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Suppression of Aβ deposition in brain by peripheral administration of Fab fragments of anti-seed antibody

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Abstract

Assembly and deposition of amyloid β-protein (Aβ) in the brain is a fundamental process of Alzheimer's disease (AD). We previously hypothesized that GM1 ganglioside-bound Aβ (GAβ) is an endogenous seed for Aβ assembly in brain. Recently, we have succeeded in generation of a monoclonal antibody specific to GAβ. Notably, this antibody, 4396C, per se substantially inhibits Aβ assembly in vitro. Here we report that the peripheral administration of Fab fragments of 4396C into transgenic mice expressing a mutant *amyloid precursor protein* gene, following the conjugation of the protein transduction domain of the Tat protein, markedly suppressed Aβ deposition in the brain. This result further supports our previous hypothesis and also provides a new insight into develop AD therapy through targeting seed Aβ in the brain, which selectively inhibits the initial step of the pathological process of AD.

Keywords: Alzheimer's disease; Amyloid; Seed; Ganglioside; Senile plaque; Fab antibody; Transgenic mouse; Tat; Protein transduction domain; Immunotherapy

A fundamental question about the early pathogenesis of Alzheimer's disease (AD) concerns how toxic aggregates of amyloid β-protein (Aβ) are formed from its nontoxic soluble form. We previously reported that Aβ adopts an altered conformation through binding to GM1 ganglioside in brain, and then, facilitates the assembly of soluble Aβ by acting as an endogenous seed [1–3]. Recently, we have successfully generated a novel monoclonal antibody (4396C) against GM1 ganglioside-bound Aβ (GAβ) purified from AD brain [4]. Our antibody specifically binds to GAβ and Aβ at the ends of growing fibrils; however, importantly, it binds to neither monomeric Aβ nor amyloid fibrils deposited as plaques [4]. Moreover, 4396C is potent to substantially inhibit the assembly of Aβ40 and Aβ42 in vitro [4]. These features of 4396C led us to expect that the administration of Fab fragments, but not the intact molecules of our antibody, is sufficient to suppress Aβ deposition in the brain.

Materials and methods

Dot blot analysis. Liposomes carrying GAβ were prepared by mixing GM1-containing liposomes with soluble Aβ (Aβ40 and Aβ42) on ice for 5 s at a weight ratio of lipid:Aβ = 100:3. The liposomes, and Aβ and GM1, in amounts equal to those contained in blotted

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liposomes (300 ng of Aβ; 2 μg of GM1), were blotted. The blots were incubated with 4396C (1:1000), 3F1, a monoclonal antibody specific to Aβ40 (1:1000), BAN052, a monoclonal antibody specific to amino terminus of Aβ [5] (1:5000), or HRP-conjugated cholera toxin subunit B (CTX) (1:20,000). The blots incubated with 4396C, 3F1 or BAN052 were then incubated with horseradish peroxidase-conjugated anti mouse IgG (Gibco-BRL). The bound-enzyme activities were visualized with an enhanced chemiluminescence (ECL) system (Amersham, Buckinghamshire, UK).

Immunohistochemistry. Cerebral hemisphere obtained from the Tg2576 mice were fixed with 4% formaldehyde solution, embedded in paraffin, and subjected to immunohistochemistry with 4396C, 3F1 or anti-AβN1, a polyclonal antibody specific to amino terminus of Aβ (IBL, Fujioka, Japan). The amyloid plaque burden was assessed

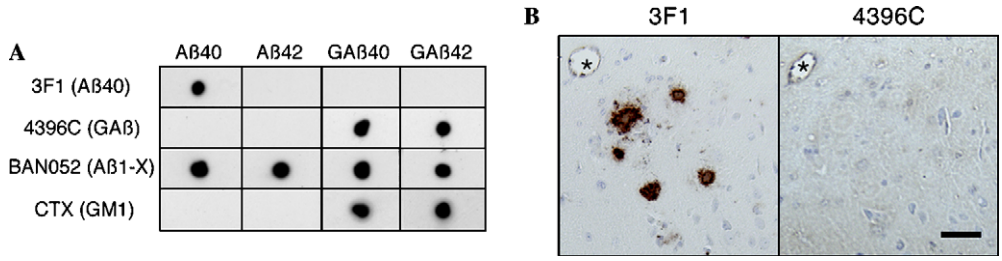

Fig. 1. Binding specificities of 4396C and 3F1. (A) Soluble or GM1-bound forms of Aβ40 and Aβ42 (Peptide Inst., Osaka, Japan) were blotted. The blots were reacted with 3F1, 4396C, BAN052, or cholera toxin subunit B (CTX). (B) Immunohistochemistry of the Tg2576 mouse brain, showing the plaque staining with 3F1, but not with 4396C. The asterisks indicate the same blood vessel in the serial paraffin sections. Bar, 50 μm.

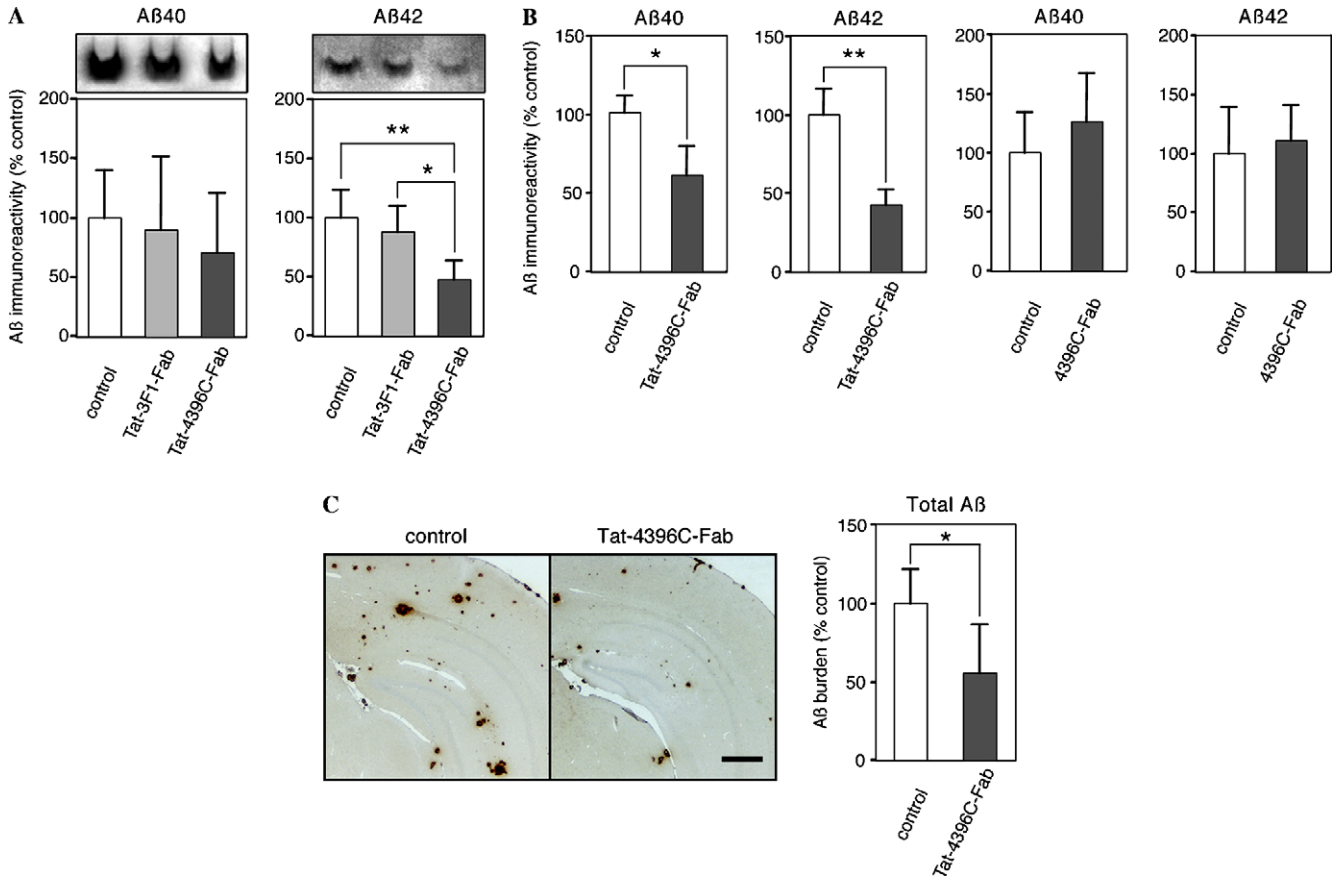

Fig. 2. Suppression of Aβ deposition in Tg2576 mice administered with Tat-4396C-Fab. (A) Western blot of homogenates obtained from mice nonadministered ($n = 4$) or administered with Tat-3F1-Fab ($n = 4$) or Tat-4396C-Fab ($n = 5$) for 5 months. Insoluble Aβ in the homogenates were precipitated following ultracentrifugation. Resulting pellets were subjected to Western blot analysis following solubilization in formic acid. The blots were reacted with BA27 or BC05, which are specific to Aβ40 or Aβ42, respectively [5]. The levels of Aβ42 deposition significantly decreased in Tat-4396C-Fab-administered mice. (B) Western blot analysis of homogenates obtained from mice nonadministered ($n = 3$) or administered with 4396C-Fab ($n = 6$) or Tat-4396C-Fab ($n = 6$) for 8 months. The blots were reacted with anti-Aβ40 and anti-Aβ42 antibodies (IBL, Fujioka, Japan). The levels of Aβ40 and Aβ42 deposition significantly decreased in Tat-4396C-Fab-administered mice. (C) Immunohistochemistry of cerebral cortex and hippocampus of mice nonadministered (control) ($n = 3$) or administered with Tat-4396C-Fab for 8 months ($n = 6$). The sections were immunostained with anti-AβN1. Amyloid burden was significantly reduced in the Tat-4396C-Fab-administered mice ($n = 6$) compared to nonadministered mice ($n = 3$). Bar, 500 μm. $^{*}p < 0.05$, $^{**}p < 0.01$, Scheffe's test.

using SimplePCI software (Compix, Cranbery Township, USA) after capturing the images by digital camera (Olympus DP50, Tokyo, Japan). Statistical analysis of the data was performed by Scheffe's test.

Preparation of Fab fragments. Fab fragments were purified by sequential chromatography using Ultrogel AcA 44 (Sigma, St. Louis, MO) and protein A–Sepharose (Amersham, Piscataway, NJ) following papain treatment of intact 4396C and 3F1. The Fab fragments were fused with a protein transduction domain of Tat [6] to facilitate the delivery of the fragments into the brain.

Treatment of Tg2576 mice with Fab fragments. We intraperitoneally injected the Fab fragments (200 μg protein) once a week into the 7-month old Tg2576 mice, expressing a mutant *amyloid precursor protein* gene [7], purchased from Taconic Farms (Germantown, NY). After 5 months (first experiment) or 8 months (second experiment), the mice were sacrificed and brain hemispheres were used for immunohistochemistry; the other samples were frozen at −80 °C until use for Western blot analysis.

Western blot analysis. The deposited Aβ in cerebral cortices was extracted with formic acid and subjected to Western blot analysis as previously reported [8]. The levels of Aβ on the blots were quantitatively assessed using NIH Image Ver/1.59 (Wayne Rasband, NIH, USA). Statistical analysis was performed by Scheffe's test.

Results and discussion

The reactivities of 4396C and 3F1 are shown in Figs. 1A and B. Notably, 4396C did not react with monomeric Aβ and senile plaque but only recognized GAβ. The binding specificities of the Fab fragments of these antibodies were not different from those of the intact forms (data not shown). In the first experiment, we intraperitoneally injected the Fab fragments of 4396C and 3F1 following conjugation of the protein transduction domain of the Tat protein (Tat-4396C-Fab and Tat-3F1-Fab). The level of Aβ42 deposited in the brain significantly decreased in mice administered with Tat-4396C-Fab for 5 months (Fig. 2A). In the second experiment, we intraperitoneally injected the Fab fragments of 4396C with or without prior conjugation of the protein transduction domain of the Tat protein. The levels of both deposited Aβ40 and Aβ42 significantly decreased following the administration of Tat-4396C-Fab for 8 months (Fig. 2B). In contrast, the administration of Tat-free 4396C-Fab did not decrease the levels of the deposited Aβ40 or Aβ42 (Fig. 2B). The suppression of Aβ deposition by Tat-4396C-Fab was further confirmed by immunohistochemistry (Fig. 2C).

Several studies have suggested that Aβ deposition in brain can be substantially suppressed by antibodies against Aβ amyloid [9–12]. In the present study, we observed, for the first time, that seed Aβ, but not the amyloid itself, can be a target for suppressing Aβ deposition in the brain. Furthermore, we showed that the administration of the Fab fragments, but not intact molecules of our antibody, is sufficient to suppress Aβ deposition in the brain. This result, together with the results of previous studies which showed non-Fc-mediated processes of experimental Aβ immunotherapy [13,14], suggests that Aβ deposition in brain can be suppressed without triggering an excessive immune response, which is likely to cause complications associated with current Aβ immunotherapies [15].

Further studies are required before we conclude that our antibody is useful for AD therapy; however, the results of the present study support our previous hypothesis that GAβ plays a critical role in the induction of Aβ assembly and deposition in brain.

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