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Beta-amyloid auto-antibodies are reduced in Alzheimer's disease

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ABSTRACT

Accumulation and cytotoxicity of amyloid beta (Aβ) are understood as the major cause of Alzheimer's disease (AD). There is evidence that naturally occurring antibodies against amyloid beta (Aβ) protein play a role in Aβ-clearance, and such a mechanism appears to be impaired in AD. In the present study, the anti-Aβ antibodies in the serum from individuals with and without late onset AD were measured using ELISA and dot-blot methods. Aβ auto-antibodies in serum were mainly targeted to Aβ1–15 epitope and its titer was significantly lower in AD patients than elderly non-AD controls (NC). The dot-blot analysis further demonstrated that auto-antibodies against fibrillar Aβ42, Aβ1–15 and Aβ16–30 epitopes were all in a lower level in AD than in NC. The isotypes of the auto-antibodies were mainly non-inflammatory IgG2 type. We also analyzed the relationship of auto-Aβ antibody levels with the genotypes of apolipoprotein E (ApoE) and ANKK1/DRD2 gene.

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1. Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disease associated with disruption of neuronal function in the hippocampus and cerebral cortex, which gradually deteriorates the cognition, function, and behavior of the patients (Schenk et al., 1999). Increased accumulation and deposition of amyloid β (Aβ) protein in the form of plaques are thought to be the leading causes, and clearance of Aβ from the brain has been a major focus for the prevention and treatment of AD. Active immunization with Aβ peptide increased blood anti-Aβ antibodies and thus decreased brain Aβ plaque burden in AD mouse models (Schenk et al., 1999; Morgan et al., 2000) as well as in AD patients (Gilman et al., 2005; Holmes et al., 2008). Administration of N-terminal Aβ antibodies was also effective in reducing plaques in AD mouse models and in AD patients (DeMattos et al., 2001; Mangialasche et al., 2010). These data indicate that pre-existing auto-antibodies against Aβ in the blood may play an important role in the plaque formation and such an immune mechanism may have been impaired in AD.

Many studies have been conducted to compare serum levels of Aβ auto-antibodies in AD and in age-matched non-AD control (NC) subjects. The results of these studies have been inconsistent: some reported that Aβ auto-antibodies in AD were lower than in normal subjects (Weksler et al., 2002; Brettschneider et al., 2005; Moir et al., 2005; Sohn et al., 2009), some unaltered (Hyman et al., 2001; Baril et al., 2004; Britschgi et al., 2009), and some increased (Nath et al., 2003; Gruden et al., 2007; Gustaw et al., 2008). The inconsistent results may have been caused by several factors including nonspecific bindings (Klaver et al., 2010), serum Aβ interference (Li et al., 2006), incorrect diagnosis (Klatka et al., 1996; Jellinger, 2009), structural conformation of Aβ1–42, and/or small sample size. Despite the relatively large number of studies being conducted, the epitope-specific binding and isotyping of the auto-antibodies against Aβ1–42 have not been reported. The present study therefore has sought to measure epitope-specific auto-antibodies against Aβ1–42 peptide in AD patients by comparison with the normal age-matched control subjects (NC). Our results indicate that naturally occurred Aβ antibodies mainly target Aβ1–15 epitope in both AD and NC subjects, as evidenced by the measurements of various Aβ epitopes with ELISA method, and in addition, its levels are significantly reduced in AD, especially in patients over 65 years of age, in comparison with those of NC subjects. Dot-blot analysis further

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demonstrated that antibody levels against fibrillar Aβ1–42, Aβ1–15 and Aβ16–30 were all significantly lower in AD than in NC subjects. The low level of Aβ1–15 auto-antibodies is also in a trend of association with ApoE 4/4 alleles and with ANKK-CC alleles.

2. Patients and methods

2.1. Patients and control subjects

We studied 113 subjects who were recruited from the Alzheimer's Disease Center at the University of Texas Southwestern Medical Center. The AD group was consisted of 53 subjects, and the control group was consisted of 60 non-cognitively impaired patients (see Table 1 for breakdown by age, race and gender). All subjects with AD had physical and neurological examinations, neuropsychological testing, laboratory studies, and brain imaging to exclude reversible causes of dementia. The average score of mini-mental state examination (MMSE) for the AD group was 19.8 ± 6.3 and for controls 28.9 ± 2.6 (mean ± SD). All patients met International Classification of Diseases (ICD)-10 criteria for dementia as well as National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria for probable AD. For some patients with AD, informants reported a family history of cognitive impairment, whereas normal controls had no reported family history. Control subjects had no significant decline or impairment in cognition on clinical examination. They had no history or evidence of neurologic disease with potential to affect cognition. All individuals or their legally authorized representatives have signed/supplied written informed consent. The blood was drawn and the serum was prepared, coded, and frozen at −80 °C within 1 h of collection.

2.2. Amyloid beta peptides

Aβ1–42, Aβ1–15, Aβ16–30 and Aβ31–42 peptides were synthesized by BioBasic Inc., Canada. The peptides were first dissolved in water and then by adding 10× PBS to make stock solution (2 mg/ml) in 1× PBS. Fibrillar Aβ1–42 was prepared by incubation of Aβ1–42 solution in 37 °C overnight (Schenk et al., 1999).

2.3. ELISA for measuring Aβ auto-antibodies in serum and Aβ1–42 peptide in plasma

The levels of anti-Aβ antibodies in sera of all subjects were measured using an enzyme-linked immunosorbent assay (ELISA). The wells of the ELISA plate (Immulon 2 HB U, Thermo Scientific) were coated with 2 μg/ml of human Aβ peptide in a 0.1 M bicarbonate–carbonate buffer (pH 9.0) at 4 °C overnight (50 μl/well). The wells were then blocked with 100 μl 1% bovine serum albumin (BSA) in PBS containing 0.05% (v/v) Tween-20 (PBST) for 1 h and then washed three times with PBST. The human serum (50 μl) was added to each well by a 1:100 dilution with 1% BSA in PBST, left for overnight at 4 °C to produce an Aβ-antibody complex. The plates were then washed three times with PBST and incubated for 2 h at room temperature with the secondary antibody (horseradish peroxidase labeled anti-human antibody, Bio-Rad) by 1:2000 dilution with 1% BSA-PBST. The plates were then washed three times with PBST. Finally, a 50 μl solution of 3,3,5,5-tetramethylbenzidine (TMB) was added to the wells, to form a colored reaction product indicating the presence of anti-Aβ antibodies, and absorbance was measured at a wavelength of 405 nm with a plate reader (ThermoMax). A mouse anti-human Aβ monoclonal antibody (4G8) was used as the standard.

For the isotyping of Aβ1–15 antibodies in the serum, highly positive samples from both AD (n = 15) and NC (n = 15) subjects were selected to add to the wells coated with Aβ1–15 peptide. The binding antibodies were detected with rabbit anti-human IgG1, IgG2 and IgG3 (Sigma-Aldrich, St. Louis, MO) followed by anti-rabbit IgG antibodies conjugated with HRP. The OD405 values were recorded.

Table 1
Subject summary.

Diagnosis	Gender	Age	Race	Vitamin	MMSE
AD (53)	33 male 20 female	55–65 (8) 66–75 (27) 76–89 (18) **72.3 ± 7.5**	2 African 3 Hispanic 48 Caucasian	22 no 31 yes	19.8 ± 6.3
NC (60)	21 male 39 female	55–65 (19) 66–75 (30) 76–82 (11) **67.6 ± 5.8**	2 African 3 Hispanic 55 Caucasian	24 no 36 yes	28.9 ± 2.6

Ages are reported as in 10 ears separate with means ± SD. AD, Alzheimer's disease, NC, non-cognitive impaired control. MMSE, mini-mental state examination (means ± SD).

2.4. Dot blot assay for detection of Aβ auto-antibodies in the serum

Aβ peptide (2 μg/2 μl) is spotted onto the nitrocellulose membrane, dried at room temperature, washed with TBST once (20 mM Tris–HCl, 150 mM NaCl, pH 7.5, 0.05% Tween 20), and blocked in 10% milk TBST at 4 °C overnight. The membrane is then incubated with human serum (1:1000) in 5% milk in TBST for 2 h, washed with TBST (3 × 5 min), incubated with secondary rabbit anti-human IgG antibody labeled with HRP in 1:2000 dilution (Santa Cruz Biotech. Dallas, TX) for 2 h at room temperature, washed three times with TBST (5 min each), one time with TBS (5 min), and one time with water (5 min). It was further incubated with ECL reagent for 1 min, covered with Saran-wrap (remove excessive solution from the surface), and exposed to X-ray film in the dark room. The spot area and intensity were quantified using ImageJ image processing and analysis software (NIH).

2.5. ApoE and ANKK1/DRD2 gene genotype

Genomic DNA was extracted from white blood cells using Qiagen DNA Blood kits (#51162; Qiagen Inc., Valencia, CA). Two TaqMan assays (Rs429358 and Rs7412, Applied Biosystems) were used for ApoE genotyping, and RS 1800497 (Applied Biosystems) for DRD2/ANKK1 Taq1A genotype.

2.6. Statistical analysis

Each blood sample was counted as an individual value for statistical analysis. The significance of difference was calculated by Student's t test (tails = 2, type = 2) expressed as the *p* value.

3. Results

We have established a sensitive, reproducible ELISA method to quantify the concentration of anti-Aβ immunoglobulins in human serum. The assay values obtained from this method are consistent with less than 10% variation between inter- and intra-assays. We first tested the binding capacity of the serum to various epitopes of the Aβ42 peptide, including Aβ1–42, Aβ1–42 fibrils (Aβ42F), Aβ1–15, Aβ16–30, and Aβ31–42 with this ELISA method. The anti-Aβ1–15 epitope antibodies were demonstrated to be in the highest reading values followed sequentially by Aβ16–30, Aβ1–42F, Aβ31–42 and Aβ1–42 in both AD and NC patients (Fig. 1A). When the antibody levels between AD and NC subjects were compared, Aβ1–15 targeted antibodies were the only one showing a significant reduction in AD compared with those in NC subjects. The average level is 0.59 ± 0.05 μg/ml serum (mean ± SEM; n = 53) in AD and 0.82 ± 0.08 μg/ml serum in NC (n = 60; $p = 0.02$, Fig. 1B). The anti-Aβ16–30 and Aβ42F antibodies also showed trend of reduction in AD compared with NC subjects, but statistical significance cannot be reached in the current sample size.