Antibodies against β-Amyloid
Slow Cognitive Decline in Alzheimer’s Disease

Christoph Hock,* Uwe Konietzko,
Johannes R. Streffer, Jay Tracy, Andri Signorell,
Britta Müller-Tillmanns, Ulrike Lemke,
Katharina Henke, Eva Moritz, Esmeralda Garcia,
M. Axel Wollmer, Daniel Umbricht,
Dominique J.F. de Quervain,
Marc Hofmann, Alessia Maddalena,
Andreas Papassotiropoulos, and Roger M. Nitsch*
Division of Psychiatry Research
University of Zurich
August Forel Strasse 1
8008 Zurich
Switzerland

Summary
To test whether antibodies against β-amyloid are effective in slowing progression of Alzheimer’s disease, we assessed cognitive functions in 30 patients who received a prime and a booster immunization of aggregated Aβ over a 1 year period in a placebo-controlled, randomized trial. Twenty patients generated antibodies against β-amyloid, as determined by tissue amyloid plaque immunoreactivity assay. Patients who generated such antibodies showed significantly slower rates of decline of cognitive functions and activities of daily living, as indicated by the Mini Mental State Examination, the Disability Assessment for Dementia, and the Visual Paired Associates Test of delayed recall from the Wechsler Memory Scale, as compared to patients without such antibodies. These beneficial clinical effects were also present in two of three patients who had experienced transient episodes of immunization-related aseptic meningoencephalitis. Our results establish that antibodies against β-amyloid plaques can slow cognitive decline in patients with Alzheimer’s disease.

Introduction
β-amyloid is a major histopathological hallmark of Alzheimer’s disease (AD) (National Institute on Aging, 1997). It is associated with age-related cognitive decline, neurotoxicity, and the formation of neurofibrillary tangles (NFT) (Naslund et al., 2000; Chen et al., 2000; Geula et al., 1998; Götz et al., 2001; Lewis et al., 2001). Therefore, several β-amyloid-lowering strategies are currently developed for clinical use. These include inhibition of the generation of amyloid β-peptide (Aβ) with β- and γ-secretase inhibitors, prevention of Aβ aggregation, and immunization against β-amyloid (Citron, 2002; Weiner and Selkoe, 2002; Sigurdsson et al., 2002; Gandy, 2002). Both passive and active immunization of transgenic mice against β-amyloid can reverse neuropathology and improve pathologic learning and memory behaviors (Schenk et al., 1999; Bard et al., 2000; Janus et al., 2000; Morgan et al., 2000; De Mattos et al., 2001).

In a human patient with AD, immunization against β-amyloid was associated with sizable brain areas devoid of β-amyloid, reduced neurotic pathology, reduced astrocytosis, and microglial cells filled with β-amyloid (Nicoll et al., 2003), as predicted by previous immunization experiments in transgenic mice. To test whether active immunization can slow the progression of dementia in patients with AD, a recent multicenter study was initiated, but active dosing of the vaccine was suspended after the transient occurrence of clinical signs of aseptic meningoencephalitis in 6% of the cases (Schenk, 2002; Orgogozo et al., 2003). After suspension of dosing, we continued to follow up our cohort of 30 AD patients who participated in this study. Patients with a clinical diagnosis of mild to moderate AD had received active prime and booster immunizations with preaggregated Aβ(1-21) (n = 24) or placebo (n = 6) in a double-blind, randomized study design (Hock et al., 2002; Schenk, 2002; Orgogozo et al., 2003). By using a specific and sensitive tissue amyloid plaque immunoreactivity (TAPIR) assay, we observed the sustained generation of antibodies against brain β-amyloid plaques in 20 of our 30 patients (Hock et al., 2002). To determine whether these antibodies were associated with modifications of the clinical course of AD, we tested cognitive functions and capacities of daily living of the patients at baseline (n = 30) and during a 1 year period (n = 28, due to two dropouts).

Results

Human Antibodies Recognized Brain β-Amyloid Plaques
Twenty of thirty patients in this study generated antibodies that specifically recognized β-amyloid plaques on brain tissue sections obtained from transgenic mice expressing in brains both human APP with the Swedish mutation and human presenilin 1 (PS1) with the M146L mutation (APPΔ6xPS1M146L) (Figure 1; Holcomb et al., 1998). The ten other patients did not generate such antibodies, or had very low serum level at baseline with unchanged levels during the study. Together, this group of patients (n = 9 observed cases at month 12) was used as the control group for comparisons. The presence, or the absence, of the antibodies against β-amyloid was unrelated to the occurrence of aseptic meningoencephalitis in a total of three patients in our cohort. Confocal microscopy images of β-amyloid plaques stained with human immune sera or immune CSF showed close to complete overlap in staining obtained with both the monoclonal antibody 4G8 against Aβ and with Thioflavin S. The overlap in staining with Thioflavin S indicated that these human antibodies recognized bona fide brain β-amyloid plaques. To score the ability of the sera to recognize β-amyloid plaques, we used our TAPIR assay (Hock et al, 2002).
Figure 1. Confocal Immunofluorescence Image of β-Amyloid Plaques Stained by Human Antibodies against β-Amyloid Obtained from a Patient with AD Who Participated in This Study

(A) Human immune serum with antibodies against β-amyloid.
(B–F) Triple-stained β-amyloid plaque.
(B) Human immune CSF, red.
(C) Monoclonal antibody 4G8, blue.
(D) Human immune CSF and 4G8, purple.
(E) Thioflavin S, green.
(F) Human immune CSF and thioflavin S, yellow.
Scale bar equals 20 μm.

Slowed Decline of Cognitive Functions and Capacities of Daily Living
AD patients who generated antibodies against β-amyloid (n = 19) performed markedly better on the Mini Mental State Examination (MMSE) 8 months and 1 year after the immunization, as compared to control patients (n = 9, p = 0.008, ANOVA) (Figure 2A). As compared to baseline, the patients who generated antibodies against β-amyloid remained unchanged after 1 year (−1.4 ± 3.5, mean ± SD n.s., median = −1.0). Patients in the control group worsened significantly by −6.3 ± 4.0 MMSE points (mean ± SD, median = −5.0; p < 0.01, Wilcoxon). This magnitude of progression of dementia is clinically relevant, and it is somewhat higher than rates of decline known for the natural history of AD of −3.9 ± 3.7 MMSE points per year, but both our mean and median values are well within one standard deviation of published data (Morris et al., 1993). In contrast, the clinical stabilization in the group of patients who generated antibodies against β-amyloid differed markedly from the known natural history of AD (Morris et al., 1993).

To determine whether beneficial effects were also noted by the patients’ caregivers, we applied the Disability Assessment for Dementia (DAD) rating scale by interviewing caregivers in a double-blinded manner (Gauthier et al., 1997). The DAD assesses activities of daily living including initiation, planning, and organization; performance in eating, bathing, grooming, dressing, and toileting; and telephone communication, paying bills, cooking, and shopping. Performance in the DAD was significantly better in patients who generated antibodies against β-amyloid, as compared to control patients (Figure 2B). After 12 months, patients who generated antibodies against β-amyloid declined by −2.9 ± 3.8 of 40 points on the DAD, as compared to −8.7 ± 10.0 in control patients (p = 0.030, ANOVA) or, in percent of applicable questions, by −6.1% ± 9.0% versus −20.1% ± 23.4%, respectively (p = 0.039, ANOVA). Thus, the cognitive stabilization translated into relevance for daily life.

Relation of Clinical Outcome to the Increase in TAPIR Scores
To determine whether the clinical outcome was related to TAPIR scores, we grouped the patients according to the magnitude of increases in TAPIR scores and ob-
Preserved Hippocampal Function
Thirteen of twenty of the patients who generated antibodies against β-amyloid and 5 of 10 of the control patients were able to complete the Visual Paired Associates Test of delayed recall from the Wechsler Memory Scale. This task is a demanding test of hippocampal memory function (Wechsler, 1987). The reasons for not completing (n = 12) this test included inability to follow instructions or to learn items for recall and refusal. Upon generation of antibodies against β-amyloid, performance of the subset of patients who completed this

Figure 2. The Generation of Antibodies against β-Amyloid Was Associated with Slowed Declines in Cognitive Functions and Activities of Daily Living
AD patients who generated antibodies against β-amyloid (filled symbols, solid lines) were compared to patients without this immune response (controls, open symbols, dashed lines).

(A) MMSE scores of dementia severity. AD patients who generated antibodies against β-amyloid (n = 19) performed significantly better (p = 0.008, ANOVA) than the controls (n = 9).

(B) DAD scores of activities of daily living. Patients who generated antibodies against β-amyloid (n = 19) performed significantly better (p = 0.030, ANOVA) than control patients (n = 9); in percent of applicable questions, patients who generated antibodies against β-amyloid declined by −6.1% ± 9.0% versus −20.1% ± 23.4% decline of controls after 12 months (p = 0.039, ANOVA).

(C) The magnitude of the immune response, as defined by increases in TAPIR scores, was related to the clinical outcome. Whereas control patients without increases in TAPIR scores (n = 9) worsened, patients with intermediate increases (n = 13) declined only marginally, and patients with strong increases (n = 6) remained stable (p = 0.008, Kruskal-Wallis test; p = 0.021, *p = 0.004, U tests versus controls).

(D) Prevention of disease progression. Upon the generation of antibodies against β-amyloid, significantly more patients did not progress to severe dementia (MMSE < 14). In contrast, the vast majority of control patients had progressed to severe dementia (*p < 0.01, χ² = 7.25, d.f. = 1).

(E) Cognitive stabilization. MMSE scores were unchanged (±3) or higher in 12 of 19 patients who generated antibodies against β-amyloid (solid bar) in contrast to 2 of 9 control patients (open bar) (p < 0.05, χ² = 4.09, d.f. = 1).

(F) Preserved hippocampal function tested by the WMS Visual Paired Associates Test of delayed recall. Only two-thirds of the study patients in either group were able to complete this task. Performance of the patients who generated antibodies against β-amyloid (n = 13) was significantly better (p = 0.029, ANOVA) as compared to control patients (n = 5).

Preserved Hippocampal Function
Thirteen of twenty of the patients who generated antibodies against β-amyloid and 5 of 10 of the control patients were able to complete the Visual Paired Associates Test of delayed recall from the Wechsler Memory Scale. This task is a demanding test of hippocampal memory function (Wechsler, 1987). The reasons for not completing (n = 12) this test included inability to follow instructions or to learn items for recall and refusal. Upon generation of antibodies against β-amyloid, performance of the subset of patients who completed this
The group of patients who generated antibodies against Aβ showed a marked and long-lasting increase in serum antibodies against aggregated Aβ42 in both IgG (Figure 3A) and IgM (Figure 3B) classes as measured by ELISA (p = 0.005 and p < 0.0001, ANOVA, two factors, repeated measurements). Titers of both anti-Aβ42-IgG and anti-Aβ42-IgM increased within the month after the prime injection, attained a maximum within the month after the booster injection, and remained high until month 12. These sustained increases could be related to the long-term stability of the Aβ42 aggregates use as the vaccine.

**Antibodies against β-Amyloid Can Reach the Brain**

In our patients, the generation of antibodies against β-amyloid was not associated with major changes in either CSF levels of Aβ42 and Aβ40 (Figures 4A and 4B) or in plasma levels of Aβ42 (Figure 4C), arguing against the possibility that sequestration of serum Aβ is an underlying principle of the therapeutic effects observed here. We do not know whether brain β-amyloid load was reduced in our study patients; in vivo imaging techniques will be required to answer this question.

**Discussion**

Here we report the results of our TAPIR analysis applied to the Zurich cohort of 30 patients who participated in a multicenter trial of β-amyloid immunization. We observed slowed cognitive decline in AD patients who generated antibodies against β-amyloid plaques. Whereas cognition of patients who did not generate such antibodies worsened, patients with intermediate increases in such antibodies declined only marginally, and patients with strong increases remained clinically and cognitively stable. The clinical stabilization was further substantiated by significantly better performance in activities of
daily living and by tests of hippocampal memory functions. These data establish the possibility that antibodies against β-amyloid are effective in halting the progression of AD.

Despite the fact that the TAPIR scores were statistically correlated with ELISA titers of serum antibodies against Aβ42 (r₁ = 0.700, p < 0.001), the TAPIR scores predicted therapeutic outcome, whereas ELISA did not. Together with our subgroup of patients with discrepant TAPIR scores and ELISA titers, these data suggest that the degree of selectivity of the antibodies for bona fide β-amyloid is an important determinant for the clinical efficacy of antibodies in AD. The difference between TAPIR and ELISA scores could be related to important qualitative characteristics of the antibodies with respect to epitope recognition, affinity, and avidity of the binding reaction with β-amyloid plaques in the physiologic brain environment. These conditions may not be mimicked adequately by preparations of synthetic Aβ on ELISA plates. Together, the data underscore the importance of using appropriate assays for antibody analyses, and they suggest using TAPIR scores instead of ELISA titers for the analysis of responders. These data also demonstrate the necessity for carefully selecting therapeutically relevant epitopes within β-amyloid and its constituents for the future development of immunotherapy for AD.

The observed clinical differences among AD patients with and without an immune response were unrelated to the AChEI treatments, because patients in both groups were on stable dosages of AChEI before and during this trial. These data therefore support the possibility that the therapeutic effects of antibodies against β-amyloid and AChEI are additive. For the formal test of this possibility, however, control groups without AChEI treatments are required. Other factors that could potentially affect rates of progression of dementia, including age, gender, medication, and head trauma, were either excluded by the selection criteria or were distributed evenly among the groups. The ApoE genotype affects the risk for getting AD as well as the age of onset, but not the rate of cognitive decline once the disease has started (Gordon et al., 1996). Nevertheless, the distribution of the common ApoE genotypes was equal among the groups (p = 0.114, χ² = 2.5; d.f. = 1 for genotypes, and p = 0.438, χ² = 0.602; d.f. = 1 for allele frequencies), and there was no carrier of an ApoE2 allele in our cohort.

During the course of the AN1792 multicenter trial, 6% of the study patients developed clinical signs of aseptic meningoencephalitis (Schenk, 2002; Orgogozo et al., 2003), and they were generally treated with corticosteroids. These signs did not correlate with the generation of antibodies against β-amyloid. Moreover, occurrence of aseptic meningoencephalitis did not predict clinical outcome: two patients with aseptic meningoencephalitis in our cohort remained cognitively stable 1 year after the immunizations, despite the transient and reversible drop in the acute symptoms. On the other hand, dementia severity in one other meningoencephalitis patient without antibodies against β-amyloid continuously declined after recovery from the acute symptoms. These data imply the possibility that the beneficial effects of antibodies against β-amyloid on cognitive functions are maintained even after transient episodes of postvaccination aseptic meningoencephalitis.

Passive immunization of mice with antibodies against soluble Aβ resulted in increased plasma and CSF levels of Aβ within 3 days (Dodart et al., 2002; De Mattos et al., 2002), suggesting that antibody binding to plasma Aβ leads to its sequestration, followed by efflux of Aβ from brain to plasma. Other data show the importance of high-affinity binding of antibodies to F₄ receptors for removal of β-amyloid from mouse brain, suggesting F₄ receptor-mediated uptake of β-amyloid by macrophages or microglia (Bard et al., 2003). Our data argue against sequestration as an underlying principle of the observed therapeutic effects, but the antibodies against β-amyloid reported here are substantially different than those used in the mouse studies, because of their relative selectivity for structural epitopes in β-amyloid in plaques (Hock et al., 2002).
How do the results of this study affect the status of the amyloid cascade hypothesis of AD? Current versions of this hypothesis claim a primary role of β-amyloid in the pathogenesis of AD (Steiner and Haass, 2000; Selkoe, 2001, 2002; Walter et al., 2001; Hardy and Selkoe 2002; Golde, 2003; Ingelsson and Hyman, 2002; Dominquez and De Strooper, 2002; Sisodia and St. George-Hyslop, 2002). In analogy to infectious disease, where the primary role in causing disease is played by an infectious agent, the characterization of the pathogenic role of β-amyloid can be accomplished by two complementary experiments: transmission and vaccination. Transmission experiments are designed to identify the disease-causing entity in a diseased tissue by isolating the minimal disease-causing entity, transmitting it to a healthy animal, and thereby causing the disease phenotype. For β-amyloid, this was largely accomplished by showing its role in neurofibrillary degeneration and in NFT formation, either by intracerebral microinjection or by transgenic expression (Gotz et al., 2001; Lewis et al., 2001). Vaccination provides a complementary approach to explore the role of a suspected disease-causing entity. The experiment uses significant parts of the suspect as a vaccine to induce antibody-mediated immunity in a host animal. If the generated antibodies selectively react with the suspect and protect against disease—after exposure to an otherwise pathogenic dose of the suspected disease-causing entity—a central pathogenic role of the suspect is highly likely. From this point of view, the use of β-amyloid as a vaccine tests the possibility that β-amyloid plays a central role in causing cognitive decline in AD. Our result that precisely the patients who developed antibodies against β-amyloid—but not patients without such antibodies—prevented the progression of AD provides, therefore, the first successful clinical evidence for a central role of β-amyloid in causing cognitive decline and dementia in AD patients. The fact that the degree of the protective effects was related to the magnitude of the immune response against β-amyloid plaques in brain tissue underscores this conclusion.

Important open questions include the relationship of the clinical efficacy to effects of antibodies against β-amyloid on the histopathology of AD. The initial observation of a single immunized case devoid of β-amyloid (Nicoll et al., 2003) is clearly supportive of antibody-mediated removal of β-amyloid, but additional histopathological analyses are required to conclusively confirm that removal of β-amyloid from brain is both necessary and sufficient for clinical efficacy.

In conclusion, our findings establish that antibodies against β-amyloid plaques can slow clinical and cognitive decline in patients with AD, and they indicate major advantages of TAPIR assays over ELISA to predict clinical outcome. Our analyses should be continued by long-term follow-up studies of the complete cohort of AD patients who generated antibodies against β-amyloid as a result of Ab immunization.

**Experimental Procedures**

**Patients and Treatments**

These experiments were done within an additional adjunct study of the Zurich cohort of 30 AD patients (9 female) who participated in the ELAN/Wyeth-Ayerst AN1792/QS-21) Phase 2A multicenter trial prior to unblinding of both treatment status and antibody responses. The study was approved by the ethics committee; written, informed consent was obtained from all patients and caregivers. The clinical diagnosis of probable AD was made according to the NINCDS-ADRDA criteria (McKhann et al., 1984), clinically relevant other diseases were excluded. MRI was done to exclude structural causes of dementia. Patients with mild to moderate dementia (MMSE 21.0 ± 3.2, range 16–26; Folstein et al., 1975) and with disease durations of 3.6 ± 2.3 years (range 1–11) were included. To exclude vascular dementia, Rosen Modified Ischemic scores were <5. The mean age was 72.1 ± 7.2 years (range 52–82). Patients were randomized in a double-blind study design: 24 patients received an active vaccine consisting of preaggregated synthetic Aβ1-42, along with QS-21 adjuvant, and 6 patients received placebo. Both active vaccine and placebo were given as a prior intramuscular injection, followed 1 month later by a boost intramuscular injection. The drug/placebo status remained blinded to patients, caregivers, clinical raters, and laboratory investigators. One patient from the placebo group died during the study from cerebrovascular hemorrhage. One patient refused testing at month 12. Therefore, our study ended with 28 observed cases after one year. The 20 patients (8 female) who generated antibodies against β-amyloid plaques were 73.6 ± 7.0 years old, had baseline MMSE scores of 21.6 ± 3.2, and had a mean duration of disease of 3.6 ± 2.4 years. Nineteen (6 female) of these completed the study (age 73.4 ± 7.1 years, MMSE 21.3 ± 3.1, duration 3.6 ± 2.5 years). The 10 control patients (3 female) were 68.8 ± 6.8 years old, had baseline MMSE scores of 19.9 ± 3.2, and had a mean duration of disease of 3.8 ± 2.3 years. Nine (3 female) of these completed the study (age 68.8 ± 7.2 years, MMSE 19.2 ± 2.5, duration 3.4 ± 2.2 years).

Twenty-eight patients received stable doses of AChEIs for at least 3 months prior to inclusion. AChEIs were continued throughout the study, except for one patient who generated antibodies against β-amyloid and who terminated AChEI at month 11. The length of treatment with AChEIs was similar among the patient groups with strong increases in TAPIR scores (3.0 ± 2.2 years), with intermediate increases (2.1 ± 0.8), or without increases (3.6 ± 1.9; p = 0.211, Kruskal-Wallis test). These time periods are beyond the 1 year period of known stabilizing effects of AChEIs (Doody et al., 2001; Giacobini, 2000). Of the patients who generated antibodies against β-amyloid, six received donepezil (5 mg per day, n = 1; 10 mg, n = 2), two rivastigmine (12 mg, n = 1; 3 mg, n = 1), and eleven galantamine (16 mg, n = 5; 24 mg, n = 6). One patient changed from galantamine (16 mg) to rivastigmine (6 mg). Among control patients, five received donepezil (10 mg, n = 3), four galantamine (16 mg, n = 1, 24 mg, n = 3), and one patient no AChEI. Other medications for cognitive enhancement were neither permitted within the trial nor during the 3 month period prior to inclusion. Nonsteroidal antiinflammatory drugs (NSAIDs), statins, estrogens, and vitamin E were evenly distributed among the two groups. Patients who generated antibodies against β-amyloid used NSAIDs (n = 11), statins (n = 3) vitamin E (n = 2), and no estrogens; patients in the control group used NSAIDs (n = 5), statins (n = 2), vitamin E (n = 1), and estrogens (n = 2).

**Tissue Amyloid Plaque Immunoreactivity (TAPIR) Assay**

For the assessment of the ability of the human immune sera to react with bona fide β-amyloid plaques in brain tissue, we developed a specific TAPIR assay. Double transgenic mice (18 months) expressing pathogenic AD-causing human mutant APP and PS1 genes (APP<sup>p<sup>125</sup> mutants; PS1<sup>amyloid</sup> mutants) were perfused and fixed. Paraffin-embedded brains sections were incubated with human sera or CSF taken at baseline (month 0) and 56 ± 5.8 days after the booster injection. The samples were used either undiluted or diluted 1:50 to 1:10,000 in 2% BSA and 10% horse serum in PBS. After washing, human IgG bound to β-amyloid plaques were detected with cy3-conjugated donkey antibodies directed against heavy and light chains of human IgG (Jackson Labs, Bar Harbor, Maine). Fluorescent secondary antibodies were imaged through a 40× objective and a TRITC filter attached to a Nikon Eclipse E800 fluorescence microscope equipped with a Kappa PS 30C CCD camera. Images of all dilutions were acquired with standardized camera settings chosen to be well.
below the saturation of 255 arbitrary units (A.U.) in 8 bit mode. The Image J software (http://www.ncbi.nlm.nih.gov) was used to quantify the mean pixel intensities (range 20 to 230 A.U.) of n = 15 β-amyloid plaques per serum dilution. Averages of the means were used for both the standard curve and the individual samples. The assay was linear for serum dilutions ranging from 1:50 to 1:10,000 (r = 0.951; p = 0.013).

For comparisons with a standard curve obtained by diluting human CSF from a responder, both preimmune and immune sera were used at 1:50 dilutions and categorized by two independent, blinded raters into the following five immunoreactivity scores: absent immunoreactivity (-); weak immunoreactivity corresponding to 1:10,000 (+); moderate, 1:5,000 (++); strong, 1:1,000 (+++); and very strong, 1:500 (++++). To determine the increase in immunoreactivity during treatment, the preimmune immunoreactivity scores were subtracted from the immune scores to generate the following group: no increase, n = 10 (n = 9 observed cases) in the control group. In the group of patients who generated antibodies against β-amyloid (n = 20), one patient dropped out because of unwillingness to participate in neuropsychological testing at month 12, leaving n = 19 observed cases. To compare the degree of the immune response to the clinical outcome, this group was further subdivided into two groups based upon the magnitude of increases in TAPiR scores as follows: strong increases representing 4+ increases from baseline to immune status (n = 6), and moderate increases representing the remaining group of 1+ to 3+ increases (n = 13) from baseline to immune status.

Neuropsychology
Clinical assessments including the MMSE were done at baseline, as well as at month 8 and month 12. Normal MMSE scores were assumed at 27–30; mild dementia at 20–26; moderate at 14–19; and severe at 0–13. The following tests were done at baseline and at months 6 and 12: the Alzheimer’s Disease Assessment Scale (ADAS) cognitive part (ADAS-Cog) (Rosen et al., 1984), the Verbal and the Visual Paired Associates Tests of immediate and delayed recall from the Wechsler Memory Scale (WMS) (Wechsler, 1987), and naming and fluency (verbal and categorical) (CERAD) (Morris et al., 1989). Global function was determined by the clinical dementia rating scale (CDRS) (Morris, 1993), as well as the clinical global impression of change (CGIC) (Kopman et al., 1994). Activities of daily living were assessed by Disability Assessment for Dementia (DAD) (Gauthier et al., 1997) rating scale ranging from 0 to 40. Because the Visual Paired Associates Test of delayed recall from the WMS is difficult, several patients were unable to complete it at month 12. The baseline scores for the patients who dropped out of this test were 1.6 ± 1.2 (n = 12), as compared to 2.8 ± 1.5 (n = 18) in patients who completed it after 1 year. The clinical raters remained blinded throughout the study to the treatment status, as well as to the immunoreactivity scores and antibody titers of the patients.

ELISA Titer Assays
Block Aβ1-40-coated (Bachem, Weil am Rhein, Germany) microplates (Nunc Maxisorp, Roskilde, Denmark) were incubated with diluted serum samples overnight at 4°C, washed, and incubated individually with goat anti-human biotinylated IgG or IgM (H+L) (Jackson Labs, Bar Harbor, ME), detected by peroxidase-conjugated streptavidin (Jackson Labs, Bar Harbor, ME) and 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma) at 450 nm on a Victor2 Multilabel microplate reader (EG&G Wallac). All samples and standards were assayed in duplicates.

Aβ1-40 and Aβ1-42 ELISAs
CSF and plasma Aβ40 were measured by the INNOTEST β-Amyloid1-42 ELISA according to the manufacturer’s protocol (Innogenetics, Belgium). For Aβ1-40 ELISA, 1 μg/ml of biotinylated 4G8 (Signet, Dedham, MA) was bound to streptavidin-coated microplates (Nunc) and incubated with CSF diluted in PBS, along with BAP-24 (courtesy Dr. Manfred Brockhaus, Roche), followed by TMB as the chromophor, sulfuric acid and reading at 450 nm. Standard curves of Aβ1-40 (Bachem) scaling from 0.15 to 40 ng/ml were used, and Aβ40 was tested as a negative control.

Statistical Analyses
Data were analyzed by ANOVA. Comparisons of two groups were done with Mann-Whitney U tests, and comparisons of three groups were done by Kruskal-Wallis tests. The distribution of categorical variables between groups was tested by using the chi-square and Fisher’s exact tests. The correlation coefficient quoted is Spearman’s rho. All p values reported are two-sided. Changes in neuro-psychosocial test scores (three data collection time points) were analyzed by observed cases analyses (OC). Changes in serum titers and plasma Aβ levels (ten data collection time points) were analyzed by intention to treat (ITT) analysis; missing values were interpolated between visits and last values were carried forward.

Acknowledgments
The preparations of active vaccine and placebo were administered to the patients within the ELAN/Wyeth-Ayerst AN1792/GO-21 trial. We thank Christin Wilde, Estelle Obrist, Andrea Walter, and Ruth Schmid for excellent clinical study support, Mohamad Jaber for technical support, Drs. Karen Duff and Karen Hisao-Ashie for transgenic mouse lines, and Dr. Manfred Brockhaus (Roche) for BAP24. We thank Drs. John H. Growdon and Dale Schenk (Elan) for critical comments on this manuscript. This study was funded in part by the National Centre of Competence in Research on Neural Plasticity and Repair (NCCR), the EU DIadem program on Diagnosis of Dementia, the Stammbach Foundation, and the University of Zurich. The authors declare that they have no competing financial interests related to Elan/Wyeth-Ayerst.

Received: March 26, 2003 Revised: April 10, 2003 Accepted: April 30, 2003 Published: May 21, 2003

References


