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Identification and characterization of natural antibodies against tau protein in an intravenous immunoglobulin product

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ABSTRACT

The latest therapeutic approaches to Alzheimer disease are using intravenous immunoglobulin (IVIG) products. Therefore, the detailed characterization of target-specific antibodies naturally occurring in IVIG products is beneficial. We have focused on characterization of antibodies isolated against tau protein, a biomarker of Alzheimer's disease, from Flebogamma IVIG product. The analysis of IgG subclass distribution indicated skewing toward IgG3 in anti-tau-enriched IgG fraction. The evaluation of their reactivity and avidity with several recombinant tau forms was performed by ELISA and blotting techniques. Truncated non-phosphorylated tau protein (amino acids 155–421) demonstrated the highest reactivity and avidity index. We provide the first detailed insight into the reactivity of isolated natural antibodies against tau protein.

1. Introduction

The concept that humoral immunity may play a particular role during the development and progression of Alzheimer disease (AD) has an effect on trends in AD therapeutic strategies. Especially, the antibody-based immunotherapy focused on structurally modified forms of amyloid peptide and tau protein has gained a greater importance mainly since AD is considered as a protein conformational disorder (Carrell and Lomas, 1997; Soto, 1999). The fact that numerous natural antibodies reactive with different neuronal proteins have been proved both in AD and control serum increases general interest in revealing their relevance and therapeutic potential (Bahmanyar et al., 1983; Bartos et al., 2012; Levin et al., 2010; Rosenmann et al., 2006; Terryberry et al., 1998; Watts et al., 1981).

As a relatively new encouraging strategy for AD, therapy proposed the use of intravenous immunoglobulin products (IVIG) prepared from plasma of healthy donors, which represent a reservoir of natural autoantibodies comprising approximately two-thirds of the human immune repertoire of IgG (Pul et al., 2011). A notable property of most natural antibodies is their polyreactivity (polyspecificity) and the associated variable, predominantly low, antigen-binding affinities (Avrameas and Ternynck, 1993; Jianping et al., 2006; Sedykh et al., 2013; Szabo et al., 2010). These polyclonal antibodies recognize multiple epitopes, and more likely have stronger therapeutic effects than the passive immunization with monoclonal antibodies (Kayed et al., 2011). Another fact supporting IVIG products in AD therapy originates from their potent immunomodulatory and anti-inflammatory effect beneficial in many diseases, e.g. immune deficiency, autoimmune to cancer diseases, and even in a number of immune-mediated neurological disorders (Fuchs et al., 2008; Kajii et al., 2014; Seite et al., 2008; Stangel and Pul, 2006). Numerous studies with various results of natural anti-Aβ antibody levels in IVIG have been published (Balakrishnan et al., 2010; Dodel et al., 2002; Du et al., 2003; Klaver et al., 2010a,b, 2013; Szabo et al., 2008).

In recent years, interest in tau protein as a therapeutic target in AD has increased. This trend is supported by facts that tau pathology better correlates with AD progression and that it may be an independent process from Aβ accumulation (Arriagada et al., 1992a, 1992b; Braak et al., 1999; Gomez-Isla et al., 1997). Two papers about natural anti-tau antibodies in IVIG are currently known (Smith et al., 2013, 2014). They have proved that three IVIG products exhibit different concentrations of anti-tau specific antibodies and even high variability in percentage of antibodies against different tau fragments relative to total levels of

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anti-tau antibody contained in these products (Smith et al., 2013, 2014). It is reasonable to characterize the target-reactive antibodies in IVIG products whether their anticipated therapeutic effect could be significantly influenced by their concentration or even by the abundance of antibodies preferentially binding with particular epitopes of pathological protein forms.

In this study, we focused on the characterization of isolated natural antibodies against tau protein (nTau-Abs) from IVIG product Flebogamma DIF (5 g/100 ml, Grifols Biologicals Inc., Los Angeles, CA, USA). We isolated antibodies against human full-length form of tau protein by low-pressure affinity chromatography. The IgG subclass distribution was assessed. Subsequently, we compared the reactivity of all fractions of the isolation procedure with different non-phosphorylated/phosphorylated human full-length and truncated tau forms by ELISA. Blotting techniques were applied to assess the reactivity of isolated nTau-Abs in comparison with two anti-tau antibodies; monoclonal tau46.1 and polyclonal rabbit anti-tau antibodies. Moreover, the avidity index of isolated antibodies was measured against full-length and truncated tau forms by using chaotropic reagent in ELISA and dot-blot assays. We observed the highest reactivity and avidity index of isolated antibodies with the truncated non-phosphorylated form, tau 155–421. Our study can partly contribute to clarify the natural antibody diversity in pooled, highly concentrated immunoglobulin product Flebogamma, which is now examined in ongoing phase III trial (Grifols Biologicals Inc., 2015).

2. Materials and methods

2.1. Materials

cDNA of recombinant human tau proteins and tau monoclonal antibody (tau-46.1) were a generous gift from Dr. Francisco Garcia-Sierra (Mexico City, Mexico) and Dr. Lester I. Binder (Chicago, USA). Recombinant human tau protein (isoform 2N4R) was purchased from rPeptide (Bogart, GA, USA). *Escherichia coli* strain BL21 (DE3) (*E. coli* B F− dcm ompT hsdS(rB− mB−) gal λ(DE3) comes from Stratagene (San Diego, CA, USA). The IVIG product Immune Globulin Intravenous (Human) Flebogamma DIF (5 g/100 ml) was ordered from Grifols Biologicals Inc. (Los Angeles, CA, USA). Recombinant protein kinase ERK2 (401 000 U/mg) and mouse cAMP-dependent protein kinase (catalytic subunit Cα, PKA, >15 U/mg) were ordered from Biaffin GmbH & Co KG (Kassel, Germany). Glycogen Synthase Kinase 3 (GSK-3, 500 000 U/ml) and adenosine 5′-triphosphate (ATP, 10 mM) were acquired from New England Biolabs (Ipswich, MA, USA). Monoclonal mouse phospho-PHF-tau pSer202/Thr205 antibody (AT8) and phospho-PHF-tau pThr231 antibody (AT180) were purchased from Thermo Scientific (Waltham, MA, USA) and polyclonal rabbit anti-tau phospho-Ser396 from GenScript (Piscataway, NJ, USA). F(ab′)2-goat anti-human IgG (Fc specific, highly cross adsorbed/HRP conjugate) was purchased from Novex, Life Technologies (Carlsbad, CA, USA) and goat anti-rabbit IgG (HRP conjugate) from Sigma-Aldrich (St. Louis, MO, USA). Isopropyl-β-thiogalactopyranosid (IPTG), bovine serum albumin (BSA; 98% electrophoresis), tetramethylbenzidine (TMB; 98% TLC), DL-dithiothreitol (DTT), ethylene glycol tetraacetic acid (EGTA) and NH_4SCN (p.a.) were purchased from Sigma-Aldrich (St. Louis, MO, USA). PVDF membrane (Immuno-Blot PVDF Membrane, 0.2 μm), nitrocellulose membrane (0.2 μm), Precision Plus Protein™ WesternC™ Standard as a molecular marker, Clarity Western ECL substrate and Immun-Blot Opti-4CN Colorimetric kit were acquired from Bio-Rad (Hercules, CA, USA). BCA protein assay kit was obtained from Thermo Scientific (Waltham, MA, USA). All other chemicals were of reagent grade. The IgG Subclass ELISA Kit was obtained from Invitrogen (Camarillo, CA, US).

Resin Labiomer 300 was ordered from Labio (Prague, Czech Republic), and Ni Sepharose 6 Fast Flow resin was acquired from GE Healthcare (Wilmington, MA, USA). Empty Econo-Pac Chromatography columns and Trans-Blot® SD Semi-Dry Transfer Cell were obtained from Bio-Rad (Hercules, CA, USA). Amicon® Ultra 15 ml filters 30K (30 kDa molecular weight cut-off) were purchased from Merck Millipore (Billerica, MA, USA). 96-well microplates (Nunc Immunoplate F96 Maxisorp) were provided by Nunc, Thermo Scientific (Waltham, MA, USA). Dot-blot DHM-96 unit manifold was purchased from Scie-Plast (Cambridge, UK). Spectrophotometer Eppendorf 6131 was from Eppendorf (Hamburg, Germany).

2.2. Methods

2.2.1. Isolation of naturally occurring antibodies against tau protein from IVIG

We purified antibodies against tau protein from Flebogamma IVIG product by low-pressure affinity chromatography. The column was prepared as follows: 25 mg of His-tagged tau 1–441 protein was reacted with 5 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 (8 ml, Flebogamma) were loaded on a column by flow 0.1 ml/min and flow-through fraction in an amount of 15 ml was collected. The column was washed with 45 ml PBS buffer and immunocaptured Abs were eluted by 10.5 ml 0.1 M glycine-HCl buffer pH 2.6. Eluted fraction was immediately neutralized by 1 M Tris-base until pH 8–9. Purified Abs were then concentrated in centrifugal filter units (Amicon-Ultra 30K) to 2 ml final volume and stored in PBS pH 7.2 with 50% glycerol at protein concentration 0.8 mg/ml at −20 °C. The IgG amount in initial IVIG fraction, flow-through fraction and concentrated isolated fraction (prior to the addition of glycerol) was spectrophotometrically determined at absorbance 280 nm with parallel to PBS buffer as a blank. Percentages of IgG subclasses were assayed using an IgG Subclass ELISA Kit, according to the manufacturer's instructions, for initial IVIG and isolated antibody samples.

2.2.2. Reactivity evaluation of isolated antibodies with tau proteins

We tested the reactivity of isolated natural antibodies with several tau forms (their preparation in Supplemental materials) by two blotting immunoassays, Western blot and dot-blot. Western blot was carried out to confirm the reactivity with both unphosphorylated and phosphorylated tau forms. Whereas the dot-blot immunoassay was carried out with only unphosphorylated recombinant tau forms due to reactivity comparison with rabbit anti-tau antibodies isolated from immunized serum.

2.2.2.1. Western blot immunoassay. Unphosphorylated and phosphorylated tau samples (Suppl): tau 1–441 rPeptide, His-tagged tau 1–441, and truncated His-tagged forms: tau 155–421 and tau 13–391 (5 μg of each) mixed with reducing sample buffer in the volume ratio 1:1, were loaded into Tricine polyacrylamide gel (10% T, 3% C) and then transferred onto nitrocellulose membrane. The membrane was blocked by 5% defatted milk in PBS-0.1% Tween 20 (PBS-T) for 1 h at RT. Isolated anti-tau antibodies (1:250) were added in PBS-T with 1% BSA and incubated with the membrane overnight at 4 °C. The unbound IgG molecules were removed by PBS-T washing five times (each 5 min). The membrane was incubated with goat anti-human IgG antibody HRP-conjugate at 1:15 000 dilution in PBS-T with 1% BSA for 2 h at RT and subsequently washed by PBS-T five times (each 5 min). The chemiluminescence detection by Clarity western ECL substrate according to the manufacturer's instructions followed. ChemiDoc™ XRS + Imaging System with Image Lab™ Software (Bio-Rad, Hercules, CA, USA) was applied for documentation.

2.2.2.2. Dot blot immunoassay. Dot-blot analysis was performed with colorimetric 4-CN detection that is more suitable for subsequently used avidity dot-blot immunoassay. Additionally, for comparison purpose