β-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy

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Inhibition of cerebral amyloid β-protein deposition seems to be an important target for Alzheimer's disease therapy. Amyloidogenesis could be inhibited by short synthetic peptides designed as β-sheet breakers. Here we demonstrate a 5-residue peptide that inhibits amyloid β-protein fibrillogenesis, disassembles preformed fibrils *in vitro* and prevents neuronal death induced by fibrils in cell culture. In addition, the β-sheet breaker peptide significantly reduces amyloid β-protein deposition *in vivo* and completely blocks the formation of amyloid fibrils in a rat brain model of amyloidosis. These findings may provide the basis for a new therapeutic approach to prevent amyloidosis in Alzheimer's disease.

A central event in Alzheimer's disease (AD) is the cerebral deposition of amyloid, an insoluble substance composed mainly of the 39–43-residue amyloid beta-protein (Aβ). Genetic, neuropathological and biochemical evidence indicate that amyloid plays an important role in the early pathogenesis of AD[1]. Therefore, inhibiting amyloid deposition in the brain would be a good target for AD therapy. The mechanism by which senile plaques and vascular amyloid are associated with dementia in AD is unknown. However, several *in vitro* studies have shown that β-pleated sheet Aβ fibrils are neurotoxic[2–4]. We have postulated that fibrillogenesis can be inhibited by short peptides partially homologous to Aβ that contain residues acting as β-sheet blockers[5] (β-sheet breaker peptides) and reported that an 11-residue β-sheet breaker peptide (iAβ11) binds to Aβ with high affinity and inhibits amyloid formation *in vitro*[5].

β-sheet breaker peptide, design and inhibitory activity *in vitro*

The central hydrophobic region in the N-terminal domain of Aβ, amino acids 17–20 (LVFF), served as a template for designing the β-sheet breaker peptide iAβ5 (LPFFD). Amino-acid substitutions in this region of Aβ produce large changes in the peptide's conformation and its ability to make amyloid fibrils[6–9]. Proline residues, well known β-sheet blockers[9,10], were included in the peptide to decrease its propensity to adopt a β-sheet structure, and a charged residue was added at the end of the peptide to increase solubility. iAβ5 inhibited, in a dose-dependent manner, amyloid formation by Aβ1–40 and Aβ1–42 *in vitro* (Fig. 1*a*). Incubation of Aβ1–40 (0.5 μg/μl) for seven days in the presence of equimolar (0.074 μg/μl iAβ5) or a 20-fold molar excess (1.5 μg/μl iAβ5) of iAβ5 produced 53.5 and 84.1% inhibitions of fibrillogenesis, respectively (Fig. 1*a*). Under the same conditions Aβ1–42 fibrillogenesis was inhibited 34.2 and 71.9% (Fig. 1*a*). Unrelated control peptides (CP1: VHVSEEGTEPA; CP2: GYLTVAAVFRG; and CP10: ISEVKMDAEF) or short Aβ fragments (Aβ17–21, Aβ1–16, and Aβ17–22) at the same concentrations either had no effect on fibrillogenesis or slightly increased amyloid formation, probably because of incorporation into the fibrils. To evaluate the ability of iAβ5 to dissolve preformed fibrils, Aβ1–40 or Aβ1–42 (0.5 μg/μl) were preincubated alone for seven days at 37 °C, and then different concentrations of iAβ5 were added. After two days of additional incubation in the presence of the inhibitor peptide, the amount of amyloid was quantitated by thioflavine T fluorometric assay (Fig. 1*b*). The results show that iAβ5 induced a disassembly of preformed Aβ fibrils. Inhibition of Aβ fibrillogenesis and dissolution of preformed fibrils *in vitro* was also evident when aggregation was quantitated by sedimentation assays[11] or Congo red-binding in solution[12] (data not shown). Qualitative examination of the effect of iAβ5 by electron microscopy showed that no fibrillar material was present when Aβ1–42 (0.5 μg/μl) was incubated with iAβ5 (1.5 μg/μl) for seven days (Fig. 1*c*, central panel). Fibrils preformed by incubation of Aβ1–42 alone for seven days (Fig. 1*c*, left panel) were completely dissolved after treatment with 1.5 μg/μl of iAβ5 for four days (Fig. 1*c*, right panel), and only amorphous aggregates were seen.

Prevention of Aβ neurotoxicity

Aβ is neurotoxic in cell culture, and this toxicity is related to the formation of amyloid fibrils[2–4]. Therefore, we studied the effect of iAβ5 on Aβ-induced toxicity in human neuroblastoma (IMR-32) cell culture. Cells were treated for two days with 50 μM aggregated Aβ1–42. Aggregation was induced by preincubation with Aβ alone, at a concentration of 310 μM, for two days at 37 °C. Under these conditions, ^{3}H-thymidine incorporation (Fig. 2*a*) and cell viability as assessed by DNA/RNA staining with acridine orange-ethidium bromide (Figs. 2*b* and 3) were approximately 30% that of control. iAβ5 at a molar ratio of 1:1 (Aβ1–42: iAβ5) was sufficient to induce a significant prevention of Aβ fibril formation. Therefore, we co-incubated Aβ1–42 (310 μM) with iAβ5 (366 μM) for 48 hours and then diluted the peptides and applied them to the cells at final concentrations of 50 and 60 μM, respectively. Co-incubation of Aβ1–42 with iAβ5 produced a marked

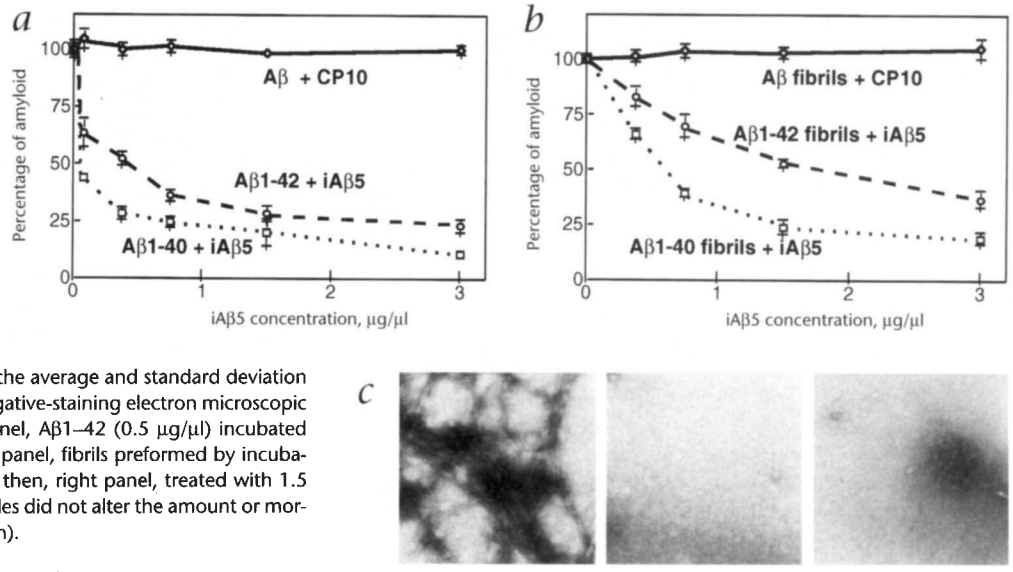

Fig. 1 Inhibition of Aβ fibrillogenesis and dissolution of preformed fibrils by iAβ5 *in vitro*. ***a***, Dose-dependent inhibition of Aβ1–40 and Aβ1–42 fibrillogenesis by iAβ5. Amyloid formation was quantitated by thioflavine T fluorometric assay and is expressed as a percentage of the amount obtained in the absence of iAβ5 or CP10. ***b***, Concentration-dependent disassembly of preformed Aβ1–40 and Aβ1–42 fibrils. Fibril formation was not affected by an unrelated peptide (CP10) and only the control experiment with Aβ1–42 fibrils is shown. In both *a* and *b*, the average and standard deviation of three different samples is shown. ***c***, Negative-staining electron microscopic analysis of the effect of iAβ5. Central panel, Aβ1–42 (0.5 μg/μl) incubated with iAβ5 (1.5 μg/μl) for seven days; left panel, fibrils preformed by incubation of Aβ1–42 alone for seven days and then, right panel, treated with 1.5 μg/μl of iAβ5 for four days. Control peptides did not alter the amount or morphology of amyloid fibrils (data not shown).

inhibition of Aβ1–42 neurotoxicity ($P < 0.01$) (Fig. 2*b*). At 60 μM, iAβ5 was not significantly toxic, as indicated by a cell loss of less than 10% compared with that of control cells. Aβ1–42 co-incubated with a control peptide (CP10) had an effect similar to that of Aβ1–42 alone (Figs. 2a, *b* and 3). The preventive effect of iAβ5 on neuronal death induced by Aβ fibrils lasted at least four days (Fig. 2*c*). The results of this study support the concept that Aβ neurotoxicity is mediated by the formation of a β-pleated sheet structure[2,3,13]. However, the relevance of Aβ neurotoxicity *in vitro* to Alzheimer's dementia is unclear at this time[4].

Inhibition of amyloid deposition in rat brain

To evaluate the effect of the inhibitor on amyloid deposition *in vivo*, we created a rat model of cerebral Aβ deposition by injecting freshly solubilized Aβ1–42 directly into the amygdala[14,15], the brain region reported to have the highest number of senile plaques in AD (ref. 16). All the rats injected with Aβ1–42 (5 nmol; 22.5 μg into each amygdala; $n = 9$) had bilateral Aβ deposits at the injection site, as determined by immunohistochemistry using EM-3, a polyclonal antibody against Aβ1–42 (ref. 17) (Fig. 4*a*). The deposits were always Congo red-positive, showing the typical apple-green birefringence under polarized light (Fig. 4*b*). The fibrillar nature of the Aβ deposits was verified by staining selected series with thioflavine S (Fig. 4*c*) and by electron microscopy analysis of EM-3-positive immunogold-labeled fibrils (Fig. 4*d*). When a 20-fold molar excess of iAβ5 was co-injected with Aβ1–42 ($n = 9$), smaller Aβ deposits (Fig. 4*e*) were observed that were always Congo red-negative (Fig. 4*f*), thioflavine S-negative (Fig. 4*g*) and had a punctuate amorphous appearance by electron microscopy (Fig. 4*h*). No deposits were found in control rats injected with vehicle ($n = 6$) or iAβ5 alone ($n = 6$) (data not shown).Image analysis of the brain sections stained with EM-3 showed that the size of the Aβ deposits in the rats injected with the mixture of Aβ1–42 and iAβ5 was 51% the size of Aβ deposits in rats injected with Aβ1–42 alone (Fig. 5) [$F(1, 32) = 7.276$, $P = 0.011$]. The area of the deposits did not differ significantly between left and right hemispheres [$F(1, 32) = 0.595$, $P = 0.446$]. Reactive astrocytosis, demonstrated by GFAP immunoreactivity, was always found associated with the cannula track in all the animals and, to a greater extent, surrounding the Aβ deposits (data not shown). Sections from several animals were stained with cresyl violet to detect neuronal damage. We did not detect neuronal loss in any of the treatment groups using gross observations of these sections. A few EM-3-positive cells that resembled phagocytes were usually seen near the deposits. The size and number of these phagocyte-like cells did not seem to differ between the fibrillar and amorphous deposits (data not shown).

The extent of astrogliosis did not seem to differ between Congo red-positive and Congo red-negative deposits, indicating that gliosis was probably a reaction to the cannula placement and peptide deposition.

Discussion

Our study demonstrates a compound that inhibits amyloid formation *in vitro*, prevents amyloid neurotoxicity in cell culture, reduces *in vivo* cerebral Aβ deposition and completely blocks amyloid fibril formation in rat brain. Although the molecular mechanism by which the peptide prevents Aβ fibrillogenesis is unknown, β-sheet breaker peptides probably inhibit amyloid formation by binding to monomeric/dimeric Aβ peptides, thereby blocking the formation of the oligomeric β-sheet-conformation precursor of the fibrils. The inhibition of *in vivo* Aβ deposition may follow the same mechanism, but because of the higher complexity of the system we can not rule out other possibilities. Enhanced clearance of the injected Aβ peptide induced by the presence of iAβ5 is an alternative. However, the number of phagocyte-like cells containing EM-3-positive material did not seem differ between animals injected with Aβ1–42 alone or in combination with iAβ5, indicating that the effect occurs during the initial deposition. A major drawback with the *in vivo* use of peptides is that they are degraded by natural enzymes and have poor blood-brain barrier permeability. We have recently designed chemical modifications of a β-sheet breaker peptide that did not alter its ability to prevent fibrillogenesis *in vitro*, but significantly increases its brain permeability and its resistance to proteolysis in rat plasma (J. Poduslo, G. Curran, B.F. & C.S., manuscript submitted).

The *in vivo* model of brain amyloidosis induced by amygdaloid injection of Aβ is an efficient and quick screening tool for Aβ inhibitors. The therapeutic utility of compounds can be determined in one to two months. Transgenic mice have been developed with amyloid deposits that more closely resemble the

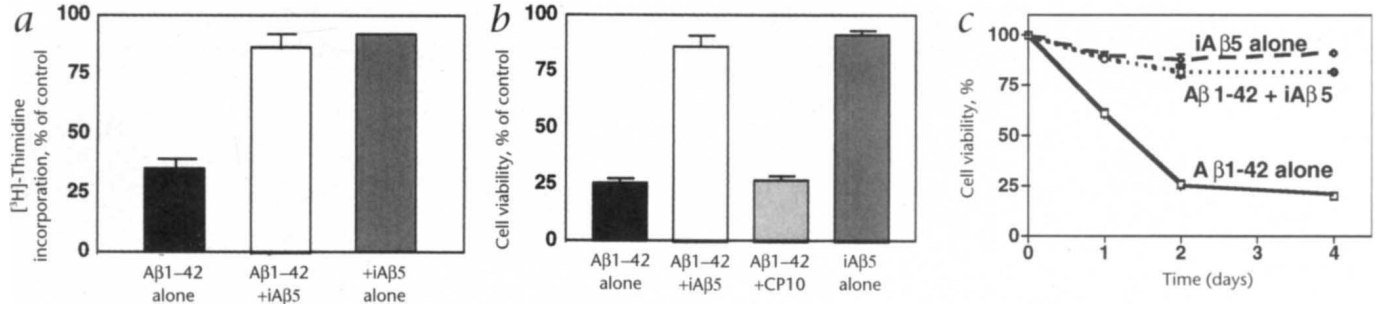

Fig. 2 Prevention of Aβ neurotoxicity by iAβ5. Viability of IMR-32 human neuroblastoma cells was evaluated by incorporation of ^{3}H-methyl thymidine (***a***) or by DNA/RNA cell staining with acridine orange-ethidium bromide (***b***). Each bar represents the mean + standard deviation of three separate experiments. ***c***, Time course of the iAβ5 inhibition of Aβ neurotoxicity.

widespread amyloidosis observed in AD[18,19], but these deposits develop slowly and the animals do not have a substantial amount of intracerebral amyloid until old age, 11 to 13 months of age. Double-transgenic mice that have both human APP and mutant presenilin 1 develop deposits earlier and in higher amounts[20]. The effect of β-sheet breaker peptides on amyloid deposition in APP transgenic mice will be evaluated soon.

Amyloid deposits occur in several different diseases in which aggregated proteins or peptides are deposited mainly in the extracellular space of various tissues producing cell damage and organ dysfunction[21]. In addition to Aβ, several other amyloidogenic proteins are toxic to cells in culture[22,23] suggesting a common mechanism of pathology induced by amyloid. However, it is not known whether amyloid fibrils are directly toxic or produce damage by mechanical disruption of tissue. Alternatively, amyloid fibril formation may be a protective process and the toxic state may consist of a soluble conformationally altered fibril precursor that induces cell death either directly or by interaction with another protein. Whether or not amyloid formation directly causes AD pathology, present data suggest that amyloid inhibition is a good target for AD therapy. Several approaches to develop inhibitors of Aβ fibrillogenesis and/or neurotoxicity have been described. Small molecules such as sulfonated dyes, nicotine, melatonin, rifampicin, anthracycline 4′-iodo-4′-deoxydoxorubicin and the cationic surfactant hexadecyl-N-methylpiperidinium bromide prevent Aβ aggregation or toxicity[24]. Synthetic peptides and cerebrospinal fluid proteins, such as transthyretin, apolipoprotein J and laminin, inhibit Aβ fibrillogenesis[25]. Our approach, using β-sheet breaker peptides, uniquely provides a potentially general strategy to treat diseases caused by a defective protein folding, such as amyloidosis-related disorders, prion diseases, disorders associated with serpin deficiency and amyotrophic lateral sclerosis[26,27]. β-sheet breaker peptides that combine a sequence similarity to the region of the protein involved in the abnormal folding with the ability to block the pathologic conformational changes offer the possibility to generate therapeutic compounds directed specifically at each of these diseases. Using this approach, we have recently developed short β-sheet breaker peptides that effectively prevent and revert the prion protein conformational changes implicated in the pathogenesis of spongiform encephalopathies[28]. At present, there is no effective treatment available for AD and for most of the conformational diseases. We propose that β-sheet breaker peptides or their non-peptidic derivatives have the potential to be therapeutic agents to prevent or retard the progression of amyloidosis in AD.

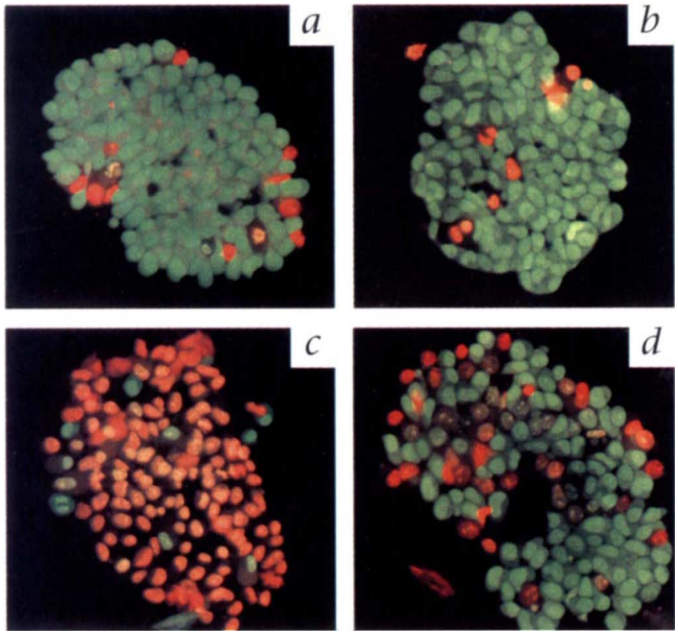

Fig. 3 Neuroblastoma cell cultures incubated with the peptide mixtures. IMR 32 cells were stained with acridine orange-ethidium bromide and visualized by fluorescent microscopy. Cells that stain green are alive and those that stain orange are dead. ***a***, Cells incubated in absence of peptide. ***b***, iAβ5 alone. ***c***, Aβ1–42 alone. ***d***, Aβ1–42 + iAβ5.

Methods

Peptide synthesis and characterization. Aβ1–40 and Aβ1–42 were synthesized at the W.M. Keck Foundation, Yale University (New Haven, Connecticut). iAβ5 (LPFFD) and control peptide (CP10: ISEVKMDAEF) were obtained from Bio-Synthesis (Lewisville, Texas). Peptides were synthesized by solid-phase technique and purified by high performance liquid chromatography. Peptide purity was evaluated by peptide sequencing and laser desorption mass spectrometry.

Fibrillogenesis assays. Amyloid formation was quantitatively evaluated by the fluorescence emission of thioflavine T (ThT) bound to amyloid fibrils, as reported[6]. Stock solutions of the peptides were prepared in 50% acetonitrile, 0.1% trifluoroacetic acid and were stored lyophilized in aliquots. Concentrations were determined by amino acid analysis using a Waters Accq-Tag amino acid analyzer. Aliquots of Aβ at a concentration of 0.5 mg/ml prepared in 0.1M Tris, pH 7.4 were incubated for seven days at 37 °C in the absence or presence of different concentrations of β-sheet breaker peptides. To evaluate the inhibition of amyloid formation and dissolution of preformed fibrils, inhibitor peptide was added at the beginning of the incubation or after seven days of incubation of Aβ alone. At the end of the incu-

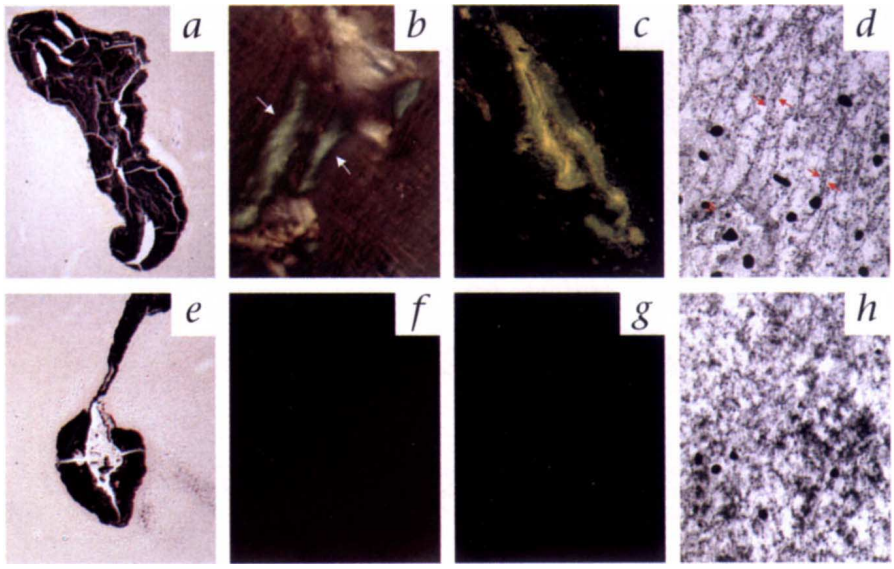

Fig. 4 Reduction of cerebral Aβ deposition and prevention of amyloid fibril formation *in vivo* by iAβ5. Immunohistochemical staining of amygdaloid Aβ deposits at eight days after surgery in rats treated with Aβ1–42 alone (***a***) or a mixture of Aβ1–42 and iAβ5 (***e***). ***b*** and ***f***, Congo red-stained sections adjacent to those shown in *a* and *e*, respectively. Arrows indicate the Congo red-positive birefringence under polarized light. Thioflavine S staining of rat brain treated with Aβ1–42 alone (***c***) or with a mixture of Aβ1–42 and iAβ5 (***g***). Electron microscopic analysis of the ultrastructure of immunogold-labeled Aβ aggregates obtained in the absence (***d***) and presence (***h***) of iAβ5. The size of the gold particles was increased by silver staining. Red arrows indicate amyloid-like fibrils. Original magnifications: *a* and *e*, ×50; *b*, *c*, *f* and *g*, ×100; *d* and *h*, ×82,000.

bation period, 50 mM glycine, pH 9.2 and 2 μM ThT were added in a final volume of 2 ml. Fluorescence was measured at excitation 435 nm and emission 485 nm in a model LS50B fluorescence spectrometer (Perkin-Elmer). Fibrillogenesis was also qualitatively analyzed by electron microscopy. Aliquots of Aβ1–42 (0.5 μg/μl) were incubated at 37 °C for seven days without or with 1.5 μg/μl of iAβ5. To study fibril disassembly, Aβ1–42 (0.5 μg/μl) was incubated alone for seven days at 37 °C and after addition of inhibitor the incubation proceeded for four more days. Samples were first centrifuged at 14,000rpm for 5 min and the pellet was resuspended in half of the initial volume and placed on carbon formar-coated 300-mesh nickel grids. The grids were stained for 60 seconds with 2% uranyl acetate and visualized on a Zeiss EM 10 electron microscope at 80 kV.

Cell toxicity assays. IMR-32 human neuroblastoma cells (American Type Culture Collection cell line 127-CC1) were grown at 37 °C and 5% CO_2 in a medium of 55% DMEM high glucose (4.5 g/l), 30% Ham F-12, 5% α-MEM, and 10% fetal calf serum, supplemented with 200 mM L-glutamine, 0.0055 μg/ml pyruvate, 50 U/L penicillin, 50 μg/ml streptomycin and 0.25 μg/ml fungizone. Confluent monolayers were treated with 0.25% trypsin, 0.03% EDTA and cells were counted and plated in 96-well tissue culture plates at 5×10^3 cells/well in 100 μl of medium or into tissue culture chamber slides at 2×10^4 cells/well in 200 μl. Two days after plating, 20 μg of Aβ1–42, pre-incubated for 48 h in 15 μl of DMEM alone or with 0.2 mg/ml iAβ5 or 0.4 mg/ml of CP10, were added to the cells. The cells were then incubated for 48 h with the peptides. Two methods were used to quantitate cell viability. Incorporation of ^{3}H-methyl thymidine was done as described[29].

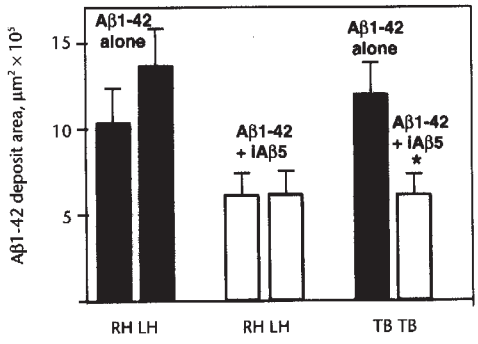

Fig. 5 Quantitative analysis of the size of Aβ deposits produced after intracerebral injection of Aβ1–42. Area of deposits in the left (LH), right hemisphere (RH) and total brain (TB) at eight days after surgery. Deposit area was measured by image analysis of EM-3 stained sections. Each bar represents the mean + standard error of the analysis of 9 rats. * $P < 0.01$.

RPMI 1640 medium (10 μl) containing 10 μCi of radiolabeled thymidine (specific activity, 2 Ci/mmol) was added to each well 24 h after the addition of the peptides. Cells were collected on a glass microfiber paper (Whatman GF/A) using an automated sample harvester. Scintillation fluid (3 ml toluene/PPO/POPOP) was added to each filter and radioactivity was measured in a 1600 TR-Packard β scintillation analyzer. Results are expressed as percentage of mean d.p.m. values of peptide-treated cells compared with the mean d.p.m. values of control cells. DNA/RNA cell staining with acridine orange-ethidium bromide was done as described[30]. Cells were visualized by fluorescent microscopy and viability was calculated by counting at least 500 cells in three different fields at low magnification. Cell count results are expressed as percentage of control values. Measurements were done in 3–5 wells per experiment in three separate experiments. Results were statistically analyzed by one-way analysis of variance (ANOVA) with post hoc Tukey's test.

***In vivo* studies using an animal model of cerebral Aβ deposition.** Male Fischer-344 rats (Taconic, Georgetown, New York) weighed 250–300 g and were 3–4 months of age at the time of arrival. The animals were housed two per cage, maintained on a 12-hour light-dark cycle with access to food and water *ad libitum*, and were habituated to their new environment for 2–3 weeks prior to surgery. Surgery was performed using sodium pentobarbital (50 mg/kg, injected intraperitoneally) as anesthesia. Atropine sulfate (0.4 mg/kg) and ampicillin sodium salt (50 mg/kg) were injected subcutaneously after the animals were anesthetized. Aβ1–42 was dissolved in dimethylsulfoxide (DMSO) and then diluted with water to 16.7% DMSO. The animal received a bilateral injection of 5.0 nmol Aβ1–42 into each amygdala by using a Kopf stereotaxic instrument with the incisor bar set at 3.3 mm below the interaural line. Injection coordinates measured from the bregma and the surface of the skull (anteroposterior, −3.0 mm; mediolateral, ±4.6 mm; dorsoventral, −8.8 mm) were empirically determined based on the atlas of Paxinos and Watson. A volume of 3.0 μl was administered during a time period of 6 min (flow rate 0.5 μl/min) using a CMA/100 microsyringe pump. The cannula was left *in situ* for 2 min after injection, withdrawn 0.2 mm and left for 3 min, and then slowly withdrawn completely. After surgery the animals were placed on a heating pad until they regained their righting reflex. To evaluate the effect of iAβ5 on amyloid deposition, animals were injected with Aβ1-42 (5 nmol) and iAβ5 (100 nmol). The peptides were mixed together immediately before the first injection, and the last animal was injected 6 h later. Vehicle-treated animals were injected with 3.0 μl of 0.1% trifluoroacetic acid, 16.7% DMSO in water.

For histological analyses, animals were anesthetized with sodium pentobarbital (150 mg/kg, injected intraperitoneally). The rats were then perfused transaortically with a phosphate buffer (pH 7.4) followed by 4% paraformaldehyde in the same buffer, eight days after surgery and their brains were processed as described[14]. Serial coronal sections (40 μm) were cut, and five series of sections at 0.2-mm intervals were saved for histologi-

cal analysis, placed in ethylene glycol cryoprotectant and stored at –20 °C until stained. Tissue sections were stained with Congo red and (immunohistochemically) with Aβ1–42 and GFAP antibodies. In addition, cresyl violet and thioflavin S staining were undertaken on selected series. Cresyl violet and Congo red staining were done as described[14,15]. For thioflavin S staining, mounted sections were defatted in xylene, hydrated in a gradient of ethyl acohol and water series, and then placed in a 1% thioflavine S aqueous solution for 30 min. The sections were then dehydrated in a gradient of water and ethyl alcohol and cleared in xylene. The tissue was subsequently coverslipped using DePex mounting medium. For GFAP staining, sections (40 μm) were incubated in GFAP primary antibody at a 1:500 dilution for 24 h at room temperature. An anti-rabbit immunoglobulin (Ig) G secondary antibody was used at a 1:1333 dilution. Aβ staining was done with an antibody (EM-3) that selectively binds to Aβ1–42 (ref. 17). Staining was done as with GFAP. EM-3 was used at a 1:1000 dilution. 'Omit' sections were obtained by not including the primary or the secondary antibody. Immunogold labelling (2 h) of Aβ deposits was done in brain sections that were incubated in EM-3 for 24 h. Gold (10 nm)-labeled goat anti-rabbit IgG was used at a 1:20 dilution. The staining was subsequently enhanced with silver and the sections were processed for electron microscopy, using standard techniques. An image analysis system (NIH Image 1.60) was used to determine the size of the Aβ deposits. The area of the Aβ deposits was measured at 0.2 mm intervals. These data were analyzed by a two-way ANOVA followed by a Newman-Keuls' multiple range test for *post hoc* comparisons. Total brain deposition was analyzed using an unpaired *t*-test, two tailed.

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1. Soto, C., Branes, M.C., Alvarez, J. & Inestrosa, N.C. Structural determinants of the Alzheimer's amyloid beta-peptide. *J. Neurochem.* **63**, 1191–1198 (1994).
2. Pike, C.J., Burdick, D., Walencewicz, A.J., Glabe, C.G. & Cotman, C.W. Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J. Neurosci.* **13**, 1676–1687 (1993).
3. Lorenzo, A. & Yankner, B.A. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc. Natl. Acad. Sci. USA.* **91**, 12243–12247 (1994).
4. Yankner, B.A. Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron.* **16**, 921–932 (1996).
5. Soto, C., Kindy, M.S., Baumann, M. & Frangione, B. Inhibition of Alzheimer's amyloidosis by peptides that prevent β-sheet conformation. *Biochem. Biophys. Res. Commun.* **226**, 672–680 (1996).
6. Soto, C., Castaño, E.M., Frangione, B. & Inestrosa, N.C. The alpha-helical to beta-strand transition in the amino-terminal fragment of the amyloid beta-peptide modulates amyloid formation. *J. Biol. Chem.* **270**, 3063–3067 (1995).
7. Hilsich, C., Kisters-Woike, B., Reed, J., Masters, C.L. & Beyreuther, K. Substitutions of hydrophobic amino acids reduce the amyloidogenicity of Alzheimer's disease βA4 peptides. *J. Mol. Biol.* **228**, 460–473 (1992).
8. Lee, J.P. *et al.* H-1 NMR of A-beta amyloid peptide congeners in water solution. Conformational changes correlate with plaque competence. *Biochemistry* **34**, 5191–5200 (1995).
9. Wood, J.D., Wetzel, R., Martin, J.D. & Hurle, M.R. Prolines and amyloidogenicity in fragments of the Alzheimer's peptide /A4. *Biochemistry* **34**, 724–730 (1995).
10. Chou, P.Y. & Fasman, G.D. Empirical predictions of protein conformation. *Ann. Rev. Biochem.* **47**, 251–276 (1978).
11. Soto, C., Castaño, E. M., Prelli, F., Kumar, R. A. & Baumann, M. Apolipoprotein E increases the fibrillogenic potential of synthetic peptides derived from Alzheimer's, gelsolin and AA amyloids. *FEBS Letters* **371**, 110–114 (1995).
12. Wood, S. J., Maleeff, B., Hart, T. & Wetzel, R. Physical, morphological and functional differences between pH 5.8 and 7.4 aggregates of the Alzheimer's amyloid peptide AP. *J. Mol. Biol.* **256**, 870–877 (1996).
13. Simmons, L.K. *et al.* Secondary structure of amyloid beta peptide correlates with neurotoxic activity in vitro. *Mol. Pharmacol.* **45**, 373–379 (1994).
14. Sigurdsson, E.M., Lorens, S.A., Hejna, M.J., Dong, X.W. & Lee, J.M. Local and distant histopathological effects of unilateral amyloid- 25–35 injections into the amygdala of young F344 rats. *Neurobiol. Aging.* **17**, 893–901 (1996).
15. Sigurdsson, E.M., Lee, J.M., Dong, X.W., Hejna, M.J. & Lorens, S.A. Bilateral injections of Amyloid-β 25-35 into the amygdala of young fischer rats: Behavioral, neurochemical and time dependent histopathological effects. *Neurobiol. Aging* **18**, 591–608 (1997).
16. Arriagada, P.V., Growdon, J.H., Hedley-Whyte, E.T. & Hyman, B.T. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639 (1992).
17. Jimenez-Huete, A. *et al.* Antibodies directed to the carboxyl terminus of amyloid beta peptide recognize sequence epitopes and distinct immunoreactive deposits in Alzheimer's disease brain. *Alzheimer's Report* **1**, 1–7 (1998).
18. Games, D. *et al.* Alzheimer-type neuropathology in transgenic mice overexpressing V717F -amyloid precursor protein. *Nature* **373**, 523–527 (1995).
19. Hsiao, K.K. *et al.* Correlative memory deficits, A elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99–102 (1996).
20. Holcomb, L. *et al.* Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Med.* **4**, 97–100 (1998).
21. Glenner, G.G. Amyloid deposits and amyloidosis. The β-fibrilloses. *N. Engl. J. Med.* **302**, 1283–1292 (1980).
22. Lorenzo, A., Razzaboni, B., Weir, G.C. & Yankner, B.A. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* **368**, 756–760 (1994).
23. Forloni, G. *et al.* Neurotoxicity of a prion protein fragment. *Nature* **362**, 543–546 (1993).
24. Esler, W.P. *et al.* Aβ deposition inhibitor screen using synthetic amyloid. *Nature Biotechnol.* **15**, 258–263 (1997).
25. Wisniewski, T., Ghiso, J. & Frangione, B. Alzheimer's disease and soluble Aβ. *Neurobiol. Aging.* **15**, 143–152 (1994).
26. Thomas, P.J., Qu, B. & Pedersen, P.L. Defective protein folding as a basis of human disease. *Trends Biochem. Sci.* **20**, 456–459 (1995).
27. Kelly, J.W. Alternative conformations of amyloidogenic proteins govern their behavior. *Curr. Opin. Struct. Biol.* **6**, 11–17 (1996).
28. Soto, C., *et al.* Reversal of disease-specific prion protein conformation and protease-resistance by peptides designed as β-sheet breakers. (Manuscript submitted).
29. Janossy, G., Greaves, M.F., Doenhoff, M.J. & Snajder, J. Lymphocyte activation. Quantitation of the proliferative response to mitogens using defined T and B cell populations. *Clin. Exp. Immunol.* **14**, 581 (1973).
30. Duke CR, *et al.* in *Current Protocols in Immunology* (eds. Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M., and Strober, W) 17.1–17.33 (John Wiley & Sons, New York, 1995).