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Changes in concentrations of tau-reactive antibodies are dependent on sex in Alzheimer's disease patients

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ABSTRACT

The presence of pre-existing natural antibodies against Alzheimer's disease (AD) pathological proteins might interfere with immune responses to therapeutic vaccination with these proteins.

We aimed to compare levels of antibodies in CSF and serum:

1) against three various tau antigens
2) between AD patients, MCI and other dementias and cognitively normal individuals
3) to find out if the antibody levels are dependent on age and sex

We observed higher reactivity of natural tau-reactive antibodies towards phosphorylated bovine tau protein than to human recombinant (non-phosphorylated) tau protein. Males with MCI-AD had higher amounts of these antibodies than corresponding controls. Concentrations of antibodies were lower in females with the MCI-AD than in control females. These findings may have implications for tau vaccination trials.

1. Introduction

Optimal targets for Alzheimer's disease (AD) immunotherapy include pathological forms of amyloid-beta and tau protein. The relationship between these molecules in development and progression of AD pathology is still controversial. The formation of amyloid-beta plaques is thought to be the initial event in the pathology of AD with recruitment of tau protein as second important player that can later propagate the pathology further (Ballatore et al., 2007; Selkoe, 1994; Selkoe and Hardy, 2016) Moreover, accumulated neurofibrillary pathology composed of tau protein correlates with cognitive decline of AD patients (Nelson et al., 2009, 2012). Current efforts to treat Alzheimer's disease (AD) include immunotherapeutic approaches that use monoclonal antibodies, active or passive vaccines and intravenous immunoglobulins products (IVIG) (Castillo-Carranza et al., 2014; Davtyan et al., 2016; Dodel et al., 2010; Gu et al., 2013; Kayed, 2010; Knight and Gandy, 2014; Kontsekova et al., 2014; Sigurdsson, 2009; Steinitz, 2009). So far, the clinical trials with intravenous immunoglobulins products Octagam and Gamagard have failed (Baxter, 2013a, 2013b; Dodel et al., 2013) and others are still under investigation (Grifols Biologicals Inc, 2015; Kile et al., 2017). The initial effort to vaccinate AD patients with amyloid-beta caused severe side effects (Fox et al., 2005; Orgogozo et al., 2003). Many more efforts to target Abeta were made after the AN1792 trial, and its targeting continues today, although because of the many negative results it remains unclear if any Abeta conformation should be considered as a legitimate therapeutic target in the AD. The continued failures in attempts to target Abeta led to increased focus on tau pathology as a potential therapeutic target.

The vaccines targeting tau protein are under investigation in clinical trials quite recently. The first findings from immunostimulation with tau-related vaccine AADVac-1 have been published (Novak et al., 2017) and results from a clinical study with ACI-35 of AC Immune SA, Janssen are awaited (ACI-35|ALZFORUM, 2018, p.). In the light of these efforts, the evaluation of character and amounts of naturally occurring antibodies against tau proteins would provide important information about the already existent immunologic background in patients with the AD and cognitively healthy elderly.

In recent years, we focused on qualitative characterization and avidity of tau-reactive antibodies from human plasma (Hromadkova et al., 2015). In our last study, we found that antibodies from the plasma of older cognitively healthy individuals reacted with pathological forms of tau protein present in the brain of AD patients unlike the antibodies from the plasma of AD patients (Krestova et al., 2017).

In the present study, we extended our previous qualitative findings by a quantitative approach. We measured concentrations of antibodies (IgG type) against tau protein simultaneously in two different compartments (serum and cerebrospinal fluid (CSF)) and calculated the estimated intrathecal synthesis (Deisenhammer et al., 2006). We aimed

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to compare serum and CSF levels of antibodies against three forms of tau antigen between AD patients and cognitively normal elderly. Tau protein forms differed in post-translational modifications that are important factors for the formation of pathological aggregates of this protein. Neither variant of our recombinant tau is phosphorylated. The first form represented recombinant human tau fragment 155–421 amino acids (aa) (cleavage sites correspond to Arg 155 specific for thrombin protease and Asp421 specific for caspase-3). The second form was native tau protein from the bovine brain with post-translational phosphorylation. As natural monomeric human tau is not readily available, we have used tau protein from the bovine brain with post-translational phosphorylation. Bovine tau is highly homologous to human tau, but less crossreactive (Alonso et al., 1995). The third form was full-length human recombinant tau protein. The aims of the current study were 1) to compare serum and CSF levels of antibodies against three various tau antigens, 2) to compare levels of antibodies between AD patients, MCI patients, individuals with other neurodegenerative disorders and cognitively normal individuals and 3) to find out if the antibody levels are dependent on age and sex.

2. Participants and methods

2.1. Participants

Paired serum and cerebrospinal fluid samples were collected from 134 participants at AD Center, Charles University, Department of Neurology or Memory Clinic, Prague, Czech Republic. The participants were divided into five groups. The first group of controls ($n = 46$) consisted of neurological patients with normal scores of the Mini-Mental State Examination according to recent Czech norms (Bartos and Raisova, 2016) and normal basic CSF findings according to European guidelines (Deisenhammer et al., 2006). The second group consisted of patients with mild cognitive impairment (MCI) not fulfilling the criteria for MCI due to AD ($n = 13$) (Petersen et al., 1999). The third groups included patients with MCI due to AD (MCI-AD) according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria ($n = 19$) (Albert et al., 2011). Both the second and the third group include MCI patients but they differ in etiology. The AD was not proved in the second one whereas AD was suggested by clinical and paraclinical findings in the third one. The fourth group consisted of patients with dementia due to the AD (ADD) according to the NIA-AA criteria ($n = 29$) (McKhann et al., 2011). The fifth group comprised patients with other types of dementia than AD (OD; $n = 26$): vascular dementia ($n = 6$, one with genetically confirmed Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), frontotemporal lobar degeneration ($n = 4$, three were autopsy confirmed), progressive supranuclear palsy ($n = 3$), Wernicke encephalopathy (n = 3, one was autopsy-confirmed), normal pressure hydrocephalus (n = 3), amyotrophic lateral sclerosis (n = 2), multiple system atrophy ($n = 1$), corticobasal degeneration (n = 1, autopsy confirmed), alcoholic dementia (n = 1) and unspecified dementia (n = 2). The diagnoses of patients with cognitive impairment were established by an experienced neurologist (AB). They were based on objective evidence of cognitive impairment, functional and neuropsychological assessments, findings on brain magnetic resonance imaging (or computed tomography in case of contraindications), single photon emission computed tomography, or concentration of total or phosphorylated tau protein and amyloid-β in cerebrospinal fluid using cut-offs established in our previous studies and others (Bartos and Raisova, 2016; Bartoš et al., 2007, 2012; Scheltens et al., 1992). We measured concentrations of total tau protein, phosphorylated tau at Thr 181 and $A\beta_{42}$ peptide in CSF of participants using ELISA kits from Fujirebio (Malvern, Pensylvania, USA) according to manufacturer's instructions and in line with our previous research (Bartoš et al., 2012).

All participants and witnesses signed an informed consent. All experiments were done in accordance with The Declaration of Helsinki and with Laws 129/2003 and 130/2003. The research was approved by the local ethics committee of the Prague Psychiatric Center, Prague/National Institute of Mental Health, Klecany, Czech Republic.

2.2. ELISA assay for measurement of serum and cerebrospinal fluid antibodies against tau antigens

Concentrations of antibodies against three tau antigens 1) recombinant human His-tagged truncated tau 155–421 aa protein, 2) bovine tau protein (Cytoskeleton, Denver, Colorado, USA), 3) recombinant human His-tagged tau 1–441 were measured by enzyme-linked immunosorbent assay (ELISA) in undiluted CSF and serially diluted sera samples (1:200, 1:600 and 1:1800) from control subjects and patients. All samples were measured in duplicate. Tau antigens in 0.1 M sodium bicarbonate buffer pH 9.5 were coated onto wells of microplate from Gama Group (Ceske Budejovice, Czech Republic) (0.05 ml of 2.5 μg/ml per well) and incubated overnight at 4 °C. The content of wells was removed, and all wells were blocked with 0.25 ml/well 1% bovine serum albumin (BSA) in phosphate buffered saline with 0.1% Tween-20 (PBS-T) pH 7.2 for 1 h at room temperature (RT). Then, the plates were washed three times with 0.3 ml/well of 0.1% BSA in PBS-T. Subsequently, 0.05 ml/well of serially diluted positive control, serum samples and undiluted CSF samples were applied to the wells and incubated 2 h in RT. The naturally occurring antibodies isolated from IVIG Flebogamma (Hromadkova et al., 2015; Krestova et al., 2017) were used as a standard and positive control for our in-house ELISA assay. The positive control was diluted in 1% BSA in PBS-T in the range from 0.05 to 36 μg/ml (stock concentration was 0.8 mg/ml) for generation of a standard curve. Each serum sample was diluted 1:200, 1:600 and 1:1800 by 1% BSA in PBS-T. The unbound primary antibodies were removed by five times washing and the incubation with 0.1 ml/well of secondary antibody goat anti-human IgG antibody HRP-conjugate (Novex, Life Technologies, Carlsbad, California, USA) at dilution 1: 20,000 for 30 min at RT followed. After five washes, the final incubation with TMB substrate for 30 min at RT in the dark was carried out. The developing color signal was stopped by 0.1 ml/well 1 M H_2SO_4 and the absorbance was measured during 30 min after stopping by a Multiskan EX ELISA reader (Thermo Scientific, Waltham, Massachusetts, USA) at wavelength 450 nm and with 620 nm as reference wavelength. The non-specific binding of antibodies to empty blocked wells was measured for each sample. The non-specific signal was subtracted from the signal obtained from coated wells. The mean optical densities of the serum and CSF samples were normalized to the mean optical density of the positive control of the corresponding ELISA plate to achieve comparable data. The concentrations (μg/ml) of tau-reactive antibodies were interpolated from the standard curve using GraphPad Prism software and for serum samples adjusted to the dilution factor (Figs. 1s and 2s). The dilutions 1:600 and 1:800 were optimal for the measurements of most samples because the concentrations corrected for the particular dilution factors corresponded well.

2.3. Evaluation of intrathecal synthesis of antibodies against tau protein antigens

We were interested not only in absolute CSF antibody levels but also in the portion of CSF antibodies that were produced locally within the CNS (intrathecal synthesis). We have estimated the relative intrathecal synthesis of specific antibodies in the CSF as follows: ((ratio between CSF and serum specific anti-tau antibodies) / (ratio between CSF and serum concentrations of total IgG)) ∗ 100 (Terryberry et al., 1998).

2.4. Statistical analysis

Data were analyzed with GraphPad Prism 6 (GraphPad Software, La Jolla, California, USA) and STATISTICA software (StatSoft, Tulsa, Oklahoma, USA). The results are presented as median with 25th–75th