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Amyloid β peptides with an additional cysteine residue can enhance immunogenicity and reduce the amyloid β burden in an Alzheimer's disease mouse model

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ABSTRACT

For the development of a safe vaccine for Alzheimer's disease (AD), we studied the immunogenicity of amyloid β (Aβ) peptides without adjuvant. Addition of a cysteine residue (Cys) to Aβ peptides enhanced immunogenicity in mice compared to those without Cys. Vaccination with the Aβ-Cys peptides reduced Aβ deposits in AD model mice. From these results, the Aβ-Cys peptides, administered without adjuvant, are considered candidates for vaccine therapy for AD.

In 1999, Schenk et al. showed that Aβ peptide vaccination with adjuvant in an AD mouse model reduced the Aβ deposits [1], and other groups subsequently reported similar results, in which AD model mice were protected from cognitive impairment [2,3]. Thereafter, a Phase II clinical trial was conducted of an AN-1792 vaccine consisting of Aβ peptide and saponin adjuvant QS21, but 6% of the vaccinated patients suffered meningoencephalitis during the trial, and it was therefore suspended. However, in the course of observing the patients after AN-1792 vaccination, it was reported that, while only a slight decline in cognitive function occurred in patients with elevated anti-Aβ antibodies, patients with no such elevation experienced the usual decline [4]. When the brains of deceased patients were analyzed, senile plaques had disappeared in the neocortex [5]. MRIs revealed greater brain volume decreases in antibody responders compared to placebo, and this was attributed to the alleviation of edema, and a decrease in brain volume, due to the removal of Aβ from the brain [6]. The AN-1792-induced meningoencephalitis was attributed to the use of a potent adjuvant and a T cell-mediated immunological response by the T cell epitope present in the Aβ sequence itself [5,7–12]. Therefore, we investigated the immunogenicity of Aβ peptides without an adjuvant to develop an AD vaccine that does not cause such side effects. As a result, it was determined that a high level of anti-Aβ antibody production is achieved by adding Cys to the C terminus of the Aβ peptides.

Materials and methods

Mice. Male C57BL/6 mice from Charles River Laboratories Japan were used in the immunization study. Immunization was initiated at 8–9 weeks of age. Female TG2576 mice from Taconic (Germantown, NY) were used as the AD model.

Peptides. Peptides having a variety of lengths in their C termini (Aβ1–6 to Aβ1–40) and Aβ peptides with a Cys conjugated to their C termini (Aβ1–6-Cys to Aβ1–40-Cys) were synthesized based on the human Aβ sequence DAEFRHHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (Aβ1–42) at Sigma–Aldrich. These peptides were dissolved in physiological saline at a concentration of 5 mg/mL and stored at less than −80 °C until use.

Immunization. The peptides were administered at 100 μg/200 μL/body subcutaneously. Immunization was performed three times at 2-week intervals.

Adjuvant. MPL + TDM (monophosphoryl lipid A [MPL]/trehalose dicorynomycolate [TDM]) emulsion produced by Corixa Corporation was used as adjuvant. MPL + TDM emulsion was mixed with Aβ peptide, and administered to the mice at 17 μg/body.

Collection of blood samples and serum separation. On day 7 after the final immunization, blood samples were collected from the

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abdominal vena cava of all mice under anesthesia with pentobarbital sodium (Somnopentyl) (Kyoritsu Seiyaku). After the blood samples were left to stand for one hour at 37 °C and adequate clotting had occurred, serum was separated by centrifugation for 10 min at 3500 rpm.

ELISA. ELISA plates (Immobilizer Amino) (Nunc) were coated overnight with human Aβ peptide (Aβ1–40) (BioSource) at 4 °C. Serum samples were diluted 50- to 10,000-fold with 0.05% Tween 20/PBS (PBST) and 100 μL/well was added. After incubation for an hour at 37 °C, each well was washed with PBST three times at 300 μL/well. HRP conjugated goat anti-mouse IgG (FC) (American Quax) that had been diluted 2000-fold was then transferred to each well at 100 μL/well. After incubation for an hour at 37 °C, each well was washed as described above. Bound antibody was detected by measuring the optical density at 450 nm (OD_{450} value), developed with TMB peroxidase substrate (TMB+) (Dako). A commercial monoclonal antibody to human Aβ (MS × Amyloid Beta Protein) (Chemicon) was used as the standard and the anti-Aβ IgG antibody titer of each serum sample was calculated from the standard curve obtained.

Evaluation using an AD mouse model. Aβ1–35-Cys was administered to the TG2576 AD mouse model, and the level of human Aβ (hAβ) in the brain was evaluated. Administration to TG2576 mice at four months of age was performed at 2-week intervals for the first four times, and thereafter at one-month intervals. Blood samples were collected at 15 months of age.

Sampling of hAβ deposited in the brain. Sampling was performed according to the method of Kawarabayashi et al. [13]. After blood collection, the brains were isolated via craniotomy. Part of the frontal lobe was sectioned and weighed. TBS (20 mM Tris, 137 mM NaCl, pH 7.6; CP/TBS) that contained protease inhibitors (complete protease inhibitor cocktail) (Boehringer Mannheim) was added to achieve a concentration of 150 mg (brain wet weight)/mL, and the mixture was homogenized in a Teflon homogenizer. Thereafter, centrifugation was performed at 12,000*g* for 10 min at 4 °C and the supernatant was used as the TBS fraction. The precipitate was resuspended in 1% Triton X-100/CP/TBS (the same amount as the CP/TBS added above) and shaken on a vortex mixer for 1 min. The supernatant obtained by centrifugation again at 12,000*g* for 10 min at 4 °C was used as the Triton X-100 fraction. The precipitate was again resuspended in 2% SDS/CP/TBS (the same amount as the CP/TBS added above) and shaken on a vortex mixer for 1 min. The supernatant obtained by centrifugation at 12,000*g* for 10 min at 4 °C was used as the 2% SDS fraction in the measurements.

Measurement of hAβ deposited in the brain. Measurement was performed using the β-amyloid (1–42) Elisa Kit Wako (Wako Pure Chemicals), per the procedures specified in the Kit.

Results

Immunogenicity of Aβ-Cys peptides

We evaluated the immunogenicity in mice of Aβ peptides having an additional amino acid residue on their C termini. Higher levels of anti-Aβ antibody production were achieved by adding Cys. Table 1 shows the titers of anti-Aβ antibodies when the Aβ peptides Aβ1–39, Aβ1–37, Aβ1–35 and Aβ1–31, and those with Cys added to their C termini, were administered to mice. Anti-Aβ antibody production was enhanced by several to several hundred fold by the addition of Cys to the C termini of these Aβ peptides. The addition of amino acids other than Cys to Aβ peptides did not result in such enhancement of immunogenicity (data not shown). In order to clarify at what sequence length anti-Aβ antibody production is enhanced by Cys, Cys was added to the C termini of Aβ1–6 to Aβ1–40 peptides (Table 2). Enhancement of anti-Aβ antibody production was observed when Cys was added to the Aβ1–28, Aβ1–29, Aβ1–30, Aβ1–31, Aβ1–35, Aβ1–36, Aβ1–37, Aβ1–38, Aβ1–39 and Aβ1–40 peptides. The addition of Cys had no enhancement effect on shorter peptides.

Table 1
Effects of addition of cysteine to the C termini of Aβ peptides.

Peptides	Anti-Aβ antibody (ng/mL) No. of animals				
	1	2	3	4	Average
Aβ1–39	257	1614	509	1544	981.1
Aβ1–39-Cys	447,512	196,147	85,137	13,445	185,560.2
Aβ1–37	23,421	222	16,477	4442	11,140.5
Aβ1–37-Cys	19,005	377,257	65,174	127,649	147,271.3
Aβ1–35	34	3584	1745	23,739	7275.7
Aβ1–35-Cys	382,533	37,390	88,795	257,920	191,659.5
Aβ1–31	56,582	158	18,302	1171	19,053.2
Aβ1–31-Cys	110,312	377,757	5809	51,904	136,445.4

Table 2
Effects of addition of cysteine to the C termini of 6- to 40-residue Aβ peptides.

Peptides	Anti-Aβ antibody (ng/mL) No. of animals				
	1	2	3	4	Average
Aβ1–6-Cys	16	9	16	8	12.2
Aβ1–7-Cys	25	27	47	42	35.2
Aβ1–8-Cys	27	14	13	17	17.7
Aβ1–9-Cys	15	31	21	50	29.2
Aβ1–10-Cys	24	12	15	11	15.5
Aβ1–11-Cys	13	14	20	11	14.4
Aβ1–12-Cys	28	35	28	35	31.4
Aβ1–13-Cys	12	14	12	10	11.9
Aβ1–14-Cys	21	34	12	11	19.7
Aβ1–15-Cys	13	11	10	17	12.5
Aβ1–16-Cys	24	28	18	14	20.8
Aβ1–17-Cys	15	11	19	15	15.0
Aβ1–18-Cys	62	23	22	31	34.5
Aβ1–19-Cys	17	14	23	15	17.3
Aβ1–20-Cys	7	8	14	11	10.0
Aβ1–21-Cys	29	17	19	19	21.0
Aβ1–22-Cys	21	31	18	19	22.3
Aβ1–23-Cys	23	25	23	73	35.8
Aβ1–24-Cys	16	17	22	32	21.7
Aβ1–25-Cys	7	16	6	5	8.5
Aβ1–26-Cys	39	19	21	19	24.5
Aβ1–27-Cys	15	19	24	19	19.0
Aβ1–28-Cys	3120	2191	115,618	86,736	51,916.1
Aβ1–29-Cys	143,754	5545	74,235	114,322	84,464.2
Aβ1–30-Cys	10,705	72	7335	1414	4881.5
Aβ1–32-Cys	27	18	213	19	69.4
Aβ1–33-Cys	44	28	35	27	33.4
Aβ1–34-Cys	24	25	74	18	35.3
Aβ1–36-Cys	36,535	16,261	44,439	13,262	27,624.4
Aβ1–38-Cys	4963	952	478	4371	2684.3
Aβ1–40-Cys	22,998	15,102	204,464	25,604	67,041.8

Comparison with adjuvant

The MPL + TDM adjuvant is widely used experimentally as a potent adjuvant, along with alum, Freund's complete adjuvant and Freund's incomplete adjuvant. We compared the immunogenicity of our Aβ1–28-Cys with or without the conventional potent adjuvant (Table 3). Anti-Aβ antibodies could not be detected following vaccination with Aβ1–28 without adjuvant, while high levels of anti-Aβ antibodies were observed with an MPL + TDM emulsion. Aβ1–28-Cys without adjuvant exhibited about the same antibody production enhancement effect as that seen with Aβ1–28 plus the potent adjuvant.

Table 3
Comparison of antibody generation effects of Aβ-Cys peptide and Aβ peptide plus adjuvant.

Peptides	Anti-Aβ antibody (ng/mL) No. of animals					
	1	2	3	4	Average	±SD
Aβ1–28	37	72	37	27	43.2	±19.94
Aβ1–28 + MPL + TDM emulsion	20,602	25,442	14,814	8832	17,422.6	±7188.6
Aβ1–28-Cys	8847	9458	10,446	48,224	19,243.7	±19,331.3

Table 4
Antibody generation of Aβ-Cys peptide in TG2576 mice.

Peptides	Anti-Aβ antibody (ng/mL) No. of animals					
	1	2	3	4	5	Average
4 M treated[a]	43,085	159,564	308,485			170,378.0
7 M treated[b]	125,039	7997	22,727			51,921.0
Non-treated[c]	242	105	660	932	510	489.6
Non-TG[d]	78	213				145.2

[a] Administration started from 4 months of age.
[b] Administration started from 7 months of age.
[c] Untreated.
[d] Non-TG mice, untreated.

Table 5
Intracerebral hAβ values in TG2576 mice.

Groups	n[a]	hAβ concentration (pmol/mL)		
		TBS	1% Triton-100[b]	2% SDS[c]
4 M treat	3	71 ± 15.9	21 ± 2.7*	1353 ± 935.2*
7 M treat	3	85 ± 67.4	34 ± 23.7*	3008 ± 1966.9
Non-treated	5	111 ± 29.1	92 ± 17.5	5031 ± 1523.8
Non-TG	2	0 ± 0.0	0 ± 0.0	0 ± 0.0

[a] Number of animals.
[b] Soluble fraction.
[c] Insoluble fraction.
* Significantly different at $p < 0.05$ with ANOVA followed by post hoc Tukey's test among 4 M treated, 7 M treated and non-treated.

Brain hA clearance by administration of A-Cys peptide to TG2576

The TG2576 mouse is a transgenic mouse expressing hAβ and is used as an AD mouse model that exhibits the accumulation of hAβ in the brain and kinetics similar to those in human AD. The Aβ1–35-Cys peptide was administered to this AD model mouse and antibody production and intracerebral hAβ were examined (Tables 4 and 5). Aβ1–35-Cys peptide showed potent antibody production even in the homozygote system. Both the soluble and insoluble fractions of the hAβ in the brains of mice with antibody elevations exhibited low values compared with those in the brains of the untreated groups (Table 5). Intracerebral hAβ decreased in the dosing groups at 4 months and 7 months of age, while administration at 4 months of age resulted in a greater percentage decrease than that at 7 months of age. The results indicated that earlier treatment with Aβ-Cys peptide is effective for reducing the accumulation of hAβ in the brain.

Discussion

This study showed that anti-Aβ antibody production induced by immunization without any adjuvant is greatly enhanced by the addition of Cys to Aβ peptide. The level of enhancement is about the same as that when a commercially available potent adjuvant is used for the original peptide. Therefore, those Aβ-Cys peptides need no adjuvant in inducing antibody responses without adjuvant-induced side effects. Antibody production can also be enhanced by the addition of Cys even to an Aβ peptide with a short C-terminal region sequence possibly missing a T cell epitope. T cell-mediated immunity caused by the T cell epitope present in the C-terminal segment may be escaped. Because intracerebral hAβ clearance in TG2576 mice by administration of Aβ-Cys peptide has been confirmed, this peptide is a promising candidate for AD vaccination.

Recent reports have shown that soluble Aβ oligomers mainly cause the neurotoxicity in AD [14,15]. In the present study, both the soluble and insoluble fractions of hAβ in the brains of AD model mice were reduced by the administration of Aβ-Cys peptide. Therefore, vaccination with Aβ-Cys peptides may reduce the neurotoxicity in AD.

It is not clear why Aβ-Cys peptides exhibited high immunogenicity. Change of antibody response by a point mutation to Cys in antigen molecules was indicated for factor VIII [16,17]. The enhancing effect of Cys addition on antibody response to Aβ peptides was recognized for larger Aβ peptides (28 and larger residues) except for Aβ1–32, 1–33 and 1–34. Cys addition to shorter peptides (6–27 residues) did not also increase antibody response. Thus, although the effect of Cys addition was dependent upon Aβ peptide length or sequence, this dependence was not simple. It has been shown that different glycosylation of a common peptide can both increase and decrease antibody binding ability through conformational change [18]. The limited enhancing effect of Cys addition on antibody response to some but not all Aβ peptides may be explained by conformational differences of Aβ1–32, 1–33 and 1–34 peptides from other Aβ peptides. The C-terminal region of Aβ has the β-sheet structure [19]. The enhancing effect of Cys addition on antibody response may be related to formation of this structure. No enhancing effect for shorter Aβ peptides can also be explained by missing such conformational characteristics in shorter length of peptides.

It is necessary to investigate cellular immunity to those peptides compared to conventional adjuvants. Aβ1–28-Cys peptide at least could avoid the side effects induced by the cellular immunity originating from the T cell epitopes in the C-terminal segment.

Ghochikyan et al. confirmed antibody production without adjuvant by conjugating mannan to the N terminus of Aβ1–28 peptide, and also demonstrated Th2 type immunity [20]. Cao et al. also reported antibody production without adjuvant using a peptide produced by introducing point mutation to a 42-residue peptide [21]. Since Aβ-Cys peptides examined in this study can be produced by a simple method,—that is, the addition of Cys to a shorter Aβ peptide than conventional Aβ—they can be expected to be widely used in research into an effective and safe AD vaccine.

Research into the use of anti-Aβ antibodies as immunotherapy for AD has been progressing apace. Antibody therapy is accompanied by little risk of the adverse reactions caused by cellular immunity that have been seen in a previous clinical trial of an Aβ peptide vaccine. However, with antibody therapy, it is possible that anti-idiotype and neutralizing antibodies to the antibodies used will be produced. According to a report by Wilcock et al., in a mouse

model, antibodies disappeared on about day 7 when anti-Aβ antibody was administered directly into the hippocampus, and frequent administration was required to maintain the antibody effects, which can cause an economic burden for the patients [22]. Pfeifer et al. reported on the risk of cerebral amyloid angiopathy-related vasculitis and cerebral hemorrhage in a study using a mouse model, noting that the risk of these diseases was increased by frequent administration [23].

Since Aβ-Cys peptides (e.g., Aβ-1–28-Cys) are shorter than conventional Aβ peptide (1–42) and are prepared using a simple method by adding Cys to the C-terminal segment, it is possible to select less invasive routes of administration, such as oral or percutaneous. It should be possible to develop this method as an extremely economical therapy for Alzheimer's disease, provided the efficacy and safety thereof can be assured.

References

[1] D. Schenk, R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, Z. Liao, I. Lieberburg, R. Motter, L. Mutter, F. Soriano, G. Shopp, N. Vasquez, C. Vandevert, S. Walker, M. Wogulis, T. Yednock, D. Games, P. Seubert, Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse, Nature 400 (1999) 173–177.

[2] C. Janus, J. Pearson, J. McLaurin, P.M. Mathews, Y. Jiang, S.D. Schmidt, M.A. Chishti, P. Horne, D. Heslin, J. French, H.T. Mount, R.A. Nixon, M. Mercken, C. Bergeron, P.E. Fraser, P. St George-Hyslop, D. Westaway, A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease, Nature 408 (2000) 979–982.

[3] D. Morgan, D.M. Diamond, P.E. Gottschall, K.E. Ugen, C. Dickey, J. Hardy, K. Duff, P. Jantzen, G. DiCarlo, D. Wilcock, K. Connor, J. Hatcher, C. Hope, M. Gordon, G.W. Arendash, A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease, Nature 408 (2000) 982–985.

[4] C. Hock, U. Konietzko, J.R. Streffer, J. Tracy, A. Signorell, B. Müller-Tillmanns, U. Lemke, K. Henke, E. Moritz, E. Garcia, M.A. Wollmer, D. Umbricht, D.J. de Quervain, M. Hofmann, A. Maddalena, A. Papassotiropoulos, R.M. Nitsch, Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease, Neuron 38 (2003) 547–554.

[5] J.A. Nicoll, D. Wilkinson, C. Holmes, P. Steart, H. Markham, R.O. Weller, Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report, Nat. Med. 9 (2003) 448–452.

[6] N.C. Fox, R.S. Black, S. Gilman, M.N. Rossor, S.G. Griffith, L. Jenkins, M. Koller, Effects of Abeta immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease, Neurology 64 (2005) 1563–1572.

[7] I. Ferrer, M. Boada Rovira, M.L. Sánchez Guerra, M.J. Rey, F. Costa-Jussá, Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease, Brain Pathol. 14 (2004) 11–20.

[8] E. Check, Nerve inflammation halts trial for Alzheimer's drug, Nature 415 (2002) 462.

[9] J.M. Orgogozo, S. Gilman, J.F. Dartigues, B. Laurent, M. Puel, L.C. Kirby, P. Jouanny, B. Dubois, L. Eisner, S. Flitman, B.F. Michel, M. Boada, A. Frank, C. Hock, Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization, Neurology 61 (2003) 46–54.

[10] A. Monsonego, J. Imitola, S. Petrovic, V. Zota, A. Nemirovsky, R. Baron, Y. Fisher, T. Owens, H.L. Weiner, Abeta-induced meningoencephalitis is IFN-gamma-dependent and is associated with T cell-dependent clearance of Abeta in a mouse model of Alzheimer's disease, Proc. Natl. Acad. Sci. USA 103 (2006) 5048–5053.

[11] M. Maier, T.J. Seabrook, C.A. Lemere, Modulation of the humoral and cellular immune response in Aβ immunotherapy by the adjuvants monophosphoryl lipid A (MPL), cholera toxin B subunit (CTB) and *E. coli* enterotoxin LT(R192G), Vaccine 23 (2005) 5149–5159.

[12] R.M. Nitsch, C. Hock, Targeting beta-amyloid pathology in Alzheimer's disease with Abeta immunotherapy, Neurotherapeutics 5 (2008) 415–420.

[13] T. Kawarabayashi, L.H. Younkin, T.C. Saido, M. Shoji, K.H. Ashe, S.G. Younkin, Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease, J. Neurosci. 21 (2001) 372–381.

[14] D.M. Walsh, I. Klyubin, J.V. Fadeeva, W.K. Cullen, R. Anwyl, M.S. Wolfe, M.J. Rowan, D.J. Selkoe, Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo, Nature 416 (2002) 535–539.

[15] D.J. Selkoe, Alzheimer's disease is a synaptic failure, Science 298 (2002) 789–791.

[16] K. Fijnvandraat, E.A.M. Turenhout, E.N. van den Brink, J.W. ten Cate, J.A. van Mourik, M. Peters, J. Voorberg, The missense mutation Arg593 to Cys is related to antibody formation in a patient with mild hemophilia A, Blood 89 (1997) 4371–4377.

[17] A.R. Thompson, M.E. Murphy, M. Liu, E.L. Saenko, J.F. Healey, P. Lollar, D. Scandella, Loss of tolerance to exogenous and endogenous factor VIII in a mild hemophilia A patient with an Arg593 to Cys mutation, Blood 90 (1997) 1902–1910.

[18] X. Huang, J.J. Barchi Jr., F.D. Lung, P.P. Roller, P.L. Nara, J. Muschik, R.R. Garrity, Glycosylation affects both the three-dimensional structure and antibody binding properties of the HIV-1IIIB GP120 peptide RP135, Biochemistry 36 (1997) 10846–10856.

[19] K. Takano, S. Endo, A. Mukaiyama, H. Chon, H. Matsumura, Y. Koga, S. Kanaya, Structure of amyloid β fragments in aqueous environments, FEBS J. 273 (2006) 150–158.

[20] A. Ghochikyan, I. Petrushina, A. Lees, V. Vasilevko, N. Movsesyan, A. Karapetyan, M.G. Agadjanyan, D.H. Cribbs, Abeta-immunotherapy for Alzheimer's disease using mannan-amyloid-Beta peptide immunoconjugates, DNA Cell Biol. 25 (2006) 571–580.

[21] C. Cao, X. Lin, M.M. Wahi, E.A. Jackson, H. Potter Jr., Successful adjuvant-free vaccination of BALB/c mice with mutated amyloid beta peptides, BMC Neurosci. 9 (2008) 25.

[22] D.M. Wilcock, G. DiCarlo, D. Henderson, J. Jackson, K. Clarke, K.E. Ugen, M.N. Gordon, D. Morgan, Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation, J. Neurosci. 23 (2003) 3745–3751.

[23] M. Pfeifer, S. Boncristiano, L. Bondolfi, A. Stalder, T. Deller, M. Staufenbiel, P.M. Mathews, M. Jucker, Cerebral hemorrhage after passive anti-Abeta immunotherapy, Science 298 (2002) 1379.