved by the Ethics Committee of the University Hospital Kralovske Vinohrady and conducted according to the Declaration of Helsinki and the Laws 129/2003 and 130/2003 of the Czech Republic. Informed consent was obtained from all individual participants included in this study.

Plasma samples were obtained from 11 patients from the Department of Neurology or Memory Clinic of the Charles University, Czech Republic. Their cognitive functions were evaluated using an updated Czech version of Addenbrooke's Cognitive Examination Revised (ACE-CZ) (Bartoš et al., 2011; Mioshi et al., 2006). We were then able to derive MMSE scores (range 0–30) from the ACE-CZ (score range 0–100). Four aged men and three elderly women were recruited as in-patients from the Department of Neurology and classified as cognitively normal. They had normal ACE-CZ (cut-off ≥ 79) and MMSE scores (cut-off ≥ 28). They mostly presented with non-inflammatory conditions such as polyneuropathy and peripheral Bell's facial palsy and the rest presented with a variety of diseases (e.g. a headache, trigeminal neuralgia and transient unconsciousness). Four AD patients (two men and two women) were diagnosed according to the NIA-AA criteria (McKhann et al., 2011).

Plasma samples were collected, centrifuged, and aliquoted in 1 ml polypropylene tubes and stored (on average within 1.5 h of sampling) at − 80 °C until analysis. The specimens were thawed just before experiments. All participants signed an informed consent.

Human brain tissues of one control individual (79 years old man whose cause of death was myocardial infarct) and two AD patients (two men at the age of 82 and 83 whose cause of death was cardiac insufficiency) were obtained by autopsy. Brains were evaluated using silver staining technique, dissected and stored according to the study of (Kriştofikova et al., 1995). The control subject was described as a nondemented and nonpsychotic patient (no marked histological changes indicative of AD pathology). The AD patients had clinically diagnosed dementia with a number of senile plaques and tangles in given areas of the cortex and in the hippocampus higher than would be expected for age. The criteria for the postmortem assessment of dementia and control subjects were consistent with those standardized by Dr. S. S. Mirra et al. from VA Medical Center, Department of Pathology and Laboratory Medicine (Decatur, GA) (Mirra et al., 1991).

2.2. Preparation of tau protein forms

2.2.1. Recombinant tau forms

The reactivity of isolated anti-tau antibodies was examined by using four recombinant His-tagged tau forms. The preparation of His-tagged full-length human tau 1–441 amino acids (tau 1–441 aa) and His-tagged human truncated tau form (tau 155–421 aa) was performed as previously described (Hromadkova et al., 2015). Both tau forms (52 µg each) were also phosphorylated by kinase mixture (500 U of GSK-3 from New England Biolabs (Ipswich, MA, USA), 125 U of ERK2 and 750 U of PKA from Biaffin GmbH & Co KG (Kassel, Germany)) in 200 µl of 40 mM HEPES buffer pH 7.2 with 5 mM $MgCl_2$, 5 mM EGTA, 2 mM DTT, and 1 mM ATP for 20 h at 30 °C. The phosphorylation was stopped by heat-denaturation of kinases (95 °C, 10 min). The phosphorylation state of tau proteins was confirmed by a mobility shift in SDS-polyacrylamide gel and by Western blots with specific anti-phospho-tau antibodies as described previously (Hromadkova et al., 2015). For all subsequent experiments, tau protein forms were transferred into PBS buffer by using Amicon® Ultra 0.5 ml filters (10 K, Merck Millipore, Billerica, MA, USA).

2.2.2. Tau protein forms in brain tissue homogenates

The reactivity of isolated anti-tau antibodies was also investigated using human brain homogenates where tau protein occurs in various native forms. The detailed characterization of the three used brain samples is summarized in the section 'Participants'. Sections of left hemisphere hippocampi from two AD patients and one control were homogenized in 1 × PBS buffer containing 2 × inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), 1 mM EDTA and 0.02% NaN_3 or with additional 2% SDS in 1:3 w/v dilution followed by 10,000 × g spin. Supernatants were collected and the total protein content of all homogenized samples was determined by BCA test according to manufacturer's instructions (Thermo Scientific, Waltman, MA, USA).

2.3. Isolation of natural tau-reactive antibodies

The antibodies were purified against tau protein by low-pressure affinity chromatography as previously described (Hromadkova et al., 2015). New column was prepared as follows: 4 mg of His-tagged tau 1–441 aa protein was reacted with 2 ml of pre-packed resin (Labiomer 300, epoxy-activated, 50 µm bead size in Econo-Pac column of 14 cm length and 1.5 cm diameter), residual reactive epoxide groups were blocked with 0.2 M ethanolamine overnight at 4 °C and then the column was equilibrated with PBS buffer (Hermanson, 2013). IVIG (3 ml, Flebogamma® 5% DIF (5 g/100 ml), Grifols Biologicals Inc., Los Angeles, CA, USA), plasma pool of samples from seven cognitively normal control subjects (3 ml; 108.5 mg/ml) and four patients with AD (3.5 ml; 107.3 mg/ml) were diluted to 10 ml in PBS and loaded onto the column. General characteristics of donors of plasma samples are listed in Table 1. The amount of protein in the initial fractions, flow-through (F-T) fractions and concentrated eluted fractions (before the addition of glycerol) was determined by BCA assay according to manufacturer's instructions (Thermo Scientific, Waltman, MA, USA).

Table 1
General characteristics of donors of plasma samples.

Groups	n	Sex (M/F)	Age (years)	MMSE (0 − 30)	ACE (0 − 100)	$A\beta_{42}$ (pg/ml)	Total Tau (pg/ml)	Phospho-Tau_{181} (pg/ml)
Controls	7	4/3	70 ± 8	28.5 ± 1	88 ± 5	737 ± 126	240 ± 65	37 ± 7
AD	4	2/2	76 ± 5	**17.5 ± 11***	**66 ± 14***	835 ± 279	**638 ± 140****	**52 ± 3****

Data are presented as the mean ± SD. Statistically significant differences highlighted in bold were calculated with respect to controls (Mann-Whitney test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).