

# Immunological and Anti-Chaperone Therapeutic Approaches for Alzheimer Disease

Thomas Wisniewski<sup>1,2,3</sup>; Blas Frangione<sup>2,3</sup>

<sup>1</sup>Departments of Neurology, <sup>2</sup>Pathology, and <sup>3</sup>Psychiatry, New York University School of Medicine.

Corresponding author:

Thomas Wisniewski, Department of Neurology, New York University School of Medicine, 550 First Avenue, New York, NY 10016  
(E-mail: thomas.wisniewski@med.nyu.edu)

**Alzheimer disease (AD) is the most common cause of dementia. Currently available therapies only provide symptomatic relief. A number of therapeutic approaches are under development that aim to increase the clearance of brain A $\beta$  peptides. These include immune mediated clearance of A $\beta$  and the inhibition of the interaction between A $\beta$  and its pathological chaperones. Both active and passive immunization has been shown to have robust effects in transgenic mouse models of AD on amyloid reduction and behavioral improvements. However, a human trial of active immunization has been associated with significant toxicity in a minority of patients. New generation vaccines are being developed which likely will reduce the potential for cell-mediated toxicity. In addition, the recent development of anti-chaperone therapy opens a new therapeutic avenue which is unlikely to be associated with toxicity.**

*Brain Pathol* 2005;15:72-77.

## INTRODUCTION

Alzheimer disease (AD) currently affects approximately 4.5 million Americans. It is an age-associated disease that typically starts insidiously with progressing memory and learning impairments, with a clinical course lasting 10 to 15 years. Currently no treatment that significantly modifies the pathological mechanisms of Alzheimer disease is available. The current clinical standard is to treat with anticholinesterase inhibitors (12), which are designed to increase the level of the neurotransmitter acetylcholine. These drugs primarily offer symptomatic therapy and are capable only of providing temporary maintenance of clinical function (11). A more recently introduced treatment is memantine, which is an N-methyl-D-aspartate (NMDA) receptor antagonist thought to act on abnormal glutamatergic activity; however, this drug also provides only symptomatic relief (55). If no highly effective treatment for AD is introduced, the incidence is projected to double and triple within the next 10 and 20 years, respectively, as a function of the increasing average age of US society (9).

The major neuropathological features of AD patients are neuronal loss, neurofibrillary tangles (NFTs) and the deposition of

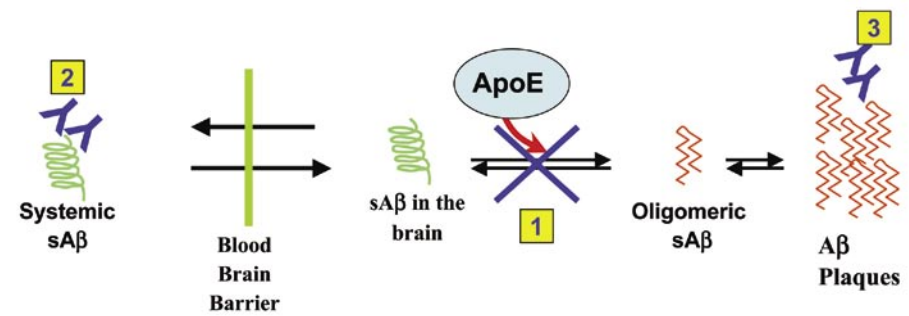
$\beta$ -amyloid (A $\beta$ ), a 39 to 42 amino acid long hydrophobic peptide, both in the walls of meningeocephalic arteries and in the form of plaques in the gray matter of the brain (43, 44, 77). The A $\beta$  peptide exists as a constituent of serum and other physiological body fluids called soluble A $\beta$  (sA $\beta$ ); it is only when it undergoes a conformational change containing high  $\beta$ -sheet content in the form of oligomers, protofilaments or plaque deposits that this peptide becomes neurotoxic. Accumulation of A $\beta$  in AD brains is associated with increased production and/or impaired clearance which have been both observed with advancing age and in sporadic AD (63). The high  $\beta$ -sheet content renders A $\beta$  insoluble, resistant to proteolytic degradation and toxic to neurons. Initially, the process of A $\beta$  peptide deposition is difficult to detect and clinically silent (27). Extensive A $\beta$  deposits can be detected in clinically asymptomatic individuals (24); however, with time the deposits of A $\beta$  become more compact and extensive, leading to synaptic damage that correlates with clinical dysfunction. sA $\beta$  circulating in the serum may also cross the BBB and co-deposit on existing plaques increasing the load of deposited fibrillar A $\beta$ .

The process of the conformational transformation of sA $\beta$  toward fibril formation can be accelerated by the presence of pathological chaperones (79). A $\beta$  can interact with a group of proteins and proteoglycans that modulate the conformational transformation of sA $\beta$  to a more proamyloidogenic state (37, 59, 78). This action contrasts with the role of physiological chaperones which act to promote normal, functional protein folding. The best example of A $\beta$  pathological chaperone is apolipoprotein E (ApoE) (80). Humans possess 3 isoforms of this protein: E2, E3, and E4, which differ at residues 112 and 158. These minor differences in the sequence result in a significant structural differences (75). The presence of the ApoE4 isoform, in an allele-dose dependant manner is associated with an increased occurrence of sporadic AD (8). So far the presence of the ApoE4 allele is the best characterized genetic risk factor for sporadic AD, which by some estimates accounts for the vast majority of sporadic AD risk (13). The risk for sporadic AD is increased among E4 heterozygotes by 3- to 4-fold and up to 14-fold for homozygotes, with an earlier age of onset, on average by 5 and 10 years, for heterozygotes and homozygotes respectively, comparing to non-E4 carriers (38, 40, 40, 56).

Hence, at least 2 processes that can enhance A $\beta$  deposition in the brain are decreased clearance and pathological chaperones that increase fibrillization. Therefore potentially therapeutic approaches for AD are the increase of A $\beta$  clearance by induction of an immune response to this peptide and by blocking the interaction of A $\beta$  with its pathological chaperones. Both of these approaches are further discussed below.

## VACCINATION APPROACHES FOR AD

Vaccination was the first treatment approach which has been shown to have genuine impact on the disease process, at least in animal models of AD. Vaccination of AD Tg mice with A $\beta$ 1-42 or A $\beta$  homologous peptides co-injected with Freund adjuvant prevented the formation of A $\beta$  deposition and as a consequence eliminated the behavioral impairments that are related to A $\beta$  deposition (26, 46, 61, 65). Similar effects on A $\beta$  load and behavior have been demonstrated in AD Tg mice by injecting anti-A $\beta$  monoclonal antibodies into the blood stream indicating that the therapeutic effect of the vaccine is based primarily on eliciting a humoral response (5, 10). The striking biological effect of the vaccine in preclinical testing and the apparent lack of side effects in AD Tg mice encouraged ELAN Pharmaceutical to launch clinical trials with a vaccine designated as AN1792. AN1792 contained pre-aggregated A $\beta$ 1-42 and QS21 as an adjuvant. This type of vaccine design was aimed to induce a strong cell mediated immune response, since QS21 is known to be a strong inducer of Th-1 lymphocytes (30). The initial safety testing of AN1792 in Phase I did not demonstrate any adverse effects. The phase II of the trial was prematurely terminated when 6% of vaccinated patients manifested symptoms of acute meningoencephalitis (49). An autopsy performed on one of the affected patients revealed an extensive cytotoxic reaction surrounding some cerebral vessels, however analysis of the A $\beta$  load in the brain cortex suggested that A $\beta$  clearance had occurred (48). It appeared that the immune reaction triggered by AN1792 was a double-edged sword, where the benefits of a humoral response against A $\beta$  were overshadowed in some individuals by uncontrolled cytotoxicity (39). Not all patients who received AN1792 responded with antibody production. The majority mounted a humoral response and showed a modest but statistically significant cognitive benefit demonstrated as an improvement on some cognitive testing scales comparing to baseline and a slowed rate of disease progression comparing to the patients who did not form antibodies (21). The follow-up data from the "Zurich's cohort," who are a subset of the Elan trial followed by Dr Nitsch's group (20, 21), indicated that the vaccination approach may be beneficial for human



**Figure 1.** A $\beta$  peptide dynamics in Alzheimer disease. In AD the levels of A $\beta$  peptides increase locally in the brain related to either increased production or decreased clearance. In the common late-onset forms of AD the primary mechanism appears to be related to decreased clearance. This leads to formation of oligomeric A $\beta$ , which over time deposits in the form of amyloid plaques. ApoE promotes the conformational change from normal sA $\beta$  to fibrillar A $\beta$ . Some potential therapeutic approaches include: 1) blocking the binding between sA $\beta$  and its pathological chaperones such as apoE (using compounds such as A $\beta$ 12-28P); 2) a humoral anti-A $\beta$  peptide immune response which clears A $\beta$  peptides in the systemic circulation. This acts as a "peripheral sink" drawing out A $\beta$  peptides from the brain; and 3) a humoral and cell mediated immune response within the brain which clears A $\beta$  deposits. However, this clearing mechanism has the potential to induce innocent bystander neuronal damage and inflammation.

AD patients, but the concept of the vaccine has to be redesigned.

It appears that a humoral response elicited by the vaccine has at least 2 mechanisms of action and both of these are thought to be involved in amyloid clearance (Figure 1) (21). Conformational specific anti-A $\beta$  antibodies may target A $\beta$  deposits in the brain (20) leading to their disassembly (2, 68) and elicit Fc mediated phagocytosis by microglia cells which is associated with a strong cell mediated response (60). The second mechanism by which anti-A $\beta$  antibodies likely prevent A $\beta$  deposition is the creation of a "peripheral sink" effect, where the removal of excess sA $\beta$  circulating in the blood stream leads to sA $\beta$  being drawn out from the brain (10, 66). This peripheral sink mechanism is likely to be the dominant means of reducing A $\beta$  peptides in the brain.

The cause of the toxicity in 6% of the Elan trial patients is not entirely known; however, it is thought from the clinical and limited autopsy data that an excessive Th1 cell mediated response within the brain was to blame (57). The concept of a redesigned AD vaccine puts emphasis on avoiding this cell mediated response in the following ways: *i*) avoiding stimulation of Th-1 lymphocytes so the vaccine could potentially elicit a purely humoral response; *ii*) using a non-toxic and non-fibrillogenic A $\beta$  homologous peptides, so that the immunogen can not produce any direct toxicity; and *iii*) enhancing the peripheral sink effect rather than central action.

Passive transfer of exogenous monoclonal anti-A $\beta$  antibodies appears to be the easiest way fulfilling the goal of providing anti-A $\beta$  antibodies without risk of uncontrolled Th-1 mediated autoimmunity. AD Tg model mice treated this way had a significantly reduced A $\beta$  level and demonstrated cognitive benefit (5, 10). Major drawbacks of this approach are the high cost, limited half-life of monoclonal antibodies (2-21 days depending on class and isoform) and the potential for inducing serum sickness with resultant complications such as renal failure or lymphomas. Nevertheless, clinical trials for passive immunization trials are under development. Alternative approaches for passive immunization which are less likely to be associated with toxicity, are use of Fv fragments or mimetics of the active antibody binding site.

Understanding the antigenic profile of A $\beta$  peptide, allows engineering of modifications that favor a humoral response and reducing the potential for a Th-1 mediated response. This approach has been termed altered peptide ligands. Computer models have predicted that A $\beta$ 1-42 has one major antibody binding site located on its N-terminus and 2 major T-cell epitopes located at the central and C-terminal hydrophobic regions encompassing residues 17-21 and 29-42 respectively (25, 45). Therefore, their elimination or modification provides a double gain by eliminating toxicity, as well as the potential for T-cell stimulation. Sigurdsson et al (64) immunized AD Tg mice with K6A $\beta$ 1-30E<sub>18</sub>E<sub>19</sub>, a non-toxic A $\beta$ -ho-

mologous peptide, where the first above mentioned T-cell epitope was modified and the second removed. Polyamino acid chains coupled to its N-terminus was designed to increase the immunogenicity and solubility of the peptide. AD Tg mice vaccinated with this peptide produced mainly IgM class antibodies and low or absent IgG titer. These animals showed behavioral improvement and a partial reduction of A $\beta$  deposits (64). One of the advantages of this design is that IgM, with a molecular weight of 900 kDa, does not penetrate the BBB and is therefore unlikely to be associated with any immune reaction in the brain. Like passive immunization, this type of vaccine focuses its mechanism of action on the peripheral sink and furthermore is reversible. Therefore, this vaccine method may potentially be safer than typical active immunization.

Mucosal vaccination can be an alternative way to achieve a primarily humoral response. This mechanism is based on the presence of lymphocytes in the mucosa of the nasal cavity and of the gastrointestinal tract. This type of response produces primarily S-IgA antibodies but when the antigen is co-administrated with adjuvants such as cholera toxin subunit B or heat-labile *Escherichia Coli* enterotoxin significant IgG titer in the serum may be achieved (34, 83). A marked reduction of A $\beta$  burden in AD Tg mice immunized this way using A $\beta$  as an antigen has been already demonstrated (74, 83). Interestingly this type of mucosal immunization has been shown to be highly effective for prion infection recently (16). This promising approach requires further exploration, especially using non-fibrillar and non-toxic A $\beta$  homologous peptides as an antigen. Mucosal immunization offers a great potential advantage in that a more limited humoral immune response can be obtained, with little or no cell mediated immunity.

Further modification of the vaccine design involves the use of specific adjuvants. Freund adjuvant, which has been used in most preclinical studies on Tg animals, is not feasible for human use due to the likelihood of inducing local granulomatous inflammation. QS-21 which was used for AN1792 is associated with strong stimulation of Th-1 lymphocytes (29, 30). Another group of adjuvants with great potential for use and an excellent safety record are the FDA-approved aluminum-based min-

eral salts (aluminum hydroxide and aluminum phosphate). These show only weak stimulation of cell-mediated immunity (17, 18). An alternative way of enhancing antibody production may be encapsulating the peptide antigen in biodegradable nanoparticles (42). Genetically engineered filamentous phages displaying the A $\beta$ 3-6 motif (EFRH) have shown to be capable of inducing a strong humoral response against A $\beta$  (67) and more recently has been shown to reduce the amyloid burden and improve cognitive behaviour in AD Tg mice (33).

### INHIBITION OF A $\beta$ FIBRILLIZATION

Formation of A $\beta$  fibrils and deposition of A $\beta$  in the brain parenchyma or in the brain's vessels occurs in the setting of increased local A $\beta$  peptide concentrations (6). Initially, conditions do not favor aggregation of fibrils; however, once a critical nucleus has been formed, conditions change to favor aggregation with fast kinetics. Any available monomer becomes instantly entrapped in an aggregate or fibril. Several compounds such as Congo red (35), anthracycline (41), rifampicin (73), anionic sulphonates (32), or melatonin (50) can interact with A $\beta$  and prevent its aggregation of into fibrils in vitro reducing toxicity. It has been further identified that certain non-fibrillogenic, A $\beta$  homologous peptides can bind to A $\beta$  and break the formation of  $\beta$ -sheet structure (19, 70, 71). Therefore these peptides were termed  $\beta$ -sheet breakers. Several modifications were used to extend serum half-life and increase BBB permeability of these peptides. Permanne et al (51), using a BBB permeable 5 amino-acid long peptide (iA $\beta$ 5), were able to demonstrate a reduction of A $\beta$  load in AD Tg mice who received this peptide comparing with age-matched control group which received placebo. Of interest, a similar concept of  $\beta$ -sheet breakers appears to be applicable to another protein conformation disorder, the prion disease (69).

A $\beta$  homologous peptides can aggregate and form fibrils in vitro spontaneously; however, in vivo this process appears more dependant on the presence of A $\beta$  pathological chaperones. This is a group of proteins promotes conformational transformation at certain concentrations by increasing the  $\beta$ -sheet content of these diseases specific proteins and stabilizes their abnormal structure (53, 79). Examples of such

proteins in AD include apolipoprotein E (apo E), especially its E4 isoform (59, 78),  $\alpha$ 1-antichymotrypsin (ACT) (37) or C1q complement factor (7, 28). In their presence, the formation of A $\beta$  fibrils in a solution of sA $\beta$  monomers becomes much more efficient (37, 78). These "pathological chaperone" proteins have been found histological and biochemically in association with fibrillar A $\beta$  deposits (15, 52, 79, 82) but not in preamyloid aggregates which are not associated with neuronal toxicity (31, 76). Inheritance of the apo E4 isoform has been identified as the major identified genetic risk factor for sporadic, late-onset AD (62) and correlates with an earlier age of onset and greater A $\beta$  deposition, in an allele-dose-dependent manner (54, 62). In vitro all apo E isoforms can propagate the  $\beta$ -sheet content of A $\beta$  peptides promoting fibril formation (15), with apo E4 being the most efficient (78). The critical dependence of A $\beta$  deposition in plaques on the presence of ApoE has also been confirmed in AD Tg APP<sup>V717F</sup>/ApoE<sup>-/-</sup> mice which have a delayed onset of A $\beta$  deposition, a reduced A $\beta$  load, and no fibrillar A $\beta$  deposits. Compared to APP<sup>V717F</sup>/ApoE<sup>+/+</sup> Tg mice, APP<sup>V717F</sup>/ApoE<sup>+/-</sup> mice demonstrate an intermediate level of pathology (3, 4, 22, 23). Neutralization of the chaperoning effect of ApoE would therefore potentially have a mitigating effect on A $\beta$  accumulation. ApoE hydrophobically binds to the 12 to 28 amino acid sequence of A $\beta$ , forming SDS insoluble complexes (47, 72, 81). Ma et al (36) have demonstrated that a synthetic peptide homologous to 12-28 amino-acid sequence of A $\beta$  can be used as a competitive inhibitor of the binding of full length A $\beta$  to apo E, resulting in reduced fibril formation in vitro and increased survival of cultured neurons. Introduction of several modifications to A $\beta$ 12-28 by replacing a valine for proline in position 18 make this peptide non-toxic and non-fibrillogenic, as well as end-protection by amidation and acetylation of the C- and N-termini, respectively to increase serum half-life have allowed us to use this peptide in vitro in the APP<sup>K670N/M671L</sup>/PS1<sup>M146L</sup> double Tg mice model. Tg mice treated with A $\beta$ 12-28P for one month demonstrated a 63.3% reduction in A $\beta$  load the cortex ( $p=0.0043$ ) and a 59.5% ( $p=0.0087$ ) reduction in the hippocampus comparing to age matched control Tg mice which received placebo (58).

No antibodies against A $\beta$  were detected in sera of treated mice, therefore, the observed therapeutic effect of A $\beta$ 12-28P cannot be attributed to an antibody clearance response. This experiment demonstrate that compounds blocking the interaction between A $\beta$  and its pathological chaperones may be beneficial for treatment of  $\beta$ -amyloid deposition in AD (58).

A similar treatment concept is being developed based on inhibiting the interaction between glycosaminoglycans (GAGs) and A $\beta$  fibrils (32). Neurochem Inc. is testing several drugs aiming at prevention of amyloid fibrils formation and deposition in AD (Alzhemed™), cerebral amyloid angiopathy (Cerebril™), and systemic amyloidosis A (Fibrillex™). These compounds are synthetic sulphated GAGs mimetics which are designed to compete with naturally occurring GAGs in binding to A $\beta$  and preventing A $\beta$  deposition. Only limited preclinical data regarding the effectiveness of these compounds have been published; however, the company has moved to clinical trials that demonstrate the safety and tolerability of these drugs (14). Recently, results of a 12-month treatment trial in patients with mild to moderate AD with Alzhemed™ were presented (1). Alzhemed™ appears to reduce A $\beta$ 1-42 concentration in the CSF and stabilize cognitive decline (monitored using mini mental state examination and ADAS-cog) compared to the placebo receiving group.

## SUMMARY

In familial AD the pathogenesis is related to overproduction of total sA $\beta$  or the more fibrillogenic A $\beta$ 1-42. However, in the most common sporadic AD, the major pathogenic process appears to be impaired clearance of brain A $\beta$  peptides. Inducing a humoral immune response to A $\beta$  appears to be a promising method of enhancing A $\beta$  peptide clearance, provided toxic side-effects can be reduced. Immunization via a mucosal route, as well as use of nontoxic, non-fibrillogenic A $\beta$  homologous peptides as altered peptide ligands are current methods under investigation to specifically induce a humoral immune response without associated cell mediated immunity, which has been linked to toxicity in the Elan trial. Another approach is to block the interaction of A $\beta$  peptides with its pathological chaperones such as ApoE. This method also

aims to increase the clearance of A $\beta$  peptides, and in animal studies is not associated with toxicity. Extensive further animal testing and evaluation in non-human primates will be needed to determine if the promise of these therapeutic approaches will translate into safe patient use in the future.

## ACKNOWLEDGEMENTS

This manuscript is supported by NIH grants: AG15408, AG05891, NS047433 and AG20245 and the Alzheimer's disease Association.

## REFERENCES

1. Aisen PS, Mehra M, Poole R, Lavoie E, Gervais F, Laurin J, Braind R, Garceau L (2004) Clinical data on Alzhemed after 12 months of treatment in patients with mild to moderate Alzheimer's disease. *Neurobiol Aging* 25:20.
2. Bacskai BJ, Kajdasz ST, Christie RH, Carter C, Games D, Seubert P, Schenk D, Hyman BT (2001) Imaging of amyloid- $\beta$  deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat Med* 7:369-372.
3. Bales KR, Verina T, Cummins DJ, Du Y, Dodel RC, Saura J, Fishman CE, DeLong CA, Piccardo P, Petegnief V, Ghetti B, Paul SM (1999) Apolipoprotein E is essential for amyloid deposition in the APPV717F transgenic mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 96:15233-15238.
4. Bales KR, Verina T, Dodel RC, Du YS, Altstiel L, Bender M, Hyslop P, Johnstone EM, Little SP, Cummins DJ, Piccardo P, Ghetti B, Paul SM (1997) Lack of apolipoprotein E dramatically reduces amyloid  $\beta$ -peptide deposition. *Nat Genet* 17:263-264.
5. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T (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.
6. Barrow CJ, Yasuda A, Kenny PT, Zagorski MG (1992) Solution conformations and aggregation properties of synthetic amyloid  $\beta$ -peptides of Alzheimer's disease. Analysis of circular dichroism spectra. *J Mol Biol* 225:1075-1093.
7. Boyett KW, DiCarlo G, Jantzen PT, Jackson J, O'Leary C, Wilcock D, Morgan D, Gordon MN (2003) Increased fibrillar  $\beta$ -amyloid in response to human C1q injections into hippocampus and cortex of APP+PS1 transgenic mice. *Neurochem Res* 28:83-93.
8. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921-923.

9. Crawford FC, Vanderploeg RD, Freeman MJ, Singh S, Waisman M, Michaels L, Abdullah L, Warden D, Lipsky R, Salazar A, Mullan MJ (2002) ApoE genotype influences acquisition and recall following traumatic brain injury. *Neurol* 58:1115-1118.

10. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM (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 U S A* 98:8850-8855.

11. Doody RS (2003) Current treatments for Alzheimer's disease: cholinesterase inhibitors. *J Clin Psychiatry* 64:11-17.

12. Doody RS, Stevens JC, Beck C, Dubinsky RM, Kaye JA, Gwyther L, Mohs RC, Thal LJ, Whitehouse PJ, DeKosky ST, Cummings JL (2001) Practice parameter: Management of dementia (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 56:1154-1166.

13. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA (1997) Effects of age, sex and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. *JAMA* 278:1349-1356.

14. Garceau D, Gurbindo C, Laurin J (2001) Safety, tolerability and pharmacokinetic profile of Fibrillex in healthy and renal impaired subjects. *Budapest Proc IXth Int Symp Amyloidosis* 116-118.

15. Golabek AA, Soto C, Vogel T, Wisniewski T (1996) The interaction between apolipoprotein E and Alzheimer's amyloid  $\beta$ -peptide is dependent on  $\beta$ -peptide conformation. *J Biol Chem* 271:10602-10606.

16. Goni F, Knudsen EL, Schreiber F, Scholtzova H, Pankiewicz J, Carp RI, Meeker HC, Brown DR, Chabalgoy JA, Sigurdsson EM, Wisniewski T (2005) Mucosal vaccination delays or prevents prion infection via an oral route. *Neuroscience* In Press.

17. Gupta RK (1998) Aluminum compounds as vaccine. *Advanced Drug Delivery Rev* 32:155-172.

18. Gupta RK, Rost BE (2000) Aluminum compounds as vaccine adjuvants. In: O'Hagan DT (ed) *Vaccine Adjuvants*. Humana Press, Totowa, NJ, pp 65-89.

19. Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K (1992) Substitutions of hydrophobic amino acids reduce the amyloidogenicity of Alzheimer's disease  $\beta$ A4 peptides. *J Mol Biol* 228:1-14.

20. Hock C, Konietzko U, Paspasotiropoulos A, Wollmer A, Streffer J, von Rotz RC, Davey G, Moritz E, Nitsch RM (2002) Generation of antibodies specific for  $\beta$ -amyloid by vaccination of patients with Alzheimer disease. *Nat Med* 8:1270-1276.

21. Hock C, Konietzko U, Straffer JR, Tracy J, Signorell A, Muller-Tillmanns B, Lemke U, Henke K, Moritz E, Garcia E, Axel Wollmar M, Umbricht D, de Quervain DJF, Hofmann M, Maddalena A, Paspasotiropoulos A, Nitsch RM (2003) Antibodies against  $\beta$ -amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 38:547-554.

22. Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, Sartorius LJ, Mackey B, Olney J, McKeel D, Wozniak D, Paul SM (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 97:2892-2897.
23. Holtzman DM, Bales KR, Wu S, Bhat P, Parsadanian M, Fagan AM, Chang LK, Sun Y, Paul SM (1999) Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J Clin Invest* 103:R15-R21.
24. Ingelsson M, Fukumoto H, Newell KL, Growdon JH, Hedley-Whyte ET, Frosch MP, Albert MS, Hyman BT, Irizarry MC (2004) Early A[ $\beta$ ] accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. *Neurol* 62:925-931.
25. Jameson BA, Wolf H (1988) The antigenic index: a novel algorithm for predicting antigenic determinants. *Comput Appl Biosci* 4:181-186.
26. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon, RA, Mercken M, Bergeron C, Fraser PE, George-Hyslop P, Westaway D (2000) A $\beta$  peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 408:979-982.
27. Jarrett JT, Berger EP, Lansbury PT, Jr. (1993) The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 32:4693-4697.
28. Johnson LV, Leitner WP, Rivest AJ, Staples MK, Radeke MJ, Anderson DH (2002) The Alzheimer's A beta-peptide is deposited at sites of complement activation in pathologic deposits associated with aging and age-related macular degeneration. *Proc Natl Acad Sci U S A* 99:11830-11835.
29. Kensil CR, Soltysik S, Wheeler DA, Wu JY (1995) Structure-function studies on Qs-21 - a unique immunological adjuvant from Quillaja-Saponaria. *Abstracts of Papers of the American Chemical Society* 210:87-AGFD.
30. Kensil CR, Wu JY, Soltysik S (1995) Structural and immunological characterization of vaccine adjuvant QS-21. *Pharm Biotechnol* 6:525-541.
31. Kida E, Golabek AA, Wisniewski T, Wisniewski KE (1994) Regional differences in apolipoprotein E immunoreactivity in diffuse plaques in Alzheimer's disease brain. *Neurosci Lett* 167:73-76.
32. Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA (1995) Arresting amyloidosis in vivo using small-molecule anionic sulphates or sulphates: implications for Alzheimer's disease. *Nat Med* 1:143-148.
33. Lavie V, Becker M, Cohen-Kupiec R, Yacoby I, Koppel R, Wedenig M, Hutter-Paier B, Solomon B (2004) EFRH-Phage immunization of Alzheimer's disease animal model improves behavioral performance in Morris water maze trials. *J Mol Neurosci* 24:105-114.
34. Lemere CA, Maron R, Selkoe DJ, Weiner HL (2001) Nasal vaccination with beta-amyloid peptide for the treatment of Alzheimer's disease. *DNA Cell Biol* 20:705-711.
35. Lorenzo A, Yankner BA (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc Natl Acad Sci U S A* 91:12243-12247.
36. Ma J, Brewer BH, Potter H, Brewer HB, Jr. (1996) Alzheimer A $\beta$  neurotoxicity: promotion by antichymotrypsin, apoE4; inhibition by A $\beta$ -related peptides. *Neurobiol Aging* 17:773-780.
37. Ma J, Yee A, Brewer HB, Jr., Das S, Potter H (1994) Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 372:92-94.
38. Maestre G, Ottman R, Stern Y, Gurland B, Chun M, Tang MX, Shelanski M, Tycko B, Mayeux R (1995) Apolipoprotein E and Alzheimer's disease: ethnic variation in genotypic risks. *Ann Neurol* 37:254-259.
39. Mathews PM, Nixon RA (2003) Setback for an Alzheimer's disease vaccine - Lessons learned. *Neurol* 61:7-8.
40. Mayeux R, Stern Y, Ottman R, Tatemichi TK, Tang MX, Maestre G, Ngai C, Tycko B, Ginsberg H (1993) The apolipoprotein epsilon 4 allele in patients with Alzheimer's disease. *Ann Neurol* 34:752-754.
41. Merlini G, Ascari E, Amboldi N, Bellotti V, Arbustini E, Perfetti V, Ferrari M, Zorzoli I, Marinone MG, Garini P (1995) Interaction of the anthracycline 4'-iodo-4'-deoxydoxorubicin with amyloid fibrils: inhibition of amyloidogenesis. *Proc Natl Acad Sci U S A* 92:2959-2963.
42. Miller D, Currie JR, Mehta P, Potempska A, Hwang YW, Wegiel J (2003) Humoral immune response to fibrillar  $\beta$ -amyloid peptide. *Biochem* 42:11682-11692.
43. Mirra SS, Gearing M, Nash F (1997) Neuropathologic assessment of Alzheimer's disease. *Neurol* 49:S14-S16.
44. Mirra SS, Hart MN, Terry RD (1993) Making the diagnosis of Alzheimer's disease. A primer for practicing pathologists. *Arch Pathol Lab Med* 117:132-144.
45. Monsonego A, Weiner HL (2003) Immunotherapeutic Approaches to Alzheimer's Disease. *Science* 302:834-838.
46. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW (2001) A $\beta$  peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982-985.
47. Naslund J, Thyberg J, Tjernberg LO, Wernstedt C, Karlstrom AR, Bogdanovic N, Gandy SE, Lannfelt L, Terenius L, Nordstedt C (1995) Characterization of stable complexes involving apolipoprotein E and the amyloid beta peptide in Alzheimer's disease brain. *Neuron* 15:219-228.
48. Nicoll JAR, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 9:448-452.
49. Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C (2003) Subacute meningoencephalitis in a subset of patients with AD after A beta 42 immunization. *Neurol* 61:46-54.
50. Pappolla M, Bozner P, Soto C, Shao H, Robakis NK, Zagorski M, Frangione B, Ghiso J (1998) Inhibition of Alzheimer beta-fibrillogenesis by melatonin. *J Biol Chem* 273:7185-7188.
51. Permann B, Adessi C, Saborio GP, Fraga S, Frossard MJ, Van Dorpe J, Dewachter I, Banks WA, Van Leuven F, Soto C (2002) Reduction of amyloid load and cerebral damage in a transgenic mouse model of Alzheimer's disease by treatment with a  $\beta$ -sheet breaker peptide. *FASEB J* 16:860-862.
52. Permann B, Perez C, Soto C, Frangione B, Wisniewski T (1997) Detection of apolipoprotein E dimeric soluble amyloid  $\beta$  complexes in Alzheimer's disease brain supernatants. *Biochem Biophys Res Commun* 240:715-720.
53. Quinn J (2003) Vascular dementia. *J Amer Med Dir Assoc* 4:S155-S161.
54. Rebeck GW, Reiter JS, Strickland DK, Hyman BT (1993) Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* 11:575-580.
55. Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ, the Memantine Study Group (2003) Memantine in moderate-to-severe Alzheimer's disease. *N Eng J Med* 348:1333-1341.
56. Relkin NR, Tanzi R, Breitner J, Farrer L, Gandy S, Haines J, Hyman B, Mullan M, Poirer J, Strittmatter W, Folstein M, Farlow M, Mayeux R, Petersen R, Roses A, Schenk D, Small G, Van Gool W, Cook-Deegan R, Fleck L, Kapp M, Karlinsky H, Pericak-Vance M, Post S (1996) Apolipoprotein E genotyping in Alzheimer's disease. *Lancet* 347:1091-1095.
57. Robinson SR, Bishop GM, Lee HG, Munch G (2004) Lessons from the AN 1792 Alzheimer vaccine: lest we forget. *Neurobiol Aging* 25:609-615.
58. Sadowski M, Pankiewicz J, Scholtzova H, Ripellino JA, Li Y, Schmidt SD, Mathews P, Fryer JD, Holtzman DM, Sigurdsson EM, Wisniewski T (2004) Blocking the apolipoprotein E/ $\beta$ -amyloid interaction reduces  $\beta$ -amyloid toxicity and decreases  $\beta$ -amyloid load in transgenic mice. *Am J Pathol* 165:937-948.
59. Sanan DA, Weisgraber KH, Russell SJ, Mahley RW, Huang D, Saunders A, Schmechel D, Wisniewski T, Frangione B, Roses AD, Strittmatter WJ (1994) Apolipoprotein E associates with beta amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. *J Clin Invest* 94:860-869.
60. Schenk D (2002) Opinion: Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning. *Nat Rev Neurosci* 3:824-828.
61. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandeventer C, Walker S, Wogulis M, Yednock T, Games D, Seubert P (1999) Immunization with

- amyloid- $\beta$  attenuates Alzheimer disease-like pathology in the PDAPP mice. *Nature* 400:173-177.
62. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90:9649-9653.
63. Shibata M, Yamada S, Kumar S, Calero M, Badling J, Frangione B, Holtzman D, Miller CA, Strickland DK, Ghiso J, Zlokovic B (2000) Clearance of Alzheimer's amyloid- $\beta$  1-40 peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* 106:1489-1499.
64. Sigurdsson EM, Knudsen EL, Asuni A, Sage D, Goni F, Quartermain D, Frangione B, Wisniewski T (2004) Enhanced cognition with a reduced immune response in an AD mouse model immunized with A $\beta$  derivatives. *J Neurosci* 24:6277-6282.
65. Sigurdsson EM, Scholtzova H, Mehta P, Frangione B, Wisniewski T (2001) Immunization with a non-toxic/non-fibrillar amyloid- $\beta$  homologous peptide reduces Alzheimer's disease associated pathology in transgenic mice. *Am J Pathol* 159:439-447.
66. Sigurdsson EM, Wisniewski T, Frangione B (2002) A safer vaccine for Alzheimer's disease? *Neurobiol Aging* 23:1001-1008.
67. Solomon B (2004) Alzheimer's disease and immunotherapy. *Curr Alz Res* 1:149-163.
68. Solomon B, Koppel R, Frankel D, Hanan-Aharon E (1997) Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proc Natl Acad Sci U S A* 94:4109-4112.
69. Soto C, Kascak RJ, Saborio GP, Aucouturier P, Wisniewski T, Prelli F, Kascak R, Mendez E, Harris DA, Ironside J, Tagliavini F, Carp RI, Frangione B (2000) Reversion of prion protein conformational changes by synthetic  $\beta$ -sheet breaker peptides. *Lancet* 355:192-197.
70. Soto C, Kindy MS, Baumann M, Frangione B (1996) Inhibition of Alzheimer's amyloidosis by peptides that prevent  $\beta$ -sheet conformation. *Biochem Biophys Res Commun* 226:672-680.
71. Soto C, Sigurdsson EM, Morelli L, Kumar A, Castaño EM, Frangione B (1998)  $\beta$ -sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy. *Nat Med* 4:822-826.
72. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* 90:1977-1981.
73. Tomiyama T, Shoji A, Kataoka K, Suwa Y, Asano S, Kaneko H, Endo N (1996) Inhibition of amyloid b protein aggregation and neurotoxicity by rifampicin - Its possible function as a hydroxyl radical scavenger. *J Biol Chem* 271:6839-6844.
74. Weiner HL, Lemere CA, Maron R, Spooner ET, Grenfell TJ, Mori C, Issazadeh S, Hancock WW, Selkoe D (2000) Nasal administration of amyloid- $\beta$  peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease. *Ann Neurol* 48:567-579.
75. Weisgraber KH, Mahley RW (1996) Human apolipoprotein E: The Alzheimer's disease connection. *FASEB J* 10:1485-1494.
76. Wisniewski HM, Sadowski M, Jakubowska-Sadowska K, Tarnawski M, Wegiel J (1998) Diffuse, lake-like amyloid-beta deposits in the paraventricular layer of the presubiculum in Alzheimer disease. *J Neuropathol Exp Neurol* 57:674-683.
77. Wisniewski HM, Wegiel J (1995) The neuropathology of Alzheimer's disease. In: Ajax E G. (ed) *Neuroimaging Clinics of North America*. W B Saunders, Philadelphia, pp 45-57.
78. Wisniewski T, Castaño EM, Golabek AA, Vogel T, Frangione B (1994) Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am J Pathol* 145:1030-1035.
79. Wisniewski T, Frangione B (1992) Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci Lett* 135:235-238.
80. Wisniewski T, Ghiso J, Frangione B (1994) Alzheimer's disease and soluble A $\beta$ . *Neurobiol Aging* 15:143-152.
81. Wisniewski T, Golabek AA, Matsubara E, Ghiso J, Frangione B (1993) Apolipoprotein E: binding to soluble Alzheimer's beta-amyloid. *Biochem Biophys Res Commun* 192:359-365.
82. Wisniewski T, Lalowski M, Golabek AA, Vogel T, Frangione B (1995) Is Alzheimer's disease an apolipoprotein E amyloidosis? *Lancet* 345:956-958.
83. Zhang J, Wu X, Qin C, Qi J, Ma S, Zhang H, Kong Q, Chen D, Ba D, He W (2003) A novel recombinant adeno-associated virus vaccine reduces behavioral impairment and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Neurobiol Dis* 14:365-379.