Towards Alzheimer's Disease Vaccination

Beka Solomon*

Department of Molecular Microbiology & Biotechnology, Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Tel-Aviv 69978, Israel

Abstract: Active and passive immunization against fibrillar β-amyloid of various mice models of Alzheimer's disease leads to the disaggregation and inhibition of plaque formation. Preliminary results showing improved behaviour and memory function obtained after administration of anti-β-amyloid vaccines to transgenic mice encourage these and related approaches for testing in the treatment and prevention of Alzheimer's disease.

GENERAL BACKGROUND

Alzheimer's disease (AD), characterized by progressive loss of memory and cognitive function, affects 15 million people worldwide. The incidence increases steadily from 0.5 percent per year at the age of 65 years to nearly 8 percent per year after the age of 85 years. Mutations in the gene for the amyloid precursor protein and the genes for presenilin 1 and 2 cause rare, dominantly inherited, forms of the disease occurring before the age of 60 years, and the ε4 variant of apoliproprotein E is associated with the sporadic form and some familial forms with onset after the age of 60 years [1]. The expected increase in the number of elderly people at risk for Alzheimer's disease and the projected costs involved have led to the consideration of methods to prevent or delay the progression of AD. Prevention of AD requires the development of safe treatments or interventions that could be used in a large number of elderly people at risk, many of whom might never have the disorder. The development of such approaches depends on increasing our knowledge of the pathophysiology of the disease.

More and more evidence shows that Alzheimer's disease, one of the most perplexing medical problems, belongs to the family of conformational diseases [2-4]. These diseases arise when a constituent protein undergoes a change in size or fluctuation in shape, with resultant self-association and tissue deposition, such as amyloid fibrils. Although such changes can occur with normal proteins, there is commonly an interacting genetic contribution, which may sometimes be dominant. The risk of self-association and aggregation, whether or not it is associated with a genetic defect, is greatly increased with proteins that are inherently able to undergo radical changes in their conformation. The conformationally modified protein may be implicated in the disease by direct toxic activity, by the lack of the biological function of the normally folded protein, or by improper trafficking [2-4].

Knowledge of the pathogenesis, treatment or prevention of these presently incurable diseases is limited due to the relative paucity of information regarding the biophysical basis of amyloid formation. At least 15 different polypeptides are known to be capable of causing different forms of amyloidosis via their deposition in particular organs and tissues as insoluble protein fibrils. Despite the diverse nature of the precursor proteins involved in amyloid formation, all amyloid fibrils have characteristic physicochemical, tinctorial and ultrastructural features. Regardless of the nature of the protein constituent, all forms of amyloid are stable assemblies based on noncovalent interactions between subunits forming non-branching fibrils composed of crossed β-sheet structure having diameters of 5-10 nm [review, see 5].

The pathology of Alzheimer's disease is characterized primarily by extracellular plaques and intracellular neurofibrillary tangles. Plaques are mainly composed of the amyloid-β peptide (AβP), whereas tangles are composed of the cytoskeletal protein tau. The relationship between these lesions and the disease process has long been debated. The current dominant theory of AD etiology and pathogenesis is related to the amyloid cascade hypothesis [6-8] which states that overproduction of AβP, or failure to clear this peptide, leads to AD primarily through amyloid deposition, which is supposed to be involved in neurofibrillary tangles formation; these lesions are then associated with cell death which is reflected in memory impairment, the hallmarks of this dementia [9,10]. Over the last eight years, the amyloid cascade hypothesis has gained strength through the observation that AD-causing mutations were identified in the amyloid-β-precursor protein (AβPP) and in the presenilin genes [11,12].

As AβP might be critical for inducing the pathology seen in AD, its accumulation may result in a cascade effect, thereby allowing for intervention at multiple different points to slow disease progression. Treatment may be directed remotely (distantly) by modulating downstream events possibly due to β-amyloid (βA), such as free radical toxicity, decreasing inflammation, preventing cell membrane damage, restoring calcium homeostasis, preventing excitotoxicity, and blocking the cellular response to injury by inhibiting neuronal apoptosis [13]. As an alternative, treatment may be directed towards decreasing AβP production, increasing its removal, and decreasing βA aggregation.

*Address correspondence to this author at the Department of Molecular Microbiology & Biotechnology, Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Tel-Aviv 69978, Israel; e-mail: beka@post.tau.ac.il

1389-5575/02 $35.00+.00

Amyloid β-peptide is a normal metabolite of ~4-kDa that is produced by processing a large transmembrane glycoprotein called amyloid β-protein precursor. Once released by proteolytic cleavage of AβPP the β-peptide may exist in solution [14,15]. The pathological conditions and mechanisms that transform soluble β-amyloid peptide into the fibrillary, toxic, β-sheet form that is found in the plaques and vessels of AD patients is not yet completely understood, but it is clear that the same amino acid sequence of AβP can have both a fibrillar and a soluble conformation.

Many investigators have studied the propensity of AβP or its fragments to assemble into insoluble aggregates [16]. AβP can exist in two alternative conformations, depending on the secondary structure adopted by the N-terminal domain [17,18] under various environmental conditions [19]. The N-terminal domain contains sequences that permit the existence of a dynamic equilibrium between the α-helix and the β-strand conformations [18]. The perturbations of the equilibrium of various conformational states of the β-amyloid peptide can be caused by local pH changes, alterations of environmental hydrophobicity, or binding of other proteins [18,20].

Monoclonal Antibodies Modulate Fibrillar β-Amyloid Formation and Disaggregation

Amyloid filaments, similar to those found in amyloid plaques and cerebrovascular amyloid, can be assembled from chemically synthesized β-peptide under well-defined experimental conditions *in vitro*, and the effect on neural cells may be neurotoxic or neurotrophic, depending on the β-amyloid fibrillar state [21,22]. *In vitro* amyloid formation is a complex kinetic and thermodynamic process and the reversibility of amyloid plaque growth *in vitro* suggests a steady-state equilibrium between AβP in plaques and in solution. [16]. The dependence of AβP polymerization on peptide-peptide interactions to form a β-pleated sheet fibril and the stimulatory influence of other proteins on the reaction suggest that amyloid formation may be subject to modulation.

Recently, the immunological approach in the treatment of conformational diseases has gained more attention. Antibody-antigen interactions involve conformational changes in both antibody and antigen that can range from insignificant to considerable. Binding of high affinity monoclonal antibodies (mAbs) to regions of high flexibility and antigenicity may alter the molecular dynamics of the whole antigen [23,24].

Appropriate mAbs interact with strategic sites where protein unfolding is initiated, thereby stabilizing the protein and preventing further precipitation [25,26]. Monoclonal antibodies were found to stabilize the conformation of an antigen against incorrect folding and recognize an incompletely folded epitope, inducing native conformation in a partially unfolded protein [27-29].

In vitro aggregation of β-amyloid peptide, induced by incubating the peptide for 3 h at 37ºC, was investigated via immunocomplexation with a panel of monoclonal antibodies raised against various β-amyloid fragments. Some of these monoclonal antibodies prevented the formation of β-amyloid and this effect may be related to the localization of the antibody binding sites. Monoclonal antibodies 6C6, 10D5 and AMY-33, which recognize epitopes spanning the amino acid residues 1-28 of β-amyloid peptide, inhibited its aggregation by 40%-90% when compared with aggregation occurring in the absence of the respective antibodies. The antibodies, 2H3 and 1C2, directed to the regions comprising peptides 1-12 and 13-28, respectively, had a considerably low effect [30-32].

Mab 6C6 and/or 10D5 raised against the N-terminal region of the AβP (residues 1-28) can disaggregate βA fibrils and restore the peptide solubility. Binding of such antibodies interfered with noncovalent interactions between the amyloid fibrils and led to deterioration of amyloid fibrillar assembly into an amorphous form, even at molar ratios Ab/peptide 1:10-100. The prevention of peptide aggregation, as well as the solubilization of already formed aggregates, required an equimolar ratio of Ab/peptide, indicating the molecular level of these interactions [30-32].

Antibody engineering methods were applied to minimize the size of mAbs (135-900 kDa) while maintaining their biological activity [33]. These technologies and the application of the PCR technology to create large antibody gene repertoires make antibody phage display a versatile tool for isolation and characterization of single chain Fv (scFv) antibodies [34]. The scFvs can be displayed on the surface of the phage for further manipulation or may be released as a soluble scFv (~25 kd) fragment.

We engineered an scFv which exhibits anti-aggregating properties similar to the parental IgM molecule [35]. For scFv construction we cloned the antibody genes from the anti-AβP IgM 508 hybridoma. The secreted antibody showed specific activity toward the AβP molecule in preventing its toxic effects on cultured PC 12 cells.

Site-directed single-chain Fv antibodies are the first step towards targeting therapeutic antibodies into the brain via intracellular or extracellular approaches. The ability of single chain antibody 508F(Fv) to dissolve already formed βA fibrils suggests that only the antigen binding site of the antibodies is involved in modulation of β-amyloid conformation.

N-terminal EFRH Sequence has a Key Role in Conformational Changes Occurring in β-Amyloid Peptide

The existence of sequences that are kinetically involved in the folding process has previously been suggested in other systems and has been demonstrated by *in vitro* denaturation-renaturation experiments [36]. Such sequences, which may play a role in the folding pathway, suggest the possibility that they serve not only for the folding process but may also contribute to conformational stability.

The disaggregation as well as the prevention of amyloid was found to be dependent on the location of the epitopes on

the β-amyloid and the binding characteristics of the mAbs [31,32].

Identifying the "aggregating epitopes" as sequences that are related to the sites where protein aggregation is initiated, and preparing monoclonal antibodies against these regions, facilitates understanding and prevention of the protein aggregation processes.

Using the phage-peptide library, composed of filamentous phage displaying random combinatorial peptides, we defined the EFRH residues located at positions 3-6 of the N-terminal AβP as the epitope of anti-aggregating antibodies within AβP [37]. The EFRH epitope is available for antibody binding when β-amyloid peptide is either in solution or is an aggregate, and locking of this epitope by antibodies affects the dynamics of all the molecules, preventing self-aggregation as well as enabling resolubilization of already formed aggregates. Identification of the epitope of mAb 2H3, which cannot affect β-amyloid formation despite the fact that it binds to the N-terminal of β-amyloid peptide, shed light on the importance of this specific sequence region, defined as anti-aggregating epitope, on the behaviour of the whole AβP molecule [38].

Immunization Against β-Amyloid with EFRH Phage as Antigen

The EFRH sequence of β-amyloid peptide was found to be involved in modulating the dynamics of aggregation as well as the resolubilization of already formed aggregates. However, such small synthetic peptides are generally poor antigens requiring the chemical synthesis of a peptide and need to be coupled to a large carrier, but even then they may induce a low affinity immune response. We developed a novel immunization procedure for raising anti-AβP antibodies, using as antigen the filamentous phages displaying only EFRH peptide. Filamentous phages are long thread-like single-stranded DNA phages which infect bacteria via sex pili. The best known, the Ff phages, are a group of three phages (M13, fd and fl). The phage protein that is responsible for binding to the pilus tip, pIII, is present in four copies, encoded by gene 3. If foreign DNA that encodes a peptide or protein is inserted downstream of the leader sequence of gene 3, it will be translated and exposed at the N terminus of the mature pIII without compromising the ability of pIII to mediate infection via the F pilus. Although pIII is the protein that has been most used for phage display, the major coat protein, pVIII, has been used in these studies. The main difference between pIII and pVIII is the copy number of the displayed protein, while PIII is present in four copies the pVIII is found in 2700 copies. Filamentous bacteriophages have been used extensively in recent years for the 'display' on their surface of large repertoires of peptides generated by cloning random oligonucleotides at the 5' end of the genes coding for the phage coat proteins [39,40]. As recently reported, filamentous bacteriophages are excellent vehicles for the expression and presentation of foreign peptides in a variety of biological systems [41,42]. Parenteral administration of filamentous phages induces a strong immunological response to the phage coat proteins [43-45].

Immunization with the EFRH-phage may, in a short period of time, raise the high concentration of high affinity (IgG) antibodies able to prevent the formation of β-amyloid and to minimize further toxic effects. The level of antibody in the sera was found to be related to the number of peptide copies per phage [46].

The antibodies resulting from EFRH phage immunization are similar regarding their immunological properties to antibodies raised by direct injection with whole β-amyloid. As shown in Table 1, the antibodies resulting from EFRH phage immunization are similar regarding their biorecognition of whole AβP to monoclonal antibodies previously studied raised against β-peptide 1-28**.** Such antibodies are able to sequestrate the peripheric AβP, thus avoiding passage to the blood brain barrier (BBB) or even, as recently shown using transgenic mice model, to overcome the BBB and to dissolve already formed β-amyloid plaques [47].

These antibodies recognize the full length β-peptide (1-40) and exhibit anti-aggregating properties as antibodies

Table 1. Competitive Inhibition by Various Peptides within AβP of Serum Antibody Raised Against f88-EFRH Compared to Amyloid Anti-Aggregating Antibody*

Peptide	Mice Serum	anti-aggregating antibody*.
FRH (4-6)	~ 10^{-3} M	3 x10^{-3} M
EFRH (3-6)	6.0x10^{-6} M	3x10^{-6} M
DA**EFRH** (1-6)	3.0x10^{-6} M	8x10^{-7} M
DA**EFRH**D (1-7)	5.0x10^{-6} M	9x10^{-7} M
DA**EFRH**DSG (1-9)	5.0x10^{-6} M	1x10^{-6} M
βAP(1-40)	3.0x10^{-6} M	8x10^{-7} M
WVLD	Nd **	Nd **

* Frenkel et. al. 1998

** IC_{50} value of less than 10^{-2} M which cannot be detected by ELISA assay.

raised against Aβ peptide 1-28 and/or β-amyloid [46,48]. The high immunogenicity of filamentous phages enables the raising of antibodies against self-antigens. Immunization of guinea pigs with EFRH-phage as an antigen, in which the AβP sequence is identical to that in humans, resulted in the production of self-antibodies [48].

The recombinant filamentous phage approach to obtain specific peptide antigens has a major advantage over chemical synthesis, as the products obtained are the result of the biological fidelity of translational machinery and are not subject to the 70-94% purity levels common in the solid phage synthesis of peptides. The phage represents an easily renewable source of antigen since more material can be easily obtained by growth of bacterial cultures.

The above data demonstrated that a recombinant bacteriophage displaying a self-epitope can be used as a vaccine to induce autoantibodies for Alzheimer's disease treatment. Filamentous phages are normally grown using a laboratory strain of *E. coli*, and although the naturally occurring strain may be different, it is reasonable to assume that delivery of phage into the gut will result in infection of the natural intestinal flora. We have found that UV inactivated phages are as immunogenic as their infective counterparts. There is evidence of long lasting filamentous phages in the guts of the immunized animals that may explain the long lasting immune response found in pIII immunized mice [49].

Due to the high antigenicity of the phage, administration can be given by the intranasal route, which is the easiest way for immunization without any use of adjuvant. As olfactory changes are proposed to play a role in Alzheimer's disease [50] mucosal immunization is an effective induction of specific AβP IgA antibodies for preventing local pathologic effect of the disease.

The efficacy of phage-EFRH antigen in raising anti-aggregating β-amyloid antibodies [51] versus whole β-amyloid can be summarized as follows:

a. the high immunogenicity of the phage enables production of high titer of IgG antibodies in a short period of weeks without need of adjuvant administration;

b. self-expression of the antigen leads to long-lasting immunization;

c. the key role of the EFRH epitope in β-amyloid formation and its high immunogenicity leads to anti-aggregating antibodies which recognize whole β-amyloid peptide, substituting the use of β-amyloid fibrils.

Performance of Anti-β-Amyloid Antibodies in Transgenic Mice Model of AD

Several labs have bred transgenic mice that develop β-amyloid plaques associated with neuron damage in their brains [reviewed in 52]. Although they do not develop the widespread neuron death and severe dementia seen in the human disease they are used as models for the study of the feasibility of vaccination against AD [47].

Production of anti-β-amyloid antibodies, by immunization with the fibrillar β-amyloid of the PDAPP mouse model of AD [47], led to inhibition of the formation of amyloid plaques and the associated dystrophic neurites in the mouse brain. The transgenic PDAPP mice, based on the amyloid-β precursor protein mini-gene driven by a platelet-derived (PD) growth factor promoter, which overexpresses one of the disease-linked mutant forms of the human amyloid precursor protein, show many of the pathological features of Alzheimer's disease, including extensive deposition of extracellular amyloid plaques, astrocytosis and neuritic dystrophy [53]. These mice were immunized before the onset of AD-type neuropathology (at 6 weeks of age), or when some amyloid deposition has occurred (at 11 months of age). At 13 months, the former group had virtually no amyloid plaques or associated histopathology. In the latter group, amyloid burden, neuritic dystrophy and astrogliosis was also significantly reduced in the βA 1-42 treated group after 4 and 7 months treatment. Another set of experiments showed that peripheral administration of antibodies against amyloid β-peptide was sufficient to reduce amyloid burden in the affected mice brains [54]. Following these reports, Weiner *et al.* [55] demonstrated that intranasal immunization with freshly solubilized βA 1-40 reduced cerebral amyloid burden in the PDAPP mouse. However, because of the low immunogenicity of the βA fibrils, repeated antigen administration in the presence of adjuvant is required to obtain the anti-AβP antibodies necessary to affect plaque formation. Moreover, immunizing with toxic fibrils may induce more accumulation of the toxic amyloid itself.

Sigurdsson *et al.* [56] injected homologue poly-lysine (K6), K6-AβP 1-30, which failed to undergo any detectable fibrillization on incubation for 2 weeks and was also non-toxic to neuronal cell cultures. This peptide was designed to reduce fibrillogenic potential and enhance immunogenicity while maintaining the major immunogenic sites of βA peptides supposed to be the residues 1-11 and 22-28. The immunization of Tg APP mice (Tg2576, APP695(K670N+M671L) for 7 months with K6AβP(1-30)-NH_2, a non-amyloidogenic, non-toxic βA homologous peptide, reduced cortical and hippocampal brain amyloid burden by 89% and 81%, respectively. These promising findings suggest that immunization with non-amyloidogenic βA derivatives represents a potentially safer therapeutic approach to reduce amyloid burden in AD, instead of using toxic βA fibrils.

Poly-L-lysine enhances immunogenicity, and the coupling of lysine residues to the C-terminus of short βA sequences within the 15-25 domain of βA has recently been proposed by Pallitto *et al.* [57] in the design of anti-β-sheet peptides or βA fibrillogenesis inhibitors.

Lemere *et al.* [58] developed an active immunization approach in which the immunogen (a combination of βA 40 and βA 42) was administered via alternative routes, including nasal mucosa. Significant titers of antibodies were achieved which, when mapped to overlapping 15-mers of

βA, were primarily directed towards the N-terminal 15 amino acids of βA. Different strains of mice showed marked differences in the titers generated against βA, a variable that should be noted as various APP transgenic mice on different background strains are subjected to these immunization protocols.

Cognitive Improvement of Immunized Transgenic Mice

Appropriate animal models were used to test the effects of anti-β-amyloid antibodies on both brain damage and cognitive losses caused by Alzheimer's disease. Indeed, immunization with β-amyloid peptide improves learning and memory, as well as diminishing brain damage in AD animal models [59,60]. Recently, Morgan *et al.* [60] provided valuable data regarding the potential safety and efficacy of βA vaccination. Specifically, they offered evidence in a mouse model of AD that inoculation with βA peptide had no deleterious effects on performance in a novel working memory task in adult mice (l l.5 months) and could prevent the age-related performance decline in this task when the mice were retested at a later age (15.5 months) following monthly inoculations. The authors also reported (without data shown) that the vaccination had no significant effects on motor performance in several tasks. Because of the potential impact of these findings on vaccine development for AD, the details of this report should be reviewed to appreciate its strengths and possible weaknesses. The results support a previously observed reduction in the formation of amyloid deposits, but they go further to show that immunization also offered to the mice some protection from the 'spatial' learning deficits that normally accompany plaque formation. Both groups [59,60] suggest that either a small or selective reduction in β-amyloid deposition may be sufficient to protect against dementia. Each group used different tests of spatial memory, in which mice had to swim to and mount a platform located invisibly beneath the surface of a pool of water. It is remarkable that both groups find that immunization with β-amyloid peptide offers significant protection from the age- and amyloid-dependent performance deficits seen in non-immunized controls.

These findings indicate that βA overexpression and/or βA plaques are associated with disturbed cognitive function and, importantly, suggest that some but not all forms of learning and memory are suitable behavioural assays of the progressive cognitive deficits associated with Alzheimer's disease type pathologies.

Putative Mechanisms of Plaque Clearance

Active and passive immunization with human amyloid β-peptide reduces plaque burden and its associated pathology. Several hypotheses have been proposed regarding the mechanism of this response.

Despite their relatively modest serum levels, the passively administered antibodies were able to enter the central nervous system, decorate plaques and induce clearance of pre-existing amyloid. Small amounts of such antibodies that cross the blood–brain barrier (0.1% of serum levels) might be sufficient to attenuate the further aggregation of these species into fibrillar βA dense-cored plaques. Because this pool of βA is small, and the antibodies to this form of βA might only be needed to inhibit the assembly of βA fibrils to have a functional effect, these antibodies need not necessarily cause large changes in total cerebral βA. These antibodies convert βA, the dense-cored plaques, to diffuse βA deposits.

These results indicate that antibodies can cross the BBB to act directly in the central nervous system (CNS) and should be considered as a therapeutic approach for the treatment of AD and other neurological disorders.

By comparison, of the antibodies tested only mAbs 10D5, 3D6 and PabA$\beta_{1\text{-}42}$, directed to the N-terminal regions of AβP demonstrated efficacy *in vivo*. In contrast, mAbs16C11, 21F12 and the control antibody TM2a, directed to other regions of AβP, were inactive. This result is consistent with the inability of these two antibodies to decorate plaques after *in vivo* administration and explains their inability to trigger plaque clearance [54]. These *in vivo* data confirm the previous *in vitro* data [30,32] that only antibodies directed to EFRH, exhibit so-called 'chaperone-like' properties in dissolving the plaques and preventing their formation. These data also confirm that only a small amount of antibodies is necessary to interfere with non-covalent interactions between βA fibrils to disaggregate them into an amorphous non-toxic configuration [32], suggesting the *in vivo* mechanism of dissolving and removal of amyloid plaques.

The antibodies are likely to have their effect by enhancing clearance of βA by a variety of mechanisms. One pathway may be via microglial activation following antibody binding to βA plaques. Their effect may also be in part caused by binding to soluble βA within the brain that alters the equilibrium between deposited βA vs. soluble βA, resulting in enhanced clearance of deposited βA.

The antibody Fc-mediated microglial clearance was demonstrated using an *ex vivo* phagocytosis assay [54]. Fab fragments clearly bind to βA deposits but do not promote βA clearance . It is conceivable, however, that immunization might modulate βA metabolism through several distinct mechanisms, including destruction of βA by microglial phagocytosis [47] or by effective function of the antibody to activate Fc receptors able to remove the whole immunocomplex of fibrillar βA with site-directed antibodies only towards sequences related to aggregation process [Solomon, B., personal communication].

Another possibility is that the antibodies neutralize βA in some restricted compartment or deplete a non-deposited form of βA (for example, a soluble form) that is responsible for the memory loss observed [60]. Recently, soluble βA has been proposed as the cause of synapse loss in APP transgenic mice, as some transgenic lines develop reductions in synaptophysin immunoreactivity in dentate gyrus without developing βA deposits. A further possibility is that microglia activated by the antibodies can clear the deposited βA, thereby permitting normal cognitive function [61]. An alternative explanation is that immunization affects βA in a

particular conformation, like β-sheet forms in protofibrils. The former is more likely because of oligomeric assemblies of βA in β-sheets ('protofibrils') as an immunogen, and the resultant antisera preferentially recognized β-sheet forms of βA. This is significant because monoclonal antibodies raised to βA epitopes that initiate fibril aggregation inhibit assembly of synthetic βA oligomeric protofibrils *in vitro* [30]. It is possible, therefore, that the antibodies induced in the transgenic mice may bind to β-sheet oligomeric aggregates and inhibit further assembly. This βA species is especially neurotoxic, a critical intermediary in fibrillogenesis and an accurate predictor of neurodegeneration.

A major pathway in the clearance of cerebral βA may be the binding of antibodies to soluble βA in peripheral fluids [62]. The subsequent reduction in peripheral βA levels alters the equilibrium between βA found within and outside the CNS that may result in efflux of βA out of the CNS. This possibility is supported by the fact that most antibodies are circulating in peripheral tissues and the Tg2576 mice have high levels of βA not only within the CNS but also in the periphery. Furthermore, antibodies to non-fibrillar βA derivatives may have higher affinity for soluble βA than those generated against fibrillar βA. It has been suggested that the effect of immunization with aged AβP 1-42 is via the generation of antibodies specifically against fibrillar βA. However, the successful generation of specific antibodies against amyloid fibrils is rare. Recent reports show that in the Tg2576 mice, plasma levels of βA decrease as cerebral plaque burden increases [56]. This suggests an interaction between these two compartments that can be manipulated. Monitoring of plasma levels of βA throughout the immunization procedure in addition to comparing the plasma half-life of AβP 1-40/42 in immunized vs. non-immunized mice may give some insight into this important issue. The clearance of βA from the circulation may be altered if soluble plasma βA is to some extent bound to antibodies in the immunized mice. This data, together with antibody affinity for soluble vs. fibrillar βA, the BBB permeability of βA antibodies and their binding motif (soluble, amorphous or fibrillar βA) will clarify possible interactions between antibodies and βA within the CNS and in the peripheral system that may affect amyloid burden within the brain. These experiments will address the feasibility of targeting βA peripherally to reduce amyloid burden within the brain, which may result in therapy with fewer side effects because CNS entry will not be needed. The importance of this view was recently confirmed by DeMattos and colleagues [62]. They used the PDAPP Tg model that expresses human AβPP within the CNS but not in the periphery. Following i.v. injection of anti-βA antibody, plasma- and cerebrospinal fluid levels of βA increased acutely whereas no antibodies were observed labeling the plaques within the brain. The overall effect of the peripheral administration of the anti-βA antibody was to reduce amyloid plaque burden within the brain, presumably by increasing efflux of βA peptide outside the brain [62]. These findings need to be confirmed in the more widely used Tg2576 model which expresses human AβPP both within the CNS and in peripheral organs. Humans express AβPP at much lower levels than the Tg mice and, therefore, have lower circulating levels of βA. Monitoring of plasma βA levels in ongoing clinical safety studies on βA immunization will be used to determine if a similar phenomenon may occur in humans.

In summary, immunization with AβP greatly reduced the development of AD-like pathology. Immunization preceding plaque development profoundly affected the occurrence of new lesions, as the progression of β-amyloidosis and associated neuropathology was essentially wholly blocked, as seen both in the entire brain of the young animals and in at-risk brain regions of the older animals. Amyloid-β immunization also significantly retarded the progression of existing pathology in affected regions of the older animals. Outcomes of βA-plaque burden, neuritic dystrophy and gliosis were all significantly improved by AβP treatment in both young and old animals. Histological examination of several organs, including brain and kidney, revealed no signs of immune-mediated complications, despite the high levels of human AβPP expressed in these tissues and the significant antibody titre to endogenous mouse βA peptide.

Nonetheless, the data presented support the hypotheses that βA plays a central role in Alzheimer's disease and that the procedures that modulate its production, assembly and/or removal might be used as treatments. In view of the absence of adverse effects on behaviour and brain functioning, and the protection of memory functions by the βA vaccines in mice [60], these and related approaches should be tested for the treatment and prevention of Alzheimer's disease.

A brief update of the status of Elan's initial (Stage I) clinical trials with AN1792 (βA 1-42) showed that a single-dose escalation study has been completed, principally designed to look at safety and tolerability. A multi-dose escalation study is under way to show how immunization with AN1792 will be tolerated.

REFERENCES

[1] Mayeux, R.; Sano, M. *The NEJM,* **1999**, *341*, 1670-1679.

[2] Thomas, P.J.; Qu B-H.; Pedersen, P.L *Trends Biochem. Sci*., **1995,** *20***,** 456-459.

[3] Kelly, J.W. *Curr. Opin. Struct. Biol*., **1996.** *6,* 11-17.

[4] Carrell, R.W.; Lomas, D.A. *Lancet,* **1997**, *350*, 134-138.

[5] Wetzel, R. *Trends. Biotechnol*., **1994,** *12*, 193-198.

[6] Hardy, J.; Allsop, D.. *Trends Pharmacol Sci*., **1991,** *12***,** 18295-18298.

[7] Selkoe, D.J. *J. Biol. Chem.,* **1996,** *271*, 18, 295-18298.

[8] Hardy, J. *Trends Neurosci.,* **1997**, *20*, 154-159.

[9] Goate, A.; Chartier-Harlin, M-C.; Mullan, M.; Brown, J.; Crawford, F.; Fidani, L.; Giuffra, L.; Haynes, A.; Irving, N.; James, L.; Mant, R.; Newton, P.; Rooke, K.; Roques, P.; Talbot, C.; Pericak-Vance, M.; Roses, A.; Williamson, R.; Rosser, M.; Owen, M.; Hardy, J. *Nature,* **1991,** *349*, 704-706.

[10] Hardy, J.; Duff, K.; Hardy, K.G.; Perez-Tur, J.; Hutton, M. *Nat. Neurosci.,* **1998**,*1,* 355-358.

[11] Sherrington, R.; Rogaev, E.I.; Liang, Y.; Rogaeva, E.A.; Levesque, G.; Ideda, M.; Chi, H.; Lin, C.; Li, G. *et al. Nature,* **1995,** *375,* 754-760.

[12] Levy-Lahad, E.; Wasco, W.; Poorkaj, P.; Romano, D.M.; Oshima, J.; Pettingell, W.H.; Yu, C-E.; Jondro, P.D.; Schmidt, S.D.; Wang, K.; Crowley, A.C.; Fu, Y-H.; Guenette, S.Y.; Galas, D.; Nemens, E.; Wijsman, E.M.; Bird, T.D.; Schellenberg, G.D.; Tanzi, R.E. *Science,* **1995,** *269***,** 973-977.

[13] Sabbagh, M.N.; Galasko, D.; Thal, L.J.. *Alzheimer's Disease Review*, **1998,** *3*, 1-19.

[14] Haass, C., Schlossmacher, M.G., Hung, A.Y.; Vigo-Pelfrey, C.; Mellon, A.; Ostaszewski, B.L.; Lieberburg, I.; Koo, E.H.; Schenk, D.; Teplow, D.B. *et al. Nature,* **1992,** *359(6393)* 322-5.

[15] Seubert, P.; Vigo-Pelfrey, C.; Esch, F.; Keem J.; Dovey, H.; Davis, D.; Sinha, S.; Schlossmacher, M.; Whaley, J.; Swindelhurst, C. et al.. *Nature,* **1992,** *359(6393),* 325-7.

[16] Maggio, J.E.; Mantyh, P.W. *Brain. Pathol.,* **1996**, *6,* 147-162.

[17] Hollosi, M.; Otvos, L.; Kaijtar, J.; Percell, A.; Andlee, V.M.Y. *Pept. Res.,* **1989** *2,* 109-113.

[18] Soto, C.; Castano, E.M.; Frangione, B.; Inestrosa, N.C., *J. Biol. Chem.,*. **1995**, *270*, 3063.

[19] Barrow, C.J.; Zagorski, M.G. *Science*, **1991,** *253*, 179-182.

[20] Kirshenbaum, K.; Daggett, V. *Biochemistry,* **1995,** *34*, 7629-7639.

[21] Lorenzo, A.; Yankner, B.A. *Proc. Natl. Acad. Sci. USA,* **1994 ,** *1*, 12243-12247.

[22] Howlett, D.R.; Jennings, K.H.; Lee, D.C.; Clark, M.S.G.; Brown, F.; Wetzel, R.; Wood, S.J.; Camilleri, P.; Roberts, G.W. *Neurodegeneration,* **1995**, *4,* 23-32.

[23] Frauenfelder, H.; Petsko, G.A.; Tsernoglou, D. *Nature*, **1979,** *280*, 558-563.

[24] Karplus, M.; Petsko, G.A. *Nature,* **1990**, *347*, 631-639.

[25] Solomon, B.; Balas, N. *Biotechnol. and App. Biochem.,* **1991**,*4,* 202-211.

[26] Katzav-Gozansky, T.; Hanan, E.; Solomon, B. *Biotechnol. Appl. Biochem.,* **1996,** *23*,227-230.

[27] Blond, S.; Goldberg, M. *Proc. Natl. Acad. Sci. USA,* **1987,** *84*, 1147-1151.

[28] Carlson, J.D.; Yarmush, M.L. *Biotechnology,* **1992**, *10,* 86-91.

[29] Solomon, B.; Schwartz, F. *J. Molec. Recognition.,* **1995,** 72-76.

[30] Solomon, B.; Koppel, R.; Hannan, E.; Katzav, T. *Proc. Natl. Acad. Sci. USA,* **1996,** *93,* 452455.

[31] Hanan, E.; Solomon, B. *Amyloid: Int. J. Exp. Clin. Invest*., **1996,** *3*, 130-133

[32] Solomon, B.; Koppel, R.; Frankel, D.; Hanan-Aharon, E. *Proc. Natl. Acad. Sci. USA,* **1997,** *94,* 4109-4112.

[33] Winter, G.; Griffiths, A.D.; Hawkins, R.E.; Hoogenboom, H.R. *Annu. Rev. Immunol.,* **1994,** *12***,** 433-455.

[34] Hoogenboom, H.R.; De Bruine, A.P.; Hufton, S.E.; Hoet, R.M.; Arends, J.W.; Roovers, R.C. *Immunotechnol.,* **1998,** *4,* 1-20.

[35] Frenkel, D.; Solomon, B.; Benhar, I. *J Neuroimmunol*., **2000a**, *106*:23-31.

[36] Silen, J.L.; Agard, D.A. *Nature,* **1989,** *341***,** 462-464.

[37] Frenkel, D.; Balass, M.; Solomon, B. *J Neuroimmunol*., **1998**, *8*, 85-90.

[38] Frenkel, D.; Balass, M.; Kachalsky-Katzir, E.; Solomon, B. *J. Neuroimmunol*., **1999,** *95*, 136-142.

[39] Scott, J.K.; Smith, G.P. *Science,* **1990**, *249***,** 386-390.

[40] Scott, J.K. *Trends in Biochem. Sci.,* **1992**, 241-245.

[41] Greenwood, J.; Willis, E.A.; Perham, N.R. *J. Mol. Biol*., **1991**, *220,* 821-827.

[42] Medynski, D. *Biol. Technol.,* **1994**, *12,* 1134-1136.

[43] Willis, E.A.; Perham, N.R.; Wraaith, D. *Gene,* **1993***, 128***,** 79-83.

[44] Meola, A.; Delmastro, P.; Monaci, P.; Luzzago, A.; Nicosia, A.; Felici, F.; Cortese, R.; Galfre, G. *J. Immunol.*, **1995,** *154,* 3162-3172.

[45] Bastein, N.; Trude, M.; Simard, C. *Virology,* **1997**, *234*, 118-122.

[46] Frenkel, D.; Katz, O.; Solomon, B. *Proc. Natl. Acad. Sci.USA,* **2000b,** *97*, 21, 11455-11459.

[47] 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.; Zhenmei, L.; Lieberburg, I.; Motter, R.; Mutter, L.; Soriano, F.; Shopp, G.; Vasquez, N.; Vandevert, C.; Walker, S.; Wogulis, M.; Yednock, T.; Games, D.; Seubert, P. *Nature,* **1999,** *400*, 173-177.

[48] Frenkel, D.; Kariv, N.; Solomon, B. *Vaccine*, **2001**, *19*, 2651-2619.

[49] Zuercher, A.W.; Miescher, S.M.; Voge, M.; Rudolf, M.P.; Stadler, M.B.; Stadler, B.M. *Eur. J. Immunol.,* **2000,** *30*, 128-135.

[50] Murphy, C. *Physiol. Behav.,* **1999,** *66***,** 177-182.

[51] Solomon, B.; Frenkel, D. *Drugs of Today,* **2000,** *36*(9), 655-663.

[52] Van Leuven, F. *Prog. Neurobiol.,* **2000,** *200061(3),* 305-12. Review.

[53] Games, D.; Adams, D.; Alessandrini, R.; Barbour, R.; Berthelette, P.; Blackwell, C.; Carr, T.; Clemens, J.;

Donaldson, T.; Gillespie, F. *et al. Nature, (London)* **1995**, *373*, 523-527.

[54] Bard, F.; Cannon, C.; Barbour, R.; Burke, R-L.; 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. *Nat. Med*., **2000**, *6*, 916-920.

[55] Weiner, H.L.; Lemere, C.A.; Maron, R.; Spooner, E.T.; Grenfell, T.J.; Mori, C.; Issazadeh, S.; Hancock, W.W.; Selkoe, D.J. *Ann. Neurol.*, **2000**, *48*, 567-579.

[56] Sigurdsson, E.M.; Scholtzova, H.; Mehta, P.D.; Frangione, B.; Wisniewski, T. *American J. Pathol.*, **2001** (In press).

[57] Pallitto, M.M..; Ghanta, J.; Heinzelman, P.; Kiessling, L.L.; Murphy, R.M. *Biochemistry*, **1996**, *38*, 3570-3578.

[58] Lemere, C.A.; Maron, R.; Spooner, E.T.; Grenfell, T.J; Mori, C.; Desai, R.; Hancock, W.W.; Weiner, H.L.; Selkoe, D.J. *Ann. N.Y. Acad. Sci.*, **2000**, *920*, 328-31

[59] Janus, C.; Pearson, J.; Mclaurin, J.; Mathews, P.M.; Jiang, Y.; Schmidt, S.D.; Azhar Chisti, M.; Horne, P.; Heslin, D.; French, J.; Mount, H.T.J.; Nixon, R.A.; Mercken, M.; Bergeron, C.; Fraser, P.E.; St. George-Hyslop, P.; Westaway, D. *Nature*, **2000,** *408*, 979-982.

[60] Morgan, D.; Diamond, D.M.; Gottschall, P.E.; Ugen, K.E.; Dickey, C.; Hardy, J., Duff, K.; Jantzen, P.; Dicarlo, G.; Wilcock, D.; Connor, K.; Hatcher, J.; Hope, C.; Gordon, M.; Arendash, G.W. *Nature*, **2000,** *408*, 982-985.

[61] Mucke, L.; Masliah, E. Yu, GO; Mallory, M.; Rockenstein, E.M.; Tatsuno, G.; Hu, K.; Kholodenko, D.; Johnson-Wood, K.; McConlogue, L.J. *Neurosci.*, **2000,** *20(11)* 4050-8.

[62] DeMattos, R.B.; Bales, K.R.; Cummins, D.J.; Dodart, J.C.; Paul, S.M.; Holtzman, D.M. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 8850-8855.