Antibody against C-terminal Abeta selectively elevates plasma Abeta

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Received 22 September 2006; accepted 18 November 2006

Accumulation of amyloid β in the brain is a pathological hallmark of Alzheimer's disease, and the reduction of amyloid β has been proposed as a primary therapeutic target. Mice immunized against amyloid β and mice infused with anti-amyloid β antibody (active and passive immunization, respectively) have reduced brain amyloid β levels, and two mechanisms have been proposed: microglial phagocytosis in the brain and enhancement of amyloid β efflux by antibodies present in the periphery (sequestration). The optimal antibody for microglial phagocytosis has been shown to be N-terminal-specific antibody; however, the potency of C-terminal-specific antibody in sequestration remains unclear. In this study, we found that anti-amyloid β 40-specific antibody induces amyloid β sequestration. These results indicate that C-terminal antibodies may be useful in amyloid β sequestration therapy. *NeuroReport* 18:293–296 © 2007 Lippincott Williams & Wilkins.

Keywords: Alzheimer's disease, amyloid β, antibody, C-terminus, immunization, passive immunization, sequestration, therapy

Introduction

Alzheimer's disease is a neurodegenerative affliction associated with memory dysfunction. Accumulation of amyloid β (Abeta) in the central nervous system (CNS) is a pathological hallmark of Alzheimer's disease, and the prevention of Abeta increase and accumulation has been proposed as a primary target for Alzheimer's disease therapy. Anti-Abeta antibodies evoked in response to active immunization with Abeta peptides reduced brain Abeta burden in amyloid-forming mice [1–4]. A clinical trial of active immunization was, however, terminated after severe encephalitis was found in 6% of patients. The specific cause of this adverse effect is not fully known, but immune reaction is presumably involved. Despite this failure, three patients who received the Abeta immunization showed apparent reduction of Abeta plaque load [5], and study results suggest that patients who had a humoral immune response to the immunization had slowing of cognitive decline [6]. More recently, intravenous infusion with pooled human immunoglobulin (which contains anti-Abeta antibody) improved cognition in an open-label study of a small number of Alzheimer's disease patients [7]. Cognitive enhancement was also reported in a Phase I study of passive immunotherapy with a humanized N-terminus monoclonal antibody, though MRI abnormalities consistent with focal brain edema were noted in some patients exposed to the highest dose of antibody. Overall, antibody-mediated Abeta reduction seems promising, but safety continues to be a significant concern.

Two possible mechanisms for antibody-mediated Abeta reduction have been proposed: the enhancement of microglial Abeta phagocytosis via Fc receptors [8], and alteration of the CNS/peripheral Abeta equilibrium by antibodies present in the blood (Abeta sequestration) [9,10]. Among anti-Abeta antibodies against various regions of Abeta, N-terminal antibodies are the most effective in the induction of microglial activity [11]. Antibodies against N-terminal and middle regions have the capacity to sequester Abeta [12]; however, the activity of C-terminal antibody in Abeta sequestration is unknown.

In this study, we investigated the activity of C-terminal Abeta antibody in Abeta sequestration using a transgenic mouse model of Alzheimer's disease.

Materials and methods

Transgenic mice expressing mutant amyloid precursor protein $(APP)_{K670N,M671L}$ (Tg2576) [13] at 9 ± 0.5 months of age (n=6 males in each group) were used in this study. We used two mouse monoclonal IgG1 antibodies: clone 1A10 (Immuno-Biological Laboratories, Gunma, Japan) [14] and 6E10 (Signet Laboratories, Dedham, Massachusetts, USA) [15], which have epitope at 36–40 (C terminus) and 3–7 (N terminus) amino acid residues of Abeta, respectively. Abeta 40-specific antibody, clone 1A10, was previously characterized [14]. In brief, with immunoblotting using Abeta 1-40 and 1-42 synthetic peptides, clone 1A10 did not detect

Abeta 1-42, whereas 1A10 strongly detected Abeta 1-40. Using a microplate assay, we determined that crossreactivities to uncleaved APP and Abeta 42 are negligible (at the background level and less than 1.8%, respectively). Clone 6E10 reacts with non-Abeta APP fragments, such as uncleaved APP, soluble APP α, in addition to Abeta. Both antibodies have similar affinity to Abeta 40; 1A10 and 6E10 have affinities of 20 and 35 nM, respectively.

Fifty micrograms IgG in 100 μl of 10 mM phosphate-buffered saline, pH 7.4, was intravenously administered through the tail vein. Blood was collected from the tail vein at 0, 3, 6 and 24 h after the injection. Ethylenediaminetetraacetic acid-treated plasma was prepared, and we determined the level of full-length Abeta 1-40 and 1-42 in plasma using the enzyme-linked immunosorbent assay (ELISA) that we have developed [14].

Abeta forms complexes with administered anti-Abeta antibody in the blood, and this Abeta/Abeta antibody complexes might interfere with the Abeta ELISA quantification. We compared Abeta levels in five samples: sample 1: synthetic human Abeta peptide only, 1200 or 260 fmol Abeta 1-40 or 1-42/ml, respectively, in plasma collected from nontransgenic mice; sample 2: sample 1 and anti-Abeta antibody, 20 μg IgG 1A10 or 6E10/ml. [We estimated total blood volume as 7% of body weight; that is, 35 g (average body weight) × 7%=2.45 ml blood/mouse. We administered 50 μg IgG/mouse, which results in 50 μg IgG/2.45 ml=20 μg IgG/ml], sample 3: 2% sodium dodecyl sulfate (SDS) was added after 15 min preincubation of Abeta and anti-Abeta antibody mixture (sample 2), sample 4: plasma prepared from Tg2576 mice and anti-Abeta antibody, 20 μg IgG 1A10 or 6E10/ml; sample 5: 2% SDS was added after 15 min preincubation of transgenic mouse plasma and anti-Abeta antibody mixture (sample 4). All samples were incubated on a rocker for 10 min at 37°C, diluted at 1 : 10 and then loaded onto an ELISA plate (the final SDS concentration in the ELISA plate is 0.13% owing to other liquid components). Statistical significance against Abeta level at 0 h time point was determined by analysis of variance (ANOVA) (SPSS, Chicago, Illinois, USA).

Results

Sodium dodecyl sulfate treatment dissociates Abeta/Abeta antibody complex in the plasma

As administered anti-Abeta antibody and Abeta form complexes in plasma that may interfere with Abeta quantification, we first established a method to dissociate the complexes to obtain accurate Abeta quantification. Our full-length Abeta 1-40 and 1-42 ELISA [14] detected 95–105% of known amounts of synthetic Abeta peptide in the plasma from nontransgenic mice (sample 1). In samples 2 and 4 (Abeta peptide or transgenic mouse plasma and 1A10), the ELISA indicated Abeta 1-40 at a level barely above background. Clone 1A10 is a component of the capture antibody in the ELISA, and we suspect that 1A10/Abeta complexes did not bind to the immobilized 1A10 capture antibody. Treatment with 2% SDS (final concentration in ELISA plate is 0.2%) dissociated complexes of synthetic Abeta/antibody and more physiologically relevant plasma Abeta/antibody, and recovered the ELISA signal (90–110%). With 6E10 antibody (epitope: 3–7) no interference was observed, because there is no overlap of the epitopes between the administered antibody and the antibodies used in the ELISA. SDS-treated samples were diluted at 1 : 10 and loaded onto the ELISA plate. We detected slightly larger variance in SDS-treated samples (90–110% in SDS-treated samples compared with 95–105% in plain samples). To maintain consistency, we treated all plasma with SDS regardless of the presence of interference.

Anti-C-terminal Abeta antibody selectively elevated plasma Abeta level

We determined full-length Abeta 1-40 and 1-42 levels in Tg2576 APP transgenic mice at 9 months of age as 1295 ± 175 and 264 ± 5 fmol/ml (mean ± SEM, n=6), respectively. We diluted the plasma 1 : 10 before loading the samples onto the ELISA plate; that is, actual ELISA measurements are 1/10th of the above levels. (Our ELISA has sensitivity to single-digit fmol/ml [14]). After injection of C-terminal Abeta 40-specific antibody, clone 1A10, plasma Abeta level was significantly elevated, reached a peak at 3 h after the

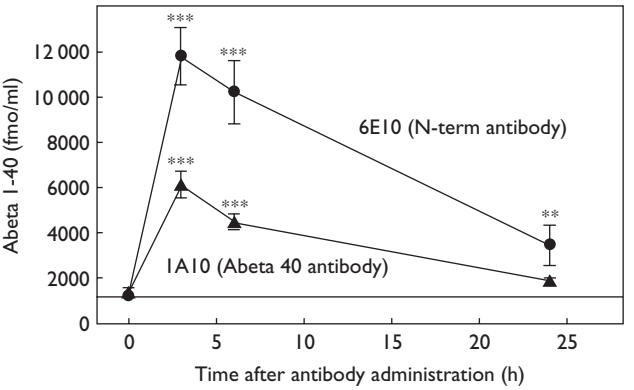

Fig. 1 Change in plasma amyloid β (Abeta) 1-40 in mice treated with intravenous injection of anti-Abeta antibodies. Anti-Abeta antibodies, clones 1AI0 and 6EI0, were intravenously injected through the tail vein of amyloid precursor protein transgenic mice, Tg2576, at 9 months of age. Blood was collected and plasma Abeta 1-40 level was determined by enzyme-linked immunosorbent assay. ***P < 0.001, **P < 0.01 using analysis of variance.

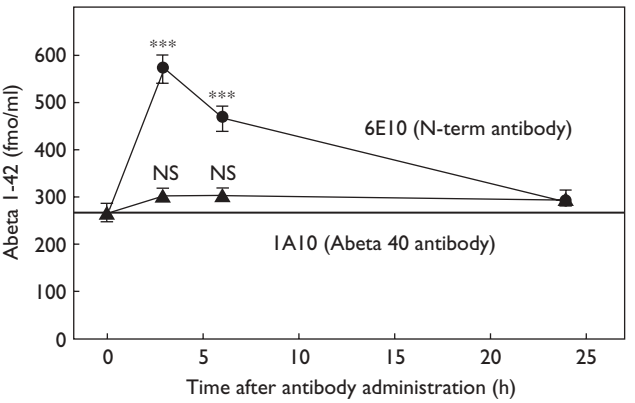

Fig. 2 Change in plasma amyloid β (Abeta) 1-42 in mice treated with intravenous injection of anti-Abeta antibodies. Anti-Abeta antibodies, clones 1AI0 and 6EI0, were intravenously injected through the tail vein of amyloid precursor protein transgenic mice, Tg2576, at 9 months of age. Blood was collected and plasma Abeta 1-42 level was determined by enzyme-linked immunosorbent assay. ***P < 0.001, NS, not significant, using analysis of variance.

injection, and then gradually declined (Fig. 1, closed triangle, $***P<0.001$, ANOVA). N-terminal region antibody, clone 6E10, also elevated plasma Abeta 1-40 level and showed similar kinetics, peaking at 3 h and then gradually decreasing (Fig. 1, closed circle, $***P<0.001$, $**P<0.01$, ANOVA).

Clone 1A10 is specific for Abeta 40; it did not alter plasma Abeta 1-42 level (Fig. 2, closed triangle, not significant at any point). N-terminal antibody, clone 6E10, binds both Abeta 1-40 and 1-42, and altered plasma Abeta 1-42 level (Fig. 2, closed circle, $***P<0.001$). Kinetics of plasma Abeta 1-42 elevation in response to the injection of 6E10 was similar to changes in Abeta 1-40; that is, reaching a peak at 3 h and then gradually declining.

Discussion

Antibodies raised in response to active immunization are against N terminus [1,10]. Bard *et al*. [8,11] compared the potency of various antibodies in microglial phagocytosis mode using accumulation of cultured microglia surrounding the Abeta plaques in APP transgenic mouse brain sections as an index. N-terminal antibodies showed the most efficient microglial accumulation, whereas antibodies against other regions, middle and C-terminal regions, did not enhance accumulation of microglia surrounding the plaques [8,11]. Antibodies are large molecules; however, peripherally administered antibodies entered the brain to some degree in mice [16]. In active immunization clinical trials, antibodies were detected in the cerebrospinal fluid, indicating that antibodies entered the brain [17]. C-terminal antibodies also enter the brain, but phagocytosis-mediated Abeta reduction may be limited, as their capability to enhance accumulation of microglia is limited [8,11]. Abeta in amyloid plaques is highly aggregated and accessible epitopes are limited, and only N-terminal antibodies can stain Abeta plaques in brain sections and promote microglial accumulation [8,11]. Antibodies against the middle and C-terminal regions of Abeta do not bind to Abeta plaques *in vivo* and presumably do not reduce brain Abeta load through phagocytosis. Abeta sequestration is an alternative mechanism of antibody-mediated Abeta reduction; it does not involve antibody binding to Abeta plaques. Instead, the antibody binds to Abeta in the plasma, altering Abeta dynamics between the CNS and periphery. In this study, we found that C-terminal Abeta 40-specific antibody, clone 1A10, has the ability to alter plasma Abeta levels. As we and other groups have shown, Abeta sequestration without any immune modulation is sufficient to reduce brain Abeta in plaque-bearing transgenic mice [18,19]. APP transgenic mice genetically lacking the Fc receptor, which were obtained by crossing APP overexpressing transgenic mice and Fc receptor knockout mice, showed reduction of brain Abeta load after immunization [20]. Fab fragments of anti-Abeta antibody reduced brain Abeta load after injection into the cerebral ventricle [21,22].

Conclusion

We found that C-terminal antibody has the ability to alter plasma Abeta levels. As C-terminal antibody does not induce microglial phagocytosis, it may represent a safe approach to antiamyloid immunotherapy. We found that the specificity of antibody determines the species of Abeta that is sequestered in response to treatment. As evidence suggests that the ratio of different Abeta species may carry more significance than total peptide levels [23], this selectivity may have therapeutic importance.

Acknowledgements

This work was supported by grants from the National Institutes of Health (AG022455, Y.M.; AG027398, K.D.) and the Alzheimer's Association (IIRG-02-3815, Y.M.).

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