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ORIGINAL RESEARCH

Autoantibodies to Amyloid β-Peptide (Aβ) are Increased in Alzheimer's Disease Patients and Aβ Antibodies Can Enhance Aβ Neurotoxicity

Implications for Disease Pathogenesis and Vaccine Development

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Abstract

Studies of amyloid precursor protein transgenic mice suggest that immune responses to amyloid β peptide (Aβ) may be instrumental in the removal of plaques from the brain, but the initial clinical trial of an Aβ vaccine in patients with Alzheimer's disease (AD) was halted as the result of serious neurological complications in some patients. We now provide evidence that AD patients exhibit an enhanced immune response to Aβ and that, contrary to expectations, Aβ antibodies enhance the neurotoxic activity of the peptide. Serum titers to Aβ were significantly elevated in AD patients and Aβ antibodies were found in association with amyloid plaques in their brains, but there was no evidence of cell-mediated immune responses to Aβ in the patients. Aβ antibodies were detected in the serum of old APP mutant transgenic mice with plaque-like Aβ deposits, but not in the serum of younger transgenic or nontransgenic mice. Serum from APP mutant mice potentiated the neurotoxicity of Aβ. Our data suggest that a humoral immune response to Aβ in AD patients may promote neuronal degeneration, a process with important implications for the future of vaccine-based therapies for AD.

Index Entries: Apoptosis; hippocampal; immune; secretase; transgenic.

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Introduction

Amyloid β peptide (Aβ), a principal component of amyloid plaques found in the brain of patients with Alzheimer's disease (AD) (Markesbery, 1997), is believed to play a major role in the dysfunction and death of neurons by inducing oxidative stress and disrupting cellular calcium homeostasis (Mattson, 1997; Selkoe, 2000). Aβ is an insoluble 40- to 42-amino acid peptide formed by the cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. Although produced at lower levels, the longer 42 amino acid form of the peptide (Aβ42) is more prone to aggregation and fibril formation, comprises a disproportionate fraction of Aβ in plaques, and is more toxic towards neurons than is the shorter Aβ40 peptide (Iwatsubo et al., 1994; Tamaoka et al., 1994; Esler and Wolfe, 2001). The process of peptide aggregation is believed to be central to the neurotoxicity of Aβ (Pike et al., 1991; Simmons et al., 1994; Estus et al., 1997). That Aβ aggregation may be causal in AD is also suggested by findings that mutations in APP and presenilins associated with familial AD lead to increased production of Aβ42 (Citron et al., 1992; Cai et al., 1993; Suzuki et al., 1994; Cairns et al., 1993; Scheuner et al., 1996) and by studies showing that Aβ, and oxidative and metabolic insults, can promote the formation of neurofibrillary tangles (Cheng and Mattson, 1992; Mattson et al., 1997; Gotz et al., 2001; Lewis et al., 2001). It is therefore important to identify factors that may modulate the aggregation and/or neurotoxicity of Aβ.

Recent studies of APP mutant transgenic mice with amyloid deposits have shown that a reduction in plaque burden can be achieved by immunization with Aβ (Schenk et al., 1999; Morgan et al., 2000; Games et al., 2000; Janus et al., 2000) and that peripheral administration of Aβ antibodies can also reduce amyloid burden (Bard et al., 2000). Despite modest serum levels, the antibodies were shown to enter the brain and bind to plaques. These studies have led to clinical trials of immunization with Aβ in patients with AD. Unfortunately, an initial phase II study was recently halted because several patients developed central nervous system (CNS) neurological complications (Birmingham and Frantz, 2002). A better understanding of immune responses to Aβ in patients with AD and of the consequences of such responses for neuronal function and survival is needed in order to establish if and how an effective vaccine might be developed. We therefore characterized the immune response to Aβ in serum, cerebrospinal fluid (CSF), and brain tissue of patients with AD and determined the effect of Aβ antibodies on Aβ aggregation and neurotoxicity. We provide evidence for a humoral response to aggregated Aβ in AD patients and show that Aβ antibodies can potentiate the neurotoxicity of Aβ, suggesting that an immune response to Aβ may contribute to the neurodegenerative process in AD.

Methods

Blood, CSF, and Tissue Collection

For analyses of Aβ antibody titers, serum was obtained from 16 patients with probable AD (mean age 76.9 ± 6.0 yr; 4 males and 12 females) and 31 control subjects (mean age 72.5 ± 6.4 yr; 13 males and 18 females). Blood samples from 11 of these AD patients and 2 control patients were also used for T-lymphocyte studies. The mini-mental status evaluation score of the patients with AD was 17.3 ± 3.8 (mean ± SD) and the control population was 29 ± 1.4 (mean ± SD). Ventricular CSF samples were obtained at autopsy within four h of death from another 17 patients clinically diagnosed with AD prior to death, and later confirmed histopathologically. CSF from 31 age-matched subjects who were clinically and pathologically normal were similarly obtained at autopsy.

*Immunohistochemistry for Antibodies to A*β

Ten micron formalin-fixed paraffin-embedded brain sections from patients with AD (University of Kentucky) were deparaffinized in xylene, hydrated in graded alcohols, and unmasked by heating slides for 10 minutes in a conventional microwave oven in 0.1 *M* sodium citrate buffer (pH 6.0). After washing in PBS (7.2 m*M* Na_2HPO_4, 2.7 m*M* KCl, 1.5 m*M* KH_2PO_4, 137 m*M* NaCl), sections were immersed in 3% H_2O_2 in PBS for 30 min to quench endogenous peroxidases. Non-specific antibody–antigen interactions were blocked by 1% (w/v) casein in bt-PBS for 30 min (58 m*M* Na_2HPO_4, 17 m*M* $NaH_2PO_4 \bullet H_2O$, 68 m*M* NaCl) followed by incubation with biotinylated goat anti-human IgG, M, A (Harlan labs; 1 : 100 in blocking solution) overnight

at 4°C (cover slips were applied to minimize evaporation). Sections were washed in PBS containing 0.05% (v/v) Tween-20 and incubated with avidin-horse radish peroxidase (HRP) (Sigma; 1 : 1000) for 30 min at room temperature. After washing in PBS/0.05% Tween-20, immunoreactive complexes were visualized using 3,3′-diaminobenzidine as the chromagen (0.05% [w/v] DAB+0.03% H_2O_2 in PBS). Thorough washing in H_2O halted the development. These same sections were again unmasked by heating in 0.1 *M* sodium citrate buffer (pH 6.0), endogenous peroxidases quenched in 3% H_2O_2, and non-specific antibody reactions blocked in 1% casein. The primary antibody for beta-amyloid staining (mouse anti-beta-amyloid IgG; 10D5; Athena Neurosciences, Inc.; 1 : 200 in blocking solution) was applied overnight at 4°C (cover slips applied to prevent evaporation). After washing with PBS+0.05% Tween-20, goat anti-mouse IgG-HRP (Chemicon; 1 : 500 in blocking solution) was applied for 90 min at room temperature. After washing, the amyloid-reactive HRP immune complexes were visualized using Vector® VIP as the chromagen (Vector Laboratories). After washing, sections were dehydrated and mounted with Permount (Fisher). Human IgG complexes develop as a reddish-brown precipitate, whereas amyloid stained by 10D5 appear a deep purple.

Antibody Assay

ELISA was performed as previously described (Nath and Wolinsky, 1990) with the following modifications. Microtiter plates were coated with soluble Aβ or Aβ that had been pre-aggregated at 37°C for 24 h. Each well was coated with 250 ng of Aβ for 16 h in bicarbonate buffer (pH 9.6) and then incubated with human sera (1 : 100) or CSF (1 : 1) for three hours in duplicate wells. The wells were then serially incubated with biotinylated antihuman IgG and streptavidin conjugated to horseradish peroxidase. ATBS was used as a chromogen. Serial dilutions of mouse monoclonal antibody 10D5 was used as a positive control. This monoclonal antibody reacts best against the aggregated form of Aβ hence it served as an internal control for confirming the aggregation of the peptide. Negative controls included wells from which either Aβ, human sera or secondary antisera was omitted. The intra-assay variability was <4% and the inter-assay variability was <10%. For CSF samples, the sample was considered positive if the optical density reading was greater than the mean + one standard deviation of the control wells from which the CSF had been omitted.

Cell Proliferation Assay

T cells were enriched from the blood by sheep red blood cell rosetting and separation on ficoll hypaque density gradients. Five × 10^5 cells were cultured in triplicate wells of a microtiter plate in the presence of media only (RPMI 1640, 10% fetal calf serum, 10 m*M* glutamine, 10 m*M* sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin) 0.5 μg/mL anti-CD3 or 0.2 μg/mL Aβ for 5 d. ^{3}H-thymidine was added during the last 18 h of culture. The cells were harvested on glass fiber filters and counted in a scintillation counter. Stimulation indices (SI) were calculated as the ratio of the mean values of the treated to untreated cultures.

Peptide Aggregation Assay

Thioflavin T was used to quantitate the amount of β-sheet formation (LeVine, 1999) since it undergoes spectral changes upon binding to the fibrils. Aβ peptide (5 μ*M*) with or without purified IgG (100 ng/well) from each patient was incubated in 50 m*M* glycine, pH 8.5 in opaque microtiter plates for 24 h at 37°C. Thioflavin T (5 μ*M*; Sigma) was added to each well for 15 min and the fluorescence was measured at λex = 450 and λem = 485 and analyzed as a ratio of the reading with and without antisera. All samples were analyzed in triplicate. For evaluation of Aβ42 aggregation in cell cultures, aggregates were visualized by phase-contrast microscopy, and the number of aggregates per microscope field (40X objective) were determined by counting 10 randomly chosen fields in each culture. Determinations were made without knowledge of treatment history of the cultures.

Hippocampal Cell Cultures and Experimental Treatments

Hippocampi were removed from embryonic d 18 Sprague-Dawley rats (Harlan, Inc.), and cells were dissociated by mild trypsination and trituration and seeded onto polyethyleneimine-coated plastic 35 or 60 mm diameter dishes at a density of approx 150 cells/mm^2 of culture surface. Cultures were

maintained in Neurobasal medium containing B-27 supplements (Gibco BRL), 2 m*M* L-glutamine, 1 m*M* Hepes, and 0.001% gentamicin sulfate (Sigma). Experimental treatments were added to the cultures by dilution from concentrated stocks. Aβ1-42 was purchased from Bachem and prepared as a 1 m*M* stock in sterile water 16 h prior to addition to cultures. Aβ antibodies included mouse monoclonal antibody (clone BAM-10 from Sigma/RBI); a rabbit polyclonal antibody (Biosource catalog number 44-348); and a mouse monoclonal antibody (Chemicon catalog number MAB5206).

Quantification of Neuron Survival

Neuronal survival was quantified as described previously (Guo et al., 1999). Briefly, viable neurons in premarked fields (10X objective) were counted before experimental treatment and at specified timepoints thereafter. Neurons with intact neurites of uniform diameter and a cell body with a smooth round appearance were considered viable, whereas neurons with fragmented neurites and vacuolated soma were considered non-viable.

Mouse Studies

Male mice overexpressing a mutant form of amyloid precursor protein (Borchelt et al., 1996) and age-matched male non-transgenic littermate control mice were maintained on a 12 h light/12 h dark cycle with free access to food and water. This line of mice exhibits increased levels of soluble Aβ and develops amyloid deposits in an age-dependent manner with diffuse deposits first appearing at approx 12 mo of age and plaque-like deposits developing later, typically by 18–22 mo of age.

Statistical Analysis

Mean responses for the AD and control groups were compared by two sample t-statistics. Correlation was measured by Pearson's coefficient. Slopes of regression lines were compared using partial F tests.

Results

Aβ Antibodies are Increased in the Serum, CSF, and Brain Tissue of AD Patients

Significantly elevated levels of IgG antibody to the aggregated form of Aβ were found in sera from the AD patient group when compared to age matched controls (Fig. 1A; $p<0.005$). Considering the possibility that antibodies to Aβ may be a nonspecific inflammatory response, we also determined antibody responses to Aβ in serum samples from patients with multiple sclerosis or HIV encephalitis. No significant differences were found in these patient groups when compared to controls (Fig. 1A). Levels of serum antibodies against the soluble form of Aβ were also significantly increased in the AD group (Fig. 1B; $p<0.05$). The antibody levels in the

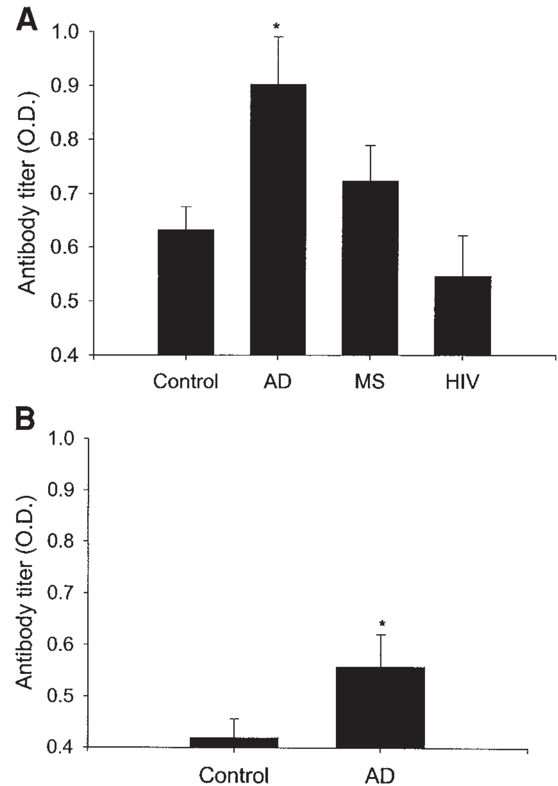

Fig. 1. Serum Aβ antibody titres are increased in Alzheimer's disease. Microtiter plates were coated with $A\beta_{1-42}$ (250ng/well) followed serially by incubation with serum (1 : 100), biotinylated antihuman IgG and streptavidin conjugated to peroxidase. ABTS was used as a chromogen. Data represents mean+SE. **(A)** Elevated titers of antibodies in the AD patients compared to age matched controls, as well as patients with MS or HIV infection against preaggregated $A\beta_{1-42}$. ($^*p<0.005$). **(B)** Antibody titers against unaggregated $A\beta_{1-42}$ were much lower in both groups but still higher in the AD patients ($^*p<0.05$).

Table 1
Antibodies to Aβ in Postmortem CSF

Patient #	Antibody titer (mean O.D.)	MMSE[a]	BRAAK[b] staging
A-2-98	0.204 +0.012 (agg A-beta)	4	6
A-26-97	0.435 +0.023 (agg A-beta)	1	6
A-83-98	0.202 +0.018 (soluble A-beta)	0	6

[a]Normal MMSE values are 26–30.

[b]BRAAK staging in autopsy ranges from 0–6, 0 being normal.

AD group against the aggregated and soluble forms of Aβ were also analyzed against the mini-mental status evaluation (MMSE) scores. No significant correlation was found.

Aβ antibodies were detected in the CSF of 3 of 11 AD patients (Table 1); in two patients the antibodies recognized the preaggregated form, while in the other patient the antibodies bound to the soluble form of Aβ. All 3 patients with CSF Aβ antibodies had severe AD with MMSE cognitive test scores of 0, 1, and 4 and pathological BRAAK stage 6 in each case. None of the CSF samples from the 31 control patients had any detectable antibodies to Aβ.

When tissue sections were dual stained with anti-human IgG and a mouse monoclonal antibody to Aβ, we found that human IgG was frequently associated with amyloid plaques. In most plaques, anti-human antibodies were localized to the center of the plaque, while in some plaques antibodies were present either in the periphery or throughout the plaque (Fig. 2). Some plaques were not immunoreactive with the anti-human antibodies. In one patient, prominent neuronal staining with the anti-human antisera was also noted (Fig. 2A). Brain sections from all AD and control patients exhibited immunoglobulin immunoreactivity in blood vessels (data not shown).

Peripheral blood lymphocytes from AD patients and normal control donors were tested for their proliferative response to anti-CD3 or Aβ. All AD patients and control patients tested showed excellent T cell proliferation in response to anti-CD3 stimulation (SI = 10 to 30; range) while no proliferation was observed in response to Aβ stimulation (SI < 2 in each case) (Table 2). These results suggest that the increased levels of circulating Aβ antibodies in AD patients is not associated with a cell-mediated immune response.

Effects of Serum IgG on Aβ Aggregation

The ability of the serum IgG to influence Aβ aggregation was measured in a thioflavin assay. No significant differences were found in the AD group (mean ± SD = 46.8 ± 22.3) vs the age-matched control population (mean ± SD = 48.0 ± 13.3). However within the AD group, an inverse correlation was present between the antibody levels to soluble Aβ and the effect of serum IgG on Aβ aggregation ($p = 0.05$). A similar trend was also seen with antibodies to aggregated Aβ. However, age-matched controls showed a positive correlation between the antibody titers to soluble Aβ and the effect of on Aβ aggregation. The difference between the slopes was significant ($p = 0.02$). These observations suggest that while increasing antibody titers to Aβ in patients with AD likely interfere with Aβ aggregation, similar increases in age-matched control populations may have an opposite effect by enhancing Aβ aggregation.

Aβ Antibodies Enhance Peptide Neurotoxicity

Although studies of APP mutant mice suggest that Aβ antibodies can promote removal of Aβ from the brain (Schenk et al., 1999; Morgan et al., 2000; Janus et al., 2000), the impact of the Aβ antibodies

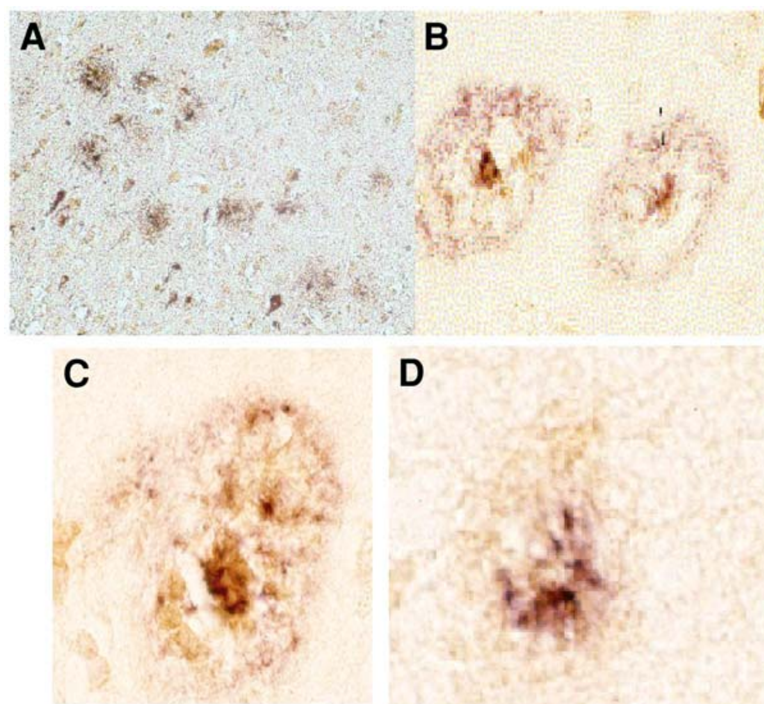

Fig. 2. Increased Aβ antibodies associated with plaque pathology in Alzheimer's disease. Paraffin embedded sections from the temporal cortex of three AD patients were immunostained with mouse monoclonal antisera (10D5) (purple) and rabbit-antihuman IgG (brown). **(A)** Several amyloid plaques immunostaining for Aβ and human antibodies; also note that some neurofibrillary tangles bind the human IgG. **(B)** Human IgG in the center of two plaques. **(C)** IgG is present in the center of the plaque and bound to the amyloid fibrils. **(D)** IgG is present in the periphery of a plaque. Color version available for viewing at **www.humanapress.com**

on the toxicity of the peptide is unknown. We exposed cultured hippocampal neurons to 1 μ*M* Aβ42 in the absence or presence of increasing concentrations of three different Aβ antibodies which included one polyclonal and two monoclonal antibodies. During a 48 h exposure period, Aβ42 caused death of approx 30% of the neurons, and this level of cell death was unaffected by co-treatment with control IgG (Fig. 3A). Each of the three Aβ antibodies tested caused a highly significant concentration-dependent enhancement of Aβ42-induced neuronal death, with 80–90% of the neurons being killed in cultures receiving an Aβ antibody at a concentration of 1 μg/mL. The enhancement of Aβ42 neurotoxicity by Aβ antibodies was associated with increased formation of Aβ aggregates associated with the cells and cell culture surface (Fig. 3B).

Reasoning that if levels of Aβ autoantibodies are increased in AD patients, then APP mutant mice with amyloid deposits might also exhibit an immune response to the Aβ, we performed ELISA analyses of serum samples from APP mutant mice and non-transgenic mice. Samples from 6 APP mutant and 6 nontransgenic mice ranging in age from 15 to 20 mo were analyzed. Autoantibodies against aggregated Aβ were detected in the serum of the oldest (20 mo) APP mutant mice, but were not detected in the serum of younger APP mutant mice or in any of the nontransgenic mice (data not shown). No autoantibodies recognizing soluble Aβ were detected in any of the serum samples. We next determined the effects of serum from APP mutant transgenic mice and non-transgenic mice on the vulnerability of hippocampal neurons to Aβ neurotoxicity. Cultures were exposed to Aβ1-42 alone or in combination with serum from non-transgenic or APP mutant transgenic mice for 48 h and neuronal survival was quantified. Significantly more

Table 2
Lack of a Primary T Cell Proliferative Response in Patients with AD

Responder[a]	Media[b]		Anti-CD3		Aβ	
C1	347	(115)	23,221	(2,063)	484	(166)
C2	374	(143)	8338	(995)	404	(173)
1	477	(215)	14,822	(2,359)	381	(65)
2	911	(366)	15,876	(486)	722	(252)
3	760	(433)	6109	(1,653)	444	(183)
4	288	(149)	14,509	(1,755)	435	(198)
5	179	(59)	14,515	(680)	242	(126)
6	380	(93)	1195	(140)	354	(128)
7	382	(34)	6913	(832)	445	(46)
8	336	(64)	5545	(250)	462	(152)
9	410	(60)	10,142	(738)	689	(93)
10	1239	(181)	9438	(631)	1762	(404)
11	660	(208)	11,307	(306)	571	(113)

[a]C1 and C2 represent two control individuals and 1–11 represent 11 different patients with AD.

[b]Peripheral blood lymphocytes were cultured with media, 0.5 μg/mL anti-CD3, or 0.2 μg/mL Aβ. (Numbers represent the mean CPM ± SD of triplicate culture wells.)

neurons were killed in cultures exposed to Aβ1-42 plus serum from APP mutant mice than were killed in cultures exposed to Aβ1-42 plus serum from nontransgenic mice (Fig. 4). Collectively, our data demonstrate that, contrary to expectations based on indirect evidence from studies of APP mutant mice immunized with human Aβ, Aβ antibodies can exacerbate Aβ neurotoxicity.

Discussion

The present findings suggest that patients with AD generate antibodies that recognize the aggregated form of Aβ and that such Aβ antibodies further promote the aggregation and neurotoxicity of Aβ. Other recent studies examined the presence of Aβ antibodies in normal and AD patients. One group found low levels in 50% of individuals with no correlation to the likelihood of developing dementia (Hyman et al., 2001). In two other studies, anti-Aβ antibodies were more prevalent in CSF (Du et al., 2001) and blood (Weksler et al., 2002) of neurologically normal patients compared to AD patients. In contrast to the latter studies, we found elevated levels of antibodies to Aβ in the blood and CSF of AD patients. A distinguishing feature of our study is that we used aggregated and soluble Aβ in the ELISA while the other groups used only soluble Aβ. We found the greatest antibody response to aggregated Aβ, suggesting that AD patients may be generating an immune response primarily to the conformational epitopes found on aggregated/fibrillar forms of Aβ. This immune response was specific for AD patients since patients with multiple sclerosis or HIV infection, known to have a polyclonal B cell activation, did not have increased titers to Aβ. There was no correlation between the antibody titers to Aβ and the MMSE scores in the AD patients. This may be due to the small sample size and the lack of even distribution of patients for varying severity of cognitive dysfunction.

For the anti-Aβ antibodies to be of any functional significance, they need to cross the blood–brain barrier and be associated with the amyloid plaques. Indeed, we found that the antibodies were frequently associated with amyloid plaques. In a small number of patients with AD, we also found the antibodies in the CSF. All of these patients had a severe form of AD. This relative lack of detection of antibodies in the CSF may be because the antibodies are tightly associated with the plaques, or, since our CSF samples were obtained at autopsy, some degradation may have occurred, even though all samples

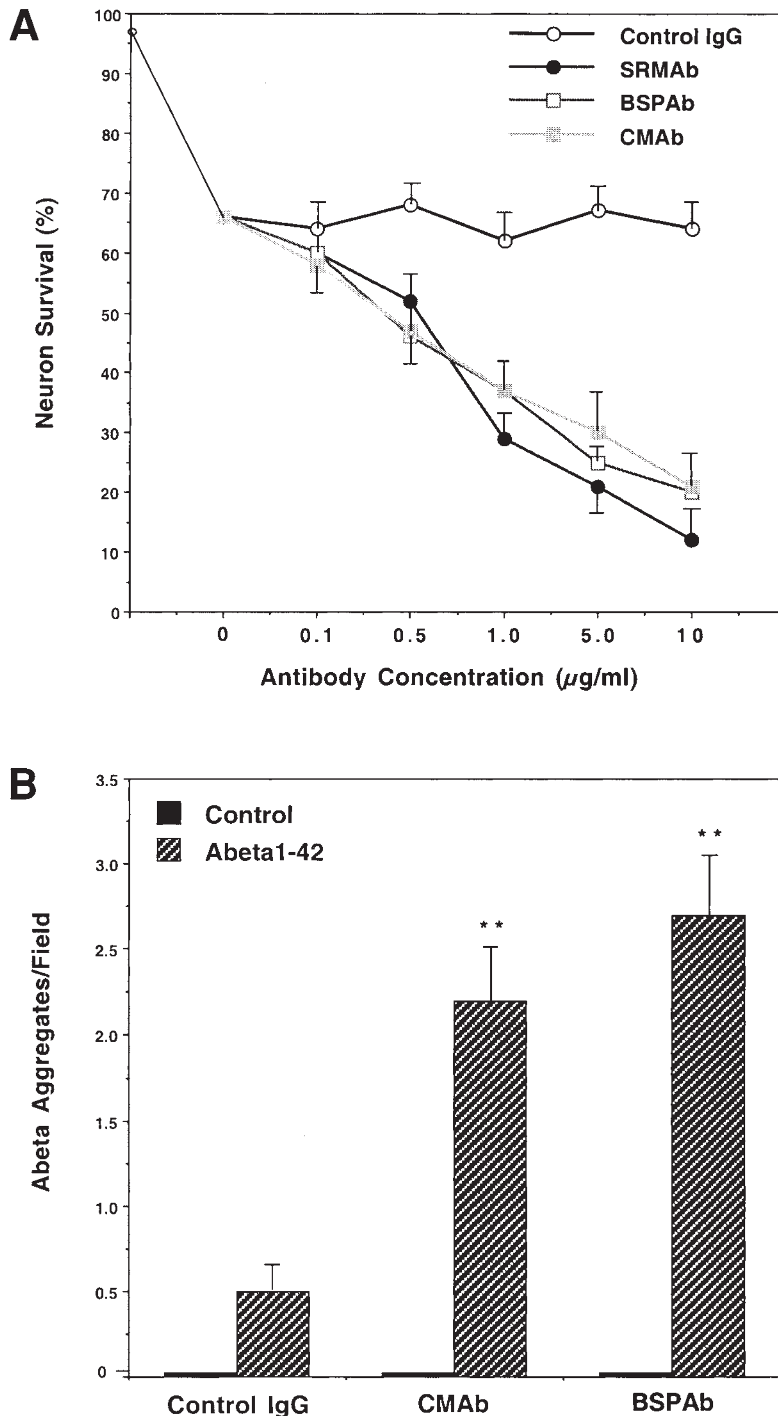

Fig. 3. Aβ antibodies enhance the neurotoxicity and aggregation of Aβ42. **(A)** Hippocampal cultures were exposed for 48 h to 1 μ*M* Aβ42 alone or in combination with increasing concentrations of Aβ antibodies, and neuronal survival was quantified. Values are the mean and SD of determinations made in 4 separate cultures. **(B)** Cultures were exposed for 48 h to the indicated antibodies alone or in combination with 1 μ*M* Aβ42 and peptide aggregation was assessed. Values are the mean and SD of determinations made in 4 separate cultures ($^{*}p<0.01$ compared to value for cultures exposed to control IgG plus Aβ42; ANOVA with Scheffe posthoc tests). SRMAb, mouse monoclonal antibody (Sigma clone BAM-10); BSPAb, rabbit polyclonal antibody (Biosource 44-348); CMAb, mouse monoclonal antibody (Chemicon, MAB206).

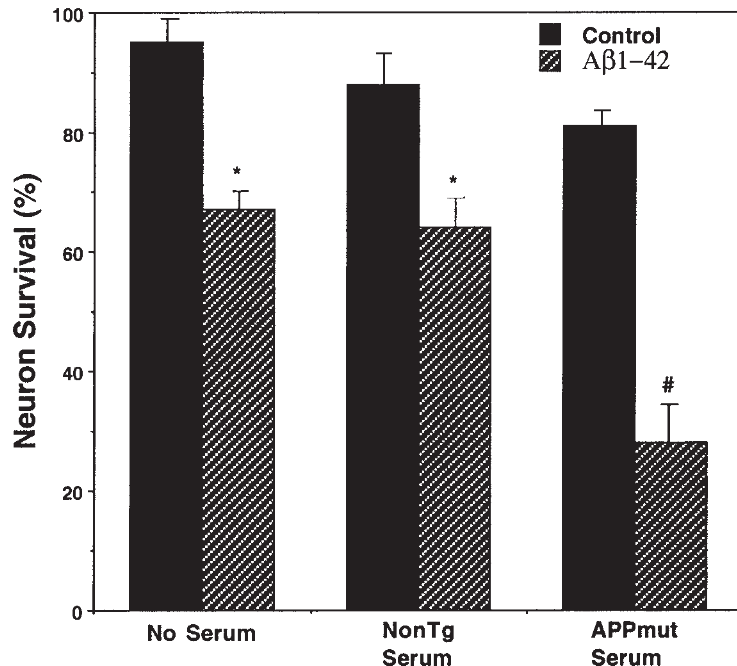

Fig. 4. Serum from APP mutant mice potentiates the neurotoxicity of Aβ. Hippocampal cultures were exposed for 48 h to 1 μ*M* Aβ42 alone or in combination with 10 μL of serum from either non-transgenic mice (n=6) or APP mutant transgenic mice (n=6), and neuronal survival was quantified. Values are the mean and SD. *p<0.01 compared to the corresponding control value. #p<0.001 compared to the corresponding control value and p<0.01 compared to the value for cultures co-treated with Aβ1-42 and serum from non-transgenic mice (ANOVA with Scheffe posthoc tests).

were obtained within four hours of death and frozen immediately. Our observations are consistent with animal studies showing that passively administered antibodies to Aβ can efficiently cross the blood–brain barrier and bind to the amyloid plaques (Bard et al., 2000). The elevated levels of antibodies in patients with AD suggest that unlike in APP mutant transgenic mice, these immune responses are ineffective in clearing the plaques.

We considered the possibilities that the antibodies may represent an immune response generated secondary to plaque formation and by itself may have no role in the pathogenesis of the disease; alternatively it may modulate plaque formation. Using an in vitro assay for Aβ aggregation, we found that an increasing titer of Aβ autoantibodies in AD patients was associated with an inhibition of aggregation of Aβ, whereas increasing antibody titers within the control group was associated with an increase in Aβ aggregation. The influence of Aβ autoantibodies on peptide aggregation and neurotoxicity therefore appear to be complex and may depend upon the particular forms of Aβ recognized by the antibodies. These observations are consistent with previous studies suggesting that relatively soluble form(s), and not the fibrillar form of Aβ, are toxic (Varadaraian et al., 2000). Thus, Aβ appears to be most toxic when it is in the process of aggregation and less toxic when it has completed the aggregation process (Mattson, 1997).

We did not find a T-cell response to Aβ stimulation. This lack of T-cell response is not due to a nonspecific abnormality in T cells, because all patients demonstrated robust T-cell proliferation in response to anti-CD3. This result is not surprising because T-cell proliferative responses to protein antigens are generally only observed in immunized individuals. However, this result does not indicate a lack of Aβ-specific T cells in AD patients. Indeed, the presence of antibodies to Aβ of the IgG class suggests

the existence of Aβ-specific T cells. Since Aβ is a self antigen, the frequency of antigen-specific T cells is likely too low to detect in a proliferation assay.

Finally we showed that antibodies to Aβ can modulate aggregation of Aβ and can also lead to enhancement of neurotoxicity in vitro. This was true with three different commercially available antibodies, two monoclonal and one polyclonal. With this limited number of antibodies, we cannot rule out the possibility that other Aβ antibodies might not potentiate, or even prevent, the neurotoxicity of Aβ. Our observations appear contrary to the results of immunization studies in mouse models where autoantibodies seemed to have a beneficial effect on learning and memory (Morgan et al., 2000; DeMattos et al., 2001; Frenkel et al., 2000). It is thus possible that several types of immune responses may occur in patients with AD, some of which may be detrimental. Importantly, our studies suggest that an autoimmune component exists in patients with AD which may play an integral role in the pathogenesis of the neurodegenerative process. Hence, caution needs to be exercised in vaccinating patients with Aβ, since the humoral immune responses may enhance the toxicity of Aβ with potential detrimental consequences, a plausible explanation for the recent halting of the initial vaccine trial in AD patients (Birmingham and Frantz, 2002).

References

Bard F., Cannon C., Barbour R., et al. (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* **6,** 916–919.

Birmingham K. and Frantz S. (2002) Set back to Alzheimer vaccine studies. *Nat. Med.* **8,** 199, 200.

Borchelt D. R., Thinakaran G., Eckman C. B., et al. (1996) Familial Alzheimer's disease linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron* **17,** 1005–1013.

Cai X. D., Golde T. E., and Younkin S. G. (1993) Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. *Science* **259,** 514–516.

Cairns N. J., Chadwick A., Lantos P. L., et al. (1993) Beta A4 protein deposition in familial Alzheimer's disease with the mutation in codon 717 of the beta A4 amyloid precursor protein gene and sporadic Alzheimer's disease. *Neurosci. Lett.* **149,** 137–140.

Citron M., Oltersdorf T., Haass C., et al. (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature* **360,** 672–674.

Cheng B. and Mattson M. P. (1992) Glucose deprivation elicits neurofibrillary tangle-like antigenic changes in hippocampal neurons: Prevention by NGF and bFGF. *Exp. Neurol.* **117,** 114–123.

DeMattos R. B., Bales K. R., Cummins D. J., et al. (2001) Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **98,** 8850–8855.

Du Y., Dodel R., Hampel H., et al. (2001) Reduced levels of amyloid beta-peptide antibody in Alzheimer disease. *Neurology* **57,** 801–805.

Esler W. P. and Wolfe M. S. (2001) A portrait of Alzheimer secretases—new features and familiar faces. *Science* **293,** 1449–1454.

Estus S., Tucker H. M., van Rooyen C., et al. (1997) Aggregated amyloid-beta protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. *J. Neurosci.* **17,** 7736–7745.

Frenkel D., Katz O., and Solomon B. (2000) Immunization against Alzheimer's beta-amyloid plaques via EFRH phage administration. *Proc. Natl. Acad. Sci. USA* **97,** 11,455–11,459.

Games D., Bard F., Grajeda H., et al. (2000) Prevention and reduction of AD-type pathology in PDAPP mice immunized with A beta 1-42. *Ann. NY Acad. Sci.* **920,** 274–284.

Gotz J., Chen F., van Dorpe J., and Nitsch R. M. (2001) Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. *Science* **293,** 1491–1495.

Guo Q., Sebastian L., Sopher B. L., et al. (1999) Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid beta-peptide toxicity: central roles of superoxide production and caspase activation. *J. Neurochem.* **72,** 1019–1029.

Hyman B. T., Smith C., Buldyrev I., et al. (2001) Autoantibodies to amyloid-beta and Alzheimer's disease. *Ann. Neurol.* **49,** 808–810.

Iwatsubo T., Odaka A., Suzuki N., et al. (1994) Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron* **13,** 45–53.

Janus C., Pearson J., McLaurin J., et al. (2000) A beta peptide immunization reduces behavioural

impairment and plaques in a model of Alzheimer's disease. *Nature* **408,** 979–982.

LeVine H., 3rd. (1999) Quantification of beta-sheet amyloid fibril structures with thioflavin T. *Methods Enzymol.* **309,** 274–284.

Lewis J., Dickson D. W., Lin W. L., et al. (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* **293,** 1487–1491.

Markesbery W. R. (1997) Neuropathological criteria for the diagnosis of Alzheimer's disease. *Neurobiol. Aging* **18,** S13–S19.

Mattson M. P. (1997) Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol. Rev.* **77,** 1081–1132.

Mattson M. P., Fu W., Waeg G., et al. (1997) 4-hydroxynonenal, a product of lipid peroxidation, inhibits dephosphorylation of the microtubule-associated protein tau. *NeuroReport* **8,** 2275–2281.

Morgan D., Diamond D. M., Gottschall P. E., et al. (2000) A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408,** 982–985.

Nath A. and Wolinsky J. S. (1990) Antibody response to rubella virus structural proteins in multiple sclerosis. *Ann. Neurol.* **27,** 533–536.

Pike C. J., Walencewicz A. J., Glabe C. G., and Cotman C. W. (1991) In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. *Brain Res.* **563,** 311–314.

Selkoe D. J. (2000) Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. *Ann. NY Acad. Sci.* **924,** 17–25.

Schenk D., Barbour R., Dunn W., et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400,** 173–177.

Scheuner D., Eckman C., Jensen M., et al. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* **2,** 864–870.

Simmons L. K., May P. C., Tomaselli K. J., et al. (1994) Secondary structure of amyloid beta peptide correlates with neurotoxic activity in vitro. *Mol. Pharmacol.* **45,** 373–379.

Suzuki N., Cheung T. T., Cai X. D., et al. (1994) An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. *Science* **264,** 1336–1340.

Tamaoka A., Kondo T., Odaka A., et al. (1994) Biochemical evidence for the long-tail form (A beta 1-42/43) of amyloid beta protein as a seed molecule in cerebral deposits of Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **205,** 834–842.

Varadarajan S., Yatin S., Aksenova M., and Butterfield D. A. (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. *J. Struct. Biol.* **130,** 184–208.

Weksler M. E., Relkin N., Turkenich R., et al. (2002) Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp. Gerontol.* **37,** 943–948.